# BENEFICIAL EFFECT OF CONSUMING MILK CONTAINING CELLS OF *LACTOBACILLUS ACIDOPHILUS* ON LACTOSE MALABSORPTION IN HUMANS

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# CHAPTER I

## INTRODUCTION

Lactose malabsorption is common in a high proportion of the world's population. The incidence of lactose malabsorption varies with race and geogrpahy. Lactose malabsorption is caused by an enzymatic deficiency in the intestines. Because of the lack of lactase, the lactose in milk is not hydrolyzed to glucose and galactose in the small intestine and passes to the large intestine where intestinal bacteria convert part of the lactose to acids and gases. In the large intestines, the lactose molecules along with acids and gases result in gastrointestinal disorders which include such symptoms as diarrhea, flatulence, and abdominal cramping. These symptoms discourage the consumption of milk by lactose malabsorbers.

Researchers have thought of ways to prevent the symptoms of lactose malabsorption. As one of the means to aid the lactose malabsorber, lactose hydrolyzed milk was introduced. In this product the lactose is hydrolyzed by lactase to glucose and galactose prior to ingestion. However, such a lactose hydrolyzed product has a different taste than normal milk and the enzyme is expensive. These two factors have reduced acceptance of lactose hydrolyzed milk. Another approach to aid lactose malabsorbers who cannot enjoy drinking milk would be more desirable.

Significance of Lactobacillus acidophilus cultures as dietary

adjuncts has been increased since Metchnikoff's first mention of the desirability of man consuming lactobacilli capable of living in the intestinal tract. Since some lactose malabsorbers may consume cultured dairy products without the symptoms of lactose intolerance (Bayless and Huang, 1969; Gallagher et al., 1974), milk containing cells of L. acidophilus might be useful in the prevention of the symptoms of lactose malabsorption in humans. No data are available showing whether or not the product is beneficial for lactose malabsorbers.

The objectives of this study were to determine if the addition of cells of L. acidophilus as a dietary adjunct to milk can prevent or reduce lactose malabsorption in humans; to find out if the number of L. acidophilus added to milk is critical in aiding lactose malabsorbers; to determine if consuming milk containing cells of L. acidophilus has an immediate effect on lactose malabsorption; and to determine if lactose in milk containing L. acidophilus is hydrolyzed during refrigerated storage.

# CHAPTER II

# REVIEW OF THE LITERATURE

## Lactose Malabsorption in Humans

Milk which is available in most parts of the world is the main food consumed by infants. However, older children and adults do not always include milk in their diet. Since it has been known for a long time that milk may cause loose stools or diarrhea in some people (Hahn, 1896), its place in the nutritional value of older children and adults has become a subject of controversy due to the milk intolerance which causes gastrointestinal disorders (Sandine and Daly, 1979; Eastman, 1977).

The inability to absorb lactose which is one of the several causes for milk intolerance has been studied for many years since several studies (Bayless and Rosensweig, 1966; Haemmerli et al., 1965; Littman and Hammond, 1965) have called attention to the association between milk intolerance and a deficiency of lactase in the intestines. In lactase deficient individuals the ingestion of one or two glasses of milk leads to a gastrointestinal disorder which is called lactose intolerance associated with lactose malabsorption.

Because of the lack of lactase, the lactose of milk is not hydrolyzed to glucose and galactose in the small intestine and goes into the large intestine where intestinal bacteria convert part of the lactose to organic acids and gases. An osmotic effect is exerted in the colon by the intact lactose and some products of bacterial metabolism resulting

from degradation of lactose and other nutrients. Some of these have a direct irritating effect on the colon mucosa stimulating intestinal motility (Haemmerli et al., 1965; Holzel et al., 1962; Weijers et al., 1961.) These result in gastrointestinal disorders which include such symptoms as diarrhea, flatulence, and abdominal cramping (Rosensweig, 1969; Sandine and Daly, 1979). These symptoms discourage the consumption of milk by lactose malabsorbers.

## Incidence of Lactose Malabsorption

Lactose malabsorption is common in a high proportion of the world's population. The incidence of lactose malabsorption varies with different races and geographical location (Kretchmer, 1971). Balyess and Rosensweig (1966) and Cuatrecasas et al. (1965) reported that approximately 70 percent of a population of American blacks could be classified as lactose malabsorbers, in contrast to 6 to 12 percent among the American white population. Further reports indicated malabsorption of lactose to be prevalent in other ethnic groups including: Indian (Davis and Bolin, 1967), American Indian (Johnson et al., 1977; Caskey et al., 1977), Cypriots (McMichael et al., 1966), Semites (Gilat et al., 1970), Orientals (Chung and McGill, 1968), Vietnamese (Anh et al., 1977), Korean (Bahk and Ahn, 1977), Japanese (Shibuya et al., 1970), Eskimos (Gudmand-Höyer and Jarnum, 1969), and East African (Cook and Kajubi, 1966). The symptoms of lactose malabsorption were age-related. With increasing age the incidence of lactose malabsorption becomes more prevalent (Caskey et al., 1977; Bahk and Ahn, 1977; Paige et al., 1979). Bayless et al. (1975) reported that lactose intolerance is common in most of the world's adults and is a clinically relevant problem.

## Diagnostic Methods of Lactose Malabsorption

Various direct and indirect clinical tests for diagnosis of lactose malabsorption due to a lactase deficiency have been developed. Some of the applicable methods include lactase assay (Newcomer and McGill, 1966), fecal analysis tests (Kerry and Anderson, 1964; Harrison and Walker-Smith, 1977), blood tests (Paige et al., 1978; Isokoski et al., 1972; Kern and Heller, 1968), and breath analysis tests (Arvanitakis et al., 1977; Newcomer and McGill, 1975; Bond and Levitt, 1976; Bond and Levitt, 1972; Levitt and Donaldson, 1970).

The lactase assay, a direct method, determines hydrolyzing capacity of intestinal mucosa obtained by peroral biopsy. A content of less than 2 lactase units (μmoles of substrate hydrolyzed per minute at 37°C) by enzymatic determination is considered a lactase deficiency. However, lactase activity of a given biopsy sample may not be representative of the enzyme distribution throughout the entire jejunum in all individuals. This lactase assay is too complicated and invasive for routine use (Torún et al., 1979).

Because of the difficulty of obtaining a biopsy specimen, especially in children, an indirect method for the detection of lactase deficiency is preferred. Fecal analysis tests include measurements of stool pH and fecal reducing sugars as means of determining lactose malabsorption. Harrison and Walker-Smith (1977) reported that these tests were only reliable on parameter of lactose maladsorption in infants. The tests are not quantitative, as stool dilution influences the concentration of both hydrogen ions and fecal reducing sugars.

The most commonly used indirect method was the blood glucose test. It determines the rise in plasma glucose concentration due to hydrolysis

of lactose after consumption of an oral dose of lactose. Plasma samples from capillary blood are taken at certain intervals after ingestion of the dose of lactose. Plasma glucose of less than 20 mg/dℓ is considered indicative of lactose malabsorbers. A more specific variation of this blood test is the blood galactose test. The chemical determination of galactose in the test is more complex than that of glucose, since ethanol was used with the lactose dose (Isokoski et al., 1972; Kern and Heller, 1968). Both tests require a lactose dose of 2 g/kg, up to a maximum of 50 g, in order to achieve the expected rise in plasma monosaccharide for persons who are not lactose malabsorbers.

A breath test in which the rate of pulmonary excretion of $^{14}CO_2$ was determined after an oral dose of labelled lactose was consumed has been reported (Arvanitakis et al., 1977; Newcomer and McGill, 1967). The $^{14}CO_2$ specific activity in the breath was measured by liquid scintillation spectroscopy following an oral dose of $^{14}C$-labelled lactose. The unabsorbed lactose is converted to $^{14}CO_2$ by colonic bacteria and $^{14}CO_2$ is excreted through the lungs. The amount of $^{14}CO_2$ excreted in the breath after consuming a lactose dose is an indication of the degree of lactose malabsorption. Application of this test is limited by the high cost of the stable isotope labelled lactose and the need of a mass spectrometer for its quantification (Torún et al., 1979).

The breath hydrogen test following consumption of an oral dose of lactose has become increasingly popular. This technique is based on the observation that hydrogen produced from unhydrolyzed lactose by bacteria in the colon is excreted through the lungs. Samples of the expired breath is obtained at intervals and analyzed rapidly by gas chromatography for quantitating hydrogen. The amount of hydrogen excreted in

the breath after consuming lactose is an indication of the degree to which a person is a malabsorber. Caskey et al. (1977) selected a response factor of 20 ppm of hydrogen as the upper limit for lactose absorbers on the basis of breath hydrogen tests in 15 control subjects with a normal dose of lactose. Those who excreted more than 20 ppm were considered to be malabsorbers.

Arvanitakis et al. (1977) compared the blood glucose test and the breath $^{14}CO_2$ test with a 50 g lactose dose and selected the breath $^{14}CO_2$ test as the more sensitive method. Bond and Levitt (1976), using an oral dietary dose (12.5 g) of lactose, found breath hydrogen excretion correlated much more closely with lactose malabsorption than the breath $^{14}CO_2$ test or fecal excretion of the isotope. Newcomer et al. (1975) compared the rise in plasma galactose, breath $^{14}CO_2$, and breath hydrogen tests. The breath hydrogen test proved to be the most sensitive method. The breath hydrogen test is currently accepted as the most sensitive method and is routinely used (Levitt and Donaldson, 1970; Bond and Levitt, 1972; Fernandes et al., 1978).

## Beneficial Role of Cultured Dairy Products for Lactose Malabsorbers

Interest has been increasing in finding a way to help the lactose malabsorber who cannot consume milk without suffering the symptoms associated with lactose malabsorption. As one means to aid the lactose malabsorbers, lactose hydrolyzed milk was introduced (Paige et al., 1975; Cheng et al., 1979; Skala et al., 1971). In this product the lactose is hydrolyzed by β-galactosidase to glucose and galactose prior to ingestion. Such a lactose hydrolyzed product, however, has a different taste

than normal milk. Furthermore, its application is limited by relatively high cost of the enzyme. Thus it has not gained wide acceptance. Another approach to aid lactose malabsorbers who cannot enjoy drinking milk is more desirable.

There have been several reports of cultured milk products being beneficial for people who are lactose malabsorbers. Bayless and Huang (1969) reported those who were intolerant to lactose could tolerate the consumption of large quantities of fermented milk. Baer (1970) indicated the possible use of yogurt for lactose malabsorbers because of its reduced content of lactose brought about by the action of bacterial fermentation. Gallagher et al. (1974) reported that three subjects who were described as being intolerant to lactose were able to consume fermented dairy products such as yogurt, butter milk, and cottage cheese without developing symptoms of lactose intolerance whereas consumption of nonfermented milk caused moderate to severe symptoms. They theorized that the bacteria in the cultured products might continue to exert lactase activity in the intestine after ingestion. Siddons and Coates (1972) have indicated that lactase activity in the chicken is associated with microorganisms in the intestinal tract. Germ-free chicks could not utilize lactose while conventional chicks could. Smythe (1958) reported that yogurt could be used to establish a normal intestinal flora and to prevent staphylococcal entero-colitis. The benefits attributed to yogurt were due partly to the stimulation of the normal intestinal lactobacilli (Orla-Jensen et al., 1945). Recently milk containing cells of L. acidophilus has been suggested as a possible source of the enzyme β-galactosidase to aid lactose malabsorbers (Sandine, 1979; Gilliland, 1979).

## Use of L. acidophilus as Dietary Adjuncts

The interest in using L. acidophilus cultures as dietary adjuncts has increased since Metchnikoff's first mention of the desirability of man consuming lactobacilli capable of living in the intestinal tract (Metchnikoff, 1908; Speck, 1980). Metchnikoff (1908) believed that life could be prolonged by the ingestion of soured milk containing cells of Lactobacillus bulgaricus. Later attempts to implant L. bulgaricus in the intestine were unsuccessful by Cheplin and Rettger (1920, 1921); however, they found that L. acidophilus would survive implantation. Increasing the numbers of lactobacilli in the human intestinal tract by consuming L. acidophilus has been reported by several researchers (Kopeloff, 1926; Morey, 1953; Gilliland et al., 1978). Beneficial roles of L. acidophilus in humans needing therapy for various types of intestinal illnesses have been studied by Kopeloff (1926) and by Rettger et al. (1935). They mentioned that constipation, diarrhea, and other intestinal disorders may be satisfactorily treated by L. acidophilus. Additional studies (Sharpe and Mattick, 1957; Tramer, 1973; Brown, 1977) demonstrated strong evidence for its beneficial effects on human health. Gilliland (1979) mentioned several characteristics of lactobacilli which would be desirable if they are to be used as dietary adjuncts. Such lactobacilli must be capable of surviving and growing in the intestine. They also must be able to resist gastric acid in the stomach and bile salts in the intestine. Another desirable property of the organism to be used as a dietary adjunct is that it be able to exert antagonistic effects toward undesirable microorganisms in the intestinal tract. L. acidophilus has been shown to exert antagonistic actions toward such organsisms as

enteropathogenic Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Clostridium perfringens (Gilliland and Speck, 1977; Hamdan and Mikolajcik, 1974; Vincent et al., 1959).

Since the earliest work on the role of the lactobacilli in the intestinal tract, L. acidophilus has been the organism of the group which has been most often mentioned as being useful for inclusion in the diet. Gilliland (1979) and Gilliland et al. (1975) mentioned that care must be taken with regard to source in selecting strains of L. acidophilus for use in dietary preparations intended to be of benefit in the human intestinal tract. Several other articles also indicated the host specificity of L. acidophilus. Morishita et al. (1971) reported that L. acidophilus isolated from a human intestinal tract could not be implanted in the intestinal tract of a chick. Mitsuoka (1969) indicated differences in characteristics of L. acidophilus isolated from man and other animals.

Recently the effect of dietary supplements of L. acidophilus on chemically-induced cancer was studied by using an animal model (Goldin and Gorbach, 1980). They found that these supplements delayed the development of colon cancer in rats exposed to a carcinogen. Goldin et al. (1980) also reported that the metabolic activities of the fecal bacteria could be favorably altered by supplementing the human diet with L. acidophilus.

## Unfermented Acidophilus Milk

Acidophilus milk containing viable cells of L. acidophilus was a fermented product. Speck (1980) said this product was used mainly for medical purposes. Since this fermented acidophilus milk had an unacceptable flavor and low number of viable cells of L. acidophilus, Myers

(1931) grew L. acidophilus in a broth medium and then added the cells to pasteurized milk to provide a more palatable product which had the same flavor as regular pasteurized milk. Duggan et al. (1959) used a frozen concentrated culture of L. acidophilus to be added by the consumer to milk immediately prior to consumption to provide a palatable unfermented acidophilus milk. The development of and availability of frozen concentrated cultures of L. acidophilus that can be added to refrigerated pasteurized milk commercially has made it possible to provide the consumers with a pleasant tasting nonfermented milk containing cells of the organism (Gilliland et al., 1978). Such a product, prepared by adding cells from frozen concentrated culture of L. acidophilus to cold pasteurized low fat milk, is currently available in many areas of the United States. Several million viable and bile resistant cells of L. acidophilus of human origin are present per mℓ and the population can be maintained for two to three weeks with proper refrigeration. The flavor of this milk is not altered by the culture. Gilliland et al. (1978) evaluated the qualities of such an unfermented low fat milk containing cells of L. acidophilus from a commercial frozen concentrated culture. The flavor of the product was the same as regular low fat milk and feeding trials showed significant increases in the numbers of facultative lactobacilli in the feces of human test subjects after consumption of this milk.

Sandine (1979) mentioned that three beneficial purposes of lactobacilli in the human intestinal tract are intestinal disease therapy, preventative therapy for maintaining intestinal health, and a possible enzyme source for lactose malabsorbers. Thus milk containing cells of L. acidophilus may be beneficial for lactose malabsorbers. No practical

data are available showing whether or not such a product is beneficial for lactose malabsorbers.

# CHAPTER III

## EXPERIMENTAL PROCEDURE

### Source of Culture and Preparation of Milk

Frozen concentrated cultures of L. acidophilus NCFM (of human origin) were obtained from Marschall Products, Miles Laboratories, Inc. (Madison, Wisconsin) and stored at -196°C in liquid nitrogen. The frozen concentrated cultures were contained in plastic cryogenic vials (5 g/vial). Regular pasteurized milk was obtained from the dairy processing plant in the Animal Science Department of Oklahoma State University. The frozen concentrated culture was thawed by placing a 5 g vial in 1 liter of tapwater at 30-35°C. The milk containing cells of L. acidophilus was prepared by adding the thawed concentrated frozen culture as follows: Five grams of the thawed concentrated culture was aseptically added to one quart of pasteurized milk to achieve a population of $2.5 \times 10^8$/mℓ. To prepare milk containing lower populations, one gram of the thawed concentrated culture was aseptically diluted with 9 mℓ of sterile distilled water. Five mℓ or 0.5 mℓ of this 1:10 dilution was then added to each quart of pasteurized milk to prepare milk containing populations of $2.5 \times 10^7$/mℓ or $2.5 \times 10^6$/mℓ. The prepared milk containing cells of L. acidophilus was stored at 5°C before delivery or consumption. The milk was not used beyond three days of storage.

## Confirmation of Identification of the Culture

The procedure using the Minitek system (Baltimore Biological Laboratories, Cockeyville, Maryland) as described by Martin (1979) was used to confirm the identity of the culture. The following tests were done: Gram stain, Catalase test, the ability to deaminate arginine, to hydrolyze esculin, and to ferment amygdalin, arabinose, cellobiose, galactose, glucose, inositol, lactose, maltose, mannitol, mannose, melezitose, melebiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. To test the ability of the culture to grow at 15°C and 45°C, two tubes of lactobacilli MRS broth (Difco Laboratories, Detroit, Michigan) were inoculated with the culture using a flame sterilized inoculating loop. One tube was incubated at 15°C for 1 week and the other tube was incubated at 45°C for 24 hr. Visual turbidity was a positive test for growth.

## Determination of Populations of *L. acidophilus* in Milk

Serial dilutions were prepared with 99 mℓ dilution blanks composed of 0.1% peptone (Difco Laboratories, Detroit, Michigan) and 0.001% Antifoam A Emulsion (Sigma Chemical Company, St. Louis, Missouri) in distilled water. The dilution blanks were sterilized by heating 15 minutes at 121°C prior to use. All dilutions in this study were made according to the procedures described in the *Compendium of Methods for the Microbiological Examination of Food* (Speck, 1976).

Total populations of *L. acidophilus* in milk were determined by a pour plate procedure. Plates containing the appropriate dilutions were poured with melted MRS agar at 45°C. MRS agar was prepared by dissolving

1.5% Bacto Agar (Difco Laboratories, Detroit, Michigan) in lactobacilli MRS broth prior to autoclaving. Numbers of bile-resistant organisms were enumerated using a pour plate procedure with MRSO agar. MRSO agar was prepared by adding 0.1% oxgall (Baltimore Biological Laboratories, Cockeysville, Maryland) to MRS agar. Duplicate plates for each dilution were prepared with each medium in all experiments. After solidification, the plates were inverted and incubated at 37°C for 48 hr. All colonies visible with the aid of a Quebec colony counter were counted.

## Determination of Hydrogen Concentration in Breath Samples

The breath hydrogen test (BHT) used to measure lactose malabsorption in human test subjects was a slight modification of that reported by Levitt and Donaldson (1970). The breath hydrogen test was conducted by using Varian Model 920 Gas Chromatograph equipped with a thermal conductivity detector, a one mℓ sample loop, and a 6-port gas sampling valve. Separation of hydrogen from other gaseous components of the breath was obtained using a column (336 cm long, 0.16 cm inside diameter [I.D.]) packed with 60-80 mesh 5A molecular sieve (Supelco, Inc., Bellofonte, Pennsylvania). Separation was achieved at a column temperature of 53°C with argon as the carrier gas at a flow rate of 18 mℓ/min. The detector temperature was 107°C.

When a breath sample was to be analyzed, one mℓ sample from the sample bag was transferred into a sample loop via the gas sampling valve by vacuum created with 25 mℓ gas tight syringe. Concentrations of hydrogen (ppm) were determined by comparing the area of the hydrogen peak obtained from each sample with a standard curve prepared using mixtures of

hydrogen and nitrogen. The purity of hydrogen obtained from Big Three Industry, Houston, Texas, was certified as 99.998%. Nitrogen was obtained from Sooner Supplies, Shawnee, Oklahoma. It contained no detectable hydrogen.

To prepare 10,000 ppm hydrogen, a 25 mℓ gas tight syringe was used to inject 10 mℓ of hydrogen into a gas sample bag containing 990 mℓ of nitrogen. The nitrogen was measured with a 1ℓ gas tight syringe (Glenco Scientific, Inc., Houston, Texas). Both hydrogen and nitrogen were taken from separate gas sample bags previously filled so that both gases were at room temperature when measured for preparing the mixture. A stock mixture containing 100 ppm hydrogen was prepared by adding 10 mℓ of the 10,000 ppm hydrogen to 990 mℓ of nitrogen in a sample bag. The 25, 50, 75, and 100 ppm hydrogen standards were prepared from the stock hydrogen concentration (100 ppm).

The material for the gas sample bag was plastic laminated aluminum obtained from Reynolds Metals Company, Richmond, Virginia. A plastic laminated aluminum sheet (28 x 60 $cm^2$) was folded and sealed by a heat sealer (Kapak Corporation, Bloomington, Minnesota). A brass threaded tubing connector obtained from a local hardware store was mounted through a hole in one side of the bag prior to sealing. The connector was secured by a threaded nut on the interior side of the bag. Rubber washers on either side of the bag material facilitated a gas tight seal. A tygon tube (3.2 mm I.D.) was attached to the tubing connector to facilitate sample collection and analysis. The tubing was sealed with a Hoffman openside pinchcock. A sketch of a sampling port for a sample bag is shown in Figure 1.

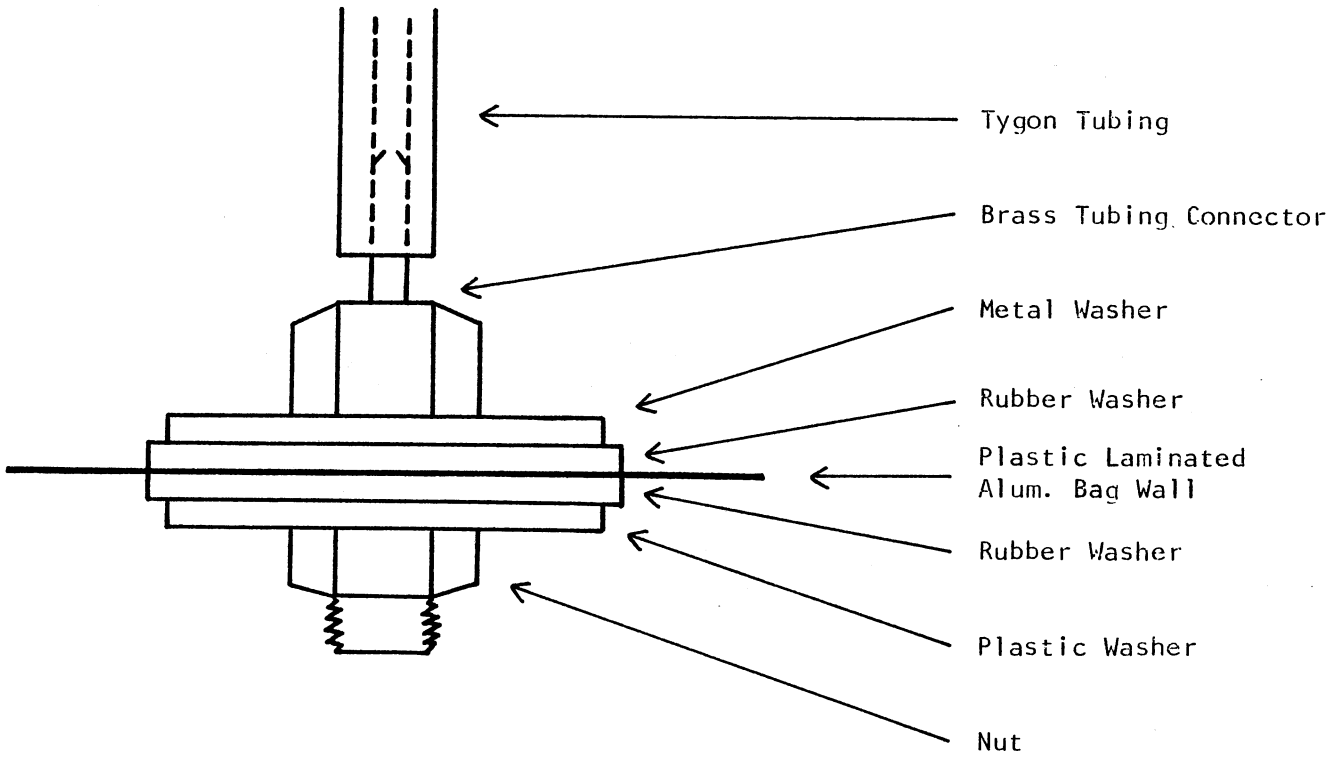


Figure 1. Sketch of Sample Port on Gas Sample Bag

## Procedure for Breath Hydrogen Test (BHT)

The breath hydrogen test was conducted in the morning following a 12-hr fasting period. Each subject was instructed to consume nothing other than water for the 12-hr period immediately preceding the test. At the start of the test the exhaled breath sample from each subject was collected in a sample bag. The subject, while standing, inhaled (deep breath) and held his breath for 10 seconds, then exhaled it into the sample bag. Prior to each use, the sample bags were flushed with nitrogen and evacuated by vacuum. They were heated in an oven (80°C for 180 min) periodically to evaporate moisture from the sample bags.

Following the initial breath sample the test subjects consumed the indicated milk product (5 mℓ/kg body weight) as the lactose dose. Additional breath samples were collected (as described for the initial sample) 60 minutes after consuming the test dose and thereafter at 30-minute intervals for 2 to 3 hr. The collected samples were analyzed immediately for hydrogen content by gas chromatography and the average hydrogen content (ppm) of the three consecutive samples exhibiting the greatest levels of hydrogen was used as the hydrogen concentration in the breath of each subject.

## Test Subjects

Test subjects determined to be lactose malabsorbers were selected from international graduate students at Oklahoma State University. Only those lactose malabsorbers excreting at least 30 ppm hydrogen were accepted as test subjects. The nationalities of subjects were 1 Nigerian, 1 American Indian, 1 Iranian, 1 Bangladeshi, 2 Iraqis, 2 Koreans, 9 Malaysian, and 12 Taiwanese. The age range of subjects was between 20 and

31. All were apparently healthy and having no recent history of gastrointestinal disturbance and were not currently using oral antibiotics. None of the subjects had consumed commercially available acidophilus milk.

All subjects gave consent for participating in the study after being informed of the nature and the purpose of the study. Figure 2 is a copy of the consent form. Each subject was asked to keep a daily record of the milk consumption, any symptoms of lactose intolerance encountered, and any medication taken during the experimental period (Figure 3). The individuals were not told whether or not the milk they were to consume contained cells of L. acidophilus.

## Influence of Daily Consumption of Milk Containing L. acidophilus on Lactose Malabsortion

To determine if the addition of cells of L. acidophilus as a dietary adjunct to milk can prevent or reduce lactose malabsorption in humans, 12 test subjects who were determined to be lactose malabsorbers were randomly assigned to one of two groups and consumed the assigned milk for six days. One group was assigned milk containing $2.5 \times 10^8$ cells of L. acidophilus per mℓ (acidophilus group) and the other was assigned milk without the cells of L. acidophilus (control group). Using the assigned milk, subjects consumed 5 mℓ per Kg body weight twice daily. The milk was delivered to them by the author. Sufficient milk for three days was delivered each time.

The intensities of lactose malabsorption for each subject in both groups were determined on day 0 using the breath hydrogen test in which pasteurized whole milk was used as the test dose. Each subject in the

Description of Experimental Objective

Some evidence has been reported that indicates cultured milk products may be consumed by people who are lactose intolerant without causing the usual symptoms associated with the consumption of milk by these individuals. There have been suggestions in recent years that a new dairy product, Sweet Acidophilus Milk, might be useful for people who are intolerant to lactose. The objective of this study is to determine if the addition of cells of Lactobacillus acidophilus to milk (resulting in a product such as Sweet Acidophilus Milk) can prevent lactose malabsorption when consumed by persons intolerant to lactose.

L. acidophilus is a bacteria that is part of the normal group of bacteria occurring in the intestinal tract of "healthy-normal" humans. It has been used in the manufacture of certain cultured milk products since the early 1900's. There has been no evidence to show it is in any way harmful to humans.

Description of Procedure

Each individual will be assigned to one of two groups. All will be subjected to a lactose malabsorption (using breath hydrogen analyses requiring about 3 to 3½ hr) test on the first day. Then each will be assigned milk containing cells of Lactobacillus acidophilus; others will receive milk without the L. acidophilus. The individual will not be told whether or not the milk contains L. acidophilus. After 7 days the individuals will repeat the lactose malabsorption test.

Each subject will be asked to keep a daily record of the milk consumption and any symptoms of lactose malabsorption that they may encounter. The usual symptoms that might be encountered are mild diarrhea, stomach cramps, and/or flatulence.

Consent Statement

My role in this experiment and discomforts I might experience have been explained to me by Dr. S. E. Gilliland, and I have been given the opportunity to ask questions concerning the experiment or procedures involving me. I understand that I may withdraw from the study at any time. I give my consent to be included as a test subject in this study.

Signature: _____

Date: _____

Figure 2. Agreement to Participate in Experiment Involving Consumption of Milk Containing Lactobacillus acidophilus

STUDY ON EFFECT OF LACTOBACILLUS ACIDOPHILUS
ON LACTOSE MALABSORPTION IN HUMANS
ANIMAL SCIENCE DEPARTMENT
OKLAHOMA STATE UNIVERSITY

Name: _____

Weight: _____

Amount of Milk: _____ twice a day

| Date | Consumed Milk[a] | | Symptoms[b] | | Medication[c] |
|---|---|---|---|---|---|
| | A.M. | P.M. | Type | Mild or Severe | |
| | | | | | |
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[a]Record time milk was consumed.

[b]Indicate symptoms (cramps, flatulence, diarrhea)

[c]If any medication was taken, indicate what it was.

Figure 3. Daily Record of Milk Consumption, Symptoms of Lactose Intolerance, and Medication

control group consumed pasteurized whole milk for six days. Individuals in the acidophilus group consumed milk containing L. acidophilus ($2.5 \times 10^8$/mℓ) for six days. On day 7, the breath hydrogen tests were repeated. The lactose test dose for the subjects in the control group was pasteurized whole milk. The test dose for the subjects in the acidophilus group was pasteurized whole milk containing $2.5 \times 10^8$ L. acidophilus/mℓ. The design for the trial is summarized in Table I.

Additional trials were conducted to determine if the number of cells of L. acidophilus in the milk was critical. Milk containing different populations of L. acidophilus cells was evaluated in a manner similar to that in the first trial. Twelve test subjects (not used in previous trial) who were determined to be lactose malabsorbers were randomly assigned to one of two groups and drank the assigned milk for six days. One group was assigned milk containing $2.5 \times 10^7$ cells of L. acidophilus per mℓ and the other received milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ. Double blind tests were used during the first and second experimental trials. Neither the test subjects nor the analyst knew which subjects received control milk or milk containing cells of L. acidophilus in the first trial. Also neither of them in the second trial knew which subjects received milk containing $2.5 \times 10^7$ cells or $2.5 \times 10^6$ cells of L. acidophilus per mℓ.

## Immediate Effect of Consuming Milk Containing L. acidophilus on Lactose Malabsorption in Humans

To determine if consumption of milk containing cells of L. acidophilus had an immediate effect on lactose malabsorption, milk containing

TABLE I

EXPERIMENTAL DESIGN FOR DETERMINATION OF INFLUENCE OF DAILY CONSUMING MILK CONTAINING CELLS OF L. ACIDOPHILUS

| | |
|---|---|
| Control Group | |
| Day 0 | Breath $H_2$ Test: Pasteurized Whole Milk as Lactose Test Dose |
| Day 1-6 | Consumed Pasteurized Whole Milk (5 mℓ/Kg Body Weight) Twice Daily |
| Day 7 | Breath $H_2$ Test: Pasteurized Whole Milk as Lactose Test Dose |
| Acidophilus Group | |
| Day 0 | Breath $H_2$ Test: Pasteurized Whole Milk as Lactose Test Dose |
| Day 1-6 | Consumed Pasteurized Whole Milk Containing L. acidophilus[a] (5 mℓ/Kg Body Weight) Twice Daily |
| Day 7 | Breath $H_2$ Test: Pasteurized Whole Milk Containing L. acidophilus[a] as Lactose Test Dose |

[a] $2.5 \times 10^8$/mℓ.

$2.5 \times 10^6$ cells of L. acidophilus per mℓ was evaluated using 5 test subjects not previously used. Breath hydrogen tests were done on all subjects twice (day 0 and day 7) using pasteurized whole milk as the lactose test dose. The breath hydrogen tests were repeated on day 14 and 21 using milk which containing $2.5 \times 10^6$ L. acidophilus/mℓ as the test dose. No milk was consumed by any of the subjects during the periods between the breath hydrogen tests.

## Statistical Evaluation

The effect of daily consumption of pasteurized whole milk on lactose malabsorption in humans was studied by using a paired experiment as statistical analysis in which comparison of breath hydrogen content of test subjects on day 0 and day 7 was determined. The effect of daily consumption of pasteurized whole milk containing L. acidophilus cells on lactose malabsorbers was also examined by using a paired experiment to determine if significant differences occur between day 0 and day 7. The t-distribution test was used to compare the effect of treatments. The paired experiment used in this trial was used also for evaluating the data collected in the second trial which was to determine if the number of L. acidophilus cells in milk was critical.

The data obtained from five subjects that consumed pasteurized milk on day 0 and day 7, and milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ on day 14 and day 21 were analyzed by a randomized complete block design, with each subject being a block, to determine if the immediate effect of consuming milk containing L. acidophilus cells on lactose malabsorbers was significant. In this analysis, it is assumed that there are no residual effects or time order effects unrelated to the treatment.

The methods for these analyses are outlined in Principle and Procedure of Statistics (Steel and Torrie, 1960).

## Lactose Content and Numbers of L. acidophilus in Milk During Refrigerated Storage

To determine if lactose in refrigerated milk containing cells of L. acidophilus was hydrolyzed prior to consumption, the amounts of lactose were quantitated periodically during refrigerated storage of the milk. The milk was assayed using an enzymatic method (Taylor, 1970) initially and after 3 and 7 days of storage at 5°C.

The number of L. acidophilus in the milk was determined at various periods during refrigerated storage using a pour plate procedure with MRS agar and MRSO agar (Mitchell, 1981). Dilutions were prepared according to procedures in Compendium of Methods for the Microbiological Examination of Food (Speck, 1976). The plates were incubated 48 hr at 37°C after which all colonies visible with the aid of a Quebec colony counter were counted.

## Influence of Bile Salts on Lactose Hydrolysis in Milk by Cells of L. acidophilus

The effect of bile salts on the hydrolysis of lactose in milk containing cells of L. acidophilus was determined by measuring the amounts of glucose after incubation of milk samples containing the organism. The samples were composed of 10% reconstituted nonfat milk solid (NFMS) and varying concentrations of oxgall. All samples were inoculated with equal numbers of L. acidophilus.

Eighteen mℓ of distilled water was added to 2 g of thawed concentrated cultures of L. acidophilus. The cells were recovered by

centrifugation of 12062 x g for 10 minutes at 0°C in a Sorvall Model 5c-5 Superspeed Refrigerated Centrifuge (Dupont Company, Newton, Connecticut). The supernatant fluid was discarded and the cells were suspended in 20 mℓ of distilled water. This washing procedure was repeated and the resuspended cells were centrifuged again. The precipitated cells were dispersed in 20 mℓ of 0.05 M sodium phosphate buffer, pH 7.0. To achieve 2.5 x $10^7$ cells of L. acidophilus per mℓ, 0.3 mℓ of the cell suspension was added to 60 mℓ of 10% reconstituted NFMS. Ten mℓ aliquots of the inoculated milk were added to test tubes containing 0.0, 0.05, 0.075, 0.1, and 0.125 g oxgall (Baltimore Biological Laboratories, Cockeysville, Maryland). All tubes were incubated for 5 hr at 37°C. Two mℓ of distilled water and 8 mℓ of 0.3 N barium hydroxide were mixed with 2 mℓ of each of the incubated samples. Then 8 mℓ of 5% zinc sulfate was added and the mixtures were mixed thoroughly to precipitate the protein. The samples were centrifuged for 10 minutes at 12062 x g. The resulting clear supernatant fluid was assayed for glucose using the PGO enzymatic method (Sigma Technical Bulletin No. 510; Sigma Chemical Co., St. Louis, Missouri).

For a glucose standard, 20 mg of anhydrous glucose was dissolved in distilled water and diluted to 1000 mℓ as stock solution. From the stock solution, concentrations of 0.004, 0.008, 0.012, 0.016, and 0.02 mg/mℓ of glucose were prepared.

The pH of each sample was measured with a Beckman Select Ion 2000 pH meter (Irvine, California) to the nearest 0.01 pH unit. The measurements were taken before and after the 5 hr incubation period to indicate whether or not the L. acidophilus grew during the assay period.

The populations of cells of L. acidophilus in 10% reconstituted NFMS were determined using a pour plate procedure with MRS agar.

# CHAPTER IV

# RESULTS

## Confirmation of Identity and Populations of the Concentrated Culture of *Lactobacillus acidophilus*

The results of tests to confirm the identity of *L. acidophilus* NCFM used in this experiment are presented in Table II. The characteristics of the culture matched those of *L. acidophilus* as described in *Bergey's Manual of Determinative Bacteriology*, 8th edition (Buchanan and Gibbons, 1974).

The population of *L. acidophilus* in the frozen concentrated culture obtained from Marschall Products, Miles Laboratories, Inc. was $5 \times 10^{10}$ per gram. This was based on counts obtained on lactobacilli MRS agar.

## Determination of Hydrogen Concentration in Breath Samples of Lactose Malabsorber

A typical recorder response resulting from gas chromatographic assay of a 1 mℓ of breath sample from a lactose malabsorber is shown in Figure 4. The retention time of hydrogen was 1.6 minutes using a column packed with 5A molecular sieve with flow rate of 18 mℓ per minute in the gas chromatographic analysis. The hydrogen peak appeared to be completely

TABLE II

BIOLOGICAL CHARACTERISTICS OF L. ACIDOPHILUS NCFM[a]

| Test | Lactobacillus acidophilus NCFM | Bergey's[b] |
|---|---|---|
| Gram Stain | + | + |
| Cellular Morphology | rods | rods |
| Catalase | - | - |
| Growth at 15°C | - | - |
| Growth at 45°C | + | ± |
| $NH_3$ from Arginine | - | - |
| Hydrolysis of Esculin | + | + |
| Acid from: | | |
| Amygdalin | + | + |
| Arabinose | - | - |
| Cellobiose | + | + |
| Galactose | + | + |
| Glucose | + | + |
| Inositol | - | - |
| Lactose | + | + |
| Maltose | + | + |
| Mannitol | - | - |
| Mannose | + | + |
| Melezitose | - | - |
| Melebiose | + | ± |
| Raffinose | + | ± |
| Rhamnose | - | - |
| Salicin | + | + |
| Sorbitol | - | - |
| Sucrose | + | + |
| Trehalose | + | + |
| Xylose | - | - |

[a]Obtained as frozen concentrated culture from Marschall Products, Miles Laboratories, Inc., Madison, Wisconsin.

[b]Characteristics of L. acidophilus as indicated in Bergey's Manual of Determinative Bacteriology, 8th Edition (Buchanan and Gibbons, 1974).

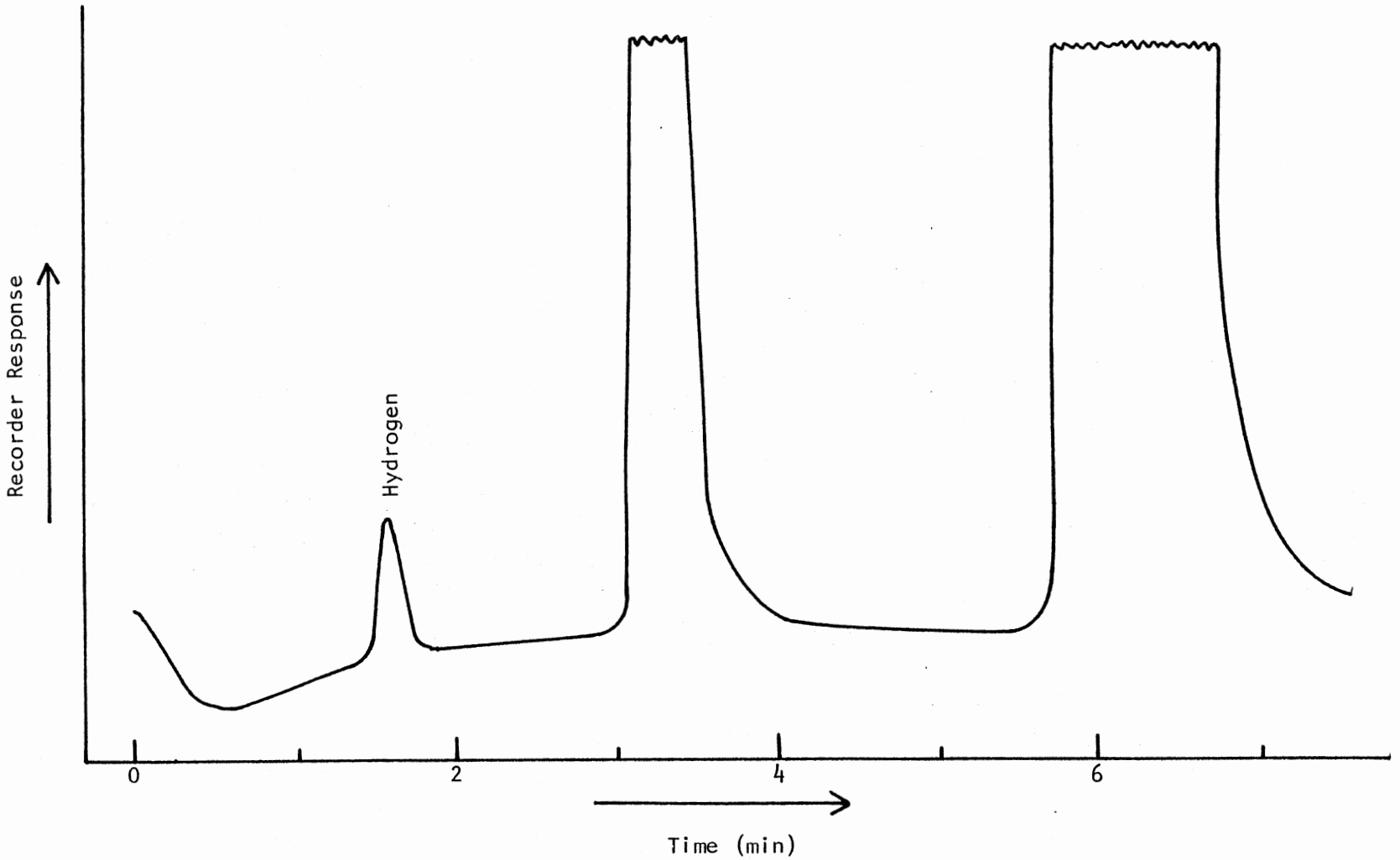


Figure 4. Gas Chromatogram Resulting From Analysis of Breath Sample From a Human Who is a Lactose Malabsorber

separated from other components in the sample. A defined peak was observed for standards containing as little as 2 ppm of hydrogen.

According to the data collected from analyses of standards on different days, the slopes of two different fitted equations--both of which passed through origin for the standard curve--were the same (OSL = 0.33), which indicated excellent repeatability (Table XII in the Appendix). The linear equation by statistical evaluation showed that $Y = 0.0136\ X$, where X is hydrogen concentration and Y is area of peak. The test for lack of fit of a straight line was not significant (OSL = 0.1). The standard curve for calculation of hydrogen concentration is shown in Figure 5. The closeness of fit of points to curve at each concentration is indicated in the graph by 95% prediction limits.

An example of a hydrogen gas excretion curve in breath samples of a lactose malabsorber after consumption of milk (5 mℓ/Kg body weight) is shown in Figure 6 in a manner similar to that of Fernandes et al. (1978). Breath samples were analyzed 60, 90, 120, 150, 180, and 210 minutes after ingestion of the milk. Hydrogen concentration (ppm) in each breath sample was plotted against sample time in this figure. The highest concentration was obtained in the range of 120 to 180 minutes after consumption of the lactose dose. An average of the three highest consecutive concentrations (at 120, 150, and 180 minutes) yielded a value of 35 ppm of hydrogen in the subject's breath.

## Feeding Trial 1: Comparison of Effect of Daily Consumption of Milk With and Without *L. acidophilus* on Lactose Malabsorption

The breath hydrogen excretion curves obtained from the six subjects

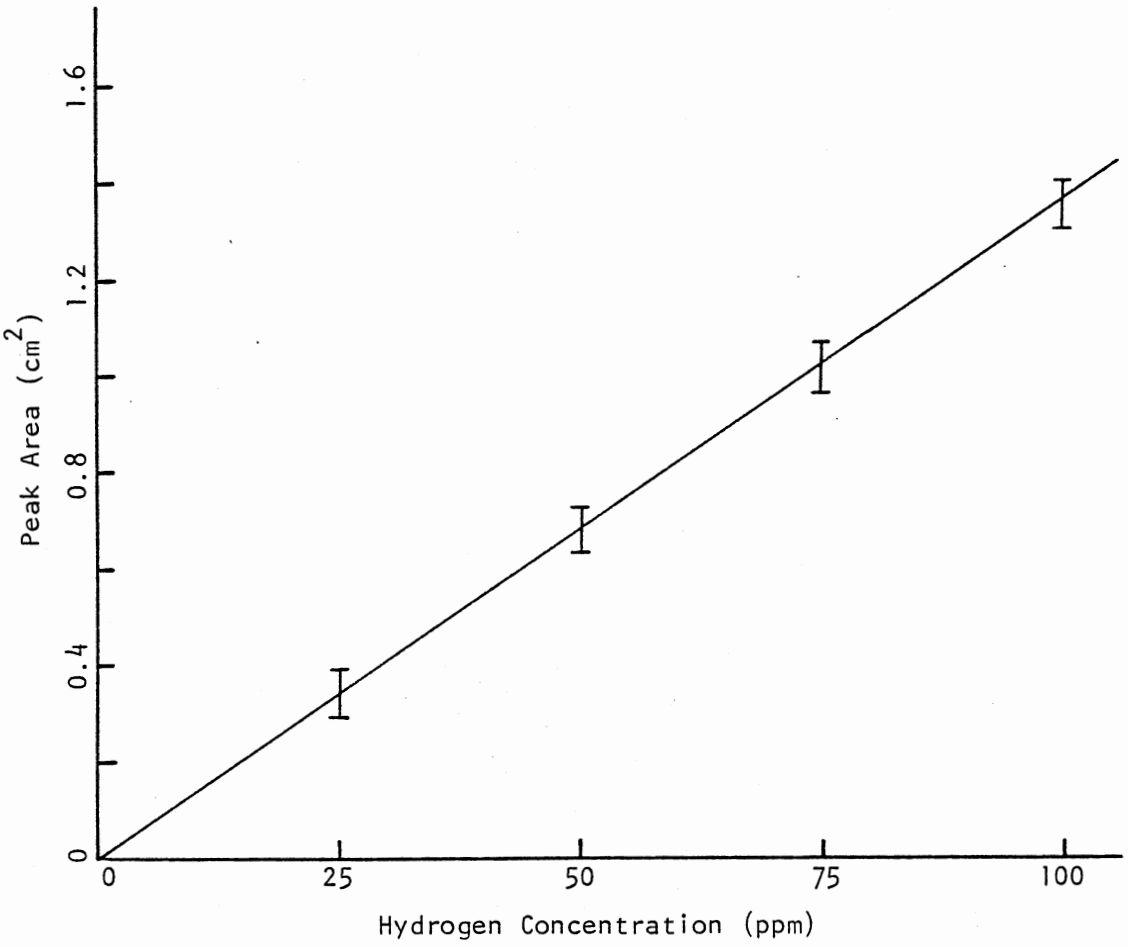


Figure 5. Standard Curve of Hydrogen Concentration

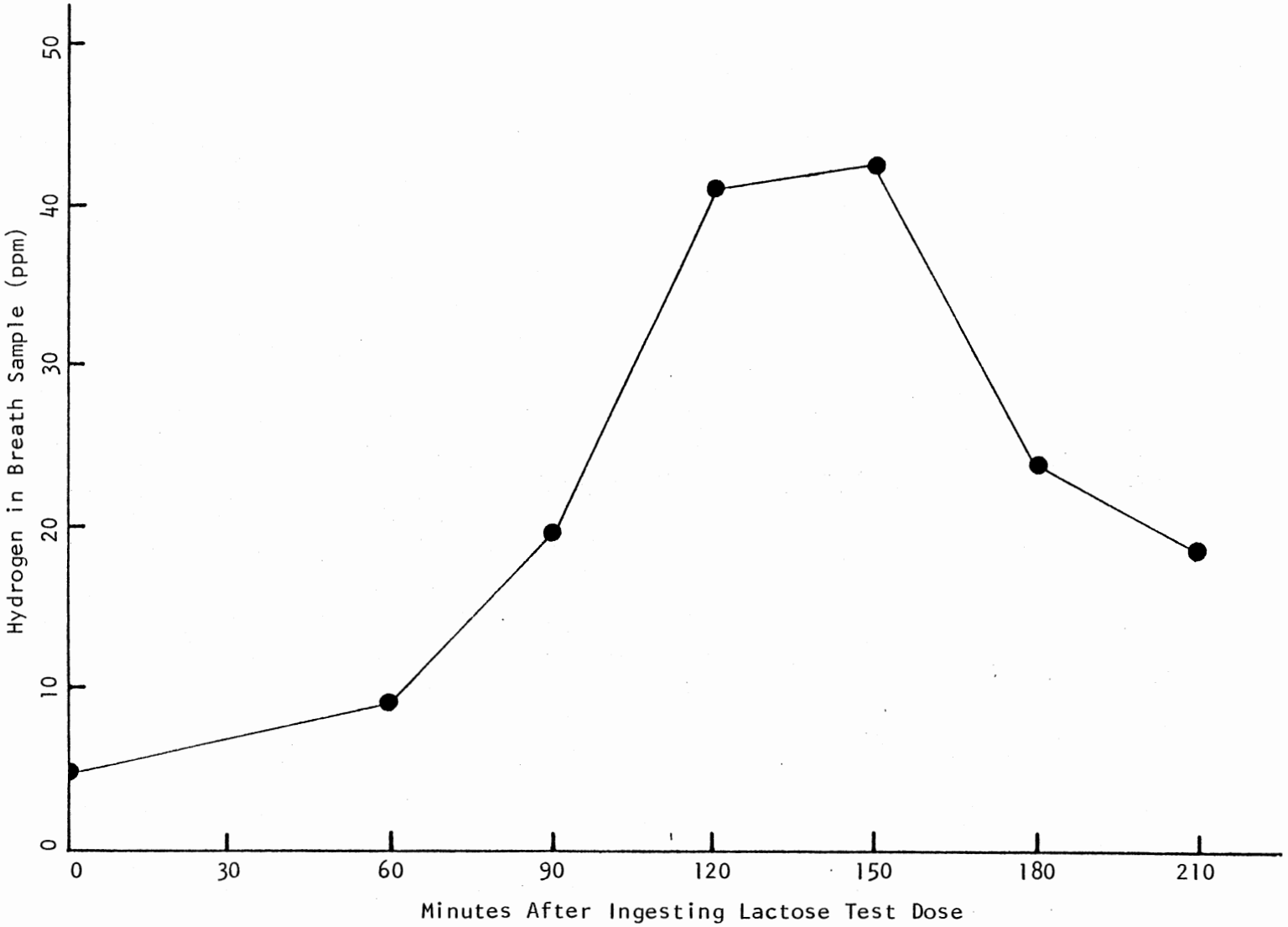


Figure 6. Hydrogen Excretion Curve Obtained During Breath Hydrogen Test for a Lactose Malabsorbing Human

before and after 6 consecutive days of consuming pasteurized whole milk (5 mℓ/Kg body weight) twice per day are shown in Figures 7, 8, and 9 in the Appendix. The test dose for the breath hydrogen test on days 0 and 7 was pasteurized whole milk (5 mℓ/Kg body weight). The age of the test subjects ranged between 24 and 31 years. Three of the six subjects complained of such intolerance symptoms as abdominal cramping and flatulence during the experimental period. Similar breath hydrogen excretion curves were observed on days 0 and 7 for each subject, especially on subjects 1, 4, 5, and 6, although considerable variations were observed in the breath hydrogen excretion curves among the six subjects.

Averages of the three highest consecutive peaks (ppm hydrogen) from each subject on day 0 and day 7 were compared to determine if significant differences occurred (Table III). The overall average hydrogen concentration in the breath of all the subjects on day 0 was similar to that on day 7. Statistical evaluation showed no significant difference ($P > 0.50$) between day 0 and day 7. Thus the level of hydrogen excretion by the lactose malabsorbers was unchanged by having the subjects consume milk for 7 days.

The breath hydrogen excretion curves obtained from the six subjects before and after six consecutive days of consuming pasteurized whole milk containing $2.5 \times 10^8$ cells of L. acidophilus per mℓ (5 mℓ/Kg body weight) twice per day are shown in Figures 10, 11, and 12 in the Appendix. The population of L. acidophilus in the milk was adjusted to $2.5 \times 10^8$/mℓ by adding the appropriate amount of concentrated culture to each quart. The population was confirmed by plating on MRS agar. The test dose for the breath hydrogen test on day 0 was pasteurized whole milk and on day 7 was milk containing $2.5 \times 10^8$ cells of L. acidophilus per mℓ. The age of the

TABLE III

EFFECT OF DAILY CONSUMPTION OF PASTEURIZED WHOLE MILK ON LACTOSE MALABSORPTION IN HUMANS

| Subject | PPM $H_2$ in Breath | |
|---|---|---|
| | Day 0 | Day 7 |
| 1 | 70.6 | 60.1 |
| 2 | 34.5 | 29.3 |
| 3 | 59.1 | 74.4 |
| 4 | 38.0 | 39.4 |
| 5 | 30.1 | 28.1 |
| 6 | 35.5 | 34.4 |
| Average | 44.6[a] | 44.3[a] |

[a]Not significantly different ($P > 0.50$).

test subjects ranged between 26 and 30 years. Two of the six subjects complained of such intolerance symptoms as abdominal cramping and flatulence during the experimental period.

Averages of the three highest consecutive peaks (ppm hydrogen) from each subject on day 0 and day 7 were compared (Table IV). The hydrogen concentrations in the breath of all the subjects except subject 2 were considerably lower than those on day 0. Statistical evaluation showed a significantly ($P < 0.01$) lower hydrogen concentration on day 7 than on day 0. Thus daily consumption of pasteurized whole milk containing $2.5 \times 10^8$ cells of L. acidophilus per mℓ significantly reduced lactose malabsorption in humans.

## Feeding Trial 2: Comparison of Daily Consumption of Two Levels of L. acidophilus on Lactose Malabsorption

Since the daily consumption of pasteurized whole milk containing $2.5 \times 10^8$ L. acidophilus per mℓ significantly reduced lactose malabsorption, additional trials were conducted to determine if the number of cells of L. acidophilus in milk was critical. The pasteurized whole milk containing $2.5 \times 10^7$ and $2.5 \times 10^6$ cells of L. acidophilus per mℓ was evaluated in a manner similar to the first trial. The populations of L. acidophilus in the milk adjusted to $2.5 \times 10^6$ and $2.5 \times 10^7$ per mℓ were confirmed by plating on lactobacilli MRS agar. Twelve test subjects, not previously used, who were lactose malabsorbers were randomly assigned to one of two groups and drank the assigned milk for six days. One group was assigned milk containing $2.5 \times 10^7$ cells of L. acidophilus per mℓ and

TABLE IV

EFFECT OF DAILY CONSUMPTION OF PASTEURIZED WHOLE MILK CONTAINING L. ACIDOPHILUS[a] ON LACTOSE MALABSORPTION IN HUMANS

| Subject | PPM $H_2$ in Breath | |
|---|---|---|
| | Day 0 | Day 7 |
| 1 | 47.0 | 12.1 |
| 2 | 56.0 | 53.0 |
| 3 | 33.8 | 13.9 |
| 4 | 45.9 | 19.4 |
| 5 | 49.1 | 33.9 |
| 6 | 53.5 | 38.0 |
| Average | 47.6[b] | 28.4[b] |

[a] $2.5 \times 10^8$/mℓ.

[b] Significantly lower on day 7 ($P < 0.01$).

the other received milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ. The age of the subjects ranged between 21 and 30 years.

The breath hydrogen excretion curves obtained from the six subjects before and after six consecutive days of consuming pasteurized whole milk containing $2.5 \times 10^7$ cells of L. acidophilus per mℓ (5 mℓ/Kg body weight) are shown in Figures 13, 14, and 15 in the Appendix. The test dose for the breath hydrogen test on day 0 was pasteurized whole milk and day 7 was whole milk containing $2.5 \times 10^7$ cells of L. acidophilus per mℓ.

Comparisons of breath hydrogen excretion of the test subjects on day 0 and day 7 are shown in Table V. The overall averages on day 0 and day 7 indicated slightly lower levels of hydrogen on day 7 than on day 0. However, examination of data for each individual showed that the level of hydrogen was lower on day 7 than on day 0 for only subjects 1, 5, and 6. The result of this paired experiment showed no significant difference ($P > 0.35$) between day 0 and day 7, mainly due to the abnormally increased hydrogen concentrations on day 7 of subjects 2 and 3.

The breath hydrogen excretion curves obtained from the six subjects before and after six consecutive days of consuming pasteurized whole milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ (5 mℓ/Kg body weight) are shown in Figures 16, 17, and 18 in the Appendix. The test dose for the breath hydrogen test on day 0 was pasteurized whole milk and day 7 was whole milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ.

Comparisons of breath hydrogen excretion of the test subjects on day 0 and day 7 are shown in Table VI. All subjects had less hydrogen (ppm) in their breath samples on day 7 than on day 0. Statistical

TABLE V

EFFECT OF DAILY CONSUMPTION OF PASTEURIZED WHOLE MILK CONTAINING 2.5 X $10^7$ CELLS OF L. ACIDOPHILUS PER Mℓ ON LACTOSE MALABSORPTION IN HUMANS

| Subject | PPM $H_2$ in Breath | |
|---|---|---|
| | Day 0 | Day 7 |
| 1 | 34.6 | 22.7 |
| 2 | 31.5 | 50.0 |
| 3 | 34.5 | 51.6 |
| 4 | 41.1 | 46.1 |
| 5 | 38.3 | 33.9 |
| 6 | 40.1 | 9.5 |
| Average | 36.7[a] | 35.6[a] |

[a]No significant difference ($P > 0.35$).

TABLE VI

EFFECT OF DAILY CONSUMPTION OF PASTEURIZED WHOLE MILK CONTAINING 2.5 X $10^6$ CELLS OF L. ACIDOPHILUS PER Mℓ ON LACTOSE MALABSORPTION IN HUMANS

| Subject | PPM $H_2$ in Breath | |
|---|---|---|
| | Day 0 | Day 7 |
| 1 | 49.1 | 43.3 |
| 2 | 58.5 | 23.0 |
| 3 | 72.8 | 44.8 |
| 4 | 37.7 | 23.8 |
| 5 | 72.7 | 69.7 |
| 6 | 43.9 | 38.8 |
| Average | 55.8[a] | 40.6[a] |

[a]Significantly lower on day 7 ($P < 0.025$).

analysis of the data showed that the mean value on day 7 was significantly ($p < 0.025$) lower than that for day 0. Thus the daily consumption of pasteurized whole milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ significantly reduced lactose malabsorption in humans.

## Immediate Effect of Consumption of Milk Containing L. acidophilus Cells on Lactose Malabsorption in Humans

To determine if the frequency of drinking milk was critical on lactose malabsorbers, milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ was evaluated by using pasteurized whole milk as the test dose for breath hydrogen test on days 0 and 7, and milk containing cells of L. acidophilus as the test dose on days 14 and 21. No milk with or without cells of L. acidophilus was consumed during the periods between breath hydrogen tests. Five test subjects not previously used were involved. The age of the test subjects ranged between 21 and 30 years. The population of L. acidophilus in the milk ($2.5 \times 10^6$/mℓ) was confirmed by plating on lactobacilli MRS agar.

The breath hydrogen excretion curves obtained from the five subjects after consumption of pasteurized whole milk on days 0 and 7, and milk containing cells of L. acidophilus on days 14 and 21 are shown in Figures 19, 20, 21, 22, and 23 in the Appendix.

Comparisons of concentrations of breath hydrogen of the test subjects on days 0 and 7 (control mild as test dose) and on days 14 and 21 (using milk containing cells of L. acidophilus as test dose) are shown in Table VII. The average of breath hydrogen concentration (ppm) on day 0 was similar to that on day 7. Also the breath hydrogen on days 14 and 21

TABLE VII

IMMEDIATE EFFECT OF CONSUMING MILK CONTAINING L. ACIDOPHILUS ON LACTOSE MALABSORPTION IN HUMANS[a]

| Subject | PPM $H_2$ in Breath | | | |
|---|---|---|---|---|
| | Control Milk | | Milk Containing L. acidophilus[b] | |
| | Day 0 | Day 7 | Day 14 | Day 21 |
| 1 | 51.3 | 46.1 | 17.4 | 35.7 |
| 2 | 57.9 | 40.9 | 45.6 | 45.3 |
| 3 | 39.5 | 38.8 | 21.3 | 21.6 |
| 4 | 54.8 | 66.0 | 58.2 | 38.7 |
| 5 | 35.2 | 37.0 | 30.3 | 29.9 |
| Averages | 47.7[c] | 45.8[c] | 31.1[c] | 34.2[c] |
| Overall Averages | 46.8[d] | | 32.7[d] | |

[a]No milk with or without L. acidophilus was consumed during the periods between breath hydrogen tests.

[b]$2.5 \times 10^6$/mℓ cells of L. acidophilus.

[c]Day 0 not significantly different from day 7 ($P > 0.70$); day 14 not significantly different from day 21 ($P > 0.95$).

[d]Significantly lower for L. acidophilus milk ($P < 0.01$).

showed fairly close values. The overall average of breath hydrogen concentrations on days 14 and 21 was considerably lower than that on days 0 and 7. The analysis of variance in the data by the completely randomized block design is shown in Table XIII in the Appendix. The analysis revealed that no significant difference ($P > 0.70$) occurred between hydrogen concentrations on days 0 and 7. Also, no significant difference ($P > 0.95$) occurred between the hydrogen concentrations on days 14 and 21. However, there was a significant difference ($P < 0.01$) between the overall average breath hydrogen concentrations for days 0 and 7 (control milk as test dose) and the overall average breath hydrogen concentrations for days 14 and 21 (milk containing L. acidophilus as test dose). Thus the beneficial influence of L. acidophilus on lactose malabsorbers does not require that the milk be consumed daily.

## Lactose Content and the Population of L. acidophilus in Milk During Storage

The amount of lactose in milk containing cells of L. acidophilus during refrigerated storage was quantitated on days 0, 3, and 7 to determine if lactose in refrigerated milk was hydrolyzed prior to consumption. The lactose content in tested milk is shown in Table VIII. The lactose content of milk was constant for seven days of refrigerated storage and no free glucose due to the hydrolysis of lactose was detected.

The population of L. acidophilus in milk during refrigerated storage was also determined on days 0, 3, and 7 to confirm if number of cells of L. acidophilus in refrigerated storage was constant (Table IX). The cells of L. acidophilus in milk did not grow during seven days refrigerated storage and showed fairly constant stability.

TABLE VIII

LACTOSE CONTENT OF MILK CONTAINING *L. ACIDOPHILUS*[a] DURING STORAGE AT 5°C

| Day | Lactose[b] (mg/mℓ) |
|---|---|
| 0 | 49.7 |
| 3 | 48.9 |
| 7 | 48.8 |

[a] $2.5 \times 10^8$/mℓ.

[b] Determined enzymatically.

TABLE IX

STABILITY OF *L. ACIDOPHILUS*[a] IN MILK DURING STORAGE AT 5°C

| Day | Numbers of Cells/mℓ[b] |
|---|---|
| 0 | $2.9 \times 10^6$ |
| 3 | $2.8 \times 10^6$ |
| 7 | $2.5 \times 10^6$ |

[a] The population of *L. acidophilus* was adjusted to $2.5 \times 10^6$/mℓ.

[b] Determined by plating on lactobacilli MRS agar.

## Effect of Bile Salts on Lactose Hydrolysis in Milk by Cells of *L. acidophilus*

The effect of bile salts on the lactase activity of the cells of *L. acidophilus* in milk was determined. The samples included 10% NFMS containing 0.0, 0.5, 0.75, 1.00, and 1.25% oxgall. All were inoculated with $2.5 \times 10^7$ *L. acidophilus* per mℓ. The glucose concentrations in the samples after 5 hr incubation at 37°C are shown for six trials in Table X. The averages from the six trials showed that as the level of oxgall was increased in the milk, the glucose concentration obtained after 5 hr incubation at 37°C increased. Thus the presence of bile salts appears to enhance the ability of the culture to hydrolyze lactose.

In two trials the samples were plated on lactobacilli MRS agar at the end of the 5-hr incubation period to determine the numbers of lactobacilli. The population of *L. acidophilus* was almost twice the initial number (Table XI) but there was no appreciable difference in the numbers among treatments. In both of these trials the pH values after the incubation period were higher in the samples containing oxgall (Table XI). As the concentration of oxgall increased, the pH values also increased.

TABLE X

EFFECT OF BILE SALTS ON THE HYDROLYSIS OF LACTOSE BY L. ACIDOPHILUS[a] IN THE MILK

| Milk Sample[b] Sample No. | Oxgall (%) | Mg Glucose in One mℓ Sample[c] I | II | III | IV | V | VI | Average |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.028 | 0.031 | 0.037 | 0.034 | 0.025 | 0.032 | 0.031 |
| 2 | 0.50 | 0.045 | 0.068 | 0.071 | 0.062 | 0.055 | 0.065 | 0.060 |
| 3 | 0.75 | 0.117 | 0.106 | 0.132 | 0.113 | 0.108 | 0.103 | 0.113 |
| 4 | 1.00 | 0.164 | 0.166 | 0.181 | 0.168 | 0.166 | 0.153 | 0.166 |
| 5 | 1.25 | 0.168 | 0.178 | 0.188 | 0.173 | 0.179 | 0.156 | 0.174 |

[a] $2.5 \times 10^7$/mℓ.

[b] 10 mℓ of 10% reconstituted NFMS with varying concentrations of oxgall.

[c] Amount of glucose after 5 hr incubation at 37°C.

TABLE XI

POPULATIONS OF L. ACIDOPHILUS AND pH CHANGES OF RECONSTITUTED NFMS SAMPLES[a] AFTER FIVE HOURS INCUBATION

| Samples[b] Sample No. | Oxgall (%) | Number of Cells Per mℓ I | II | pH I | II |
|---|---|---|---|---|---|
| 1 | 0.00 | $5.0 \times 10^7$ | $3.9 \times 10^7$ | 6.54 | 6.58 |
| 2 | 0.50 | $4.8 \times 10^7$ | $3.4 \times 10^7$ | 6.60 | 6.62 |
| 3 | 0.75 | $4.5 \times 10^7$ | $3.6 \times 10^7$ | 6.62 | 6.64 |
| 4 | 1.00 | $4.4 \times 10^7$ | $3.4 \times 10^7$ | 6.64 | 6.68 |
| 5 | 1.25 | $4.1 \times 10^7$ | $3.1 \times 10^7$ | 6.66 | 6.70 |

[a] Initial population was $2.6 \times 10^7$ (I) and $3.1 \times 10^7$ (II) per mℓ, and pH of the reconstituted NFMS was 6.72 without cells of L. acidophilus.

[b] 10 mℓ of 10% reconstituted NFMS with varying concentrations of oxgall.

# CHAPTER V

# DISCUSSION

Milk and milk products, which have a good nutritional value, are important components of the human diet in most parts of the world. The consumption of milk is not limited to infants; milk is one of the mainstays of the human diet. Lactose malabsorption, however, is very common in a high proportion of the world's population. The incidence of lactose malabsorption has been reported for various races and geographical distributions (Bayless et al., 1966; Kretchmer, 1971). Torún et al. (1979) clarified the definition of lactose intolerance and lactose malabsorption. Lactose malabsorption is the incomplete absorption of an oral dose of lactose due to the lack of lactase, which hydrolyzes lactose, in the small intestine. Lactose intolerance is the development of such clinical signs and symptoms as abdominal cramping, bloating, flatulence, and diarrhea following an oral ingestion of lactose. These lactose intolerance symptoms can be reduced or prevented by reducing lactose malabsorption. The term lactose intolerance should be avoided when discussing lactose malabsorption. It is desirable to find a way to help lactose malabsorbers who cannot consume milk without suffering the symptoms associated with lactose malabsorption.

Comparisons of various methods of determining lactose malabsorption have been done (Newcomer et al., 1975; Bond and Levitt, 1976). The breath hydrogen test is currently accepted as the most sensitive method

and is routinely used (Fernandes et al., 1978; Torún et al., 1979). The main problems associated with other methods were the sensitivity of the tests and the lactose dose required for the test. Torún et al. (1979) pointed out that a 50 g lactose dose, commonly used in clinical tests, corresponds to one quart of milk, which is in most cases too large an amount of milk to drink at one time. The higher proportion of lactose malabsorbers in various races determined by the blood glucose test which involves a high dose of lactose should be reconsidered. The rate of gastrointestinal transit of unabsorbed lactose and amount of dose play a role in the occurrence of symptoms of lactose intolerance (Douwes et al., 1978). The majority of the data suggests that many individuals who are classified as lactose malabsorbers on the basis of clinical tests involving a standard lactose dose (50 g) can consume dietary amounts of lactose, such as contained in a glass of milk, without experiencing symptoms of lactose intolerance. The selection of a lactose dose for determining lactose malabsorption should be a physiological dose such as 5 mℓ milk per Kg body weight, which corresponds to 12.5 g of lactose dose for 50 Kg body weight. This would be the amount of lactose in an 8- to 9-oz glass of milk. This amount of milk (5 mℓ/Kg body weight) used as test doses in the present study was selected for this reason. This amount of milk should not have resulted in the digestive system merely being "overloaded" with lactose.

The possible mechanism of lactose intolerance due to lactose malabsorption has been discussed (Haemmerli et al., 1965). Dietary lactose is normally split by lactase into glucose and galactose in the small intestinal mucosa cells. The unabsorbed lactose of a lactose malabsorber passes into the large intestine, where it is partly hydrolyzed by

intestinal bacterial lactase and undergoes bacterial fermentation. In the feces of a lactose malabsorber, small amounts of lactose, glucose, and galactose could be found (Thornton et al., 1962). A direct irritating effect on the colon mucosa stimulating intestinal motility due to osmotic effect (Holzel et al., 1962; Weijers et al., 1961), as well as considerable amounts of organic acids and gases in the intestinal tract due to the bacterial fermentation could be the main factors for symptoms of lactose intolerance.

Consumption of milk containing Lactobacillus acidophilus reduced the hydrogen excretion in the breath of lactose malabsorbers. Therefore, L. acidophilus added to milk as a dietary adjunct was beneficial in reducing or preventing lactose malabsorption in humans.

The possible mechanisms whereby L. acidophilus could reduce lactose intolerance symptoms associated with lactose malabsorption are the following: (1) L. acidophilus could supply a source of lactase in the intestinal tract. Lesser amounts of lactose, due to its hydrolysis, reaching the large intestine can reduce such lactose intolerance symptoms as flatulence, abdominal cramping, and even diarrhea. Sandine (1979) and Gilliland (1979) suggested that L. acidophilus might serve as a source of lactase for lactose malabsorbers. (2) L. acidophilus has been shown to exert antagonistic effects in vitro on bacteria that could produce gases in the intestine (Gilliland, 1979). The activities of the gas-producing organisms might be depressed partly due to the production of antibiotic-like substances by the lactobacilli. The antagonistic action produced by L. acidophilus may be due to a combination of substances that include acids, hydrogen peroxide, and antibiotics (Gilliland, 1979). (3) This organism is a homofermentative bacteria and thus does not

produce gas. When the ratio of L. acidophilus to other bacteria in a human intestine increases, the total gas production due to the action of heterofermentative bacteria would perhaps be decreased.

It seems that daily consumption of pasteurized whole milk containing $2.5 \times 10^7$ cells of L. acidophilus per mℓ should have shown a significant difference between the hydrogen excretions on days 0 and 7, since milk containing both $2.5 \times 10^8$ and $2.5 \times 10^6$ cells per mℓ both effectively reduced lactose malabsorption. The unexpected result was probably due to the abnormally increased hydrogen excretion on day 7 of subjects 2 and 3. The possible explanations of this unexpected result include variations in diet and other activities of the test subjects that may have imposed stresses on the digestive system. Donaldson (1964) indicated that the composition of the intestinal flora depends on the environment of the host. The fecal microflora of the mice in the different laboratory conditions showed dramatic variations in the bacterial populations (Dubos and Schaedler, 1962). Personal body conditions including stresses due to excess of alcohol and intestinal diseases vary the condition of intestinal microflora which produce hydrogen in a human intestine (Dudgeon, 1926). Gudmand-Höyer and Simony (1977) mentioned a considerable variance of the individual sensitivity to lactose partly associated with other gastrointestinal diseases. Diet has been recognized as one of the factors affecting the presence and maintenance of intestinal microflora. The numbers and species of intestinal bacteria can be altered by feeding different diets (Porter and Rettger, 1940; Wilbur et al., 1960). Different ratios and different species and strains of intestinal microflora can exist due to the different diets of various nationalities of

the subjects. Changes in the diet were shown to influence the degree of symptoms of lactose intolerance (Skala and Lamacora, 1971).

The immediate effect of consuming milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ was also significant. Lactose malabsorption was reduced by a one-time intake of milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ. Within two to three hours the lactase produced by the $2.5 \times 10^6$ cells of L. acidophilus per mℓ apparently hydrolyzed enough of the lactose in the intestine to reduce malabsorption in most of the subjects.

The individual variations among the test subjects appeared to influence the degree of hydrogen excretion as well as the effects of consuming milk containing cells of L. acidophilus. For some subjects, the effect was very beneficial; for others it was not. Thus we might expect that every lactose malabsorbing person who consumes milk containing L. acidophilus might not realize total relief from symptoms associated with lactose malabsorption.

Some of the subjects who had reduced hydrogen excretion after consuming milk containing cells of L. acidophilus still complained of experiencing symptoms of lactose intolerance. Since hydrogen excretion was generally reduced, a psychological effect should be considered. Even though they had no way of knowing, some of the subjects could have been reacting to the type of milk they thought they consumed rather than the type of milk they actually consumed. It is impossible to eliminate all preconceptions of the test subjects. Lacassie et al. (1978) conducted regression analyses on 436 conventional lactose tolerance tests and found that intolerance symptoms were a very poor predictor of the adequacy of lactose malabsorption as measured by rise in blood glucose, with a

correlation coefficient of only 0.008. The questionnaires for recording symptoms of the subjects in these experiments also showed large variance. Since the perception and tolerance of discomfort vary depending on subjects, reliance on intensity scoring of symptoms is hazardous. Psychological effects of the subject and bias by the observer are hard to control in such a subjective test (Torún, et al., 1979). Objective measurements such as the breath hydrogen tests used in the present study provide much more reliable data than would subjective observations on symptoms relative to the effect of various factors on lactose malabsorption.

In an effort to search for the mechanism whereby L. acidophilus reduces lactose malabsorption, the effect of bile salts on L. acidophilus was determined in vitro. Preliminary experiments indicated that L. acidophilus when growing in milk did not hydrolyze more lactose than was needed for the organism to grow, since free glucose did not accumulate during growth. The results of experiments involving the addition of oxgall to milk indicated that bile salts could increase the ability of the organism to hydrolyze lactose beyond its need for growth. This was based on the accumulation of free glucose when oxgall was added to milk inoculated with L. acidophilus. The amounts of bile salts used in these experiments would not exceed that encountered in the human intestine (Sjövall, 1959). After a 5-hr incubation period, the population of L. acidophilus was almost twice the initial number, although there was a slight decrease in numbers obtained as the concentration of bile salts increased. Thus the larger amounts of free glucose in the samples containing oxgall were not due to increased growth of the culture. The increase in free glucose which coincided with increasing oxgall was presumably due to enhanced lactose hydrolyzing activity of the cells of L. acidophilus.

This was probably due to altered permeability of the cells caused by reduced surface tension because of bile salts in the oxgall. Intestinal conditions, including presence of bile salts, could enhance lactase activity of L. acidophilus by changing in the permeability of the cell membrane. This could be an important factor in prevention of lactose malabsorption.

To reduce the variability of data in this type of study, environmental factors should be properly controlled. Maintaining the same body condition for all test subjects throughout the experimental period and controlling their diets would be highly desirable. The use of reconstituted milk prepared from a single lot of dried milk solids could be used to reduce the possible variations in lots of milk. Careful control of fasting time is also necessary, because more than 12 hours fasting might adversely affect the intestinal microflora and shorter fasting times could result in the digestive system retaining food from previous meals or snacks. Different races within one trial could have created varying breath hydrogen excretion patterns after consuming lactose doses due to the different incidences of lactose intolerance (Kretchmer, 1971; Bayless and Rosensweig, 1966).

Future work on the mechanism of lactose hydrolysis by L. acidophilus in the intestine and the factors which influence it are needed. This would provide information to help insure that such cultures used as dietary adjuncts contain optimum activity with respect to hydrolyzing lactose. No information is currently available about the stability of the lactose hydrolyzing system of L. acidophilus during freezing, frozen storage, refrigerated storage, or drying and dried storage. Information relative to providing the best protection to the organism under such conditions

would be very useful. It may be possible to add cells of L. acidophilus to milk powder to provide a dried product which would be useful to lactose malabsorbers. This would be especially true for developing countries in which refrigeration is inadequate for proper storage of the type of nonfermented milk supplemented with cells of L. acidophilus used in this study.

# CHAPTER VI

## SUMMARY AND CONCLUSIONS

Lactose malabsorption in humans is indicated by increases in amounts of hydrogen in the breath following ingestion of lactose or milk. The breath hydrogen test is used to measure lactose malabsorption in humans. The amount of hydrogen in breath samples in this study was determined by gas chromatography.

Experiments were conducted to determine if cells of L. acidophilus added to pasteurized whole milk could prevent lactose malabsorption in humans. These experiments involved feeding trials in which lactose malabsorbing humans were used as test subjects. Since the cells of L. acidophilus did not alter the flavor of the milk, the experiments were designed so that neither the test subjects nor the analyst knew which subjects received control milk or milk containing certain populations of L. acidophilus.

Breath hydrogen tests performed on lactose malabsorbing test subjects initially and after a 6-day interval, during which each person consumed pasteurized whole milk as control (5 mℓ/Kg body weight) twice daily, revealed no significant ($P > 0.50$) difference in hydrogen content of the breath samples. However, analysis of breath samples from test subjects who consumed similar amounts of nonfermented pasteurized whole milk containing $2.5 \times 10^8$ cells of L. acidophilus per mℓ for 7 days, twice daily, showed significant ($P < 0.01$) reduction in the amounts of hydrogen

excreted in their breath samples. Thus daily consumption of pasteurized whole milk containing $2.5 \times 10^8$ cells of L. acidophilus per mℓ significantly reduced lactose malabsorption in humans.

The effect of daily consumption of pasteurized whole milk containing $2.5 \times 10^6$ cells of L. acidophilus per mℓ on lactose malabsorbers also was significant ($P < 0.025$) while the effect of $2.5 \times 10^7$ cells of L. acidophilus per mℓ was not significant ($P > 0.35$). The lack of a significant effect in the latter group of test subjects was probably due to large increases in excreted hydrogen after the 7-day trial by two of the six test subjects.

The immediate effect of consumption of milk containing cells of L. acidophilus was determined. The breath hydrogen test was performed on each subject on day 0 and day 7 using pasteurized whole milk as the test dose and again on days 14 and 21 using milk containing $2.5 \times 10^6$ cells of L. acidophilus as the test dose. No milk with or without L. acidophilus was consumed during the periods between breath hydrogen tests. Comparison of the breath hydrogen test on day 0 and day 7 did not show significant difference ($P > 0.70$). The analysis of data collected from the breath hydrogen tests on day 14 and day 21 also revealed no significant difference ($P > 0.95$). However, when the overall average from days 0 and 7 were compared to the overall average from days 14 and 21, there was a significant difference ($P < 0.01$). Less hydrogen was in the samples on days 14 and 21 than on days 0 and 7. Thus the beneficial influence of L. acidiophilus on lactose malabsorbers does not require that the milk be consumed daily.

Storage of pasteurized whole milk containing viable cells of L. acidophilus at 5°C for 7 days did not result in hydrolysis of the lactose.

Thus the effect of L. acidophilus on lactose malabsorption was not due to hydrolysis of the lactose prior to consumption. This indicated that the beneficial effect must have occurred in the digestive tract after consuming milk containing L. acidophilus.

Preliminary experiments were conducted to determine the effect of bile salts on the lactase activity of the cells of L. acidophilus in milk. The samples included 10% NFMS containing varying levels of oxgall (0 to 1.25%). All were inoculated with $2.5 \times 10^7$ L. acidophilus per mℓ. The glucose concentrations in the samples after 5 hr incubation at 37°C increased as the level of oxgall was increased. The numbers of L. acidophilus after a 5-hr incubation period showed no appreciable difference among samples, which indicated that the large amounts of free glucose in the samples containing oxgall were not due to increased growth of the culture. Thus the presence of bile salts could enhance the ability of the culture to hydrolyze lactose by changing in the permeability of the cell membrane. So it could be assumed that the bile salts in the human intestine could favorably influence the hydrolysis of lactose by cells of L. acidophilus. This could be an important factor in prevention of lactose malabsorption.

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# APPENDIX

TABLE XII

DATA FOR STANDARD CURVE AND ANALYSIS OF DATA FOR DETERMINING LINEAR MODELS

Data for Standard Curve

| Hydrogen Concentration (ppm) | Peak Area[a] Day 1 | Day 2 |
|---|---|---|
| 100 | 1.34 | 1.34 |
| 75 | 1.03 | 1.06 |
| 50 | 0.66 | 0.68 |
| 25 | 0.33 | 0.35 |
| 25 | | 0.37 |

General Linear Models Procedure

| Source | DF | S.S. | M.S. | F Value | PR > F |
|---|---|---|---|---|---|
| Model | 2 | 7.03854 | 3.51927 | 7119.47 | 0.0001 |
| Error | 7 | 0.00346 | 0.00049 | | |
| Uncorrected Total | 9 | 7.04200 | | | |
| Contrast, Day | 1 | 0.00053 | 0.00053 | 1.08 | 0.3335 |

[a]Peak area was calculated from hydrogen peak on recorder response after gaschromatographic analysis.

TABLE XIII

ANALYSIS OF VARIANCE OF DATA FROM TRIALS EVALUATING THE IMMEDIATE EFFECT OF CONSUMING MILK CONTAINING CELLS OF *L. ACIDOPHILUS* ON LACTOSE MALABSORBERS

| Analysis of Variance | | | | |
|---|---|---|---|---|
| Source | df | SS | MS | F |
| Total | 19 | 3214.2575 | | |
| Block (Person) | 4 | 1635.5945 | | |
| Date | 3 | 772.6690 | | |
| $D_0$ vs $D_7$ | 1 | 9.8010 | 9.8010 | 0.14590[a] |
| $D_{14}$ vs $D_{21}$ | 1 | 0.2560 | 0.2560 | 0.00379[b] |
| $1/2\ (D_0 + D_7)$ vs $1/2\ (D_{14} + D_{21})$ | 1 | 762.6125 | 762.6125 | 11.35410[c] |
| Error | 12 | 805.9940 | 67.1662 | |

[a]No significant difference ($P > 0.70$).

[b]No significant difference ($P > 0.95$).

[c]Significant difference between the average of day 0 and day 7, and the average of day 14 and day 21 ($P < 0.01$).

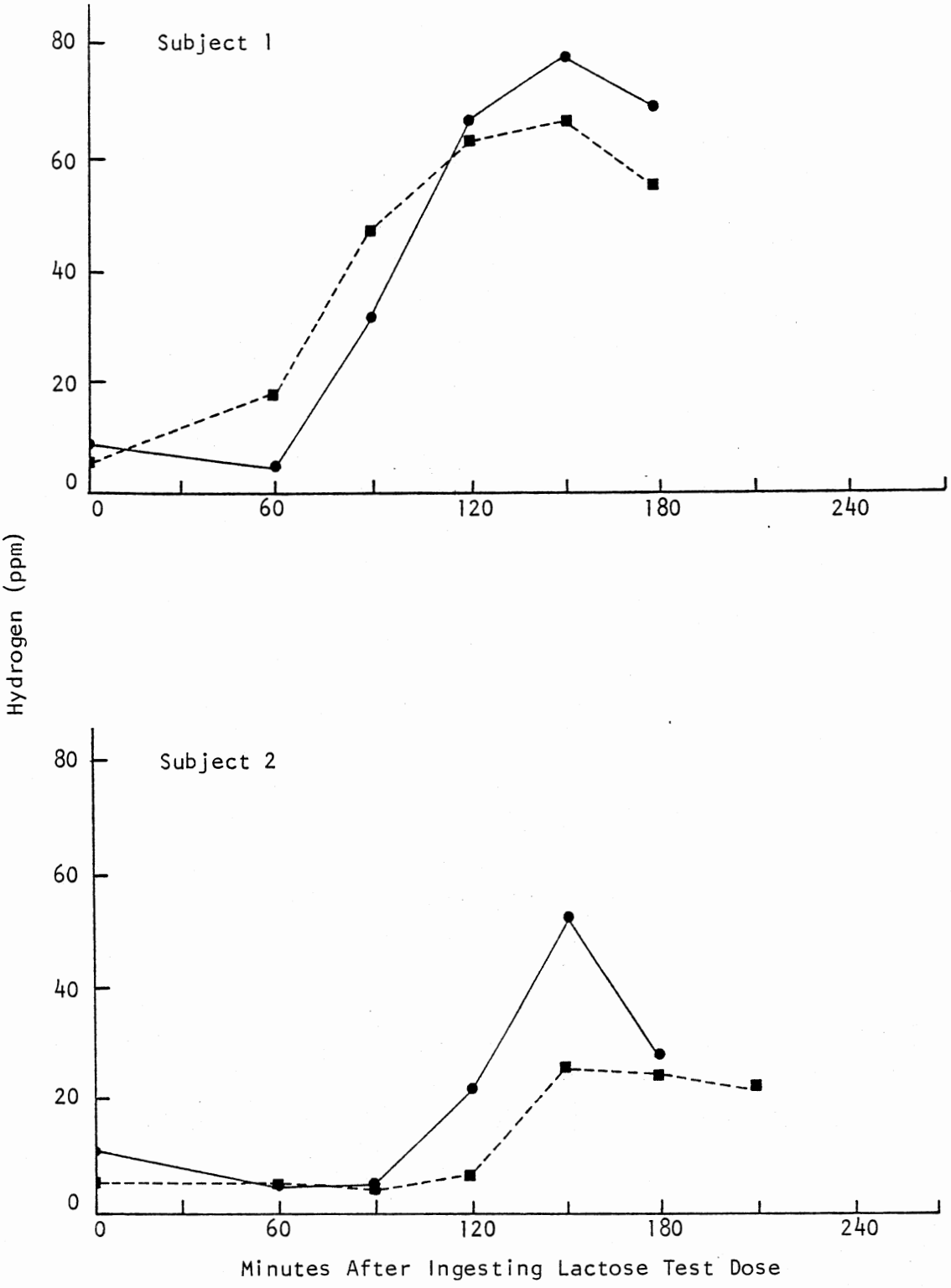


Figure 7. Breath Hydrogen Excretion Curves for Subjects 1 and 2 Before and After Consuming Whole Milk for Six Days (●: Before; ■ : After)

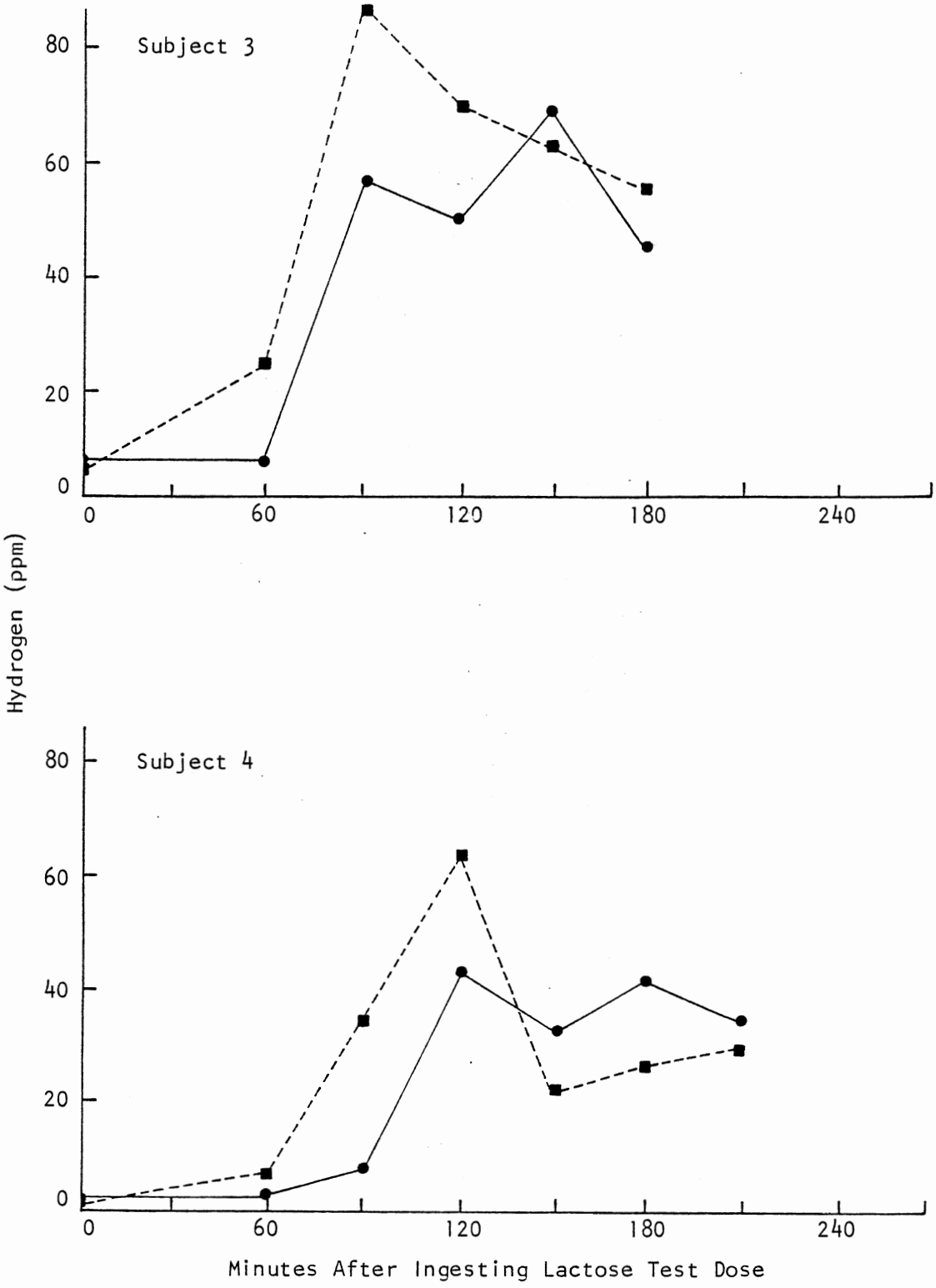


Figure 8. Breath Hydrogen Excretion Curves for Subjects 3 and 4 Before and After Consuming Whole Milk for Six Days (●: Before; ■: After)

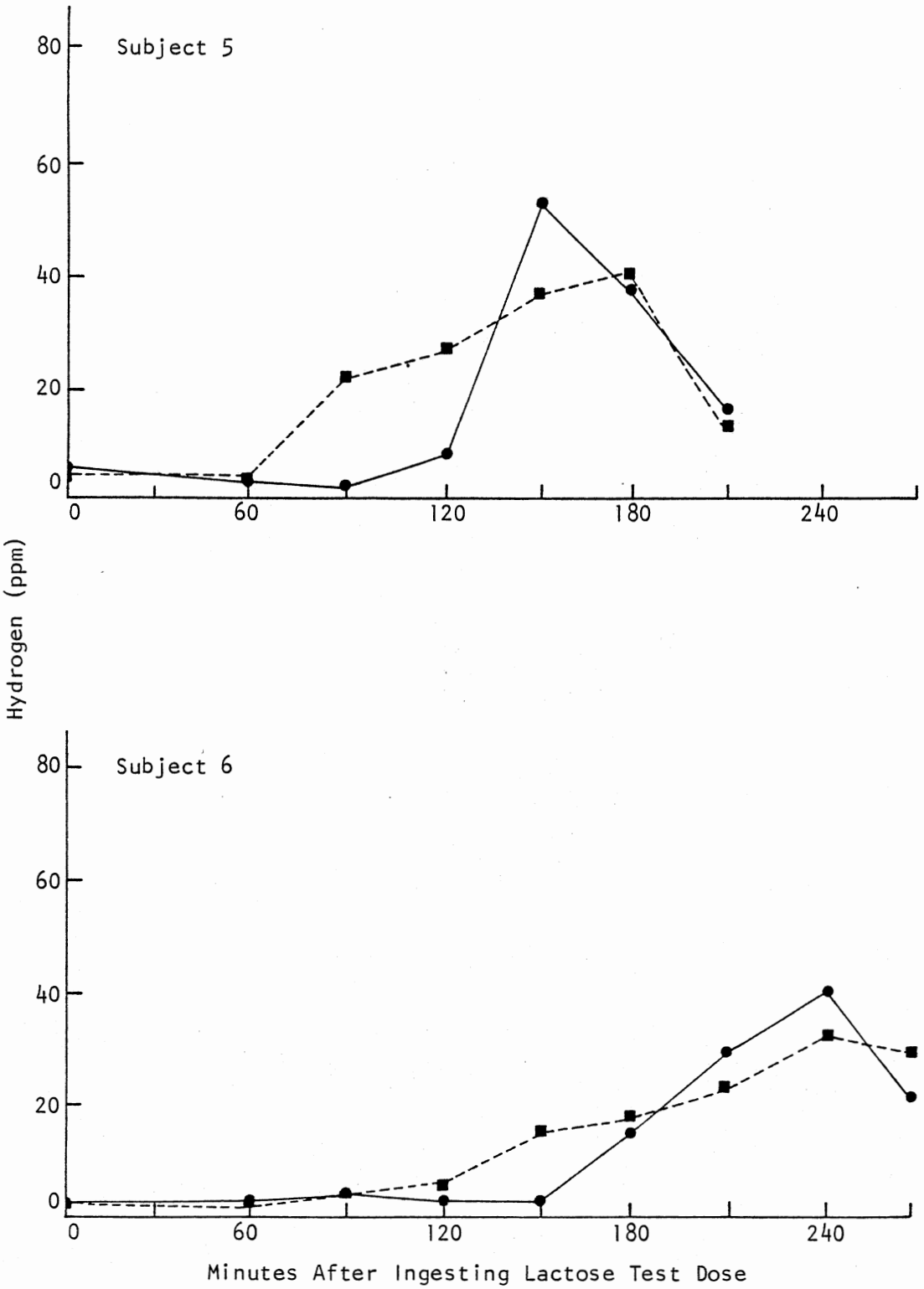


Figure 9. Breath Hydrogen Excretion Curves for Subjects 5 and 6 Before and After Consuming Whole Milk for Six Days (●: Before; ■: After)

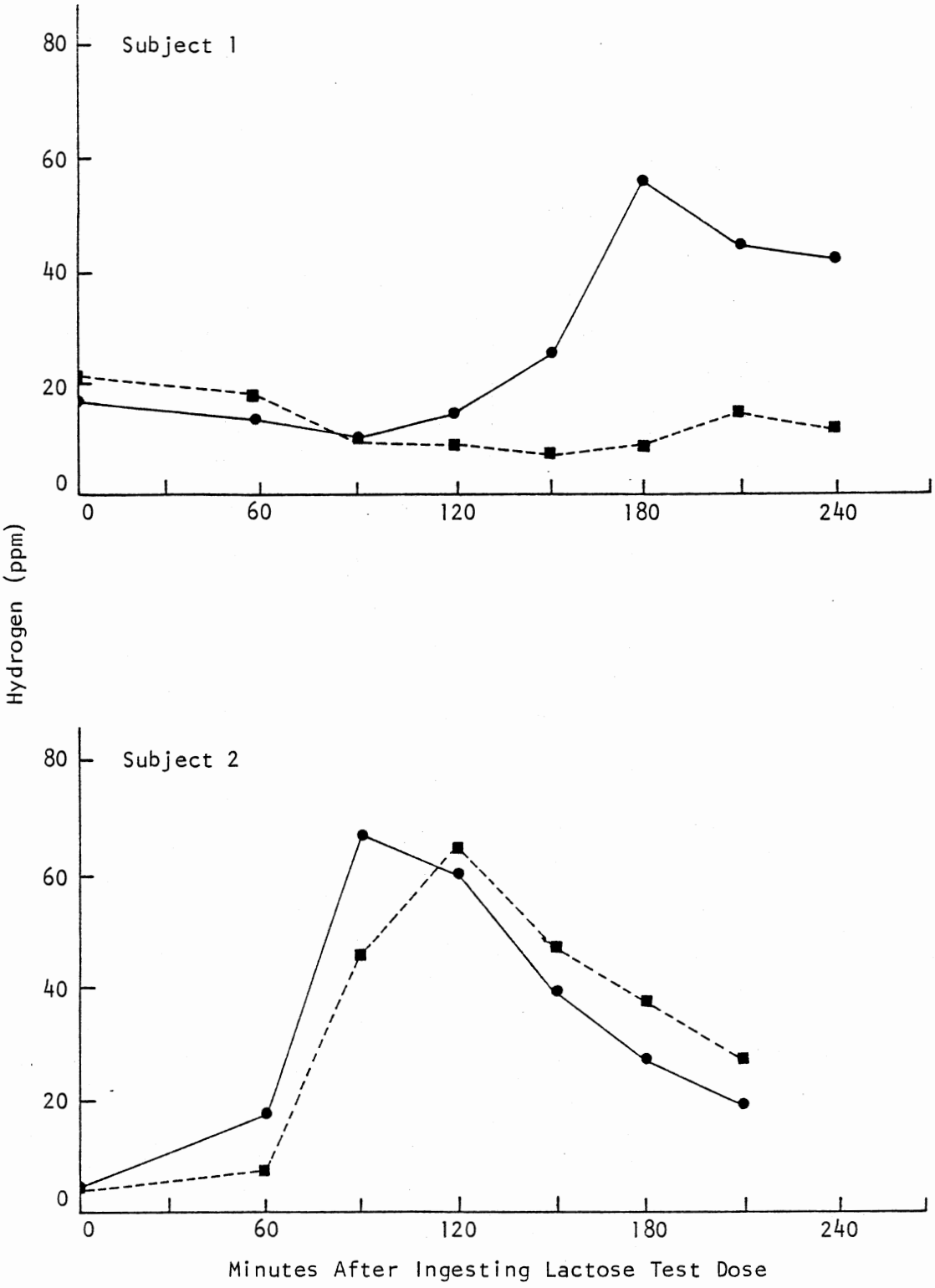


Figure 10. Breath Hydrogen Excretion Curves for Subjects 1 and 2 Before and After Consuming Whole Milk Containing $2.5 \times 10^8$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

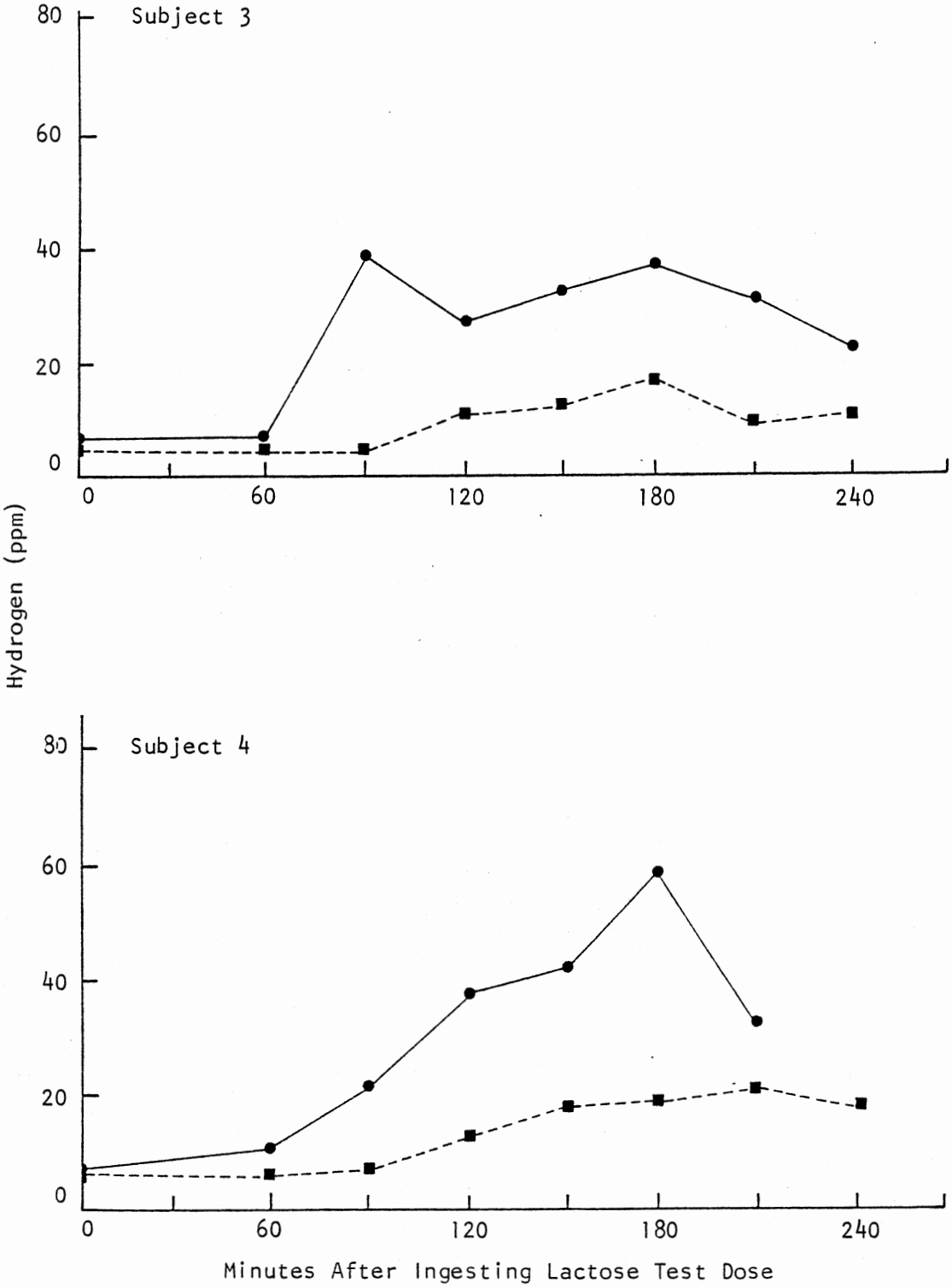


Figure 11. Breath Hydrogen Excretion Curves for Subjects 3 and 4 Before and After Consuming Whole Milk Containing $2.5 \times 10^8$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

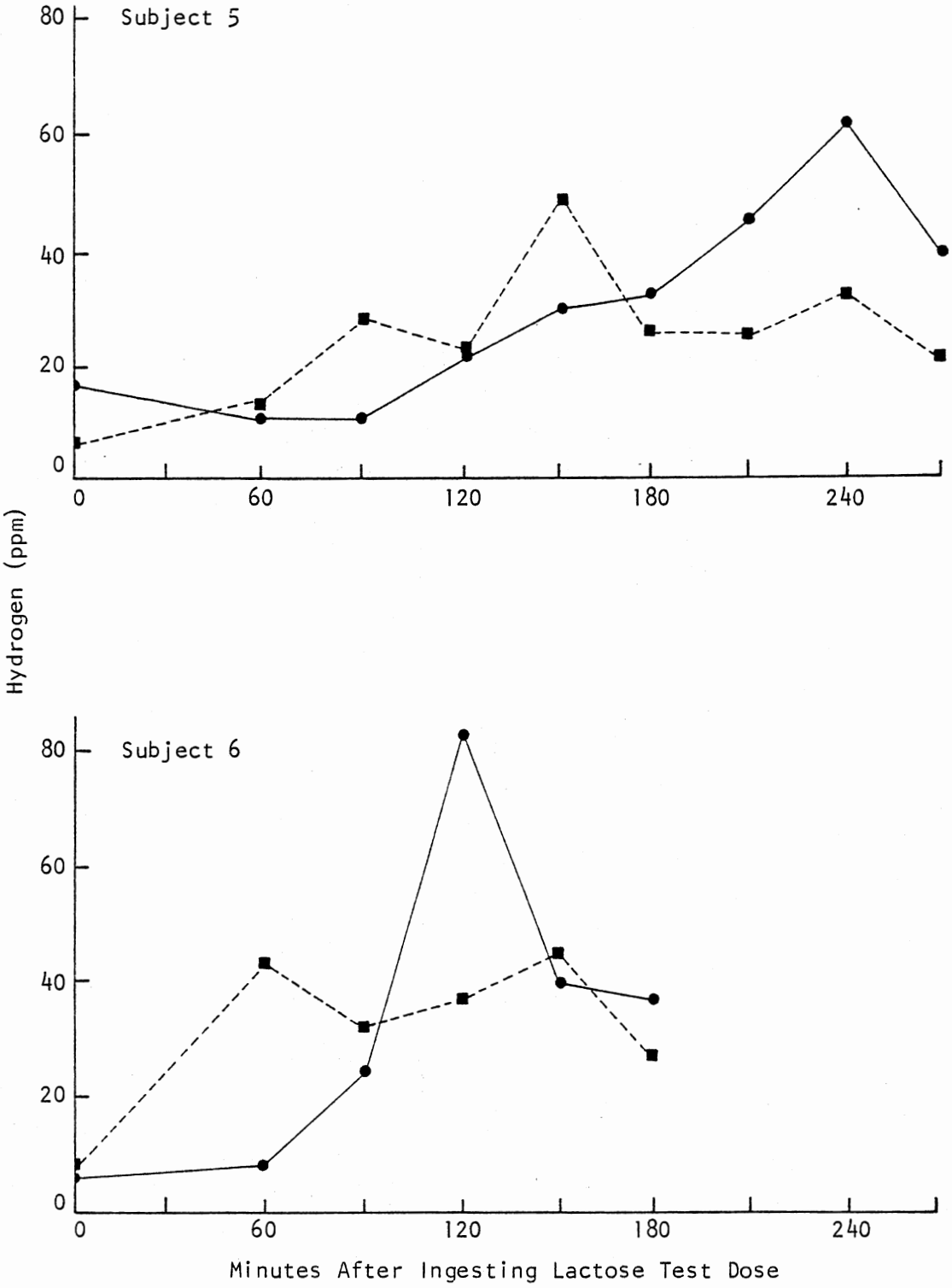


Figure 12. Breath Hydrogen Excretion Curves for Subjects 5 and 6 Before and After Consuming Whole Milk Containing $2.5 \times 10^8$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

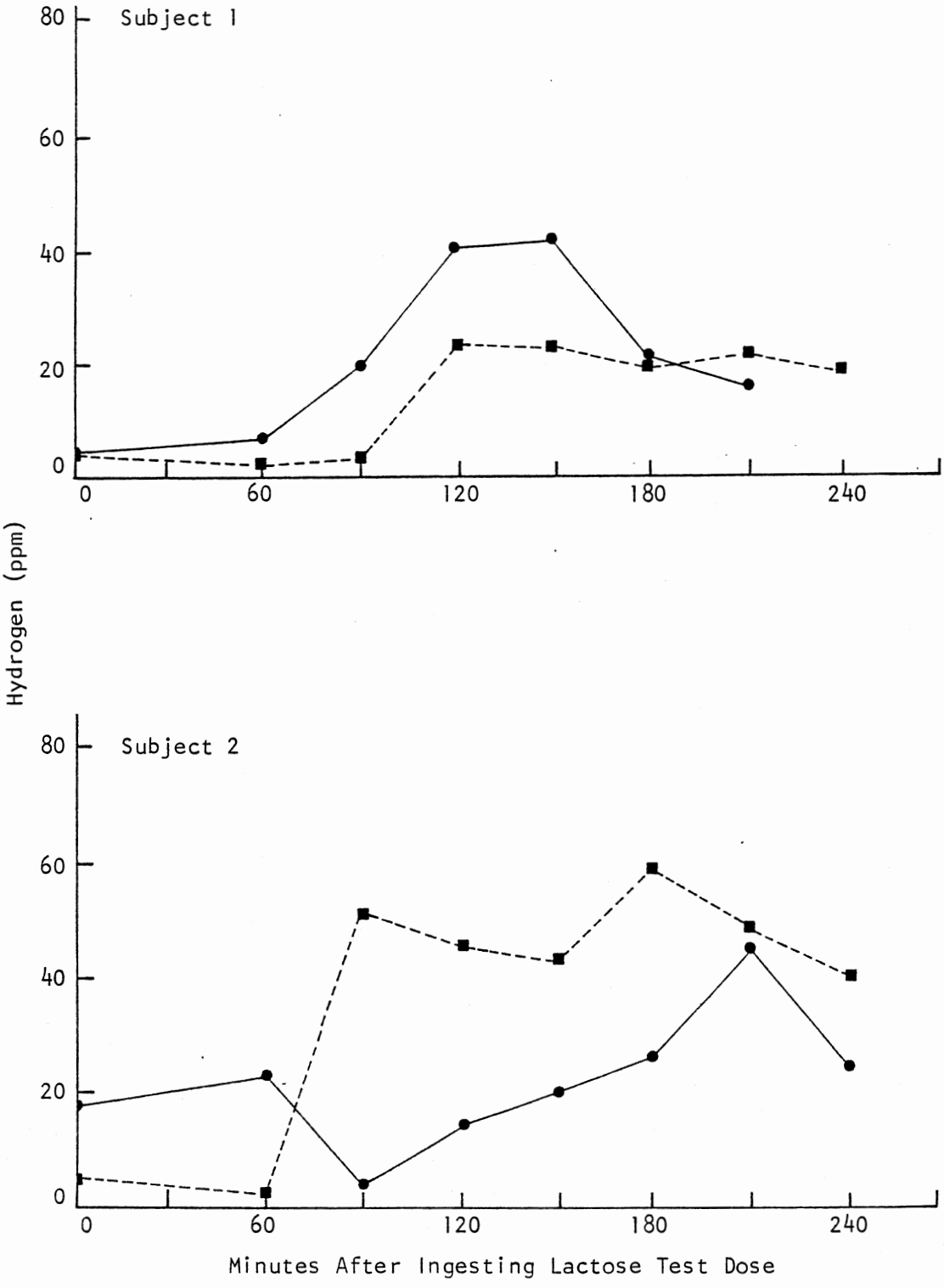


Figure 13. Breath Hydrogen Excretion Curves for Subjects 1 and 2 Before and After Consuming Whole Milk Containing $2.5 \times 10^7$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

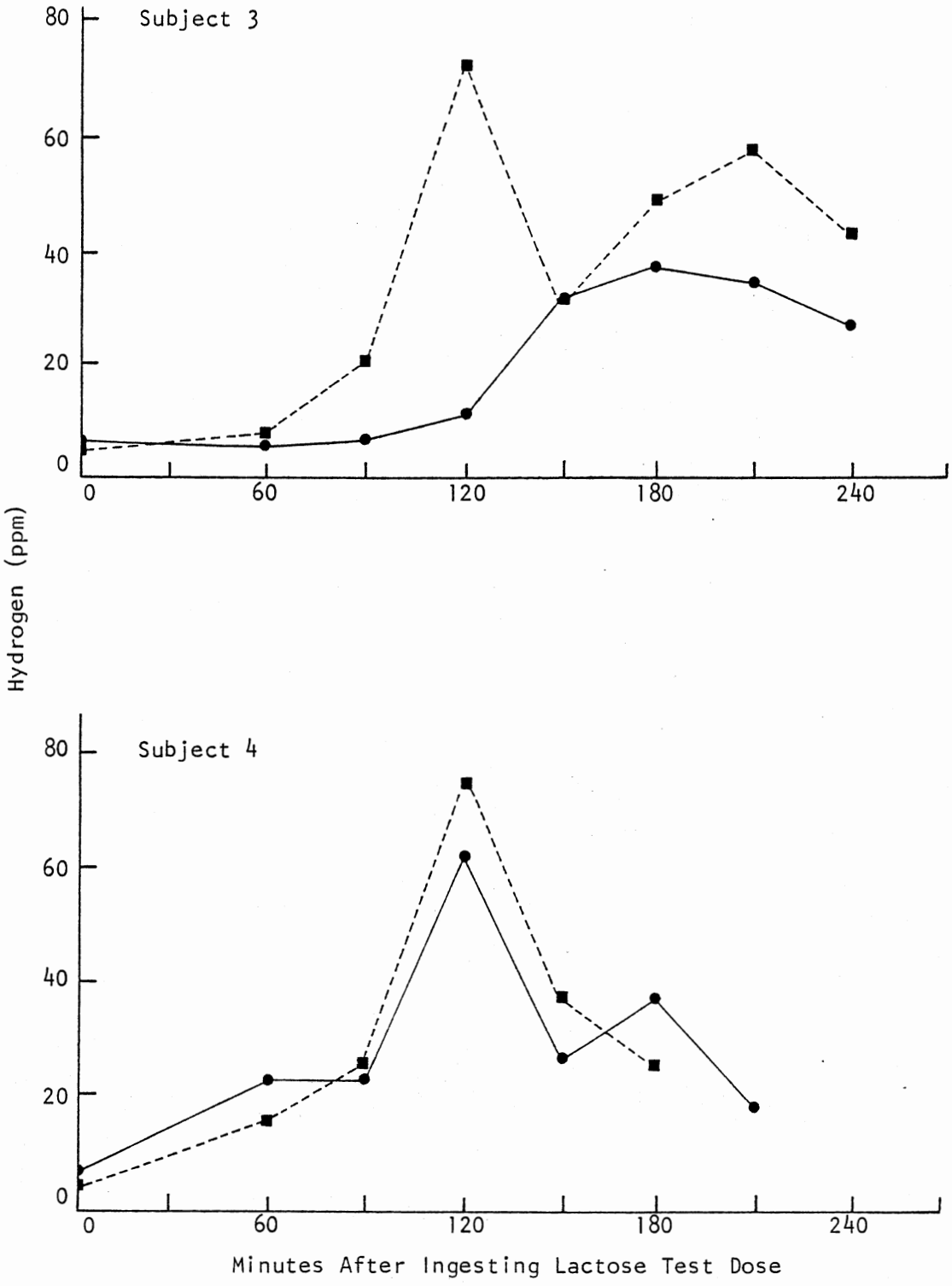


Figure 14. Breath Hydrogen Excretion Curves for Subjects 3 and 4 Before and After Consuming Whole Milk Containing $2.5 \times 10^7$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

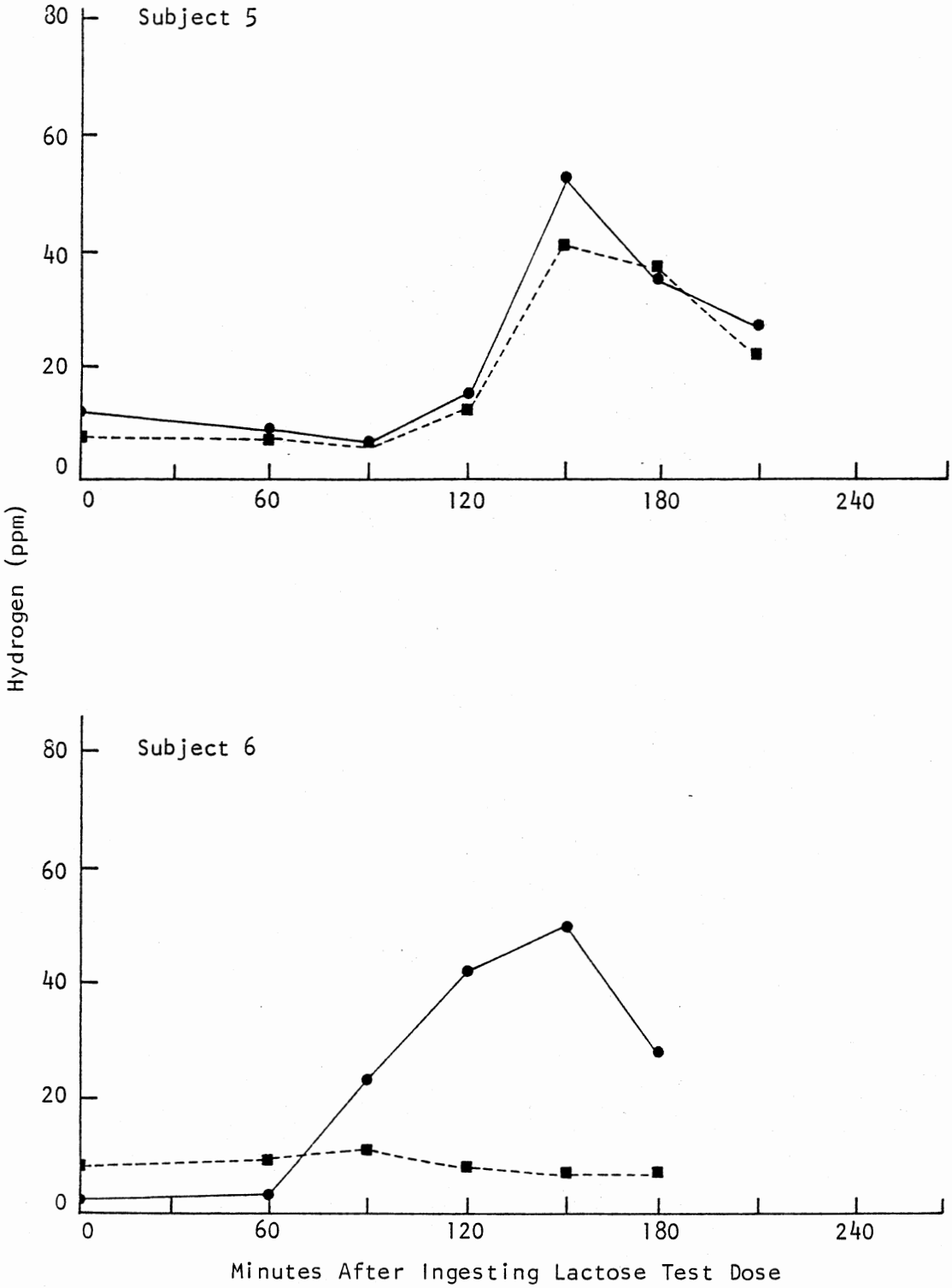


Figure 15. Breath Hydrogen Excretion Curves for Subjects 5 and 6 Before and After Consuming Whole Milk Containing $2.5 \times 10^7$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

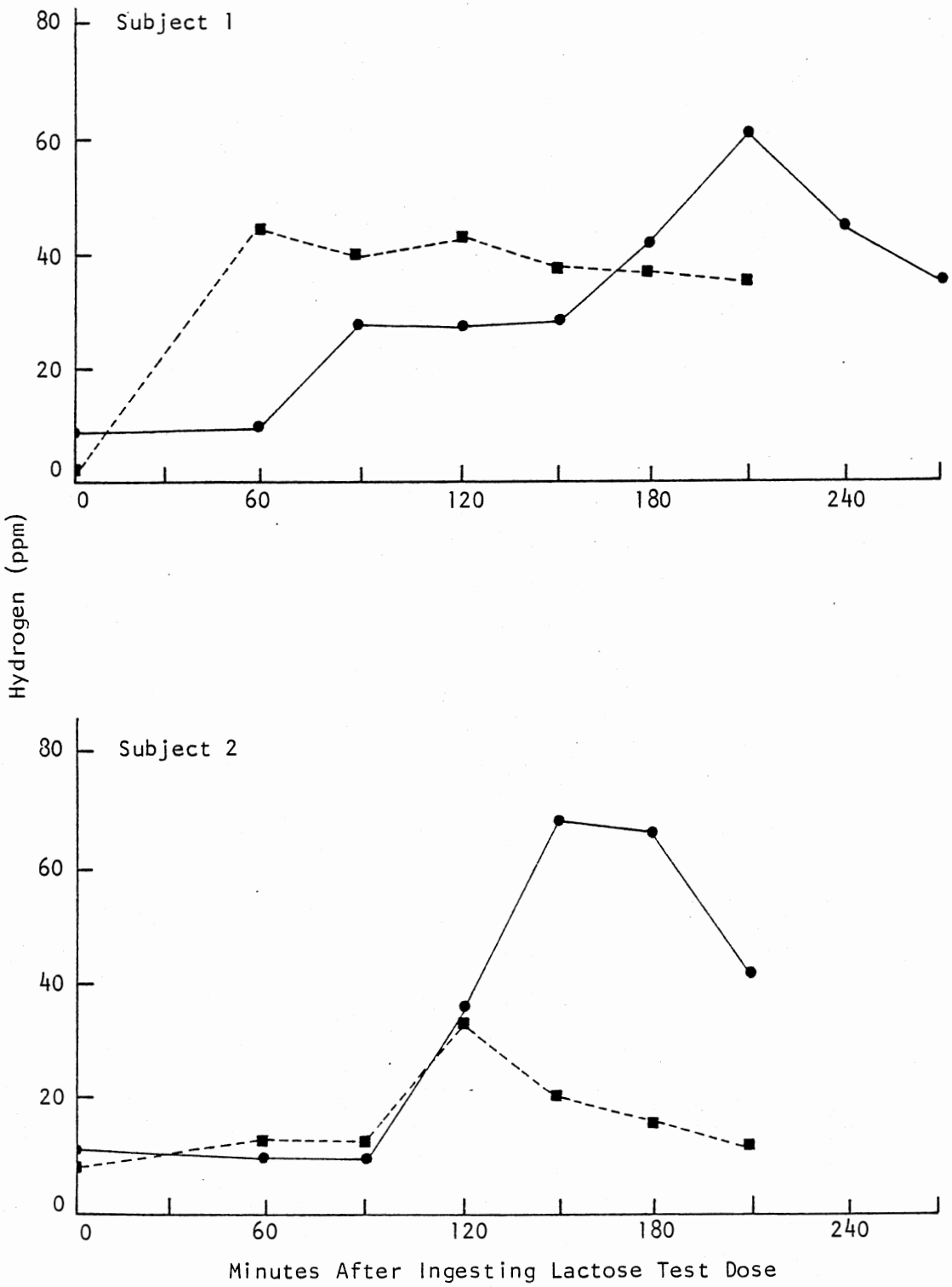


Figure 16. Breath Hydrogen Excretion Curves for Subjects 1 and 2 Before and After Consuming Whole Milk Containing $2.5 \times 10^6$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

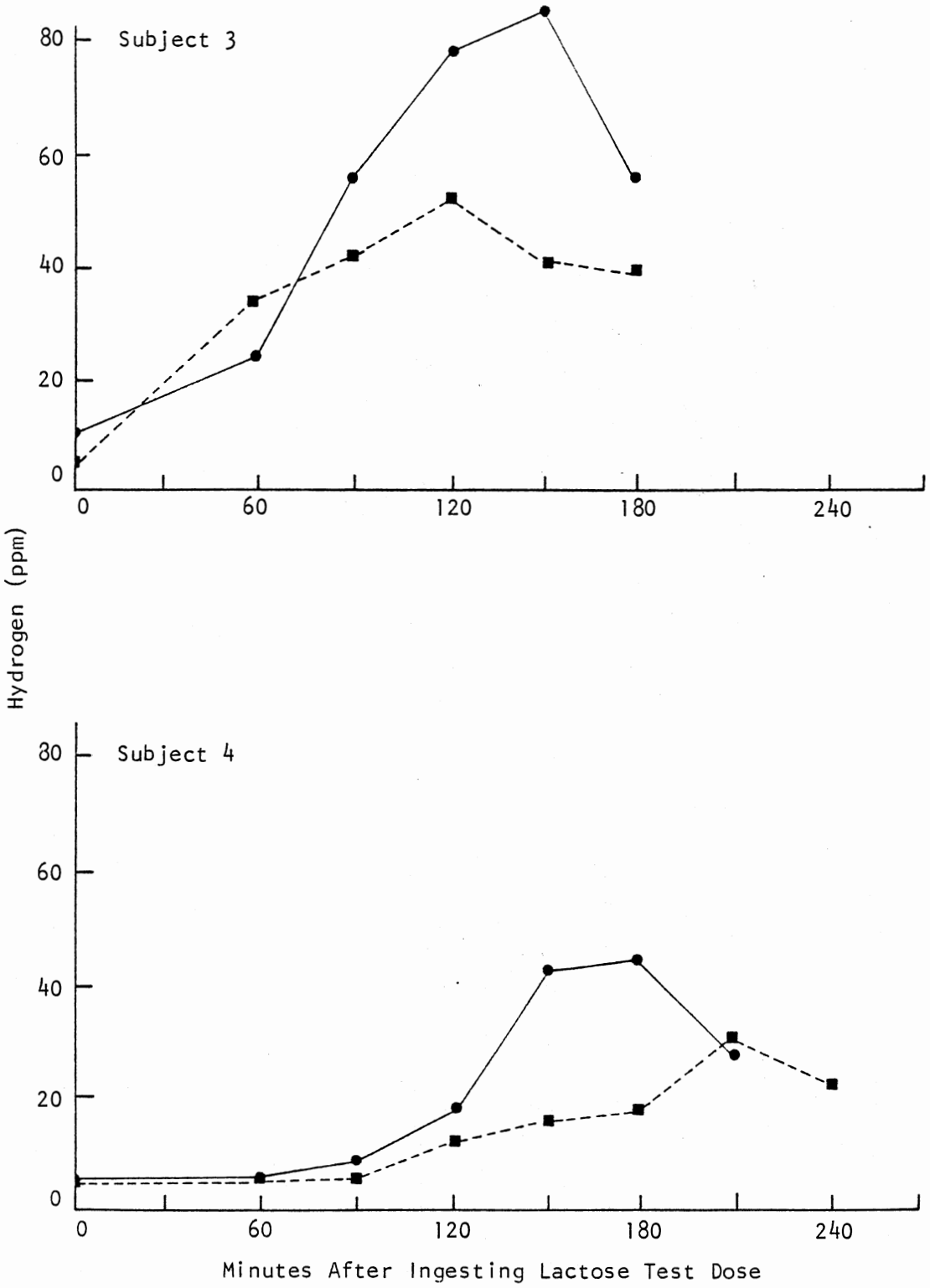


Figure 17. Breath Hydrogen Excretion Curves for Subjects 3 and 4 Before and After Consuming Whole Milk Containing $2.5 \times 10^6$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

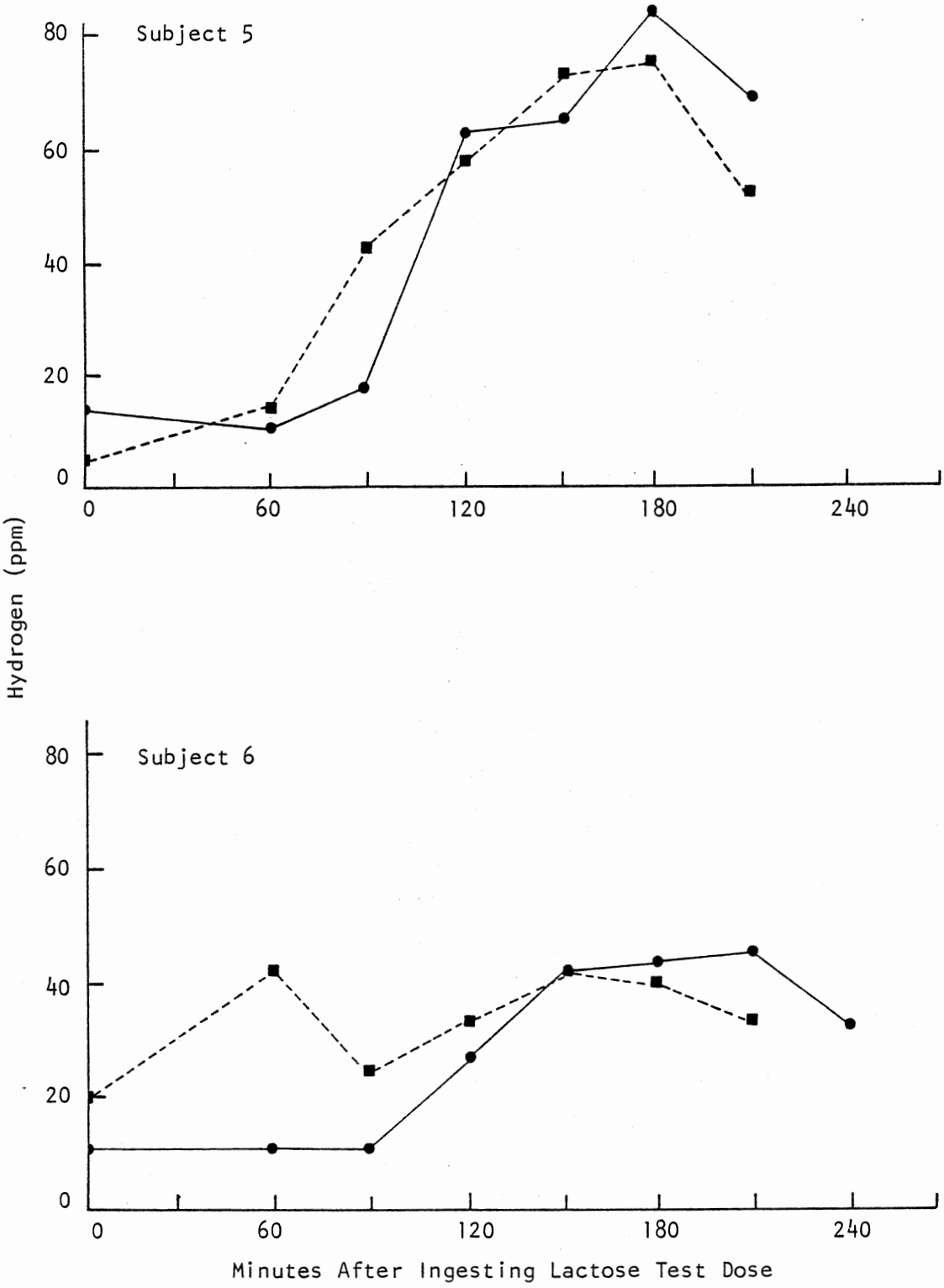


Figure 18. Breath Hydrogen Excretion Curves for Subjects 5 and 6 Before and After Consuming Whole Milk Containing $2.5 \times 10^6$ Cells of L. acidophilus Per mℓ for Six Days (●: Before; ■: After)

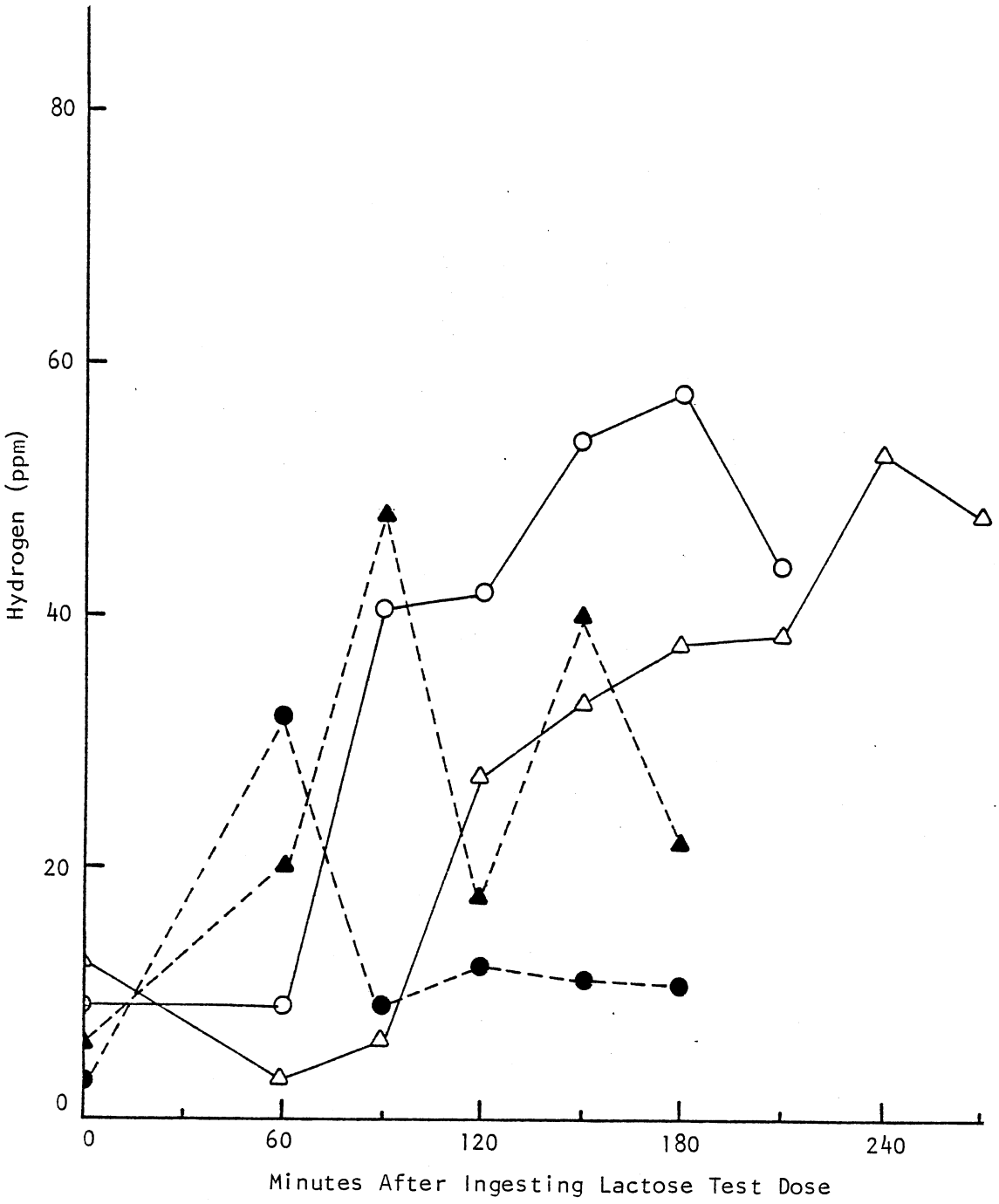


Figure 19. Breath Hydrogen Excretion Curves for Subject 1 After Consumption of Whole Milk on Day 0 (○) and Day 7 (△), and Milk Containing Cells of L. acidophilus on Day 14 (●) and Day 21 (▲)

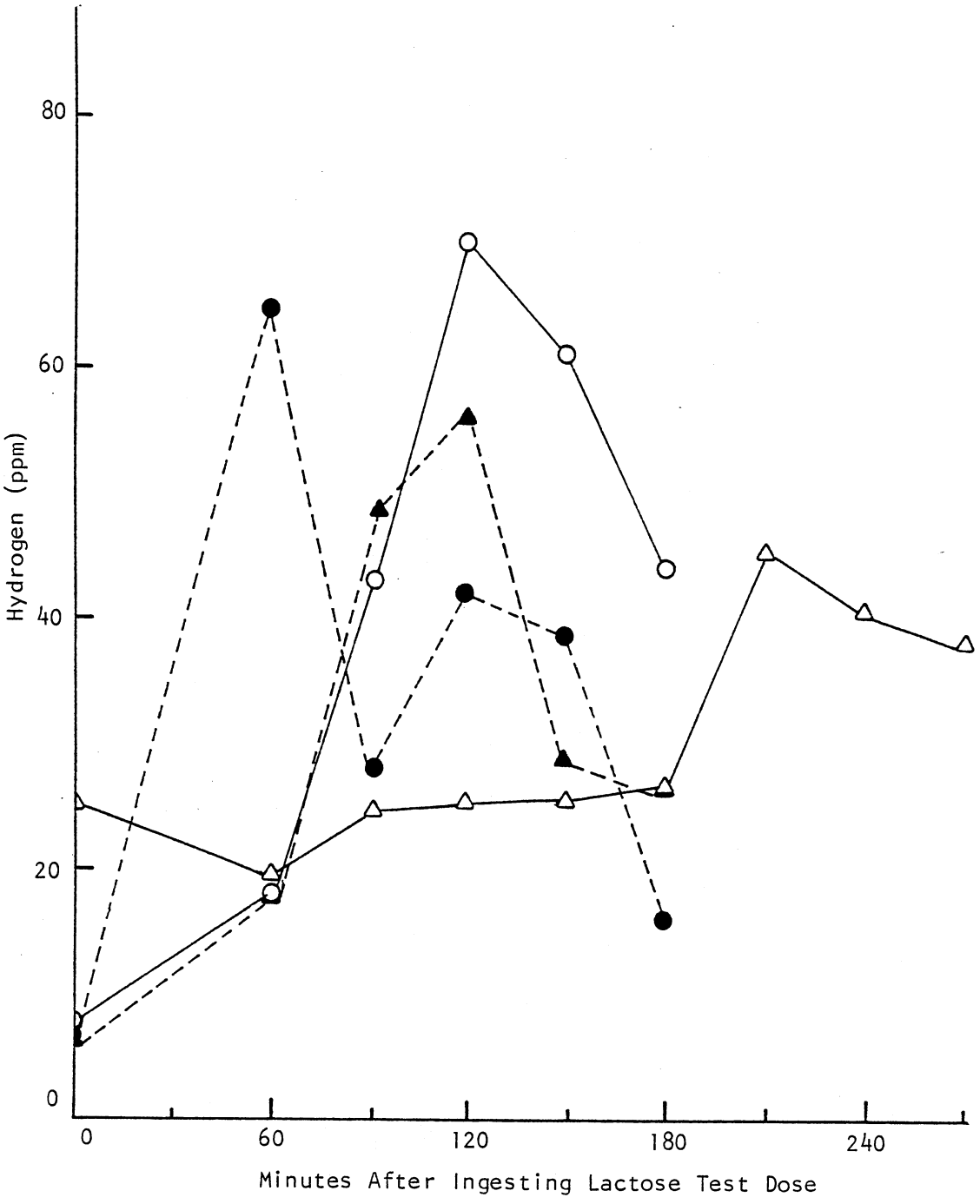


Figure 20. Breath Hydrogen Excretion Curves for Subject 2 After Consumption of Whole Milk on Day 0 (○) and Day 7 (△), and Milk Containing Cells of L. acidophilus on Day 14 (●) and Day 21 (▲)

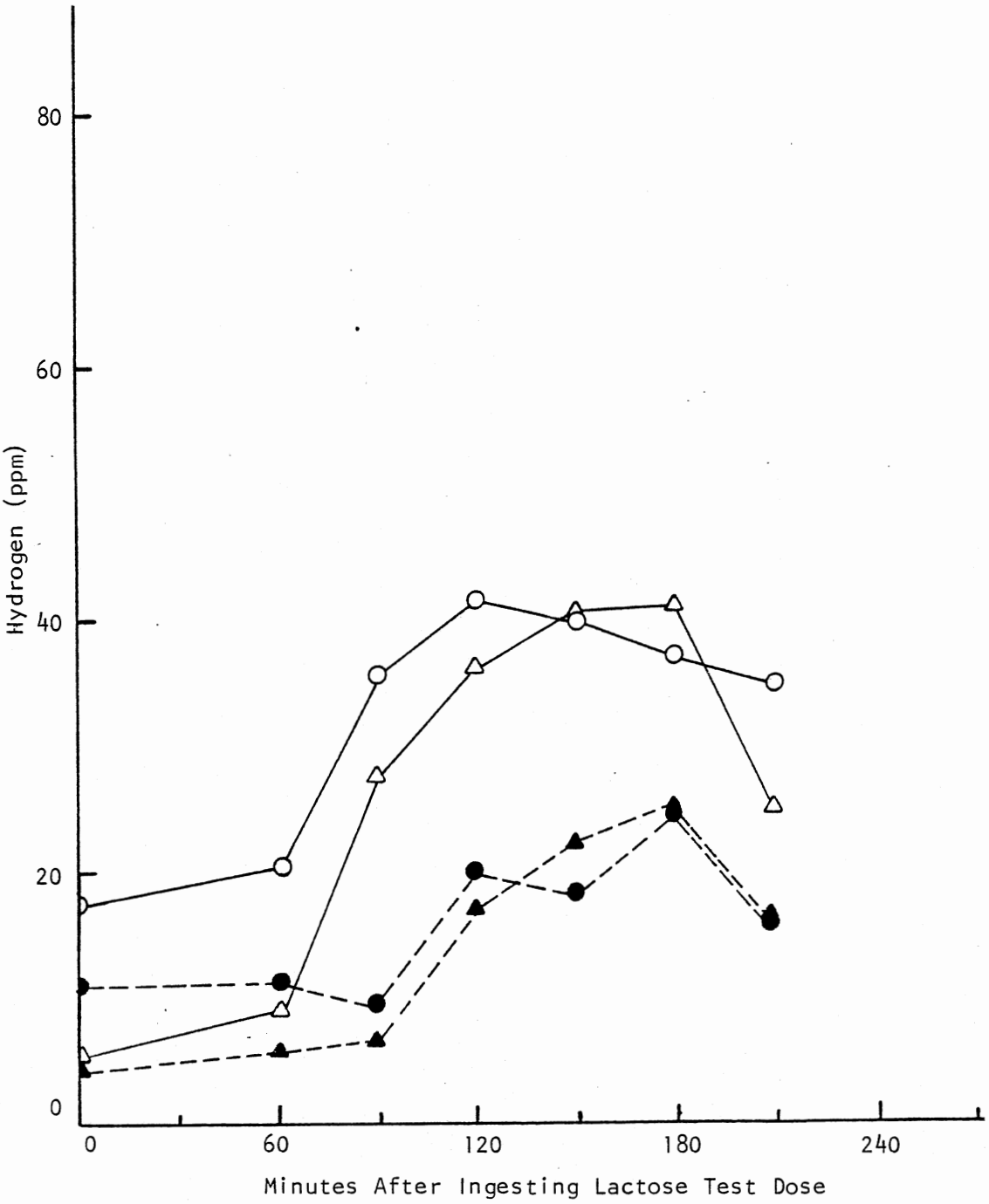


Figure 21. Breath Hydrogen Excretion Curves for Subject 3 After Consumption of Whole Milk on Day 0 (○) and Day 7 (△), and Milk Containing Cells of L. acidophilus on Day 14 (●) and Day 21 (▲)

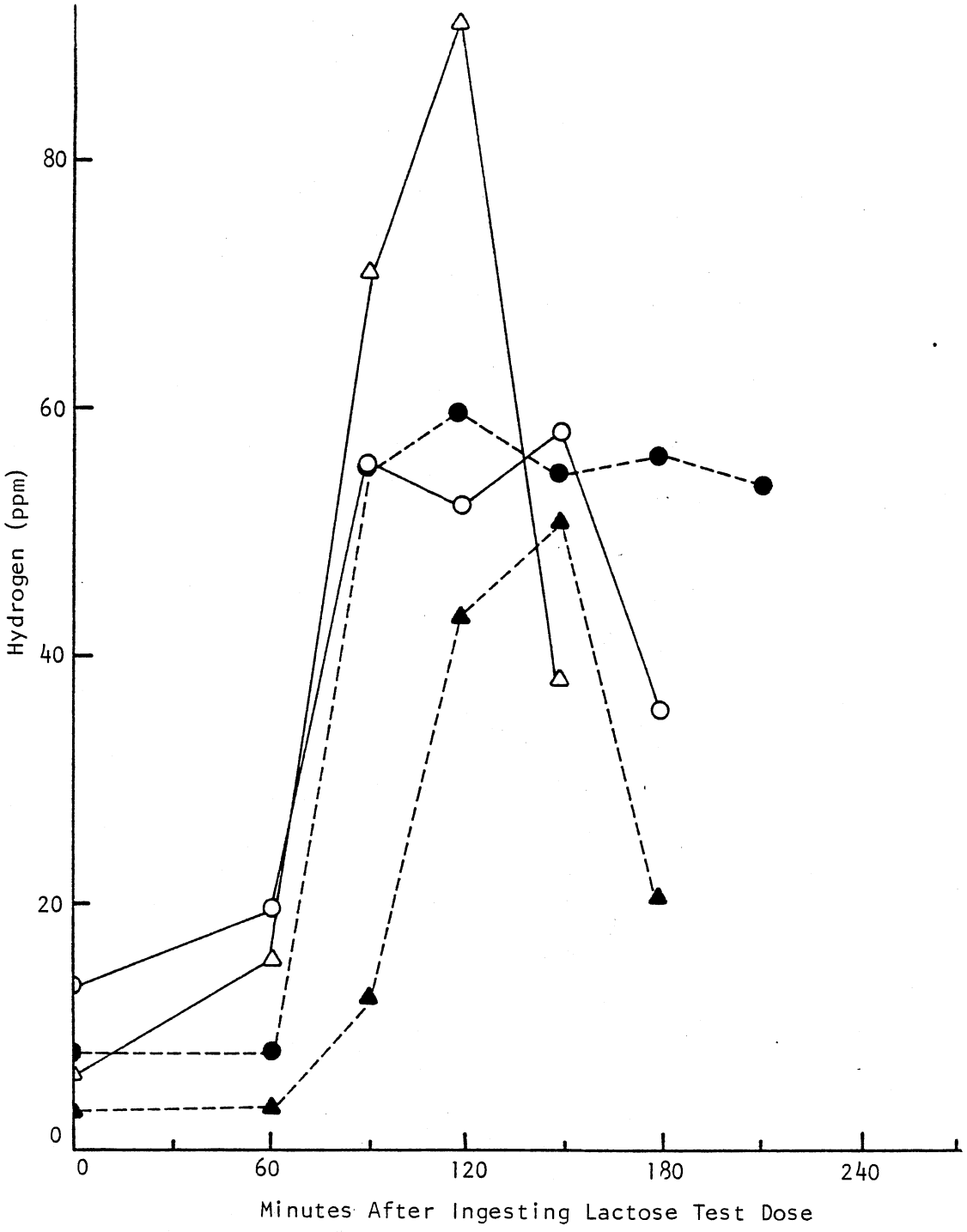


Figure 22. Breath Hydrogen Excretion Curves for Subject 4 After Consumption of Whole Milk on Day 0 (○) and Day 7 (△), and Milk Containing Cells of L. acidophilus on Day 14 (●) and Day 21 (▲)

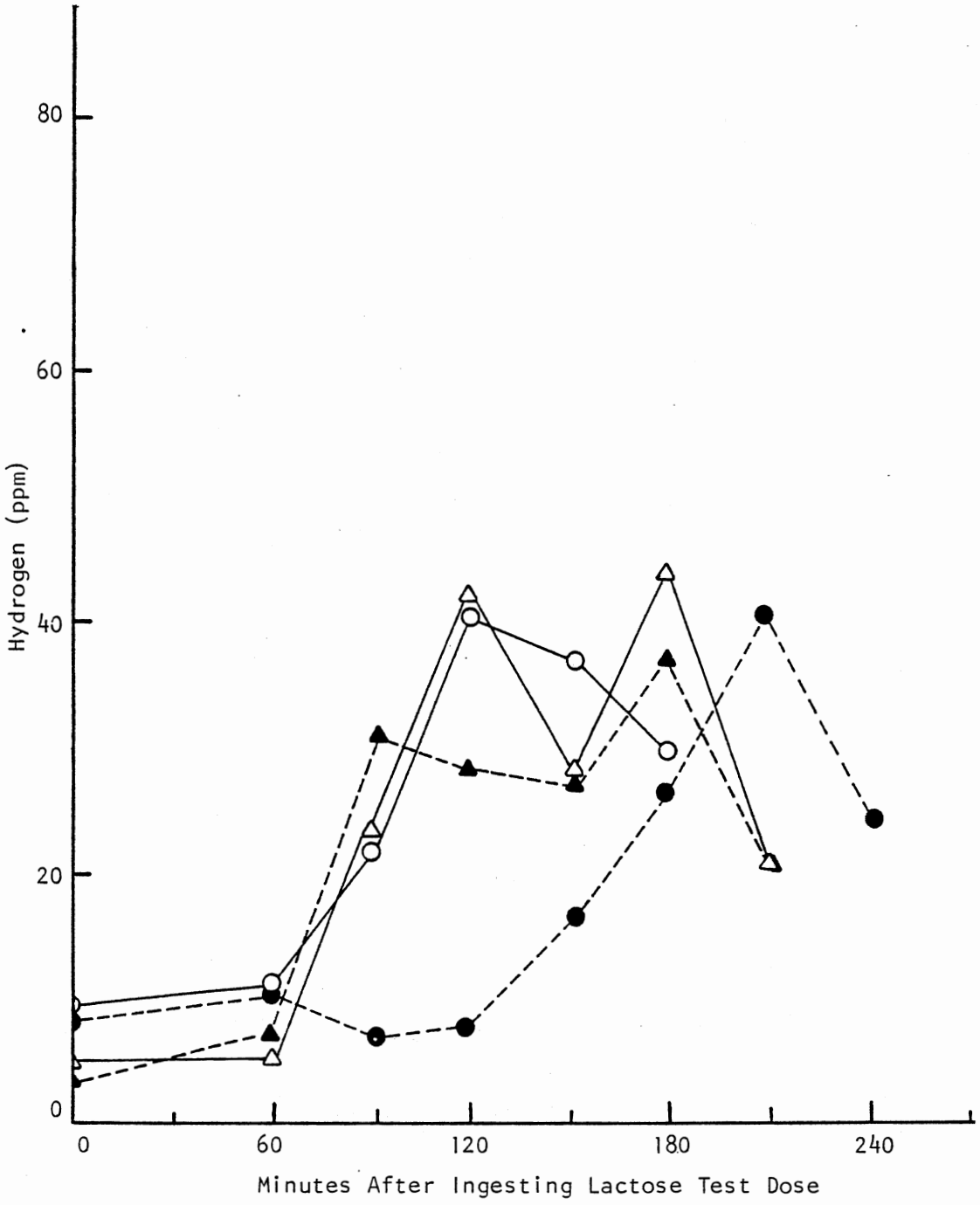


Figure 23. Breath Hydrogen Excretion Curves for Subject 5 After Consumption of Whole Milk on Day 0 (○) and Day 7 (△), and Milk Containing Cells of L. acidophilus on Day 14 (●) and Day 21 (▲)

VITA[2]

Hyungsoo Kim

Candidate for the Degree of

Doctor of Philosophy

Thesis: BENEFICIAL EFFECT OF CONSUMING MILK CONTAINING CELLS OF LACTOBACILLUS ACIDOPHILUS ON LACTOSE MALABSORPTION IN HUMANS

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