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Title: EFFECT OF MERCURY ON β-GALACTOSIDASE ACTIVITY: POSSIBLE RELATIONSHIP TO LACTOSE INTOLERANCE

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The ability of mercury to inhibit β-galactosidase activity from cell-free extracts of Streptococcus lactis, Escherichia coli and intestinal homogenates of hogs, female rats, and humans was studied. Mercuric acetate was added in vitro to all sources of β-galactosidase except for the rats, which were placed on a diet of 6ppm or 300ppm mercuric chloride. The rat study also included the determination of the mercury content of blood, liver, kidney and intestinal fractions and food consumption and weight gain patterns.

The β-galactosidase from hog mucosa and E. coli had to be partially purified before the assay would detect the near 50% inhibition resulting from 1.0ppm and 0.16ppm mercuric acetate, respectively.

Intestinal homogenates from both rats and humans contained a low-molecular weight substance that interferred with the assay

results. Once this effect was overcome by modifying the reagents or dialysis of the samples, approximately 50% inhibition of β -galactosidase was observed from rat and human mucosa by less than 0.005 ppm mercuric chloride (determined by atomic absorption spectrometry) and 33ppm mercuric acetate, respectively.

Rats on a mercuric chloride diet showed a cyclic inhibition response pattern of β -galactosidase activity. In addition, rats on a 6ppm mercuric chloride diet showed no appreciable difference in food consumption or weight gain in comparison to the controls, whereas the rats on a 300ppm mercuric chloride diet showed approximately a 20% decline in both food consumption and weight gain. The cyclic phenomenon and partial protective effect against mercuric chloride poisoning observed was considered to be the result of hormonal influence.

The data suggest that the enzyme assay techniques employed are sufficiently sensitive for use in establishing a clinical test to diagnose mercurialism at a very early stage, if samples are partially purified.

Effect of Mercury on β-Galactosidase Activity: Possible Relationship to Lactose Intolerance

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INTRODUCTION

Within recent years, people have become increasingly aware of various forms of pollution and one of the primary concerns has been the presence of heavy metals in the environment. Mercury, a protoplasmic poison, has received a lot of publicity because it can be lethal to man, animals and plants. Its ultimate effect is the inhibition of various enzyme systems (Stahl, 1969).

Currently, the United States consumes 3,000 tons of mercury per year (Stahl, 1969) which is used in the manufacture of: (1) electrical apparatuses (23 percent); (2) chlorine and caustic soda (23 percent); (3) mildew proofing compounds (14 percent); and (4) others (40 percent) (FDA, 1970). The major source of mercury pollution in the air is coal burning (Joselow, Louria and Browder, 1972) and chemical plant effluents are the major source of mercury pollution in waterways (FDA, 1970).

Serious illness has resulted from inhalation of air comtaminated with mercury (Heimann, 1967), ingestion of seed treated with mercurial fungicides (Jalili and Abbasi, 1961), ingestion of meat from an animal which had been fed contaminated seed (Curley, Sedlak and Girling, 1971) and consumption of fish from contaminated

waters (Kurland, Faro and Siedler, 1960). The latter form of poisoning can occur after the various forms of mercury discharged into the water is converted to organic mercurial compounds by aquatic microorganisms. In this manner, mercury can enter the food chain and subsequently be ingested by the fish (Dales, 1972).

One of the major problems in treating mercury poisoning is the difficulty of diagnosis because the symptoms are often vague. Currently, there are no efficient, confirmatory laboratory tests to facilitate diagnosis of this disease (Goldwater, 1964; Rentos and Seligman, 1968; and Ladd, Goldwater and Jacobs, 1963).

Recently, the treatment of a patient with chronic mercurial intoxication led to a secondary diagnosis of lactose intolerance, a deficiency in the enzyme β-galactosidase (Enzyme Commission Number 3.2.1.23; β-D galactoside galactohydrolase) which splits lactose (milk sugar) into glucose and galactose. In retrospect, the patient noted that milk intolerance was one of the first symptoms exhibited while exposed to mercury and one of the last symptoms to regress after removal from the source of mercury. This medical observation is interesting because it may represent an enzymatic defect that can be used to diagnose mercury poisoning before other symptoms develop.

This paper concerns the effect of mercury on β-galactosidase and whether or not this enzyme can be used as a method for the

determination of mercurial intoxication. The effects of mercury on the β-galactosidase activity of Streptococcus lactis 7962, Escherichia coli and intestinal tissue of rats, hogs and humans were studied. In addition, the rat study also included determination of the mercury content of blood, liver, kidney and intestinal fractions as well as food consumption and weight gain patterns.

LITERATURE REVIEW

Mercury is ubiquitous in nature, occurring in air, water, soil, plants, animals, man (FDA, 1970) and even lunar soil (Reed, Goleb and Javanovic, 1971). There is evidence that mercury was mined at least two or three milleniums ago in China, the Cyclades and Peru. The Chinese first used mercury as a red pigment in inks and paints (Goldwater, 1971). By 1000 A.D., mercury compounds were being used to treat chronic skin ailments (Bligh, 1972) and by the middle ages it was used as a cathartic and for the treatment of syphilis (Goldwater, 1971).

Mercury has many unique and useful properties: liquid at normal temperatures; high density and surface tension; uniform volume of expansion; alloys readily; good electrical conductivity; chemical stability and toxicity of its compounds (Bligh, 1972). These characteristics have led to its use in electrical apparatuses, paints, industrial and control instruments, pharmaceuticals, catalysts, amalgamation, dental preparations, agricultural chemicals, electrolytic preparation of chlorine and caustic soda and redistillation (Stahl, 1969).

As the result of large scale use in agriculture and industry, a considerable amount of mercury is being released into the environment (Goldwater, 1971). Severe cases of mercury poisonings in

Japan (Kurland, Faro and Siedler, 1960) from the comsumption of fish from contaminated waters prompted investigations of the extent and control of environmental pollution in many countries, including the United States (Bligh, 1972; FDA, 1970; and Stahl, 1969).

Sources of Mercury

Natural

Mercury is not abundantly distributed in the earth's crust. It is estimated that the outer 16 KM-thick layer contains 2.7×10^{-6} percent mercury, which occurs primarily as mercury sulfide (red cinnabar) (FDA, 1970). The major deposits in the world are located in Spain, Italy, the United States, Mexico, China and the Union of Soviet Socialist Republic (Stahl, 1969). The natural background level of mercury in the environment is: 0.01 to 0.06 ppm in soil; 0.01 to 0.09 ppm in rocks; 2 to 5 ng/M^3 in air; 0.002 to 0.006 ppm in water and beverages; and 0.003 to 9.8 ppm in foods (FDA, 1969).

Pollution Sources

Probably the major source of environmental pollution is mercury vapors which originate from the burning of coal (Joselow, Louria and Browder, 1972), mining (Stahl, 1969) and city incinerators (cited in Science News 99:280, 1971). Another hazard is the spillage of mercury and the difficulty often experienced in recovery.

Droplets of mercury scatter and become entrapped in crevices and the amount of mercury vapor released is greatly increased because of the increased surface area. The rate of vaporization of mercury greatly increases with an increase in temperature. Atmospheric concentration will nearly double with a 10° C increase. At room temperature the saturation concentration of mercury vapor in the air is approximately 130 to 180 times greater than the accepted Threshold Limit Value (TLV) for an 8-hour work day. Consequently, it is possible that the concentration of mercury in the air could reach a toxic level (Stahl, 1969).

Until the 1970's, the major source of mercury pollution in water was effluents from chlor-alkali plants using mercury as a cathode. Legislation has now sharply curtailed the release of mercury compounds into waterways (Wallace, Fulkerson and Shults, 1971). In addition, most mills in the pulp and paper industry have discontinued using mercury compounds as slimicides in the preparation of wood. Agriculture has also largely discontinued the use of mercurial compounds as antifungal agents. Even though preventive legislation has been enacted, a mercury water pollution problem still exists. A large reservoir of mercury has accumulated which can be slowly released by conversion to organic mercury compounds by aquatic microorganisms. It has been estimated that it will take

at least 100 years for polluted waters to become naturally cleaned (Joselow, Louria and Browder, 1972).

Toxicity of Mercury and Derivative Compounds

Elemental mercury and derivative compounds can be absorbed through the usual routes of inhalation, ingestion and skin absorption (Joselow, Louria and Browder, 1972). The toxicity of mercury is dependent on the amount and rate of absorption, physiochemical properties of the compound and individual susceptibility (Stahl, 1969). Toxicologically, mercury compounds are usually classified as inorganic or organic (Joselow, Louria and Browder, 1972).

Inorganic Mercury

Elemental mercury passes across the cell membrane and localizes in tissues with a high lipid content (Hughes, 1957). It readily passes through alveolar tissue (Berlin, Nordberg and Serenius, 1969; and Teisinger and Fiserova, 1965) and can readily diffuse from the blood into tissues (Berlin, Fazackerley and Nordberg, 1969). For long-term exposure, the central nervous system is first affected after elemental mercury is oxidized to mercuric ions (Clarkson, 1968; and Rothstein, 1971). This conversion favors the involvement of the kidneys as a storage and elimination site (Friberg, Hammarstrom and Nystrom, 1953; and Hunter, 1969).

The salts of inorganic mercury compounds are readily absorbed through the lungs, alimentary tract or skin. Mercury is eliminated primarily by urinary and fecal routes (Joselow, Ruiz and Goldwater, 1968) and the highest concentration is normally found in the kidneys (Friberg, Hammarstrom and Nystrom, 1953; and Hunter, 1969). The symptoms of inorganic mercury poisoning are oral cavity disorders, tremors and erethism (Joselow, Louria and Browder, 1972). Sometimes a patient also exhibits a variety of non-specific symptoms such as: fatigue, weakness, pallor, anorexia, weight loss, and gastrointestinal problems (Bidstrup, 1964).

Organic Mercury

Organic mercury compounds can be divided into two categories: (1) those that are easily broken down to yield inorganic mercury; and (2) those that maintain the carbon-mercury bond (Joselow, Louria and Browder, 1972). The former group is more easily absorbed than the inorganic salts because of greater solubility and volatility (Clarkson, 1968; and Hughes, 1957). After the carbon-mercury bond is cleaved, these compounds are distributed in a similar manner as the inorganic mercury compounds (Ellis and Fang, 1967; Prickett, Laug and Kunze, 1950, and Ulfvarson, 1962). Clarkson (1968) has shown that the toxicity of these compounds may be directly related to their breakdown to inorganic mercury.

The alkyl mercury compounds have at least one strong covalent bond with a carbon atom and this bond does not readily dissociate within the body (Ulfvarson, 1969). These compounds readily pass through membranes and bind to sulfhydryl groups of proteins (Hughes, 1957; and Rothstein, 1971) and also pass through the placental barrier and accumulate in the fetus (Suzuki, Matsumoto and Miyama, 1967; and Suzuki, Miyama and Katsunuma, 1971). On ingestion, about 90 percent of the mercury is absorbed through the intestine (Ekman, Greitz and Magi, 1968; and Ekman, Greitz and Persson, 1968). Organic mercury concentrates in the liver, blood, brain, hair and epidermis (Ekman, Greitz and Magi, 1968; Gage and Swan, 1961; and Lundgren, Swensson and Ulfvarson, 1967). Elimination of alkyl mercury compounds occurs primarily through the feces (Ekman, Greitz and Persson, 1968).

Symptoms of alkyl mercury poisoning are: motor incoordination; loss of position sense and the sense of distinguishing the form of objects by touch; concentric constriction of visual fields; hearing loss of the central type; emotional disturbance; muscular spasticity or rigidity; and loss of intellectual capacity (Dales, 1972).

Diagnosis

Diagnosis of mercurialism is difficult because the correlation between the levels of mercury found in the blood or urine and the

clinical symptoms is poor (Goldwater, 1964; Ladd, Goldwater and Jacobs, 1963; and Rentos and Seligman, 1968). Thus, a person suffering from mercury poisoning who exhibits neurological symptoms may never be diagnosed as having mercurialism (Stahl, 1969). At present, the diagnosis of mercury poisoning is commonly based on the clinical symptoms plus a proven history of exposure to mercury.

Treatment

Inorganic Mercury Poisoning

Elimination of mercuric ion is the objective in treatment of mercurialism. Patients suffering from chronic mercurial intoxication are often removed from the source of mercury to see if the symptoms will regress with normal urinary and fecal excretion rates. Metal chelating agents have been used to treat cases of mercurialism by increasing the urinary excretion rate of mercuric ion. The compounds being used currently are: dimercaprol (2,3-dimercaptropropanal, BAL); calcium disodium edetate, and n-acetyl-DL-penicillamine (Joron, 1963; Smith and Miller, 1961; and Swensson and Ulfvarson, 1967).

Alkyl Mercury Poisoning

At present, there is no specific treatment for alkyl mercury poisoning. Since alkyl mercury compounds do not readily dissociate

to yield inorganic mercuric ions, the metal chelating agents have been of little use. Even in cases where the chelating agents did increase urinary excretion of mercury, symptoms did not always regress; implying that irreversible damage had occurred (Ahlborg and Ahlmark, 1949; Lundgren and Swensson, 1960; and Swensson and Ulfvarson, 1967).

Limits

The United States governmental limits for mercury compounds in the environment are: (1) air-TLV for 8-hour work day - 10 $\mu g/m^3$ alkyl mercury, 50 $\mu g/m^3$ elemental mercury and 100 $\mu g/m^3$ inorganic mercury; (2) water--near waste outfalls 1 to 5 ppb and U. S. Public Health Service drinking water standard 5 ppb; and (3) fish and other food products--Interim guideline set by the Food and Drug Administration -0.5 ppm. It is recognized that these values have been determined from limited data and may be subject to revision at a later date (Joselow, Louria and Browder, 1972).

Lactose Intolerance

A recent medical observation has associated lactose intolerance as a symptom in a case of chronic mercurial intoxication. In the normal individual, digestion of lactose begins in the duodenum and is completed in the jejenum. The enzyme β-galactosidase, located in

the brush border of the small intestine, hydrolyzes lactose to glucose and galactose (Kretchmer, 1971; 1972), which are carried to the blood stream by active transport and used in various metabolic processes (Wohl and Goodhart, 1971). The activity of β -galactosidase is quite high in prenatal life through infancy and decreases by 90 percent in the adult. When this enzyme is deficient, the lactose is not hydrolyzed and accumulation in the gut occurs. Inability to hydrolyze lactose is known as lactose intolerance (Kretchmer, 1971; 1972).

Symptoms

This disease is characterized by abdominal cramping, flatulence, borborygmia (stomach growling) and diarrhea (Lutwak, 1969). Symptoms develop 30 to 90 minutes after ingestion of a lactose containing product, and are gone within 2 to 6 hours (National Dairy Council, 1971). Usually the symptoms are not very severe, but variations may occur in conjunction with other gastrointestinal disturbances such as ulcerative colitis (Gudmand-Høyer, Dahlqvist and Jarnum, 1970).

Incidence

The incidence of lactose intolerance varies with different races and geographical distribution. In the United States lactose intolerance occurs at a rate of 6 to 12 percent in Caucasians (Kretchmer, 1971;

1972). Surveys indicate that lactose intolerance occurs at a rate up to 20 percent in Caucasians of Scandanavian or Western European descent. A 60 to 90 percent rate of lactose intolerance occurs in populations of Greek Cypriots, Arabs, Jews, American Negroes, African Bantus, Japanese, Thai, Formosans, and Filipinos tested (Bayless, Paige and Ferry, 1971; and Lutwak, 1969).

Etiology and Types

Lactose intolerance, which may be caused by a genetic mutation or result as an acquired defect, has been divided into four classes based on its etiology (Lutwak, 1969).

A. Congenital
   1. Physiologic β-galactosidase deficiency in premature infants; only lasts for a few weeks, then the enzyme increases to a normal level.
   2. Genetically determined.
   3. Bovine protein sensitivity associated with β-galactosidase deficiency; if milk is replaced by a non-bovine source, lactose is well tolerated.
   4. Genetically determined, secondary β-galactosidase deficiency in older children and adults.

B. Acquired, secondary to mucosal damage and associated to some extent with other disaccharidase deficiencies.

1. Kwashiorkor (a dietary deficiency disease of infants and children due primarily to an inadequate intake of protein).
2. Acute gastroenteritis.
3. Chronic intestinal diseases.
4. Neomycin administration, which damages the microvilli (Cain, Reiner and Patterson, 1968).
5. Giardia lamblia infestation, which erodes the epithelium of the duodenum (Burrows, 1968).
6. Post-bowel surgery on infants.

C. Acquired, secondary to alterations of intestinal transit time.

1. Small bowel resection.
2. Post gastroectomy.

D. Associated with disease.

1. Ulcerative colitis.
2. Regional enteritis.
3. Irritable colon syndrome.
4. Osteoporosis.
5. Peptic ulcers.

Adult Lactose Intolerance

Four hypotheses have been suggested to explain the variations

in lactose intolerance in older children and adults from different populations: it is a hereditary factor occurring at a defined frequency in a given population, it is an acquired defect resulting from the lack of dairy products (lactose) in the diet; it is the result of a high incidence of diseases that damage the small intestine and inhibit the production of β -galactosidase; and it is the result of ingestion of substances that inhibit the production of β -galactosidase (Simoons, 1969).

The first and most widely accepted hypothesis suggests that low β -galactosidase levels are under genetic control. Since the incidence of lactose intolerance is very high in most cultures, it has been suggested that the few cultures with a low incidence in adults are the mutants, rather than the populations with a high rate of lactose intolerance (Bayless, Paige and Ferry, 1971). Perhaps, in cultures with high rates of lactose intolerance, another gene product is formed that may serve a useful function and confer a selective advantage to the group (Flatz and Saengudom, 1969). This view is consistent with almost all other land mammals whose β -galactosidase level is negligible after weaning (Kretchmer, 1971; 1972). Based on this premise, a given culture should have a defined rate of lactose intolerance regardless of location. One study reveals a lactose intolerance rate of 72 percent in Black Africans and 70 percent in Black Americans who descended from the Black Africans about 300 years ago, even

though they live in completely different environments (Rosensweig, 1971). Another study reveals the same phenomenon with Orientals living in Asia and Orientals living in the United States. In East Africa, a study of tribal groups has shown that the Ganda people have high levels of lactose intolerance while the "Hamitic" Hima and Tussi peoples have low levels of intolerance to milk sugar. Intermarriage of these two groups have produced progeny with intermediate levels of lactose intolerance (Simoons, 1969). In addition, studies conducted in the United States and Great Britain demonstrated that many individuals with lactose intolerance had a family history of intolerance (Huang and Bayless, 1968; McMichael, Webb and Dawson, 1965; Welsh et al., 1968; and Zschiesche and Welsh, 1968). The ability to explain group differences and evidence of a family basis have further strengthened the support for the genetic hypothesis.

The second hypothesis suggests that lactose intolerance is an acquired defect resulting from the lack of dairy products in the diet. After many centuries, genetic selection for a very low β-galactosidase level may have occurred in cultures that included very little, if any, dairy products in the diet (Bolin and Davis, 1969). It has been demonstrated in animals that the intestinal disaccharidases do adapt to the sugar content of the diet (Deren, Broitman and Zamcheck, 1967; and Rosensweig and Herman, 1968). The induction of these enzymes can occur by the stimulation of epithelial mucosal cells in

the small intestine (Fischer, 1957). However, this stimulation is a composite of many factors involving a variety of substrates, end products, number of calories, and substances not directly involved in the reaction (Rosensweig and Herman, 1968; Rosensweig, Stifel and Herman, 1968; and Stifel, Rosensweig and Zakim, 1968). Experiments conducted with rats demonstrated that if they were kept on a lactose-containing diet after weaning, the β -galactosidase level increased within 5 to 10 weeks. Previous experiments had not been continued long enough to see the increase in enzyme level (Bolin, McKern and Davis, 1971). Other workers have shown (Fischer, 1957) that the weight of the intestinal mucosa of rats increases as much as 50 percent when they are kept on a lactose-containing diet after weaning; thus, there was an overall increase in total β -galactosidase activity even though there was no increase in the units/mg wet weight of enzyme.

Other correlative evidence was obtained in a study conducted on Chinese individuals born in China or Australia. The native born Chinese had a 95 percent incidence of lactose intolerance, whereas the Australian born Chinese had an incidence of 56 percent. The former group had not included lactose products in the diet after weaning, whereas the latter group had (Bolin and Davis, 1970). However, other studies have shown that normal individuals deprived of milk products for 42 days did not show a decrease in β -galactosidase

activity (Knudsen et al., 1968) and individuals suffering from galactosemia who had not consumed lactose since infancy had normal enzyme activity (Kogut, Donnell and Shaw, 1967). On the other hand, some studies have shown that individuals who were lactose intolerant could consume dairy products (Chung and McGill, 1968; and Huang and Bayless, 1967; 1968). These studies suggest that lactose does not induce β -galactosidase in humans.

The third hypothesis suggests that lactose intolerance occurs secondary to malnutrition and is due to protein deficiency, which results in severe damage to the mucosa. Diseases, such as kwashiorkor and marasmus, flatten out the villi and reduce the absorption capacity of the small intestine and can result in adult lactose intolerance (England, 1968). However, this hypothesis does not explain the difference in the incidence of lactose intolerance in different races living in the same environment. For example, in the United States there is approximately a 70 percent incidence of lactose intolerance in blacks and a 6 to 12 percent incidence in Caucasians (Kretchmer, 1971; 1972). No answer has been found to explain why lactose is the only carbohydrate malabsorbed when there is no evidence of damage to the small intestine (Simoons, 1969).

The fourth hypothesis suggests that lactose intolerance is the result of ingestion of substances that inhibit the production of β -galactosidase. The drug, colchicine, inhibits the production of β -

galactosidase, resulting in lactose intolerance. Other inhibitory substances may be present in foods or drugs and different dietary patterns could result in different rates of lactose intolerance in groups (Nelson, 1969). Highly spiced foods or betel nut consumption, for example, could alter the mucosa and lead to lactose intolerance (Sprinz et al., 1962). Currently, diet per se has not been investigated as a predisposing factor for lactose intolerance (Simoons, 1969). However, heavy metal intoxication has been implicated in this anomaly (Wagner et al., 1972), suggesting that environmental pollution may contribute to the problem.

Pathology

When lactose intolerance occurs, the undigested sugar passes into the colon where it serves as an osmotic load and attracts water into the lumen. The sugar is partially hydrolyzed by bacterial β-galactosidase and fermented to lactate, acetate, formate, isobutyrate, propionate, and butyrate. These organic acids are irritating to the colon and cause increased intestinal motility. The bacterial content of the intestinal tract increases which results in a lowering of the pH of the feces to about 4.5 (Haemmerli et al., 1965).

Diagnosis

Lactose intolerance is a newly recognized disease and its

diagnosis is often complicated by the fact that some individuals not on a milk diet will still react to extremely low levels of lactose found in bread. Since the patient's history does not suggest a lactose intolerance problem, it could be incorrectly diagnosed as psychosomatic bile syndrome or gall bladder disease, unless a sugar test were administered. As lactose intolerance becomes more widely known, diagnosis of this disease should be facilitated.

There are several different methods being used as diagnostic criteria for lactose intolerance. One method is to give a fasting individual 50 g of lactose (test dose) and wait for symptoms to develop. A second method (McGill and Newcomer, 1967) is to give a fasting individual 50 g of lactose and measure the blood glucose concentration; a 20 mg% or more increase in blood glucose is considered normal while a 15 to 20 mg% increase is inconclusive. A A 15 mg% or less increase in blood glucose is characteristic of lactose intolerance, and the development of symptoms should also be monitored (Newcomer and McGill, 1966). This method is an index of absorption and hydrolysis of the lactose. A third method developed by Isokoski, Jussila and Sarno (1972) is to give a fasting individual 300 mg of ethyl alcohol per kg of body weight, then 50 g of lactose 15 minutes later. After 40 minutes, a capillary blood sample is taken and the galactose level measured. All three of the above methods have been criticized for the large amount of lactose given

in the tests (Hersh, 1972; and Protein Advisory Group, 1972). Fifty-grams of lactose is the amount present in a liter to a liter and a half of milk. It is not the normal dietary pattern to consume so much milk in one sitting, especially on an empty stomach (Protein Advisory Group, 1972). The lactose administered is in a water solution, whereas milk, often taken with food, is a mixture of water, protein, fat, carbohydrate, minerals, and vitamins. Some of these components may have an influence on the digestion of lactose (Wohl and Goodhart, 1971). Also, the absorption rates of the monosaccharides, glucose and galactose, should be checked to rule out a monosaccharide malabsorption syndrome (National Dairy Council, 1971). Other problems have arisen with the measurement of the level of glucose in the blood. According to this procedure one can use either capillary or venous blood to measure glucose concentration. McGill and Newcomer (1967) have shown that 30 percent of the normal individuals on a venous blood test will now show a 20 mg% or more increase in blood glucose. On the other hand, Gray (1967) has demonstrated that although a capillary blood test eliminates false negatives, it produces a falsely normal increase in blood sugar in 40 percent of β -galactosidase deficient patients. Since venous blood does not give a false high blood glucose level, it is the preferred sample. In addition, Newcomer and McGill (1966) demonstrated that some normal individuals respond abnormally to the test because they have a slower stomach

emptying time. Thus, the lactose is slower to reach the hydrolytic sites and slower to be absorbed. Another method being used to determine lactose intolerance is the direct determination of the activity of the enzyme β-galactosidase using a peroral biopsy technique (Messer and Dahlqvist, 1966). If an individual has less than 2 units of enzyme activity, he is considered lactose intolerant (Protein Advisory Group, 1972). This method has also become suspect in the light of recent evidence from a survey involving Indian adults and children that was conducted by Reddy and Pershad (1972) to determine the incidence of lactose intolerance and the activity of the enzyme β-galactosidase. The group had a high incidence of lactose intolerance and β-galactosidase deficiency and, therefore, was placed on a diet supplemented with skim milk for four weeks. At the end of this time, some members of the group showed milder symptoms of lactose intolerance and others had no symptoms at all. The activity of β-galactosidase did not increase with the disappearance of symptoms and the mechanism of this adaptation has not been explained. Some members of the group who were diagnosed as lactose intolerant when given 50 g of lactose could drink a quart of milk a day, if given in 200 ml-quantities throughout the day. Based on these data, lactose intolerance is not synonymous with milk intolerance and perhaps unmerited significance has been attached to all of the diagnostic tools mentioned.

These results can have far reaching effects. Milk is a rich protein source often given in aid by the United States to underdeveloped countries. The use of skim milk as a food supplement has been questioned because the majority of the world's population is lactose intolerant as diagnosed by the above mentioned techniques. However, if many of these lactose intolerant people can enjoy a diet virtually unrestricted in regards to dairy products, it would seem incorrect to terminate the foreign aid supply of milk in any form (Protein Advisory Group, 1972).

Therapy

If an individual suffers from lactose intolerance, his consumption of dairy products should be adjusted so that he does not suffer from gastrointestinal disturbances. If the severity of this syndrome necessitates the exclusion of most dairy products from the diet, care should be taken to include supplementary sources of calcium, riboflavin and protein (National Dairy Council, 1971).

Prognosis

An individual with lactose intolerance will suffer no permanent disabilities if a well-balanced diet is maintained. There is evidence for osteoporosis as a result of lactose intolerance. A study conducted by Birge et al. (1967) revealed that nine osteoporotic patients

had excluded dairy products from the diet because of gastrointestinal problems when they were consumed. Thus, osteoporosis may have developed because the patients had been on a long-term calcium-restricted diet due to lactose intolerance.

MATERIALS AND METHODS

Sources of β -Galactosidase

The intestinal tissue from hogs was obtained from the Oregon State University (OSU) Meat Laboratory and the intestinal tissue from rats was obtained from the OSU Small Animals Laboratory. The human intestinal biopsies were provided by Good Samaritan Hospital, Corvallis, Oregon. Cultures of Streptococcus lactis 7962 and Escherichia coli A189 were obtained from the stock culture collection, Department of Microbiology and the partially purified β -galactosidase from E. coli was purchased from Sigma Chemical Company.

Preparation of Tissue

Intestinal Tissue from Hogs

The intestinal tissue from hogs was cut into one-foot segments and washed with distilled water. It was then incised and the mucosa was removed with a glass slide. All the mucosa from one hog was pooled and mixed in a Waring blender that was cooled to 4° C in an ice bath. Five-gram samples were removed, diluted with distilled water (4:1, V/W), and homogenized for 5 minutes in a Braun homogenizer with a bottom siphon liquid CO_2 tank attachment to keep the samples cold. Cell debris was removed with a RC-2 Sorvall centrifuge operated at 4,000 RPM for 10 minutes at 1° C and the β -

galactosidase activity of the supernatant was determined.

Intestinal Tissue from Rats and Humans

Intestinal tissues from rats and humans were washed with distilled water and cut into 1-g segments. All samples, homogenized by hand until they were uniform in consistency, were then diluted with distilled water (20:1, V/W). The diluted samples were then homogenized as described above and the β -galactosidase activity of the supernatants determined.

Culture Conditions, Collection and Preparation of Cell-Free Extracts

S. lactis 7962 and E. coli A189 were grown in lactose broth (Citti, Sandine and Elliker, 1965) and E.C. medium (Difco), respectively, for 12 hours at 32° C. After incubation the cells were immediately chilled and collected (Citti, Sandine and Elliker, 1965) with a RC-2 Sorvall centrifuge operated at 4,000 RPM for 10 minutes at 1° C. The cells were washed twice with cold 0.05 **M** sodium phosphate buffer, re-suspended in 30 ml of buffer and ruptured in a Raytheon sonic oscillator for 20 minutes. Cell debris was removed with a RC-2 Sorvall centrifuge operated at 4,000 RPM for 10 minutes at 1° C and the supernatants were then diluted 10-fold with buffer and the β -galactosidase activity determined.

Protein Determination

The protein content of the samples was measured by the Folin phenol method (Lowry et al., 1951). The protein content (mg/ml) of each sample was determined from a standard curve, which was prepared by measuring the change in absorbancy produced by varying concentrations of crystallized bovine serum albumin (Pentex Incorporated).

Methods for the Determination of Enzyme Activity

Dahlqvist Method for the Assay of β -Galactosidase

The method of Dahlqvist (1964) was used to assay β -galactosidase activity from intestinal tissues of hogs, E. coli A189, and S. lactis 7962. The substrate solution consisted of 0.056M lactose in 0.1 M maleate buffer (pH 6.0). The buffer contained 1.16 g of maleic acid in 15.3 ml of 1N NaOH and was diluted to 100 ml with distilled water. Toluene, 1 ml/100 ml of lactose solution, was added as a preservative. The Tris-buffer (0.5 M; pH 7.0) consisted of 61.0 g of tris (hydroxymethyl) aminomethane (Tris) in 85 ml of 5 N HCl diluted to 1000 ml with distilled water. The detergent solution consisted of 10 ml of Triton X-100 (Sigma) in 40 ml of 95 percent ethyl alcohol. The Tris-glucose oxidase (TGO) reagent was prepared from Glucostat-glucose reagent (Worthington Biochemical

Company). The chromogen was dissolved in 1 ml of detergent solution and 3 ml of Tris-buffer. The contents of the glucostat vial were dissolved in 5 ml of Tris-buffer. The two solutions were then mixed and diluted with Tris-buffer to 100 ml. A standard glucose solution was prepared containing 100 mg of glucose and 2.7 g of benzoic acid in distilled water and the volume was adjusted to 1000 ml.

A 0.1 ml aliquot of the appropriate dilution of the enzyme solution was mixed with 0.1 ml of the lactose solution and 1 drop of toluene. The solution was incubated in a 37° C water bath for 60 minutes. At the end of incubation, 0.8 ml of distilled water was added. The enzyme reaction was terminated by boiling in water for 2 minutes and the tube cooled to room temperature. A blank was prepared with the same composition and immersed in boiling water immediately.

To determine the amount of glucose liberated, 0.5 ml of the solution was withdrawn and mixed with 3.0 ml of TGO reagent. It was then incubated in a 37° C water bath for 60 minutes for color development. At the same time, a standard series containing 0.0, 0.1, 0.3 and 0.5 ml of the standard glucose solution was incubated and distilled water was added to obtain a total volume of 0.5 ml. Each solution was then mixed with 3.0 ml of TGO reagent and incubated as previously described.

After incubation, the absorbancy at 420 nm was read on a

Bausch and Lomb spectronic 20. The amount of glucose in the sample did not exceed 50 μg. β -Galactosidase activity was calculated as follows:

$$\beta\text{-Galactosidase activity (units/ml)} = \frac{(a - b)}{540} \cdot d$$

where a = amount of glucose (μg) found in enzyme solution incubated, b = amount of glucose (μg) in corresponding blank and d = dilution factor of enzyme solution. Units of activity were defined as μmoles of lactose hydrolyzed/minute at 37° C. Data were expressed as μmoles of lactose hydrolyzed/minute/g of protein at 37° C.

Lederberg Method for the Assay of β -Galactosidase

The method of Lederberg (1950) was used to assay β -galactosidase activity from intestinal tissue of hogs, E. coli A189, and partially purified β -galactosidase from E. coli (Sigma). The chromogenic substrate, o-nitrophenyl-β -D-galactopyranoside (ONPG), was used to measure β -galactosidase activity. A solution of 0.005 M ONPG in 0.05 M sodium phosphate buffer was prepared. One milliliter of the enzyme solution was incubated with 4 ml of the ONPG solution for 15 minutes at 37° C. The enzyme reaction was terminated by addition of 5.0 ml of cold 0.5 M sodium carbonate and the absorbancy at 420 nm was read on a Bausch and Lomb Spectronic 20. The milligrams of o-nitrophenol (ONP) liberated from ONPG were determined from a standard curve, prepared by measuring the

change in absorbancy produced by varying concentrations of ONP. One unit of activity was defined as μg of ONP liberated/minute at 37° C. The specific activity of β-galactosidase was expressed as μg of ONP liberated/minute/mg protein at 37° C.

A Modification of the Messer and Dahlqvist Method for the Assay of β-Galactosidase

The method of Messer and Dahlqvist (1966) was used to assay β-galactosidase activity from intestinal tissues of rats and humans. The substrate solution consisted of 0.028 M lactose in 0.22 M sodium phosphate buffer at pH 6.0. The phosphate-glucose oxidase (PGO) reagent contained 0.2 g of glucose oxidase (Sigma) in 9.8 ml of 0.5 M sodium phosphate buffer (pH 6.0) with 0.1 ml of 1 percent o-dianisidine (Sigma) in 95 percent ethyl alcohol and 0.1 ml of 0.1 percent peroxidase (Sigma) in buffer. A standard glucose solution was prepared containing 200 mg of glucose and 2.7 g of benzoic acid in distilled water and the volume was adjusted to 1000 ml.

A 0.2 ml aliquot of the sample was mixed with 1.0 ml of the PGO reagent and placed in a 37° C water bath. After 2 minutes, 1.0 ml of the lactose solution was added, the mixture was incubated for 75 minutes and the reaction was stopped by addition of 1.0 ml of 50 percent sulfuric acid. A second test tube containing an identical mixture was incubated for only 15 minutes after the substrate

solution was added before the reaction was stopped by addition of sulfuric acid. The absorbancy at 530 nm was read on a Bausch and Lomb Spectronic 20.

The amount of glucose liberated in the samples was caluclated from a standard curve made by measuring the change in absorbancy produced by varying amounts of glucose. The PGO reagent used for the standard curve contained only 0.1 g of glucose oxidase. β-Galactosidase activity was calculated as follows:

$$\beta\text{-Galactosidase activity (units/ml)} = \frac{(G75 - G15)}{216} \cdot d$$

G75 and G15 are the amounts of glucose (μg) present after 75 and 15 minutes of incubation and d is the dilution factor of the enzyme solution.

Data were expressed as μmoles of lactose hydrolyzed/minute/g of protein at 37° C.

Partial Purification of β-Galactosidase

Intestinal tissues from both hogs and humans were partially purified according to the method of Gray and Santiago (1969). After tissue preparation, samples were centrifuged at 39,000 RPM for 60 minutes at 1° C in a Beckman L2-65 ultracentrifuge. The supernatant was assayed directly or after precipitation with 60 percent $(NH_4)_2SO_4$.

Dialysis Procedure

Several samples of enzyme solution from rats and PGO reagent were dialyzed. Dialysis tubing, containing 5 ml of enzyme solution, was placed in a 250 ml graduated cylinder containing 0.5 M sodium phosphate buffer and stirred overnight at 4° C. The buffer was changed four times during this period. The enzyme solutions were then assayed and the PGO reagent was used to assay undialyzed enzyme solutions.

Atomic Absorption Analysis of Rat Tissue and Blood

Female rats were fed mercury for three months to demonstrate the effect of this metal on β -galactosidase activity. Three groups were used as follows: Group I (12 animals) received a standard diet (control), Group II (24 animals) was fed 6 ppm mercuric chloride (low level) and Group III (24 animals) was fed 300 ppm mercuric chloride (high level). One control rat, two low-level rats and two high-level rats were sacrificed at weekly intervals. In addition, total mercury in blood, liver, kidney and intestinal fractions was determined by flameless atomic absorption spectrometry (see Figure 1) following exhaustive acid digestion. The sample sizes were as follows: (1) blood up to 1.0 g; (2) kidney up to 0.8 g; (3) liver up to 3.0 g; (4) intestinal fractions (liquid 10 g and solid 0.5 g). Each

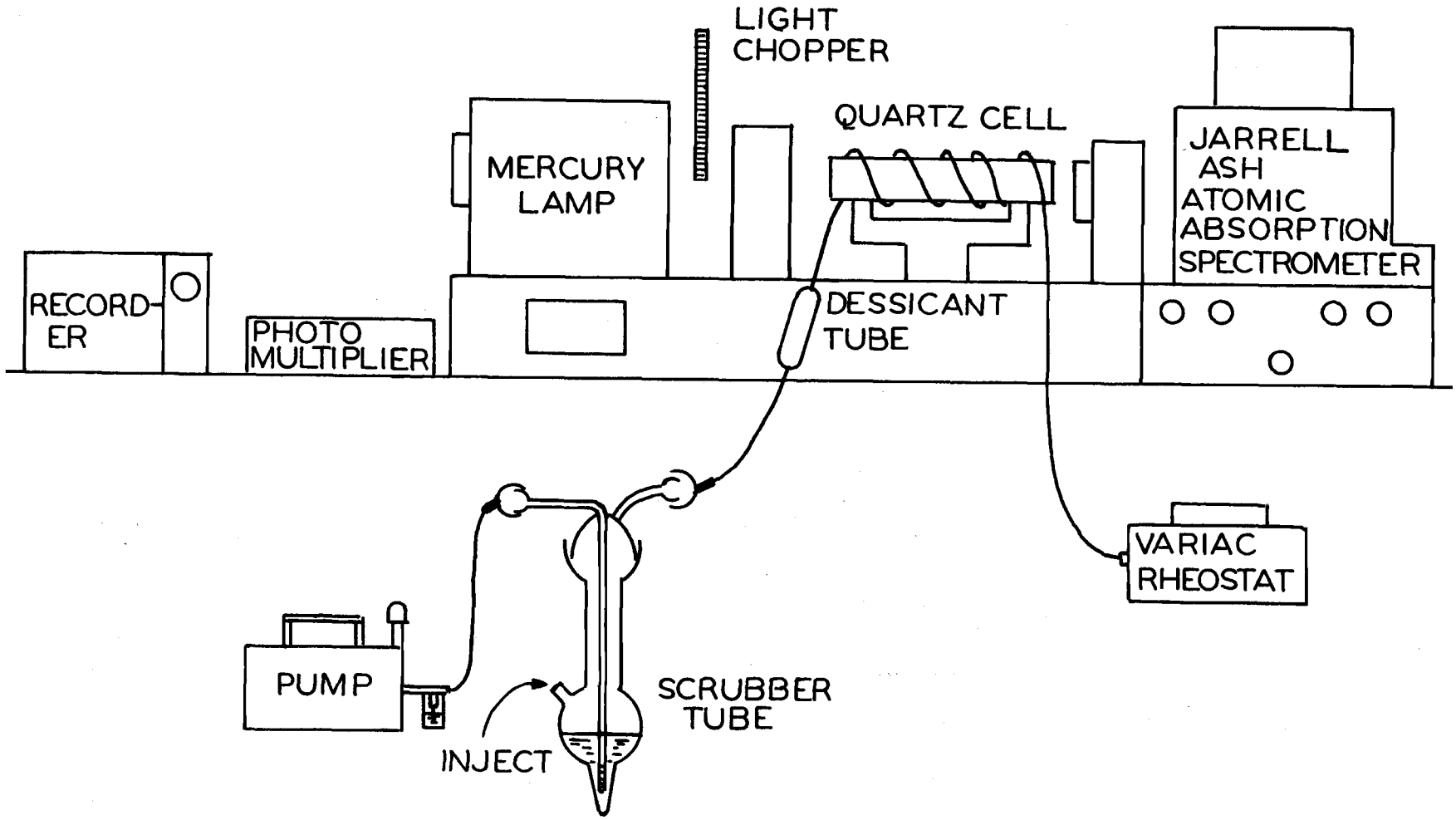

Figure 1. Drawing of Jarrell Ash Atomic Absorption Spectrometer.

sample was placed in a 24/40 T 125 or 250 ml round bottom flask with a straight bore water-cooled condenser attached. Concentrated nitric acid (1 ml/g) was added through the condenser. The sample was then heated to 75° C. After one hour the same quantity of 1:1 concentrated sulfuric acid and concentrated nitric acid was added through the condenser and heated for three hours. Then, through the condenser, sufficient $KMnO_4$ (6 percent aqueous solution) was added to keep the sample solution purple over the 1.5 hour of supplementary heating. The flask was then allowed to cool and the condenser was washed with concentrated nitric acid and the flask removed. The digested sample was clarified with a 10 percent H_2O_2 solution, poured through a glass wool plug funnel into a 100-ml graduated cylinder and the total volume recorded. The sample was then injected with a syringe through the rubber stopper of the scrubber flask, which contained a reductant solution. The mercury (II) was reduced and volatilized at room temperature in a stream of air, which was then pumped through a heated quartz cell and the atomic absorption measure of the mercury vapor was recorded on a Jarrell Ash Atomic Absorption Spectrometer at 2537-2538 Å. The sample peak area was compared to a standard peak area obtained by injecting a known volume of a standard solution of mercuric chloride in 1N sulfuric acid into the scrubber flask and aerated as described

above. The peak area was calculated with a compensating polar planimeter. The minimum sensitivity was 5 ng of mercury.

RESULTS

Effect of Mercury on β -Galactosidase from Hog Mucosa

Using the method of Dahlqvist (1964), the specific activity of β -galactosidase from hog intestine was 28.5 μmoles of lactose hydrolyzed/minute/g of protein at 37° C. This experiment was performed several times with varying concentrations of mercuric acetate, but inhibition was never observed. The enzyme solution prepared in this manner was only a crude extract. It appeared that the mercury was binding to some foreign protein thereby preventing interaction with the enzyme. If the enzyme was partially purified (Gray and Santiago, 1969) before the addition of mercuric acetate, inhibition was observed as shown in Table 1. Mercuric acetate (10 ppm) completely inhibited enzyme activity, whereas at 1 ppm there was 41.2 percent inhibition and at 0.5 ppm there was no inhibition.

Effect of Urine with Added Mercury on β -Galactosidase from Hog Mucosa

Experiments were conducted with urine to determine the effect of residual mercury on β -galactosidase activity and to assess the value of the procedure as a possible clinical test for mercurialism.

Since urine had no effect on β -galactosidase or the pH of the enzyme, varying concentrations of mercuric acetate were added

Table 1. Effect of mercuric acetate on specific activity of partially purified β -galactosidase from hog intestine.

Tube	Mercuric Acetate, ppm	Specific Activity[a]	Inhibition[b] %
1	0	42.5	control
2	.5	42.5	0
3	1	25.0	41.2
4	5	13.0	69.3
5	10	0	100
6	15	0	100
7	25	0	100
8	50	0	100
9	100	0	100

[a] Specific Activity = μmoles of lactose hydrolyzed/min/g of protein at 37° C.

[b] % inhibition = 100 - sample/control x 100.

prior to the addition of β -galactosidase. Urine is a reducing medium and apparently the added salt was reduced to mercurous acetate and then combined with chloride or perhaps bicarbonate, forming an insoluble complex. Inhibition was never observed under the above conditions.

Effect of Mercury on β -Galactosidase from Streptococcus lactis 7962

Cell-free extracts were obtained from S. lactis 7962 as described by Citti, Sandine and Elliker (1965) and used to assay for

β -galactosidase activity. Varying concentrations of mercuric acetate were added to the enzyme solution and the results appear in Table 2. At a level of 100 ppm of mercuric acetate, the enzyme was completely inhibited; at 50 ppm there was approximately 96 percent inhibition and at 25 ppm inhibition ranged from 48 to 81 percent.

Table 2. Effect of mercuric acetate on specific activity of β -galactosidase in crude extracts of S. lactis 7962.

Mercuric Acetate ppm	Trial I		Trial II		Trial III	
	Specific Activity[a]	Inhibition[b], %	Specific Activity	Inhibition, %	Specific Activity	Inhibition, %
0	265	0	139	0	219	0
25	139	48	26	81	70	68
50	13	95	7	95	6	97
100	0	100	0	100	0	100

[a] Specific Activity = μmoles of lactose hydrolyzed/min/g of protein at 37° C.

[b] % inhibition = 100 - sample/control x 100.

Effect of Mercury on β -Galactosidase from Escherichia coli A189

Experiments similar to those performed with S. lactis 7962 were conducted with E. coli A189 because the latter organism demonstrates significant β -galactosidase activity. Varying concentrations of mercuric acetate were added to the enzyme solution to assess

inhibition and the results appear in Table 3. Addition of 100 ppm of mercuric acetate resulted in 100 percent inhibition, 50 ppm approximately 99.6 percent inhibition and 25 ppm no inhibition.

Table 3. Effect of mercuric acetate on specific activity of β-galactosidase in crude extracts of E. coli.

Mercuric Acetate ppm	Trial I		Trial II		Trial III	
	Specific[a] Activity	Inhibition,[b] %	Specific Activity	Inhibition, %	Specific Activity	Inhibition, %
0	5201	0	5406	0	5300	0
25	5195	0	5400	0	5294	0
50	16	99.7	22	99.6	21	99.6
100	0	100	0	100	0	100

[a] Specific Activity = μmoles of lactose hydrolyzed/min/g of protein at 37° C.

[b] % inhibition = 100 - sample/control x 100.

Effect of Salts on Partially Purified β-Galactosidase from E. coli

Effect of Mercuric Acetate on β-Galactosidase

Partially purified β-galactosidase from E. coli was used because the excess foreign protein present in the crude extracts apparently interfered with the interaction of mercury and the enzyme. Varying concentrations of mercuric acetate were added to the enzyme solution and the results obtained are shown in Table 4. Addition of

Table 4. Effect of mercuric acetate on the specific activity of partially purified β -galactosidase from E. coli.

Tube	Mercuric Acetate, ppm	Specific Activity[a]	Inhibition[b] %
1	0	1920	control
2	.10	1920	0
3	.16	960	50
4	.20	0	100
5	1	0	100
6	5	0	100
7	15	0	100
8	25	0	100
9	50	0	100
10	100	0	100

[a] Specific Activity = μmoles of lactose hydrolyzed/min/g of protein at 37° C.

[b] % inhibition = 100 - sample/control x 100.

0.2 ppm of mercuric acetate resulted in 100 percent inhibition of the enzyme, 0.16 ppm 50 percent inhibition and 0.1 ppm no inhibition.

Effect of Sodium Acetate on β -Galactosidase

Experiments were performed with varying concentrations of sodium acetate added to the β -galactosidase solution to ensure that the acetate portion of mercuric acetate had no deleterious effect on enzyme activity. The results obtained are shown in Table 5.

Table 5. Effect of sodium acetate on the specific activity of partially purified β -galactosidase from E. coli.

Tube	Sodium Acetate, ppm	Specific Activity[a]	Inhibition[b] %
1	0	600	control
2	25	760	0
3	50	720	0
4	100	600	0

[a]Specific Activity - μmoles of lactose hydrolyzed/min/g of protein at 37° C.

[b]% inhibition = 100 - sample/control x 100.

Concentrations of 25 and 50 ppm of sodium acetate resulted in higher enzyme activity than observed in the control. This was probably due to the stimulation or activation of the enzyme by sodium ions. A concentration greater than 50 ppm sodium acetate is inhibitory to β -galactosidase activity.

Rat Study

Mercury feeding experiments with female rats were conducted over a three-month period to demonstrate the effect of mercury on β -galactosidase activity and to determine: (1) total mercury in blood liver, kidney and intestinal fractions (atomic absorption analysis); (2) food intake per week; and (3) weight.

Atomic Absorption Analyses

At the beginning of the experiment the animal feed was analyzed. There was 5.7 ppm of mercury present in the 6 ppm mercuric chloride ration and 290 ppm of mercury present in the 300 ppm mercuric chloride ration. The total amounts of mercury (ppm) present in the various tissues and blood are shown in Table 6. Atomic absorption analyses were conducted only on the control rats on the diet for 1, 2, 6 and 12 weeks since no detectable amount of mercury was found in the various tissues, with the exception of the kidney (0.70 ppm) at 12 weeks. Of the tissues examined, the kidney had the highest concentration of mercury. The solid intestinal fraction contained the nuclei and cell debris precipitated out of the sample by centrifugation after homogenization and the liquid intestinal fraction was the enzyme solution used in the assays. The average total mercury content (ppm) of the liquid intestinal fraction of rats on a low-level diet was 0.0042 ppm and on a high-level diet 0.0725 ppm.

Food Consumption and Weight Gain Patterns

The food consumption and weight data were collected only from rats on the diet for 9, 10, 11 or 12 weeks. The average food intake and weight per week for all three groups of rats are shown in Tables 7 and 8. Rats fed 6 ppm of mercuric chloride showed no appreciable decrease in food consumption or weight in comparison to the controls,

Table 6. Levels of mercury found (ppm) in various samples taken from rats receiving no, 6 or 300 ppm mercuric chloride in the feed. Animals were fed three times per week.

Number of Weeks on Diet	Sample	Blood	Liver	Kidney	Intestinal Fractions	
					Liquid	Solid
I	Control	ND[a]	ND	ND	ND	ND
	6 ppm	0.15	0.21	3.35	ND	ND
	300 ppm	0.29	2.83	124.6	0.195	1.95
II	Control	ND	ND	ND	ND	-
	6 ppm	ND	0.60	5.0	ND	2.15
	300 ppm	0.58	2.125	119.2	0.0425	2.485
III	Control	-	-	-	-	-
	6 ppm	ND	0.10	9.18	0.005	ND
	300 ppm	0.21	2.105	99.6	0.0265	1.165
IV	Control	-	-	-	-	-
	6 ppm	ND	ND	ND	ND	0.65
	300 ppm	0.32	1.29	93.5	0.155	4.595
V	Control	-	-	-	-	-
	6 ppm	ND	ND	ND	ND	0.23
	300 ppm	0.335	1.34	93.9	0.03	2.7
VI	Control	ND	ND	ND	-	-
	6 ppm	ND	ND	19.53	0.005	0.29
	300 ppm	0.255	1.53	72.575	0.11	2.275
VII	Control	-	-	-	-	-
	6 ppm	0.062	-	23.66	-	0.33
	300 ppm	0.40	1.38	74.15	0.05	2.06

Table 6 (continued)

Number of Weeks on Diet	Sample	Blood	Liver	Kidney	Intestinal Fractions	
					Liquid	Solid
VIII	Control	-	-	-	-	-
	6 ppm	0.09	-	34.23	0.006	0.48
	300 ppm	0.48	1.125	128.55	0.05	5.555
IX	Control	-	-	-	-	-
	6 ppm	0.05	-	36.82	0.009	0.32
	300 ppm	0.30	0.84	106.95	0.09	3.4
X	Control	-	-	-	-	-
	6 ppm	0.27	-	96.18	0.012	0.23
	300 ppm	0.23	0.65	116.5	0.05	8.33
XI	Control	-	-	-	-	-
	6 ppm	0.11	0.885	77.62	0.005	0.75
	300 ppm	0.85	1.40	112.7	0.04	5.67
XII	Control	ND	ND	0.70	ND	ND
	6 ppm	0.089	0.565	94.81	-	4.235
	300 ppm	0.33	2.0	98.4	0.03	11.78

ND = non-detectable

[a]ND = < 0.05 ppm for blood
< 0.06 ppm for kidney
< 0.02 ppm for liver
< 0.10 ppm for solid intestinal fraction
< 0.005 ppm for liquid intestinal fraction

Table 7. Weekly averages of feed consumed in grams by control and test animals.

Number of Weeks on Diet	Control	$HgCl_2$ 6 ppm	% of Control	$HgCl_2$ 300 ppm	% of Control
I	99	90	91%	57	58%
II	88	75	85%	68	77%
III	125	120	96%	100	80%
IV	127	124	98%	109	86%
V	109	117	107%	106	97%
VI	121	126	104%	101	83%
VII	121	111	92%	111	92%
VIII	126	124	98%	103	82%
IX	130	120	92%	106	82%
X	101	108	107%	80	79%
XI	90	117	130%	65	72%
XII[a]	-	-	-	-	-
			Av. 100%		Av. 81%

[a]No data because animals were sacrificed at beginning of week.

Table 8. Weekly averages of weight in grams for control and test animals.

Number of Weeks on Diet	Control	$HgCl_2$ 6 ppm	% of Control	$HgCl_2$ 300 ppm	% of Control
I	95	90	95%	67	71%
II	118	114	97%	85	72%
III	136	134	99%	102	75%
IV	163	160	98%	127	78%
V	182	179	98%	145	80%
VI	198	194	98%	160	81%
VII	209	204	98%	170	81%
VIII	209	215	103%	178	85%
IX	219	216	99%	178	81%
X	230	235	102%	187	81%
XI	236	246	104%	180	76%
XII	226	228	101%	163	72%
		Av.	99%	Av.	78%

whereas rats fed 300 ppm of mercuric chloride showed a decrease of approximately 20 percent in food intake and weight. These patterns are shown in Figures 2 and 3, respectively. Initially all rats weighed 64 ± 1g.

Effect of Mercury on β -Galactosidase

The method of Messer and Dahlqvist (1966) was used to determine the intestinal β -galactosidase activity in the samples. While actual values with time ranged from 860.48 to 27.82 units, repeated analyses on single samples differed by no more than 1%. Difficulty was experienced with this technique because there is a substance present in both the rat and human homogenates that inhibits color development in the reaction. To overcome this effect, a 20-fold (0.2 g) higher concentration of glucose oxidase was used in the PGO reagent for the samples than was used for the standard curve. After dialysis of the samples, a lower concentration of glucose oxidase could be used to run the assays. This suggested that the inhibitor was a low-molecular weight compound which inhibited the glucose oxidase.

The enzyme activity of the control rats for each week is shown in Table 9 and Figure 4. The β -galactosidase activity, inhibition and mercury content of the liquid intestinal fractions from rats on a low-level or high-level diet are shown in Table 9. A cyclic pattern of

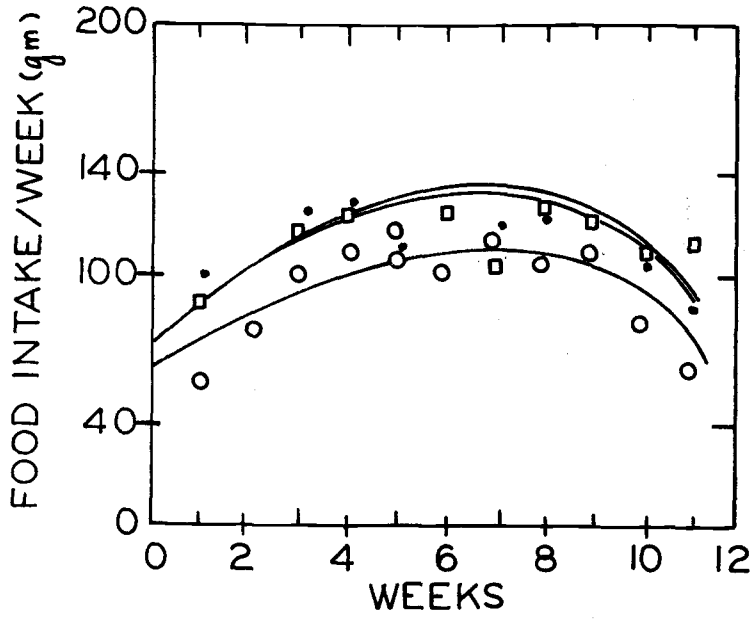

Figure 2. Comparison of food eaten per week by rats on a diet of no $HgCl_2$ (· ·), 6 ppm $HgCl_2$ (□ □), or 300 ppm $HgCl_2$ (O O).

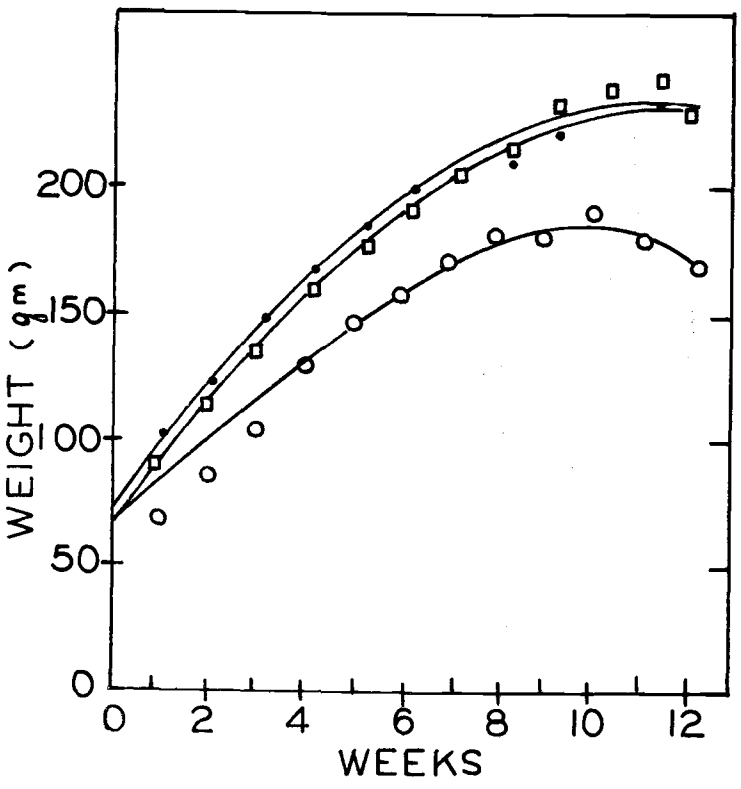

Figure 3. Comparison of weight per week of rats on a diet of no $HgCl_2$ (· ·), 6 ppm $HgCl_2$ (□□) or 300 ppm $HgCl_2$ (O O).

Table 9. Weekly specific activity and average % inhibition of β-galactosidase and total mercury content of rats fed 6 ppm or 300 ppm $HgCl_2$.

Week	Specific Activity of Controls[a]	6 ppm $HgCl_2$			300 ppm $HgCl_2$		
		Sp. Act.	Av. % Inhibition[b]	Total Hg (AA)	Sp. Act.	Av. % Inhibition	Total Hg (AA)[d]
0	790.92	-	-	-	-	-	-
I	790.92	498.90	36.92	ND[c]	414.02	47.66	0.195
II	860.48	420.54	51.12	ND	202.20	76.51	0.0425
III	229.60	123.88	46.05	0.005	105.00	54.28	0.0265
IV	243.36	133.00	45.35	ND	75.86	68.63	0.155
V	125.52	104.80	16.51	ND	131.72	0	0.03
VI	92.62	100.30	0	0.005	111.76	0	0.11
VII	304.54	201.44	33.86	-	198.92	34.68	0.05
VIII	115.24	79.82	30.74	0.006	27.82	69.69	0.05
IX	418.40	240.92	42.42	0.009	224.30	46.39	0.09
X	349.38	191.66	45.14	0.012	173.96	50.21	0.05
XI	377.86	334.28	11.54	0.005	282.70	29.77	0.04
XII	335.78	362.80	0	-	324.00	3.51	0.03

[a] Specific Activity = μ moles of lactose hydrolyzed/min/g of protein at 37°C; values are averages of 5 determinations each on one rat per sample.

[b] % inhibition = 100 - sample/control x 100.

[c] ND = non-detectable.

[d] AA = Atomic absorption analysis.

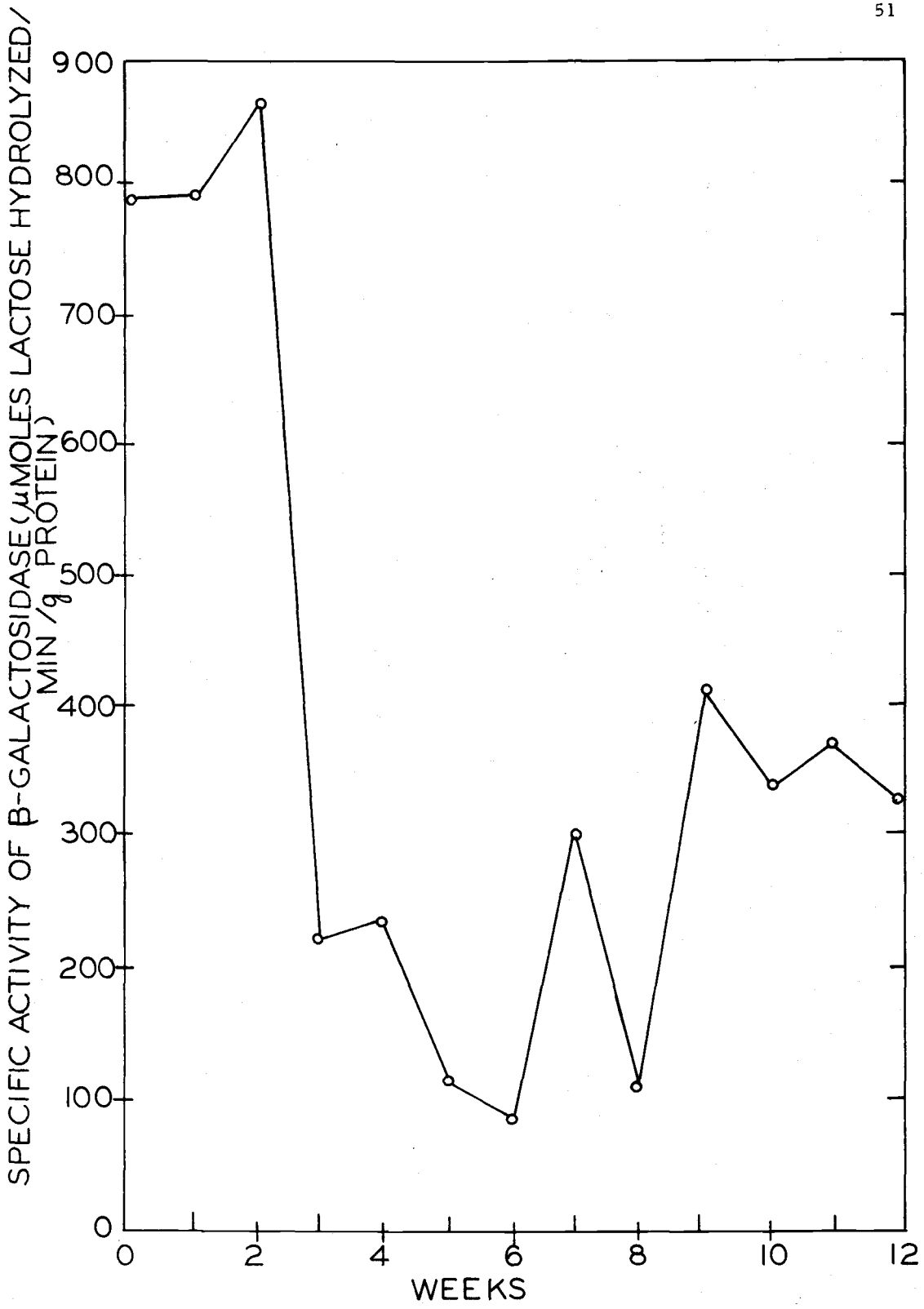

Figure 4. Weekly specific activity of β-galactosidase from control rats.

enzyme inhibition by 6 and 300 ppm of mercuric chloride is shown in Figure 5. Rats fed the 6 ppm mercuric chloride diet for 6 or 12 weeks or the 300 ppm mercuric chloride diet for 5 or 6 weeks had normal enzyme activities and only slight inhibition (3.51 percent) was observed at 12 weeks. There does not appear to be any correlation between enzyme inhibition and mercury content of the liquid intestinal fraction. For example, β-galactosidase activity for rats on a 6 ppm mercuric chloride diet for two weeks was inhibited 51.12 percent, although no detectable amount of mercury was measured in the enzyme solution. On the other hand, β-galactosidase activity for rats on a 6 ppm mercuric chloride diet for eleven weeks was inhibited 11.54 percent and 0.005 ppm of mercury was present in the enzyme solution. A similar lack of correlation was demonstrated with rats on a 300 ppm mercuric chloride diet. No gross pathological differences of the small intestine were noted between control animals and mercury fed animals.

Effect of Mercury on β-Galactosidase from Human Biopsies

β-Galactosidase assays were performed on whole intestinal tissue from normal individuals using the method of Messer and Dahlqvist (1966). The enzyme activities from seven individuals are shown in Table 10. The average activity was 318.38 μmoles of lactose hydrolyzed/minute/g of protein at 37° C. Varying concentrations

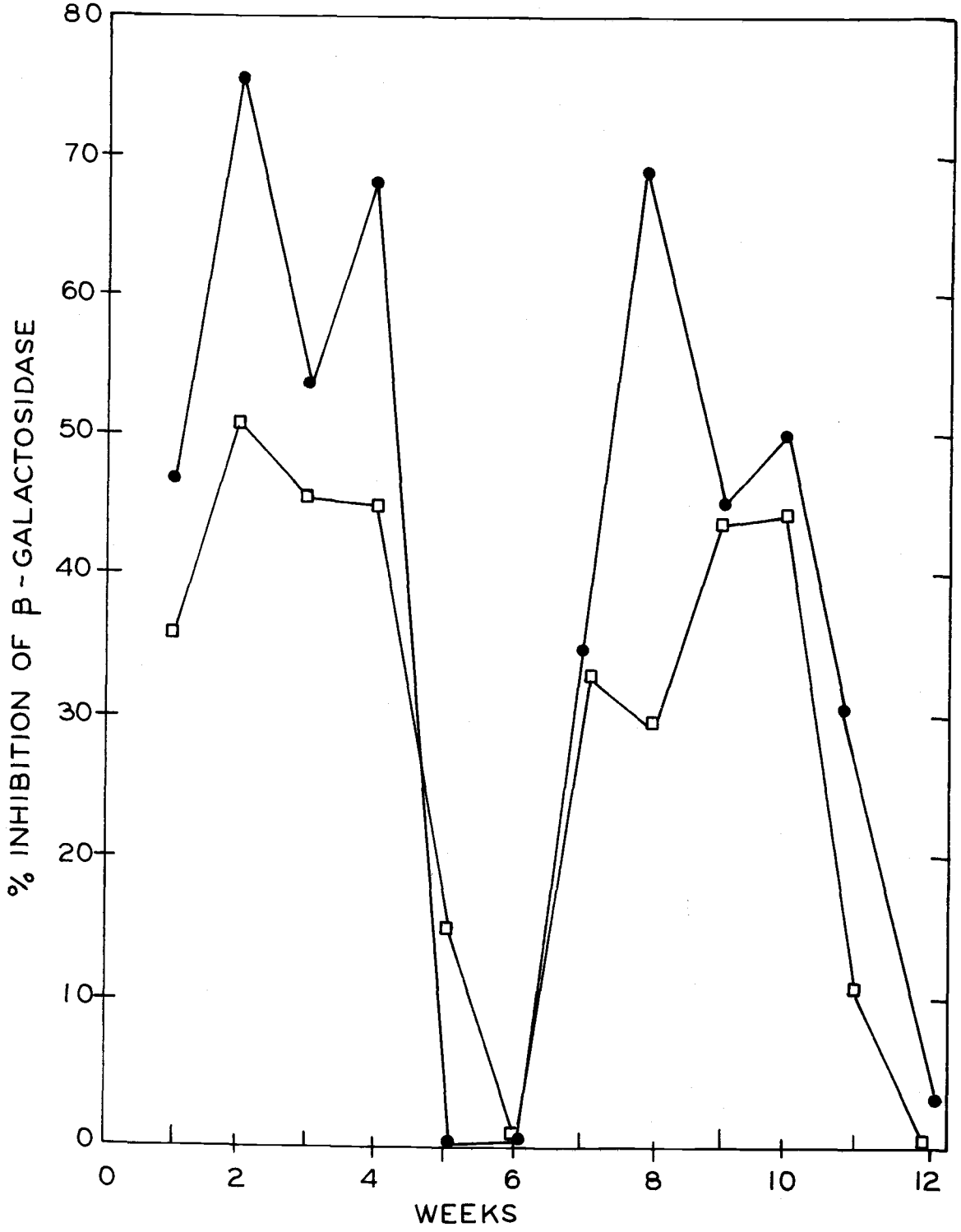

Figure 5. Weekly % inhibition of β-galactosidase from rats on a 6 ppm (□ □) or 300 ppm (· ·) $HgCl_2$ diet.

Table 10. Specific activity of β-galactosidase from human intestinal biopsies.

Patient Number	Specific Activity[a]
I	322.96
II	359.82
III	284.24
IV	312.38
V	323.08
VI	291.58
VII	334.54
	Av. 318.38

[a]Specific Activity = μmoles of lactose hydrolyzed/min/g of protein at 37° C.

of mercuric acetate were added to one of the enzyme preparations and the results are shown in Table 11. Addition of 100 ppm of mercuric acetate resulted in 100 percent inhibition, 33 ppm gave 43.78 percent inhibition, and 17 ppm gave 23.24 percent inhibition. Partial purification (Gray and Santiago, 1969) of the samples made no appreciable difference on the effect of mercury on the enzyme.

Table 11. Effect of mercuric acetate on specific activity of β - galactosidase from human intestinal biopsies.

Mercuric Acetate ppm	Specific Activity[a]	Inhibition[b] %
0	334.54	Control
.1	328.72	2.00
.3	328.72	2.00
5	337.44	0
8	325.82	2.61
17	256.78	23.24
33	188.08	43.78
100	0	100

[a] Specific Activity = μmoles of lactose hydrolyzed/min/g of protein at 37° C.

[b] % inhibition = 100 - sample/control x 100.

DISCUSSION

The procedures of Dahlqvist (1964) and Lederberg (1950), used to determine β-galactosidase activity of Streptococcus lactis, Escherichia coli and intestinal homogenates, seemed unsatisfactory when first used; when mercury was added to intestinal homogenates of hogs or bacterial extracts, no inhibition of β-galactosidase from hog mucosa was observed and inhibition of the E. coli enzyme only occurred in the presence of high-levels of mercury (50 to 100 ppm). However, when these samples were partially purified (Gray and Santiago, 1969), 1.0 ppm of mercuric acetate inhibited the β-galactosidase from hog intestine by 41.2% and 0.16 ppm of mercuric acetate inhibited the enzyme from E. coli by 50%. Thus, it is clear that the presence of excess protein in enzyme preparations markedly reduced the effectiveness of the assay.

Difficulty was also experienced with the Messer and Dahlqvist (1966) procedure; apparently this was due to a low-molecular weight substance present in the intestinal homogenates of rats and humans which inhibited the PGO reagent and prevented color development. Attempts were not made to identify the material, but when samples were dialyzed or when more glucose oxidase was added to the PGO reagent, this effect was overcome. β-Galactosidase activity of rats on a 6 ppm or 300 ppm mercuric chloride diet was 50% inhibited when

no detectable (less than 0.005 ppm) mercury was present in the samples, but 33 ppm of mercuric acetate was required to inhibit β-galactosidase from whole human tissue by 43.78%. Partial purification (Gray and Santiago, 1969) made no appreciable difference on the effect of mercury on β-galactosidase from either rats or humans. These findings suggest that intestinal enzymes are very sensitive in vivo but that in vitro reveal less sensitivity probably because active sites become masked due to destruction of tissue organization during extraction. Since the human biopsy sample used had been frozen and thawed prior to mercury sensitivity testing, additional studies should be made on fresh unfrozen samples. It is likely that inhibition at low-levels of mercury would be realized under these conditions.

The rat study also showed no appreciable difference in food consumption and weight gain patterns between control animals and animals fed 6 ppm mercuric chloride in the diet. This could be due to the length of time the experiment was run, small amounts of mercury absorbed (Clarkson, 1968) and/or a protective effect produced by estrogen (Harber and Jennings, 1965; and Lehotzky, 1972). However, rats on a 300 ppm mercuric chloride diet showed approximately a 20% decrease in food consumption and weight compared to the controls. This decline is considerably lower than that reported by Emerick and Holm (1972) in male rats on a similar diet. Their animals showed a 41% decline in weight and gross symptoms of

hypertension and dermatitis with partial hairlessness in survivors. During these studies, it was observed that rats fed 6 ppm or 300 ppm mercuric chloride in the diet, showed a cyclic inhibition of β-galactosidase activity (Figure 5). In the initial phase of the cycle, from one to five weeks, enzyme inhibition fluctuated and at 6 weeks, no enzyme inhibition was observed. A similar pattern occurred for the second phase, 7 through 12 weeks. Since female rats were used, the cyclic phenomenon observed may be the result of hormonal influence. In this regard, the recent study of Lehotzky (1972) is noteworthy. This worker fed male rats methoxy-ethyl mercuric chloride (MEMC) and administered weekly injections of estrogen. The hormone prevented the destruction of epithelial cells of tubuli and also prevented a rise in urinary transaminase level, which appear to be suitable criteria for use as indices of subacute mercury poisoning. Maximum protection occurred between 5 and 6 weeks, which in the present study, corresponded to the increase in β-galactosidase activity from female rats on the mercuric chloride diet. Selye (1970) has demonstrated a similar protective effect produced by the steroid spironolactone. The mechanism of protection might be due to the production of sulfhydryl compounds, a renotropic effect or induction of non-specific enzymes by the hepatic microsomes.

In 1965, Harber and Jennings reported that male rats were more susceptible to the nephro-toxic action of mercury compounds

than female rats. Male sex hormones given to female rats prior to injection with mercury, altered the usual benign response and a marked, coagulative, renal tubular response occurred. Injection of female sex hormones into male rats and castration with and without hormones, prevented renal damage. Therefore, the data in this study further substantiate previous reports that estrogen may account for the partial protective effect observed against mercuric chloride poisoning in rats. In addition, this study also points out the need to use both male and female rats and to extend the experiment beyond 12 weeks.

The data are encouraging and supportive that the inhibition of microbial β-galactosidase can be used as a clinical test for heavy metal intoxication. The procedures used in this study are not difficult to perform, yet appear to be sensitive enough to detect inhibition of β-galactosidase by low levels of mercury, if the samples are purified. In addition, the reagents used are relatively inexpensive and approximately 20 samples can be run in 75 min. In the future, it needs to be determined what biological specimens of humans can be conveniently used to test for inhibition of β-galactosidase or other enzymes and consequently heavy metal intoxication.

This study is related to the increased concern over the presence of heavy metals in the environment. With the recent publicity and influx of knowledge, it has been suggested that continued

exposure to very small doses of heavy metals, previously reported as harmless, may lead to toxic levels (Dales, 1972, and Joselow, Louria and Browder, 1972). Accumulation of the heavy metals may inhibit very sensitive enzyme systems, such as β-galactosidase, resulting in damage to the gastrointestinal tract and central nervous system. A variety of general and minor symptoms or severe disabilities may develop (Joselow, Louria and Browder, 1972).

No systematic epidemologic studies have been conducted for associations between chronic low-level intake of mercury and genetic, teratogenic or neurologic disturbances. The possibility of long-term exposure to low-levels of mercurial compounds exists in the form of air pollution (Joselow, Louria and Browder, 1972). It is estimated that coal burning alone releases 1,800 tons of mercury into the air per year in the United States (Wallace, Fulkerson and Shults, 1971). Furthermore, air measurements of particulates in New York City revealed that the mercury concentration of indoor samples was as high as 40 $\mu g/m^3$, which is several times greater than the safe level for animals (Stahl, 1969). Russian studies have demonstrated a mercury health hazard to animals at levels as low as 0.3 $\mu g/m^3$ over an extended period of time (Stahl, 1969). It has been suggested that a 24-hr limit for mercury be no higher than 10 $\mu g/m^3$ (Stahl, 1969). The average levels of mercury in the air at various locations is: over the ocean--0.001 $\mu g/m^3$, over nonmineralized

areas--0.003 to 0.009 $\mu g/m^3$, over ore deposits--0.2 to 20 $\mu g/m^3$ and over cities--0.01 to 0.17 $\mu g/m^3$ (Joselow, Louria and Browder, 1972). Consequently, a relationship between heavy metal intoxication as a result of breathing polluted air is suggested.

Although the etiology of adult lactose intolerance is unknown, genetic, dietary and environmental factors have been implicated as being responsible for this carbohydrate malabsorption syndrome. In 1966, Haemmerli et al. suggested that specific enzyme inhibitors may be responsible for the increasing frequency of carbohydrate intolerance being diagnosed in our society, rather than a genetic or aging effect. It has been demonstrated that the administration of both neomycin and colichine inhibit the production of β-galactosidase (Cain, Reiner and Patterson, 1968; and Nelson, 1969). No studies have been conducted that implicate heavy metals as a cause of lactose intolerance, but it is known that heavy metals are potent enzyme inhibitors, especially of the sulfhydryl type, as is the case for β-galactosidase.

SUMMARY

This investigation studied the effect of mercury on β-galactosidase activity and whether or not this enzyme could be used as a clinical test for the detection of mercury poisoning. The sources of β-galactosidase were cell-free extracts of Streptococcus lactis, Escherichia coli and intestinal homogenates of hogs, female rats and humans. Mercuric acetate was added in vitro to all sources of β-galactosidase except for the rats, which were placed on a 6 ppm or 300 ppm mercuric chloride diet. In addition to the β-galactosidase assays, the rat study also included the determination of the mercury content of the blood, liver, kidney and intestinal fractions by atomic absorption spectrometry and food consumption and weight gain patterns.

Excess protein present in crude samples of β-galactosidase from hog mucosa and E. coli interferred with the assay procedure and high levels of mercury (50-100 ppm) were required for enzyme inhibition. However, after partial purification approximately 50% inhibition of β-galactosidase from hog mucosa and E. coli was observed by low levels of mercury, 1.0 ppm and 0.16 ppm mercuric acetate, respectively.

The intestinal homogenates of rats and humans contained a low-molecular weight substance that inhibited the PGO reagent in

the assay procedure. After dialysis of the samples or modification of the PGO reagent, 50% inhibition of β-galactosidase from rat tissue was observed by less than 0.005 ppm mercuric chloride. However, 33 ppm mercuric acetate was required to inhibit β-galactosidase from human tissue by 43.78%.

While this work was in progress, it was observed that rats on a mercuric chloride diet exhibited a cyclic inhibition pattern of β-galactosidase. In addition, rats on a 6 ppm mercuric chloride diet showed no appreciable decrease in food consumption or weight gain in comparison to the control animals and rats on a 300 ppm mercuric chloride diet showed only a 20% decline in food consumption and weight gain and no gross pathological symptoms of the small intestine. The cyclic phenomenon and partial protective effect against mercuric chloride poisoning was considered to be the result of estrogen production.

The data suggest that procedures employed in this study to detect inhibition of β-galactosidase are sensitive enough for use in developing a confirmatory laboratory test to diagnose mercurialism, if samples are partially purified.

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