BREATH ANALYSIS AS A TECHNIQUE TO DETERMINE LOW LACTASE ACTIVITY IN NATIVE AMERICAN INDIANS

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INTRODUCTION

Significance of the Study

It has been found that when many ethnic groups consume large amounts of milk within a short time period, many undesirable side effects are produced. The major cause of these side effects is the milk sugar, lactose, a disaccharide that some ethnic groups cannot readily digest. This lactose malabsorption has been found among Asians, Africans, American Negroes, native American Indians, and other non-white populations (2, 15, 28, 55, 60, 69). A lactose tolerance test (LTT) is the usual means by which lactose malabsorption is determined. The LTT procedure requires a subject to fast about 10 hours and ingest one gram of lactose per kilogram of body weight or 50 g for an adult (15, 83). Fifty grams of lactose is approximately equivalent to the amount of lactose found in one quart of milk (80). Ingesting this amount of lactose does produce several clinical symptoms in a malabsorbing adult such as intestinal distension, cramps, diarrhea, irritable colon symdrome and loose stool (2). Namdi and Parham (69) and Leichter and Lee (60) noted that lactose malabsorbers could drink smaller quantities of milk with no ill side effects.

Calloway et al. (17, 19, 20) have developed a breath hydrogen test that can detect the hydrogen produced in the gut. When lactose is not absorbed in the small intestine, bacteria in the large intestine use

the sugar and release hydrogen gas that diffuses through the intestinal wall into the blood stream and lungs (61). This test determines the hydrogen gas present in the lungs at a given time.

Additional studies are needed to determine the accuracy of the breath hydrogen test as a method for determining lactose malabsorption. There is also a need to establish a standard procedure and method of analysis for this breath test.

Purpose

The purpose of this pilot study was to determine if the breath hydrogen test could be used to detect lactose malabsorption using 0.25 g of lactose per kilogram of body weight as found in reconstituted, skim milk. Five North American Indian females who had been determined to be lactose malabsorbers by the LTT were used as subjects for this study. Since the breath hydrogen test was a relatively new technique, it was necessary to determine the optimum experimental procedure and reliable response variable.

CHAPTER II

REVIEW OF LITERATURE

Milk is often referred to as one of the most perfect foods and the main source of calcium in the American diet. Milk is also rich in protein and the amino acid lysine. It compliments the protein value of breads and cereals that either lack or have low lysine amounts. All known vitamins are also present in milk to some degree. Milk is especially rich in riboflavin, and has a fair amount of thiamine and vitamin A (35). Because of its nutritional value, the recommended daily consumption for adults is two cups, and four cups are recommended for children (35, 43). Yet many people do not drink milk nor do they consume dairy products.

Many of these people who do not drink milk believe it is not fit for human consumption. The most common view is that any animal secretion is unclean; it smells and tastes bad, and it causes distension and diarrhea (79).

This latter view may have some sound reasoning behind it. Physicians have recognized the fact that many people are "allergic" to milk. Dr. John Howland, a pediatrician, made the astute observation in 1921, that many of his patients who showed signs of intolerance, had an abnormal response to carbohydrates. This intolerance expressed itself in the form of diarrhea and excessive fermentation (75). It was found upon further investigation that many people who could not tolerate milk,

could eat things such as cheese, yogurt, and buttermilk with no ill side effects. This was because the lactose in these products had been removed or broken down by the product processing procedure. When lactose malabsorbers drank milk with a meal, they did not get as severe a response to the milk as they did when they drank the milk alone (6).

Origin of the Milk Drinking Habit

Lactase activity in modern man is apparently not related to his current diet. Evidence strongly suggests that lactase activity variation among adult ethnic groups has a genetic basis; probably related to domestication of animals and use of their milk by various groups. The historical use of milk is considered to be an important factor in etiology of adult lactase deficiency and is a topic not readily found in literature. Therefore, the origin of milk drinking is considered here.

As early as 1896, Edward Hahn (79), a German geographer, recorded various lands where populations used beef products, but did not drink milk. He hypothesized that the milk drinking habit originated in Mesopotamia and spread to parts of Asia and Africa where it was not well accepted. Nor did it exist in pre-European times in the New World.

Early representations of milking have been found in the Ur Valley dating back to 2900 B.C. (16). Evidence indicates that the nomatic Eurasian tribesmen spent days hunting food for the community; and later when man settled down to communal living, he learned to domesticate animals. Many young animals were captured and suckled on human milk while being raised in the settlement. As the animals grew older, they were allowed to mate with wild animals and their young were raised in the settlement to become part of a growing herd. Goats and sheep were

probably the first animals to be domesticated in this manner since they are small and easy to maintain on pasture land. Greeks and Romans have recorded that rich foods were made better with goat's milk. It also made a good beverage when mixed with blood (16, 82).

The Mongels drank mare's milk, and made a liquor called kumiss using milk as a base. There were few fresh vegetables and fruits available on the steppes. These hardy horsemen should have showed signs of ascorbic acid deficiency or at least laziness or lethargy, but this was not the case. Mare's milk has twice the vitamin C as found in human milk and four times the amount found in cow's milk.

They were also one of the first societies to process milk into dairy products. They dried the milk and reconstituted it when on long journeys, and made such products as curd, cheese, yogurt, and butter.

When the Mongels conquered China, they brought their dairy habits with them. The Chinese accepted these habits to a degree. The emperor, Tia-tsung, used a pepper cooked in milk as a cure for an intestinal disorder. Milk seemed to have been a rarity in China and something of an acquired taste. With land at a premium, people were somewhat hesitant to allow animals to graze on planted fields. About 2000 B.C. the Chinese leaders declared that milk was an unclean or tainted liquid, not to be consumed by humans.

Moving west to India, the land of vegetarianism, there is a natural reluctance to use the cow for food. The Hindus' attitude toward the cow came from an ancient story. In the human world, it was said that the sacred Mount Meru was surrounded by a succession of seven "ring-like" oceans. The content of each successive ocean was as

follows: salt, dark brown sugar, wine, clarified butter, milk, curds, and fresh water. The oceans represent all the Indian staples except grain products. Clarified butter is used to purify food and protect the higher castes from ritual pollution. This would tend to make the cow more sacred, and its products would assume a higher value.

In Europe, during the Middle Ages, sheep were considered the best commercial investment, supplying milk, meat, and wool for clothing; skins were sold to parchment makers. They were raised all over Europe. During the 1200's there were more sheep in England than people which may explain why cheese was in abundance among the people.

Yet 300 years later, despite the increase in sheep farming, the English peasant seemes to have given up sheep dairy products. It appears that he came to prefer the flavor of cow's milk and its products (82). This is perhaps an indication of how cultural habits have influenced many nationalities' use and consumption of dairy products.

Geographic Location of Lactose Malabsorption

Primary lactase deficiency of the world's adult population appears to be the rule rather than the exception (80). Adult lactose malabsorption studies using the LTT and/or the intestinal biopsy, are listed in Table I. The subjects used in these studies were all healthy adults.

Definite patterns can be seen among the populations when grouped by geographic origin. The lowest incidence of lactose malabsorption, 0 to 24 percent, was found among people of Scandinavian and western Europe origin where dairy products and milk were used as a staple food by their ancestors (79). Caucasian male milk consumption for those 15

TABLE I

PREVALENCE OF ADULT LACTOSE MALABSORPTION

Population	Location	Number Tested	% of Subjects Intolerant		Reference
			LTT	Biopsy	
African	Nigeria				
	Fulani Tribe	33	20.6		55
	Hausa Tribe	17	76.0		
	Yorba Tribe	41	2.4		
	Uganda				
	Bantu Tribe	12		75.0	24
		55	89.0		25
	Batutsi + Bahutu	27	44.0		
	Bahimas + Balku	11	54.5		
	Hamitic Tribe	6		0.0	24
	Hamitic Origin				26
	Hutu	36	30.5		
	Shi	28	34.6		
	Nilote Tribe				
	Tutsi	25	0.0		
	Zambian Tribe	26	100.0	100.0	22
Arabs	Israel	67	80.6		39
Egyptians	Egypt	14		93.0	45
Europeans	Mixed Group	44		31.8	67
		9	0.0		34
	Finland	159	17.0		49
	Switzerland	17	29.0		44
	Greek Continent	600	44.7		50

TABLE I (Continued)

Population	Location	Number Tested	% of Subjects Intolerant		Reference
			LTT	Biopsy	
Europeans (Continued)	Crete	50	56.0		
	Cyprus	50	66.0		
		17	88.2		66
Caucasian	Australia	23	8.7		7
		23	0.0		8, 12
		65		4.6	12
		100	6.0		
	North America	50	2.0		79
		100	6.0		70, 71
		145	19.0		84
		19	12.0		28
		7		87.5	30
		25	32.0		80
		93		19.4	63
	Italian Extraction	53	20.7		81
	Slavic Extraction	38	24.0		59
	Mixed Ethnic Group	58	24.0		53
		23		0.0	
Jewish	Israel	167	63.0		37
		217	71.0		38
	North America	32	68.8		58
		41	71.0		81
Eskimos	Greenland	25	88.0		42
	Alaska	17	65.0		6

TABLE I (Continued)

Population	Location	Number Tested	% of Subjects Intolerant		Reference
			LTT	Biopsy	
Alaskan Eskimos and Canadian Indians		36	92.0		31
Indians	India	5	80.0		7, 8, 29
	Native South America	24	58.3		1
	Peru	30	66.6	66.6	32
	North America				
	Oklahoma	36	80.0		15
	Canada	30	63.3		60
Mexican-Americans	Mexico	11		100.0	30
Mexican-Caucasian-Indians	Mexico	401	73.8		62
Negro	North America	41	72.0		28
		24		75.0	63
		22	77.0		84
Orientals	Hong Kong	21	85.7		7
		30	100.0		8
	Thailand	22	68.2		10
		22	100.0		34
		56		100.0	51
		140	97.0		53
		74		100.0	
	Australian born and raised	34	56.0		9

TABLE I (Continued)

Population	Location	Number Tested	% of Subjects Intolerant		Reference
			LTT	Biopsy	
Orientals (Continued)	Lived in U.S.A. over 5 years	11		100.0	21
Others	New Guinea	8	100.0		7, 8
	Phillipines	10	100.0		47

years of age or less is about one pint per day. The female consumes the same amount until 11 years old, and then tends to drop to less than half a pint per day (12). This is considerably more milk consumed per day than by most Negroes (5, 46, 73), Orientals (69) and other races (27).

Among the Europeans, Greeks have the highest incidence of lactose malabsorption. However, in a recent study (50) the incidence of lactose malabsorption on the Greek mainland was lower than the level found in Cyprus and Crete. This was attributed to the fact that the islanders raise sheep and goats and use the milk primarily for cheese.

There appears to be a great lactose malabsorption difference among African tribes. The Hamitic (26), Yorubua and Fulani tribes (55) raise cattle and use dairy products with more regularity than other tribes. The American slave came from the Nigerian tribes so the similar incidence of intolerance found in the American Negro (28, 63, 84) may indicate a genetic determination of lactose malabsorption. Milk products are not consumed in Africa to a great extent. The tsetse fly causes trypanosimiasis among dairy cattle and makes raising cows difficult (5).

Lactose malabsorption among Jews seems to average 70 percent for people raised in different geographic locations. The Yemenites, natives of Israel, and those from the Mediterranian area seem to have a 60 percent incidence of intolerance. Those from Europe, Iraq, and Oriental countries, have an 84 percent incidence of lactose intolerance (38).

Very few lactose malabsorption studies have been done on native North American Indians. Two studies (15, 60) found the malabsorption level to be between 63 and 80 percent. This is very close to the

malabsorption recorded among South American Indians (1, 32) and Mexicans (62).

The highest incidence of lactose intolerance with Indians and Eskimos was reported by Duncan and Scott (31). Both of those groups consume few dairy products, usually one glass of milk per day and some fermented dairy products. When they ingest this small amount of milk, no clinical lactose malabsorption symptoms are present (6, 60). There has been some controversy as to whether the Eskimo should be considered a member of the Indian race, and the incidence of lactose intolerance found among Eskimos (6, 42) has shown a wider range than that found among native Indians.

Orientals as a group have the highest reported incidence of lactose intolerance (7, 8, 9, 10, 12). However, those Orientals raised in milk drinking countries consumed more milk as children and appeared to absorb lactose more efficiently than those raised on other places (9). They also seemed to drink more milk products when in high milk producing areas, but not enough to make them show clinical symptoms (69).

It may be that some environmental circumstances such as malnutrition and ratio of protein to calories may lead to loss of lactase activity among world populations at an early age. This may be coupled with a genetic determination (52).

Lactase, the Enzyme

Adult lactose malabsorption is usually caused by lack of the enzyme that breaks the one to four α linkage of lactose which is the only disaccharide found in milk. When the lactose has been broken down into its two monosaccharide components, glucose and galactose, it can be

readily absorbed into the body and used for energy (54).

The exact location of the enzyme and how it functions in the healthy human being is still not fully understood. In 1965, Semenza, et al. (79) located two lactases in the small intestine. The first one appeared to have a higher turnover rate and was labeled lactase I. The second enzyme, which appeared to have a slower rate was labeled β disaccharidase or lactase II. Other researchers (24, 48) confirmed this by determining the optimum pH of each of the lactases. They found that lactase I hydrolized lactose while lactase II did not split lactose as efficiently as it hydrolized synthetic lactose substrates. There are two theories concerning lactase II activity on lactose. One is that it has a secondary effect when lactase I is absent (85). The other possibility is that lactase II has nothing to do with lactose digestion (23).

Lactase I has been found to be confined to the brush border of the small intestine while the location of the other lactase is undetermined (2, 23, 54, 85). Lactase I is not evenly distributed in the small intestine; maximum enzyme activity tends to take place on the tip of the individual villi. In the human, this activity increases from the proximal to the distal duodenum with the peak activity occurring in the jejunum or proximal ileum. There has been no lactase enzyme activity recorded in the stomach or colon, and very low activity in the distal ileum (71, 83).

Lactose hydrolysis in man occurs along the brush border. It does so at a much slower rate than hydrolysis of sucrose or maltose. It is the lactose hydrolysis that appears to be the rate limiting step to the

overall process of lactose hydrolysis and monosaccharide absorption (2, 36, 83). Small amounts of lactose may be absorbed intact into the blood stream, depending on the intraluminal load; since it cannot be hydrolyzed in the body, it is excreted in the urine (83).

Lactase development in the fetus takes place at a much slower rate than maltase and sucrase. The latter enzymes reach their full activity level by the 28 to 32 week of gestation. Lactase activity is at a minimum level at 28 weeks and then, just at term, increases two to three fold to its maximum level (3).

Lactose Malabsorption

Malabsorption of lactose has been classified into three broad groups: congenital, primary, and secondary (2, 12, 83).

Congenital lactase deficiency is very rare. The enzyme is absent at birth and symptoms appear after the first milk is drunk. This condition will persist throughout life, and it is treated with a milk product in which lactose is hydrolyzed or with a soy milk product that contains no lactose.

Secondary lactase deficiency occurs when there has been intestinal mucosal damage, the intestine has been shortened, or transit time through the small intestine has been increased. This may be brought about by a great variety of conditions such as surgery or intestinal diseases, some of the most common being parasites, sprue, enteritis, ulcerative colitis, gastroentritis, and kwasiorkor (2, 45, 63, 79).

Primary lactose malabsorption occurs sometime after weaning with persons who have had normal intestinal lactase activity as infants. For some unknown reason, the lactase enzyme production decreases while the

sucrase and maltase levels remain stable (24, 71, 83).

Lactose malabsorption varies in adults from one geographic area compared to another. There are three theories that explain this observation. The most accepted theory has been that the infantile enzyme, lactase I, becomes deficient or is no longer produced, thus causing a high level of lactose malabsorption in the adult. Huang and Bayless (46) supported this theory since none of their subjects showed any signs of lactase II deficiency when they were determined to be lactose malabsorbers. Bolin et al. (10) also supported these findings in a study with Oriental children. They determined that a universal lactose intolerance was quite common at the age of three years. Other researchers have broadened the age to ten years or younger (34, 51).

Another theory has been that as people age, the lactase becomes deficient and causes a high level of lactose malabsorption (8). A third theory has been that there is a genetic modification that causes high lactase activity to cease when one is an adult. Ability to absorb lactose as an adult would be considered a mutant trait. Bayless, Paige, and Ferry (4) were the first to observe that the majority of the world population showed signs of lactase deficiency. McCracken (64, 65) and Simoons (79) supported this theory when they theorized that all populations had a high lactase deficiency prior to cattle domination.

In several studies using rats, after weaking, dietary lactose was decreased gradually for five to ten weeks. It was found that the lactase activity was slightly decreased; but when lactose was returned to the diet, the enzyme lactase I could still digest the lactose present (11, 40, 74). In other rat studies it was discovered that with large lactose intakes, more lactase I was present in the intestine (13,

33, 57). The diet and the hormonal activity appeared to determine intestinal lactase activity (40, 74).

In studies with humans with varying lactose intake over a period of ten days to four months, there was no significant lactase activity increase or decrease (23, 53, 57). Nor was there an increase in the lactase activity when a high lactose diet was given to a subject for two years (41).

Paige, Bayless, and Ferry (73) suggested that milk consumption would be lower among some groups because of poor financial status and poor health habits. These authors in another study found that environmental factors such as malnutrition, parasites, sprue, and other diseases that cause damage to the intestinal mucosa, interfere with lactose absorption. These diseases caused permanent intestinal damage to children and caused them to be malabsorbers from that point forward (4).

Lactose Malabsorption Detection Methods

There are several methods used to determine lactose malabsorption. The subject must fast before the tests are given so that the only food available to the body is the lactose the subject consumes. The lactose tolerance test (LTT) is the most common method. An oral lactose load is given to the subject based on body weight (15, 83). The sugar load is about 50 g for an adult which is approximately equivalent to the amount found in one quart of milk (80). Following the ingestion of that much lactose, a malabsorber will usually have one or more of these symptoms: abdominal bloating or distension, cramps, diarrhea, irritable colon syndrome, and loose stool (2). Blood samples are taken at zero time

and at half-hour intervals after the lactose is ingested for a two to three-hour period. The blood glucose level may be determined by the Nelson-Somogi method, glucose oxidase method, or with the ferric autoanalyzer (26). The maximum rise is subtracted from the fasting blood level and the difference reported. A rise of 20 mg/100 ml or less of blood would indicate a flat curve, and lactose malabsorption would be present. The normal rise would be expected to be 50 to 60 mg/100 ml of blood (83).

A person with a flat LTT curve would also be expected to have inadequate jejunal lactase levels as indicated by intestinal biopsy (3). Intestinal mucosal specimens are obtained usually with a capsule that the subject swallows, and it has a tube attached to the end. The capsule is placed at the ligament of Tretiz; its position can be confirmed with a fluoroscope (63). The capsule usually remains in the small intestine for about four hours. When the tissue from the capsule is examined for lactase, the results are expressed in units of disaccharidase per gram protein of the mucosa. When the lactase levels are less than two units per gram of wet weight, most persons will have clinical symptoms if they ingest a large lactose dose. This method may not be too successful for a number of reasons. First, the subject may not be able to swallow the capsule. The capsule may fail to get enough tissue for examination. The subject may chew the tubing and break it which makes the capsule non-retrievable by mouth. The capsule could be placed at the proper site and the wrong tissue be obtained (79).

Radiological diagnosis is being explored to determine lactose malabsorption quickly and easily. The subject is given barium to drink

that contains lactose. The subject should not ingest any food or drink 12 hours before the test is begun. When a malabsorber is given 25 g of lactose the following observations are made:

1. a dilation of contrast in the small intestine,
2. an increased transit speed to the cecum, and
3. a slight dilation of the gut.

However, it is difficult to distinguish between glucose transport and impaired hydrolysis. One of the advantages to the test is that it is completed in one hour and very few clinical symptoms result (55, 68).

Two breath analysis tests have been developed to detect lactose malabsorption. The carbon-14 test is one that requires the collection and measurement of expired $^{14}CO_2$ after ingesting 50 g of ^{14}C lactose. Breath samples are obtained over a four-hour period, with a peak in the $^{14}CO_2$ at the fourth hour. This rise is the result of the ingested lactose that has been hydrolyzed and carried to the epithelial cells of the body. The expired $^{14}CO_2$ is then dried, neutralized and marked with an indicator. The radioactivity is determined by a liquid scintillation counting vial and compared to a known standard. The higher the $^{14}CO_2$ curve, the more lactose is being absorbed by the subject. The expired $^{14}CO_2$ is expressed as a percentage of the administered dose per millimole of CO_2 (76).

The second test, the breath hydrogen test, is a positive measure of lactose malabsorption rather than a negative test measurement (20). Expired hydrogen is collected and analyzed using a gas chromatograph. The expired hydrogen is compared to a known standard. When lactose is not absorbed, bacteria in the large intestine use the sugar and release hydrogen gas that diffuses through the intestinal wall into the blood

stream and lungs (61). After the ingestion of 48 g of lactose, hydrogen concentration rises have been recorded from zero to 230 parts per million (ppm). An average of 30 ppm or higher indicates lactose malabsorption; 10 to 20 ppm is indicative of a lactose absorber. The hydrogen peak usually occurs two to three hours after the lactose is ingested. This test is simple to administer, entirely without risk, requires no sterile procedure, is sensitive, and can be repeated as frequently as needed (20).

CHAPTER III

METHODS AND PROCEDURE

The possibility of measuring lactose malabsorption by the hydrogen gas produced in the gut was investigated in this study. It is known that consumption of one gram of lactose per kilogram of body weight will determine lactose malabsorption, and if the person is a malabsorber, clinical symptoms previously mentioned will likely appear. It has also been estimated that between 60 and 80 percent of the native North American population are lactose malabsorbers, but they consume smaller amounts of lactose with no ill side effects. The breath hydrogen test can detect small hydrogen gas amounts produced by bacteria in the large intestine in the presence of lactose. This study was designed to measure the hydrogen produced by the bacteria when 0.25 g lactose/kg of body weight was ingested by subjects. It was assumed that the hydrogen produced was due to the presence of lactose in the milk because subjects were screened by questionnaire for food allergies and other malabsorbtive deficiencies (see Appendix A, pp. 45, 47). The test was repeated five times by each subject to determine the day effect as well as establish the reliability of the breath hydrogen test.

Description of Subjects

The five subjects used in this study were 63 percent or more native North American women who had been labeled lactose malabsorbers by the

LTT. Their native American heritage was recorded (see Appendix A, p. 47). Their height was measured to the nearest 0.5 cm and weight was recorded to the nearest 0.1 kg. These measurements were taken with the subject in street dress and without shoes. This information was recorded on the data sheet along with the ml of milk given, the bag number, and time each bag was taken (Appendix A, p. 48). All subjects were between 18 and 27 years of age (see Table II). Care was taken from a medical standpoint in that volunteers who had a recent history of an antibiotic usage or gastrointestinal disturbances were not included in the study.

TABLE II

DESCRIPTION OF SUBJECTS

Subject	Age (yr)	HT (cm)	Wt (kg)	Native American Heritage (%)	Milk Consumed for BHT (ml)	LTT (mg/100 ml)
1	24	163	78.6	63-Pawnee	393	0
2	27	165	73.2	75-Ponca and Creek	366	0
3	18	170	59.5	75-Ponca and Creek	298	4
6	26	156	59.2	75-Pawnee	296	18
9	19	165	50.6	100-Creek	253	11

Due to the nature of the study, the sample size was small. There may have been some bias in sample selection because some uncontrollable variances such as emotional stress, and where random selection was not possible.

Interview and Test Procedure

Each subject was familiarized with the lounge area where they were to spend six hours of each test day. They chatted with the technician in charge at least one hour before the first breath sample was taken. This interval was to allow for emotional response which is known to raise the breath hydrogen level (20).

The technician briefly explained the routine and obtained a signed consent form (see Appendix A, p. 43). A sample breath was collected at this time but no analysis was performed. During the test the subject diverted her attention in the lounge area by reading, listening to the radio, watching television, etc. She did not assume a reclining position since body position may affect the breath-hydrogen level (16, 20).

Each subject was instructed to fast from 8:00 p.m. the night preceding the test day. The test was begun between 8:00 a.m. and 9:00 a.m. with the subjects having risen at least one hour before the basal breath sample was taken to allow the breath-hydrogen to reach body equilibrium. Upon rising the breath-hydrogen is high (16, 20). No food or beverage was allowed the morning of the test with the exceptions noted below.

Basal breath was sampled as follows: Subject initially blew several breaths through a glass cylinder designed to remove carbon dioxide and water. Minimal instructions were offered as to how to

inflate the four liter bag with expirations. Once the subject had blown through the cylinder to allow for gas exchange that was in the cylinder, a hydrogen gas diffusion-proof bag was attached to the cylinder to trap expirations. When the subject had placed about three liters of air in the bag, it was clamped off, tagged and stored for analysis within a 24-hour period. To prepare the bags before use by a subject, and to clean the bags for reuse by another subject, the bags were evacuated with reactor grade helium gas. Each bag was flushed three times with this gas to ensure the removal of hydrogen gas.

Following the filling of the first bag, the subject then drank reconstituted, nonfat dry milk that had been carefully measured according to her body weight. Five ml of milk were given for each kg of the subject's body weight. The milk was drunk within a 15-minute period. Breath samples were taken, as described above, one hour after the ingestion of the milk and at 15-minute intervals thereafter for two and one-half hours. During the next two and one-half hours, breath samples were taken every 30 minutes. Preliminary work (18, 20) had indicated that the hydrogen peak was expected to occur during the first two and one-half hours.

Snacks and beverages were provided since the test period was a minimum of six hours. One hour after the milk was consumed, an ounce of toasted French bread with half an ounce of prepackaged grape jelly and decaffeinated coffee or tea with lemon were consumed by the subject. After the fourth hour a second snack was served consisting of a hard cooked egg, one and one-half ounces of French bread with decaffeinated coffee or tea with lemon. The subject was limited to four cups of beverage between snacks. All snacks and beverages were prepared by the

technician.

A second interview followed the test (see Appendix A, p. 49). The subject was asked if she felt unusual in any way. Symptoms were recorded as none, intestinal gas, abdominal cramping, diarrhea, and/or loose stools.

Repeated tests were required for an individual over a period of one and one-half weeks to measure the hydrogen peak over a set time period for each test day. Each subject started the test on Tuesday and subsequent tests were made the following Thursday, Saturday, Monday and Wednesday.

Analysis of Breath Samples

Breath hydrogen was analyzed using a helium-carrier, gas chromatograph from which quantitative analysis was done using a calibration curve technique. The bags were stored and analyzed within a 24-hour period after they were filled. The gas samples from the bags were run through the gas chromatograph and the hydrogen concentration was recorded for each bag. The order in running the bags was ignored.

Statistical Analysis

The study was set up in a randomized block design where the subject was considered as a block and the treatment as days. Each subject was considered her own control. The statistical design gave a way to study subject variation due to successive period effect as well as measure individual differences.

Calloway, Murphy, and Bauer (20) found that the hydrogen peak occurred about two and one-half hours after lactose consumption. In

this study the hydrogen concentration was recorded over a five-hour period to cover subject variation. Analysis of variance (AOV) was made on the data obtained from the initial reading up to and including the 180-minute reading. Statistical analyses were made on three response variables:

1. the total area under the curve during this period,
2. the maximum hydrogen peak height that occurred during this period, and
3. the average of three consecutive values that included the highest peak during this time period.

The base line used for the AOV was varied in three ways. One was to use the absolute value as the base line. Another variation was to subtract the initial or basal hydrogen concentration from the area or height, and the third variation subtracted the 60-minute reading from the absolute value.

CHAPTER IV

RESULTS AND DISCUSSION

Five native North American Indians that had been determined to be lactose malabsorbers were selected and tested for their lactose malabsorption using the breath-hydrogen test (BHT). These five subjects were between 18 and 27 years of age, and were under no medical care. The subjects were given reconstituted skim milk as the only lactose food source. The hydrogen produced by bacteria in the small intestine was assumed to diffuse through the intestinal wall to the blood stream which carries the hydrogen to the lungs where the rise in the hydrogen gas present at a given time was measured.

The Maximum Hydrogen Rise

It was found that all subjects had a maximum hydrogen peak over 30 ppm during the test days. This peak occurred one and one-half to three hours after the milk had been consumed. This was the level Calloway et al. (18, 20) used to distintuish an absorber from a malabsorber when 0.5 g lactose per kg body weight was consumed, over twice the dosage used here. The breath hydrogen concentrations for the subjects are listed by test day in Table III.

If the breath hydrogen concentrations (absolute value of three consecutive hydrogen readings, Table IV) were considered for the total of the five-day test series, subject 1 had the highest average of the

TABLE III

LACTOSE MALABSORPTION--THREE CONSECUTIVE H_2 CONCENTRATIONS AFTER SKIM MILK CONSUMPTION

Subject	Day	Minutes After Lactose Consumption	3 Consecutive Readings ppm*
1	Tuesday	105	53, 57, 54
	Thursday	90	93, 90, 87
	Saturday	90	76, 82, 82
	Monday	90	77, 85, 61
	Wednesday	105	61, 67, 62
2	Tuesday	150	18, 30, 45
	Thursday	150	44, 43, 55
	Saturday	135	68, 51, 51
	Monday	135	44, 51, 49
	Wednesday	105	57, 61, 57
3	Tuesday	135	39, 40, 39
	Thursday	135	44, 45, 41
	Saturday	90	39, 45, 57
	Monday	120	37, 33, 33
	Wednesday	105	33, 45, 45
6	Tuesday	90	60, 53, 50
	Thursday	105	78, 72, 68
	Saturday	135	59, 83, 52
	Monday	90	59, 58, 55
	Wednesday	105	53, 52, 42
9	Tuesday	90	48, 17, 27
	Thursday	150	46, 39, 36
	Saturday	90	54, 50, 48
	Monday	75	46, 44, 39
	Wednesday	150	48, 49, 49

*These three readings included the maximum hydrogen peak.

group with 72.4 ppm. Subject 6 had the second highest average at 59.6 ppm. The other subjects' breath hydrogen concentration averages were 41.0, 42.5, and 48.2 ppm. Bond et al. (14) and Calloway et al. (18, 20) attributed the breath hydrogen rise as an indicator of carbohydrate malabsorption. This variation found among the subjects in this study shows the sensitivity of the breath hydrogen to lactose malabsorbers.

Individual and Day Effects

Individual and day effects are shown in Tables IV and V. Figures 1 through 5 in Appendix B show individual responses for each sample for each test day. There was a trend for the breath hydrogen to increase gradually from Tuesday through Saturday and then decrease as the last test day approached. Various physiological and/or psychological factors not controlled in this study are a possible cause of these individual differences. Day effects were not as pronounced as individual effects. All individuals showed a net rise of at least 30 ppm as did those subjects used by Calloway et al. (18, 20), although the subjects in this study ingested less than half the lactose dosage relative to body weight as that given in the Calloway studies.

The subjects' lactose malabsorption symptoms were slight. All subjects except subject 9 reported only slight flatulence at about 60 minutes on each test day. Subject 9 reported intestinal gas and slight nausea on the first and last test days respectively with no symptoms reported on other days. It is interesting to note that three of the subjects had consumed virtually no milk since childhood and the other two drank very little; and their breath hydrogen concentration was

TABLE IV

MEAN RESPONSE VALUES FOR THE BREATH-H_2 METHOD USING THREE DISTINCT RESPONSE FACTORS

	Source	Obs.[a]	Maximum H_2 Concentration			Average of 3 H_2 Concentrations			Area		
			Abs.[b] Value	60 min.[c] Corr.	Basal min.[d] Corr.	Abs. Value	60 min. Corr.	Basal min. Corr.	Abs. Value	60 min. Corr.	Basal min. Corr.
Day	Tu	5	50.0	37.4	40.8	42.0	29.2	32.8	3879	2383	2775
	Th	5	63.4	50.0	52.4	58.7	44.8	47.7	5423	2893	4098
	S	5	69.2	57.4	61.4	59.9	47.8	52.1	5389	3873	4453
	M	5	55.6	55.0	48.8	51.4	39.9	44.6	4764	3257	3962
	W	5	55.0	42.2	48.8	51.8	39.2	45.6	4859	3348	4115
Ind[e]	1	5	76.8	66.2	69.2	72.4	61.6	64.8	7205	5710	6293
	2	5	56.0	40.8	46.8	48.2	33.5	39.0	3993	2463	2892
	3	5	44.8	31.6	31.6	41.0	28.0	27.8	3646	2114	2068
	6	5	66.6	56.0	60.4	59.6	47.8	53.0	5399	3897	4655
	9	5	49.0	37.4	44.2	42.5	30.0	37.7	4071	2569	3495

[a]Number of observations. [b]Absolute value. [c]60-minute correction. [d]Zero-minute correction.

[e]Subject identification number.

TABLE V

ANALYSIS OF VARIATION FOR THE BREATH-H_2 METHOD SHOWING MEAN SQUARE VALUES USING THREE DISTINCT RESPONSE FACTORS

Source	D.F.	M. S. Max. H_2 Concentration[a]			M. S. Average of 3 H_2 Concentrations[b]			M. S. Area (x 10^4)[c]		
		Abs.[d] Value	60 min.[e] Corr.	Basal min.[f] Corr.	Abs. Value	60 min. Corr.	Basal min. Corr.	Abs. Value	60 min. Corr.	Basal min. Corr.
Day	4	289.1*	293.2*	277.88**	290.4*	251.4*	258.2*	196.0*	188.9*	207.4*
Ind.[g]	4	855.7**	1019.5**	1072.8**	872.8**	1013.9**	1060.2**	1079.7**	1099.1**	1354.1**
Error	16	61.9	81.0	58.1	55.4	72.5	60.4	84.7	85.3	112.1
CV[h]		14%	20%	14%	15%	22%	20%	18%	24%	24%

Note: Data marked * denotes F test significant at 5% level; data marked ** denotes F test significant at 1% level.

[a]Mean squares of maximum H_2 concentration. [b]Mean squares of the average of the 3 highest consecutive H_2 concentration. [c]Mean squares of area under the curve. [d]Absolute value. [e]60-minute correction. [f]Zero-minute correction. [g]Individual. [h]Coefficient of variation.

always above 30 ppm when given 0.25 g of lactose per kg of body weight.

Procedure and Response Variables

A randomized block design in which a subject was considered as a block and days the treatments was used to estimate the effects due to subject and day effects. Mean values for all subjects on a given test day and for the five test days computed for each subject are shown in Table IV. The three chosen response variables are listed with the three variations used in base line values.

When examining the coefficient of variation (CV) in Table V, the absolute values gave equal or lower values than the 60-minute correction and the basal correction. The error mean square (EMS) maximum hydrogen concentration response variables had the lowest CV of 14 percent when considering the absolute value or the basal time correction. However, the EMS was high at 61.9 and 58.1 percent, respectively. The lowest EMS was 55.4 using the absolute value of the average of the three consecutive values that included the maximum hydrogen peak. It was concluded that the three consecutive hydrogen concentrations were the best response values because they had the lowest EMS and next to the lowest CV of all other response variables. Using an average might also minimize uncertainty due to technician error. Using the EMS of the area under the curve as a response variable might also be exepcted to eliminate unknown sources of variation but, since both the CV at 18 percent and the EMS at 84.7 were higher than the recommended method, it was not chosen.

One of the concerns when using this test was the sample bag hydrogen leak diffusion over a 24-hour period. Six sample bags were

randomly selected. Three bags were used for calibration curve and the others were filled with a known concentration of hydrogen. Within a 24-hour period, the three sample bags were randomly sampled at four preset intervals. Each time the sample bags were sampled, a new calibration curve was made. It was found that the CV was 1.5 percent, over the 24-hour period, indicating very little hydrogen diffusion from the sample bags and little machine variation.

If the BHT were to be used for clinical lactose malabsorption determination, the average of the three highest consecutive readings could easily be obtained. The first and last sample bags would not be analyzed as these two times appear to vary possibly with emotional status, more than the regular test time. The exclusion of these samples would not be known by the subject. The 15-minute time interval could be lengthened to 20 minutes or more but the chances of catching the hydrogen peak at its greatest height are decreased since this peak lasts about 10 minutes (16). Shortening the time interval would give more precise data, but might lead to subject discomfort because subjects complained that their lungs were taxed when breath samples were taken at less than 15-minute intervals.

Some subjects consuming 0.25 g lactose per kg of body weight had only slight gas during the test. One subject complained of cramping but stated that it only lasted a few minutes. Gas symptoms appeared during the first two hours of the test. There appeared to be no relation between the time the subjects peaked and the time the symptoms were reported. However, these symptoms were much less severe than found when subjects ingested twice as much lactose at one given time (60, 69). Since the BHT can detect these small differences, it appears to be a

good test to use as a standard clinical procedure for lactose malabsorption determination. The patient's environment can be easily controlled in his room and the patient's symptom discomfort should be a prime consideration.

CHAPTER V

SUMMARY AND CONCLUSION

Five native North American females were selected to participate in this study. The main purpose of this study was to determine if the BHT is a desirable instrument to use for lactose malabsorption determination.

All subjects were lactose malabsorbers according to the LTT, and were in good health. Each subject started the test on Tuesday, and breath samples were collected at specified time intervals over a five-hour time period. The sample bags were analyzed within 24 hours after being taken.

Sample bags were tested for hydrogen diffusion over a 24-hour period. It was found that hydrogen diffusion loss through the sample bags was at a minimal level.

The BHT was repeated the following Thursday, Saturday, Monday, and Wednesday. This enabled each subject to act as her own control.

The data was analyzed as a randomized block design. The hydrogen produced by the subjects was found to be significantly different at the one percent level, and there was also a day effect which was significant at the five percent level.

Subjects ingested less than half the lactose amount given in previous BHT (18, 20) yet still had hydrogen peaks over 30 ppm two and one-half hours after ingestion. This substantiates the findings of

Calloway et al. (18, 20) that the BHT is a sensitive method to determine lactose malabsorption. One test procedure was suggested and three methods of analyses for this test procedure were discussed. The three consecutive hydrogen reading was determined to be the best response variable. More research using this instrument as the sole diagnostic tool for lactose malabsorption in a larger population is needed.

Suggestions for the further study of the BHT for lactose malabsorption are the following:

1. Measure lactose malabsorption among a larger group of individuals of one known lactose malabsorbing race.

2. Investigate the correlation between subject's response to the LTT using 1 g of lactose per kg of body weight, and the BHT using 0.25 g of lactose per kg of body weight.

3. Determine the maximum amount of time between breath samples that would still yield a meaningful hydrogen value.

4. Determine the shortest fasting time required by subjects before using the BHT.

5. Investigate the various physiological effects such as menustration, pregnancy, and birth control pills.

6. Determine the effects, if any, of age, sex, or psychological stress on the BHT.

7. Determine whether the BHT could be used to diagnose mucosal damage as found in enteritis, and other malabsorptive conditions.

SELECTED BIBLIOGRAPHY

1. Alzate, H., Gonzalez, H., and Guzman, J.: Lactose intolerance in South American Indians. Am. J. Clin. Nutr. 22:122, 1969.

2. Bayless, T. M.: Disaccharidase deficiency. J. Am. Dietet. A. 60:478, 1972.

3. Bayless, T. M. and Christopher, N. L.: Disaccharidase deficiency. Am. J. Clin. Nutr. 22:181, 1969.

4. Bayless, T. M., Paige, D. M. and Ferry, G. D.: Lactose intolerance and milk drinking habits. Gastroent. 60:605, 1971.

5. Bayless, T. M. and Rosensweig, N. S.: A racial difference in incidence of lactase deficiency. J. Am. Dietet. A. 197:968, 1966.

6. Bell, R. R., Draper, H. H. and Bergan, J. G.: Sucrose, lactose and glucose tolerance in northern Alaskan Eskimos. Am. J. Clin. Nutr. 26:1185, 1973.

7. Bolin, T. D., Crane, G. G. and Davis, A. E.: Lactose intolerance in various ethnic groups in South-East Asia. Aust. Ann. Med. 17:300, 1968.

8. Bolin, T. D. and Davis, A. E.: Asian lactose intolerance and its relation to intake of lactose. Nature. 222:382, 1969.

9. Bolin, T. D. and Davis, A. E.: Lactose intolerance in Australian-born Chinese. Aust. Ann. Med. 19:40, 1970.

10. Bolin, T. D., Davis, A. E., Seah, C. S., Chua, K. L., Yong, K., Kho, K. M., Siak, C. L. and Jacob, E.: Lactose intolerance in Singapore. Gastroent. 59:76, 1970.

11. Bolin, T. D., McKern, A. and Davis, A. E.: The effect of diet on lactose activity in the rat. Gastroent. 60:432, 1971.

12. Bolin, T. D., Morrison, R. M., Steel, J. E. and Davis, A. E.: Lactose intolerance in Australia, Med. J. Aust. 1:1289, 1970.

13. Bolin, T. D., Pirola, R. C. and Davis, A. E.: Adaptation of intestinal lactase in the rat. Gastroent. 57:406, 1969.

14. Bond, J. H. and Levitt, M. D.: Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption. J. Clin. Invest. 51:1219, 1972.

15. Bose, D. P. and Welsh, J. D.: Lactose malabsorption in Oklahoma Indians. Am. J. Clin. Nutr. 26:1320, 1973.

16. Brothwell, D. and Brothwell, P.: Food in Aniquity. New York: Frederick A. Praeger, Inc., 1969.

17. Calloway, D. H.: Respiratory hydrogen and methane as affected by consumption of gas-forming foods. Gastroent. 51:383, 1966.

18. Calloway, D. H. and Chenoweth, W. L.: Utilization of nutrients in milk and wheat based diets of new men with adequate and reduced abilities to absorb lactose I. Energy and nutrition. Am. J. Clin. Nutr. 26:939, 1973.

19. Calloway, D. H. and Murphy, E. L.: The use of expired air to measure intestinal gas formation. Ann. N. Y. Acad. Sci. 150:82, 1968.

20. Calloway, D. H., Murphy, E. L. and Bauer, D.: Determination of lactose intolerance by breath analysis. Am. J. Dig. Dis. 14:811, 1969.

21. Chung, M. H. and McGill, D. B.: Lactose deficiency in Orientals. Gastroent. 54:225, 1968.

22. Cook, G. C., Asp, N. G. and Dahlquist, A.: Activities of brush border lactase, and β-galactosidase, and hetero-β-galactosidase in the jejunum of the Zambian African. Gastroent. 64:405, 1973.

23. Cook, G. C., Asp, N. G. and Dahlquist, A.: Lactose absorption kinetics in Zambian African subjects. Brit. J. Nutr. 30:519, 1973.

24. Cook, G. C. and Dahlquist, A.: Jejunal hetero-β-galactosidase activities in Ugandans with lactase deficiency. Gastroent. 55:328, 1968.

25. Cook, G. C. and Kajubi, S. K.: Incidence of lactose deficiency in Uganda. Lancet. 1:725, 1966.

26. Cox, J. A. and Elliott, F. G.: Primary adult lactose intolerance in the Kivu Lake area: Rwanda and the bush. Am. J. Dig. Dis. 19:714, 1974.

27. Crawford, M. A.: Lactase deficiency. Nature. 223:742, 1969.

28. Cuatesasas, P., Lockwood, D. H. and Caldwell, J. R.: Lactose deficiency in the adult. Lancet. 1:14, 1965.

29. Davis, A. E. and Bolin, T. D.: Lactose intolerance in Asians. Nature. 216:1244, 1967.

30. Dill, J., Levy, M., Wells, R. F. and Weser, E.: Lactase deficiency in Mexican-American males. Am. J. Clin. Nutr. 25:869, 1972.

31. Duncan, I. W. and Scott, E. M.: Lactose intolerance in Alaskan Indians and Eskimos. Am. J. Clin. Nutr. 25:867, 1972.

32. Figueroa, R. B., Melgar, E., Jó, N. and Garcia, O. L.: Intestinal lactase deficiency in an apparently normal Peruvian population. Am. J. Dig. Dis. 16:881, 1971.

33. Fischer, J. E.: Effects of feeding a diet containing lactose upon β-D-galactosidase activity and organ development in rat digestive tract. Am. J. Physiol. 188:49, 1957.

34. Flatz, G., Saengudom, C. H. and Sanguanbaokhai, T.: Lactose intolerance in Thialand. Nature. 221:758, 1969.

35. Fleck, H.: Introduction to Nutrition. New York: The MacMillan Co., 1971.

36. Gary, G. M. and Santiago, N. A.: Disaccharide absorption in normal and diseased human intestine. Gastroent. 51:489, 1966.

37. Gilat, T., Benaroya, Y., Malachi, E. and Adam, A.: Genetics of primary adult lactase deficiency. Gastroent. 64:562, 1973.

38. Gilat, T., Kuhn, R., Gelman, E. and Mizrahy, O.: Lactase deficiency in Jewish communities in Israel. Am. J. Dig. Dis. 15:895, 1970.

39. Gilat, T., Malachi, E. G. and Shochet, S. B.: Lactose tolerance in an Arab population. Am. J. Dig. Dis. 16:203, 1971.

40. Goldstein, R., Klein, T., Frier, S. and Menczel, J.: Alkaline-phosphatse and disaccharidase activities in rat intestine from birth to weaning. Am. J. Clin. Nutr. 24:1224, 1971.

41. Gilat, T., Russco, S., Malachi, E. G. and Aldor, T. A.: Lactose in man: a nonadaptable enzyme. Gastroent. 62:1125, 1972.

42. Gudmand-Høyer, E. and Jarnum, S.: Lactose malabsorption in Greenland Eskimos. Acta Med. Scand. 186:235, 1969.

43. Guthrie, H. A.: Introductory Nutrition. St. Louis: The C. V. Mosey Co., 1971.

44. Haemmerli, U. P., Kistler, H., Ammann, R., Marthaler, T., Semenza, G., Auricchio, S. and Prader, A.: Acquired milk intolerance in the adult caused by lactose malabsorption due to a selective deficiency in intestinal lactase activity. Am. J. Med. Sci. 38:7, 1965.

45. Halsted, C. H., Sheir, S., Soural, N. and Patwardhan, V. N.: Small intestinal structure and absorption in Egypt. Am. J. Clin. Nutr. 22:744, 1969.

46. Huang, S. and Bayless, T. M.: Lactose intolerance in healthy children. N. Engl. J. Med. 276:1283, 1967.

47. Huang, S. S. and Bayless, T. M.: Milk and lactose intolerance in healthy Orientals. Science. 160:83, 1968.

48. Hsia, D., Makler, M., Semenza, G. and Prader, A.: β-galactosidase activity in human intestinal lactases. Biochim. Biophys. Acta. 113:390, 1966.

49. Jussila, J., Isokoski, M. and Launiala, K.: Prevalence of lactose malabsorption in a Finnish rural population. Scand. J. Gastroent. 5:49, 1970.

50. Kanaghinis, T., Hatzioannou, J., Deliarguris, N., Danos, N., Zografos, N., Katsas, A. and Gardikas, C.: Primary lactose deficiency in Greek adults. Am. J. Dig. Dis. 19:1021, 1974.

51. Keusch, G. T.: Subclinical malabsorption in Thailand. Am. J. Clin. Nutr. 25:1062, 1972.

52. Keusch, G. T., Troncale, F. J., Miller, L. H., Promadhat, V. and Anderson, P. R. Acquired lactose malabsorption in Thai children. Pediatrics. 43:540, 1969.

53. Keusch, G. T., Troncale, F. J., Thavaramara, B. Prinyanont, P., Anderson, P. R. and Bhamarapravahi, N.: Lactase deficiency in Thailand: effects of prolonged lactose feeding. Am. J. Clin. Nutr. 22:638, 1969.

54. Kretchmer, N.: Lactose and lactase. Sci. Am. 227:71, 1972.

55. Kretchmer, N., Ransome-Kuti, O., Hurwitz, R. and Dyngy, C.: Intestinal absorption of lactose in Nigerian ethnic groups. Lancet. 2:393, 1971.

56. Laws, J. W. and Neale, G.: Radiological diagnosis of disaccharidase deficiency. Lancet. 2:139, 1966.

57. Lebenthal, E., Sunshine, P. and Kretchmer, N.: Effect of prolonged nursing on the activity of intestinal lactase in rats. Gastroent. 64:1136, 1973.

58. Leichter, J.: Lactose tolerance in a Jewish population. Am. J. Dig. Dis. 16:1123, 1971.

59. Leichter, J.: Lactose tolerance in a Slavic population. Am. J. Dig. Dis. 17:73, 1972.

60. Leichter, J. and Lee, M.: Lactose intolerance in Canadian west coast Indians. Am. J. Dig. Dis. 16:809, 1971.

61. Levitt, M. D. and Ingelfinger, F. J.: Hydrogen and methane production in man. Ann. N. Y. Acad. Sci. 150:75, 1968.

62. Lisker, R., Lopez-Habib, G., Daltabuit, M., Rostenberg, I. and Arroyo, P.: Lactose deficiency in a rural area of Mexico. Am. J. Clin. Nutr. 27:756, 1974.

63. Littman, A., Cady, A. B. and Rhodes, J.: Lactase and other disaccharidase deficiencies in a hospital population. Israel J. Med. 4:110, 1968.

64. McCracken, R. D.: Adult lactose tolerance. J. A. M. A. 213:2257, 1970.

65. McCracken, R. D.: Origins and implications of the distribution of adult lactose deficiency in human populations. Environ. Child Health. 17:7, 1971.

66. McMichael, H. B., Webb, J. and Dawson, A. M.: Jejunal disaccharidases and some observations on the cause of lactase deficiency. Brit. Med. J. 2:1037, 1966.

67. McMichael, H. B., Webb, J. and Dawson, A. M.: Lactase deficiency in adults: a cause of functional diarrhea. Lancet. 1:717, 1965.

68. Morrison, W. J., Christopher, N. L., Bayless, T. M. and Dana, E. A.: Low lactase levels: evaluation of the radiologic diagnosis. Radiology. 111:513, 1974.

69. Nandi, M. A. and Parham, E. S.: Milk drinking by the lactose intolerant. J. Am. Dietet. A. 61:258, 1972.

70. Newcomer, A. D. and McGill, D. B.: Disaccharidase activity in the small intestine: prevalence of lactase deficiency in 100 healthy subjects. Gastroent. 53:881, 1967.

71. Newcomer, A. D. and McGill, D. B.: Distribution of disaccharidase activity in the small bowel of normal and lactase deficient subjects. Gastroent. 51:481, 1966.

72. Olatunbosun, D. A. and Adadevoh, B. K.: Lactase deficiency in Nigerians. Am. J. Dig. Dis. 16:909, 1971.

73. Paige, D. M., Bayless, T. M., Ferry, G. D. and Graham, G. G.: Lactose malabsorption and rejection in Negro children. Johns Hopkins Med. J. 129:163, 1971.

74. Reddy, B. S., Pleasants, J. R. and Wostmann, B. S.: Effect of dietary carbohydrates on intestinal disaccharidases in germ free and conventional rats. J. Nutr. 95:413, 1968.

75. Rosenweigh, N. S.: Dietary sugars and intestinal enzymes. J. Am. Dietet. A. 60:483, 1971.

76. Sasaki, Y., Iio, M., Kameda, H., Ueda, H., Aoyagi, T., Christopher, N. L., Bayless, T. M. and Wagner, H. N.: Measurement of ^{14}C-lactose absorption in the diagnosis of lactose deficiency. J. Lab. Clin. Med. 76:824, 1970.

77. Semenza, G., Auricchio, S. and Rubino, A.: Multiplicity of human intestinal disaccharidases. Biochim. Biophys. Acta. 96:487, 1965.

78. Sheely, T. W. and Anderson, P. R.: Disaccharidase activity in normal and diseased small bowel. Lancet. 2:1, 1965.

79. Simoons, F. J.: Primary adult lactose intolerance and the milking habit: a problem in biological and cultural interrelations I-review of the medical research. Am. J. Dig. Dis. 14:819, 1969.

80. Stephens, L. S. and Latham, M. C.: Lactose intolerance and milk consumption: the relation of tolerance to symptoms. Am. J. Clin. Nutr. 27:296, 1974.

81. Tandon, R., Mandell, H., Spiro, H. M. and Thayer, W. R.: Lactose intolerance in Jewish patients with ulcerative colitis. Am. J. Dig. Dis. 16:484, 1971.

82. Tannahill, R.: Food in History. New York: Stein and Day Publishing Company, 1973.

83. Welsh, J. D.: Isolated lactose deficiency in humans: report on 100 patients. Medicine. 49:157, 1970.

84. Welsh, J. D., Rohrer, V., Knudsen, K. B. and Paustian, F. F.: Isolated lactase deficiency. Arch. Intern. Med. 120:261, 1967.

85. Zoppi, G., Hadorn, B., Gitzelmann, R., Kistler, H. and Prader, A.: Intestinal β-galactosidase activities in malabsorption syndromes. Gastroent. 50:557, 1966.

APPENDIX A

STATEMENT OF INFORMED CONSENT

Research, Experimentation or Demonstration
Involving Human Subjects

OKLAHOMA STATE UNIVERSITY

I hereby acknowledge that I have been informed of the nature of the research for which I am to participate as part of the project, Development of Carbohydrate Malabsorption as follows:

I. Statement of procedures and identification of those which are experimental:

You will arrive about 8:30 a.m. after having a good nights sleep and no food or drink (except water) since 9:00 p.m. the previous evening. Testing will start approximately 20 to 30 minutes after the arrival in the laboratory. You should become familiar with the surroundings and feel relaxed and comfortable in the lab and lounge area. Please feel free to ask the person or persons conducting the study any questions that may concern you or that may have aroused your curiosity.

The study will start by collecting a breath sample. This is done by blowing three breaths into a bag. Next you will eat or drink food or milk containing a known amount of sugar. One hour later another breath sample will be taken. Breath samples will be taken every 30 minutes after the first hour for the next five hours.

To avoid any discomfort from hunger during the time of the experiment a serving of toast and jelly will be given after the first hour, and a hard boiled egg and French bread after the fourth hour. A diet and family history will be taken sometime during the test period.

You will need to remain in the area of the lounge during the whole testing period unless other arrangements have been made previously.

II. Description of discomforts:

There should be few if any discomforts experienced. If you are intolerant to the sugar given you may experience mild stomach cramps, gas, and diarrhea. These discomforts should last only a short time, 1/2 to 2 hours. With this new method to determine if a person is lactose intolerant, we give a very small amount of the lactose sugar as it occurs naturally in milk so that one will experience few or possibly no symptoms at all. If any of the above discomforts are experienced, please tell the person giving the test so she may record the symptoms.

III. Descriptions of benefits to be expected:

This test establishes whether or not the individual is tolerant to milk

INITIALS: _____

sugar. This should be valuable information to the person, as they will be able to avoid future discomfort which may arise due to lactose intolerance. The test may indicate the presence of diabetes (breath acetone).

IV. I have been given an opportunity to ask and receive answers to any questions concerning procedures.

V. I have been informed that I am free to withdraw my consent and to discontinue participation at any time. Furthermore, I agree that there has been no attempt, either written or oral, to get me to waive any of my legal rights or to hold any person or other entity blameless except as provided by law.

VI. I hereby give my informed consent to participate.

_____ _____

Date Signature

QUESTIONNAIRE
(Ask before test)

Name: _____ ID No.: _____

Address: _____ Home Phone: _____

Date: _____ Office Phone: _____

Sex: _____ Birth Year: _____

Some of these questions may be of a personal nature, please answer them truthfully and to the best of your ability because the validity of our data depends on how you answer the following questions. All information on this sheet will be confidential.

Have you eaten any food or had any drink with the exception of water in the last 12 hours? If so when? _____

What time did you get out of bed this morning? _____

Are you a diabetic? If yes, what medication do you take? _____

Have you been ill during the last seven days? _____

If yes, how many days since you have been feeling better? _____

Have you taken any of these drugs in the last ten days? If yes, name drug please.

Antibiotics: _____ When last taken: _____

Sulfa drugs: _____ When last taken: _____

Other: _____ When last taken: _____

Have you had an emotional upset recently? For example a death in the family, fight with a spouse or boyfriend (girlfriend), car accident, failed a test, unwanted pregnancy, or lost a large amount of money? _____

Are you taking any unprescribed drugs. If yes, what kind? _____

Are you taking cold capsules? If yes, please name them. _____

Are you taking antihistamines? If yes, please name them. _____

Page 2 ID No.: _____

Date: _____

Study: _____

Have you taken any of the following in the last 48 hours?

1) Aspirin _____ When? _____
2) Cope _____ When? _____
3) Mydol _____ When? _____
4) Vanquish _____ When? _____
5) Darvon _____ When? _____

Do you use any of the following tobaccos:

Cigarette: _____ How many packs/week? _____ How long? _____

Cigar: _____ How many cigars/week? _____ How long? _____

Chewing: _____ How many plugs/week? _____ How long? _____

Snuff: _____ How many tins/week? _____ How long? _____

Pipe: _____ How many pouches/week? _____ How long? _____

Have you had any of the following gastrointestinal problems during the last two weeks?

Diarrhea: _____ Severity? _____ When? _____ (days ago)

Constipation: _____ Severity? _____ When? _____ (days ago)

Other: _____ Severity? _____ When? _____ (days ago)

Are you having your monthly period at this time? _____

What day of your period is it? _____

Do you notice any discomfort having to do with the:

Stomach? _____ When? _____

Intestine? _____ When? _____

What (if any) medication or pain killers have you taken in the last 24 hours? _____

Are you taking birth control pills? _____

How long have you taken the birth control pills? _____

Comments: _____

_____

_____

QUESTIONNAIRE

ID No.: _____ Date: _____ Sex: _____

How many cups of milk do you drink each day? _____

How many cups of milk did you drink daily while in high school? _____

How many cups of milk did you drink daily while in grade school? _____

Do you like to eat these dairy products?

cottage cheese _____
ice cream _____
custard _____
cheese _____
yogurt _____
Sego _____
Carnation Instant Breakfast _____
Metrical _____
buttermilk _____
skim milk _____
chocolate milk _____
whole milk _____
malts or shakes _____

Are you allergic to any foods? _____

When you drink milk does it cause any symptoms? _____

_____

Approximately how many cups of milk would cause you to experience any discomfort after drinking the milk? _____

FAMILY HISTORY

Trace your family history back two generations. Please list the name, tribe and native American percentage of each family member. Include adopted and half brothers and sisters. Example: Mary Wolfe--Creek 4/4.

Maternal Grandparents

_____

_____

Paternal Grandparents

_____

_____

Parents

_____

_____

Children

_____

_____

DATA SHEET

Name: _____ Bag Color Code: _____

ID No.: _____ Height: _____

Date: _____ Weight: _____

Arrival Time: _____ Age: _____

Time Milk Ingested: _____ Sex: _____

Ml. of Milk Given: _____ % Indian: _____

Dosage and Sugar: _____ Tribe: _____

Study: _____ Birth Year: _____

Bag No.	Time	Minutes	ppm H_2	Comments

How did you feel during the test? _____

_____

_____

_____

QUESTIONNAIRE
(Ask after test)

Sex: _____

Date: _____

ID No.: _____

_____, I want to ask you these few simple questions and this will conclude our test for today. I want to thank you for your time and interest in this particular project.

I would like to set up another day for us to do a test exactly like this one, except the test meal will consist of a different item. Which of these two days will be best for you? _____ or _____.

Decided day: _____. Decided time: _____.

Please phone the lab if you have a change in plans so that we might work in another person to test.

How did you feel during the test? _____

_____

_____

_____

APPENDIX B

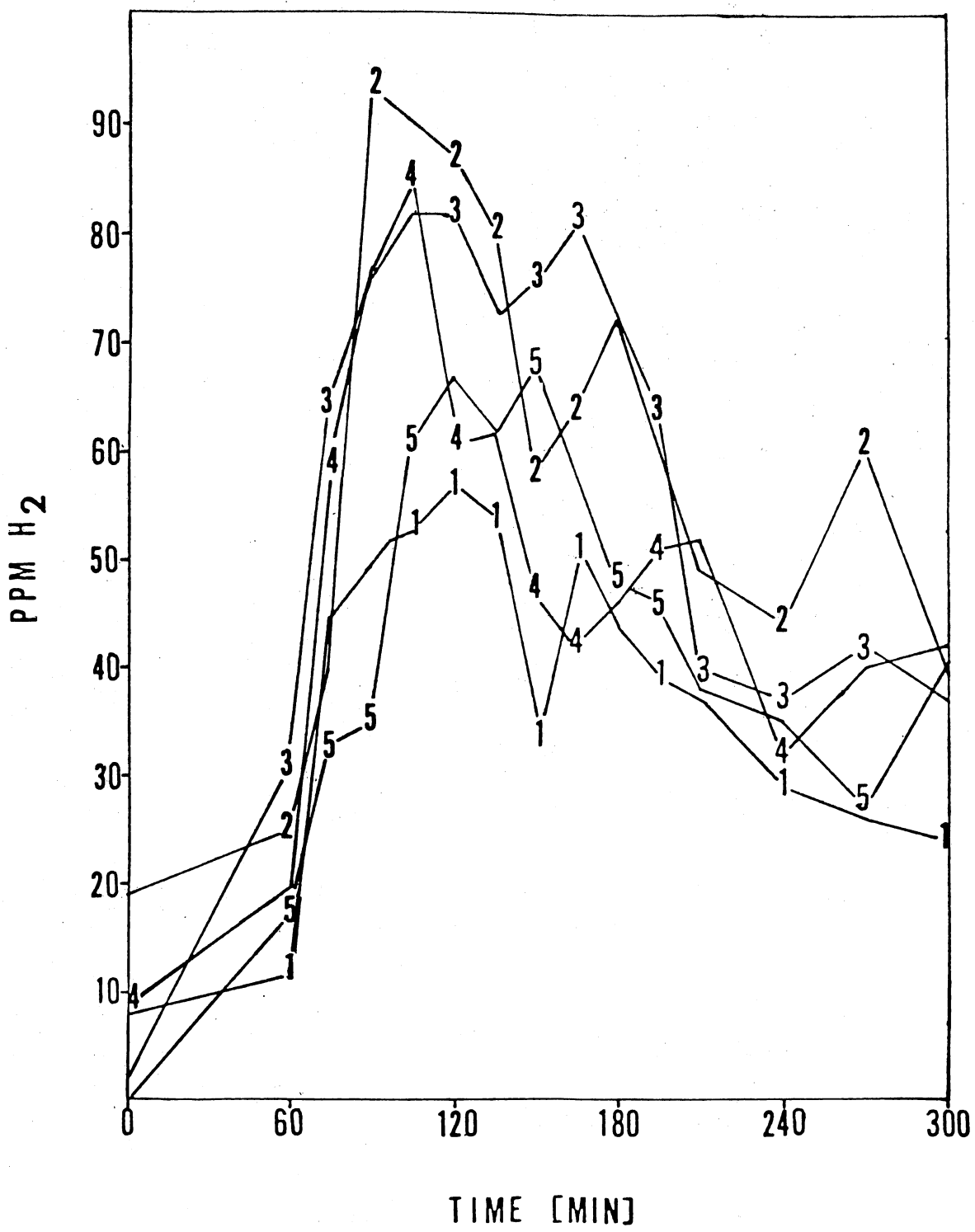

Figure 1. Subject 1--Breath H_2 Concentration Versus Time After Consumption of 5 ml Reconstituted Skim Milk/kg Body Weight (1, Tuesday; 2, Thursday; 3, Saturday; 4, Monday; 5, Wednesday)

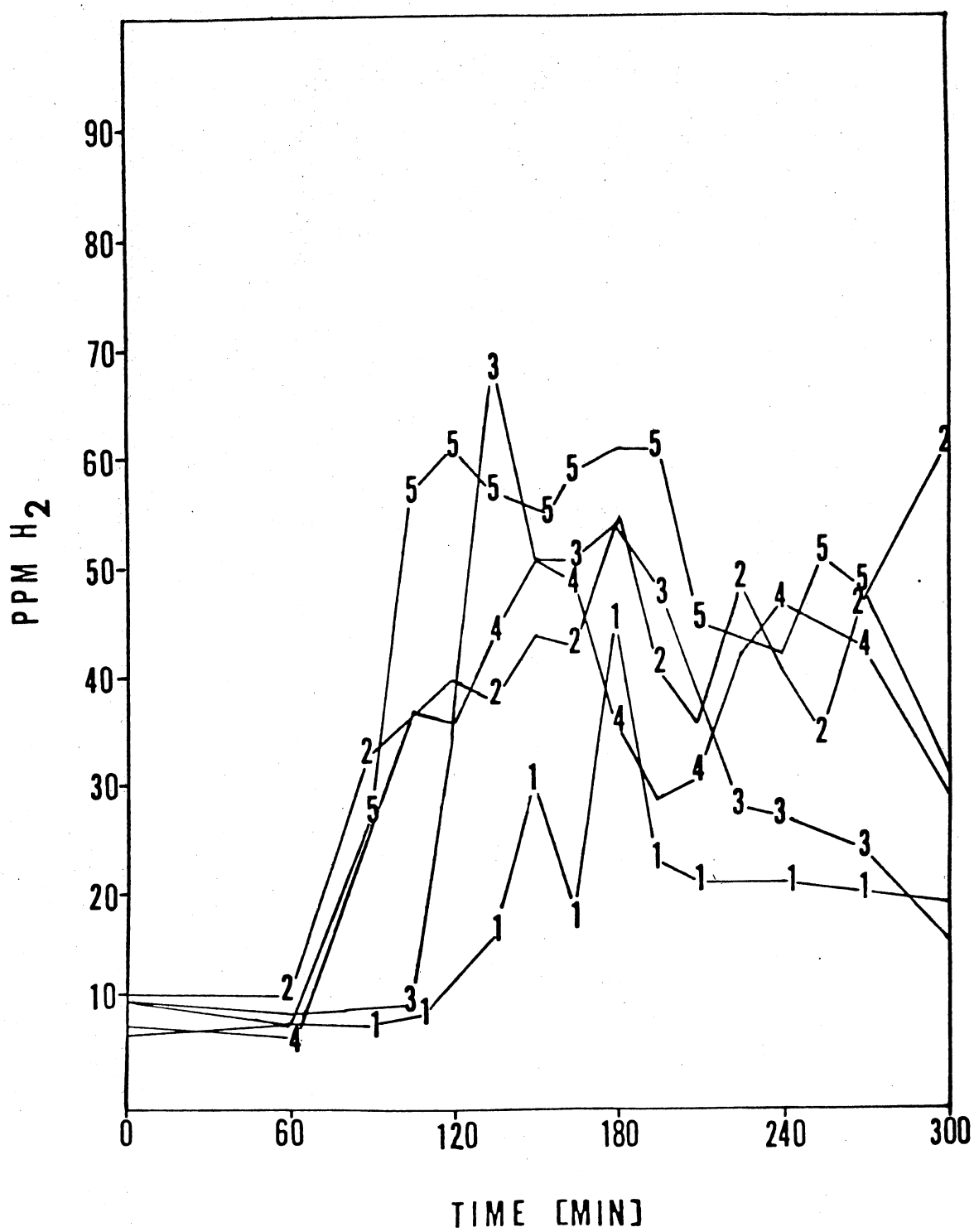

Figure 2. Subject 2--Breath H_2 Concentration Versus Time After Consumption of 5 ml Reconstituted Skim Milk/kg Body Weight (1, Tuesday; 2, Thursday; 3, Saturday; 4, Monday; 5, Wednesday)

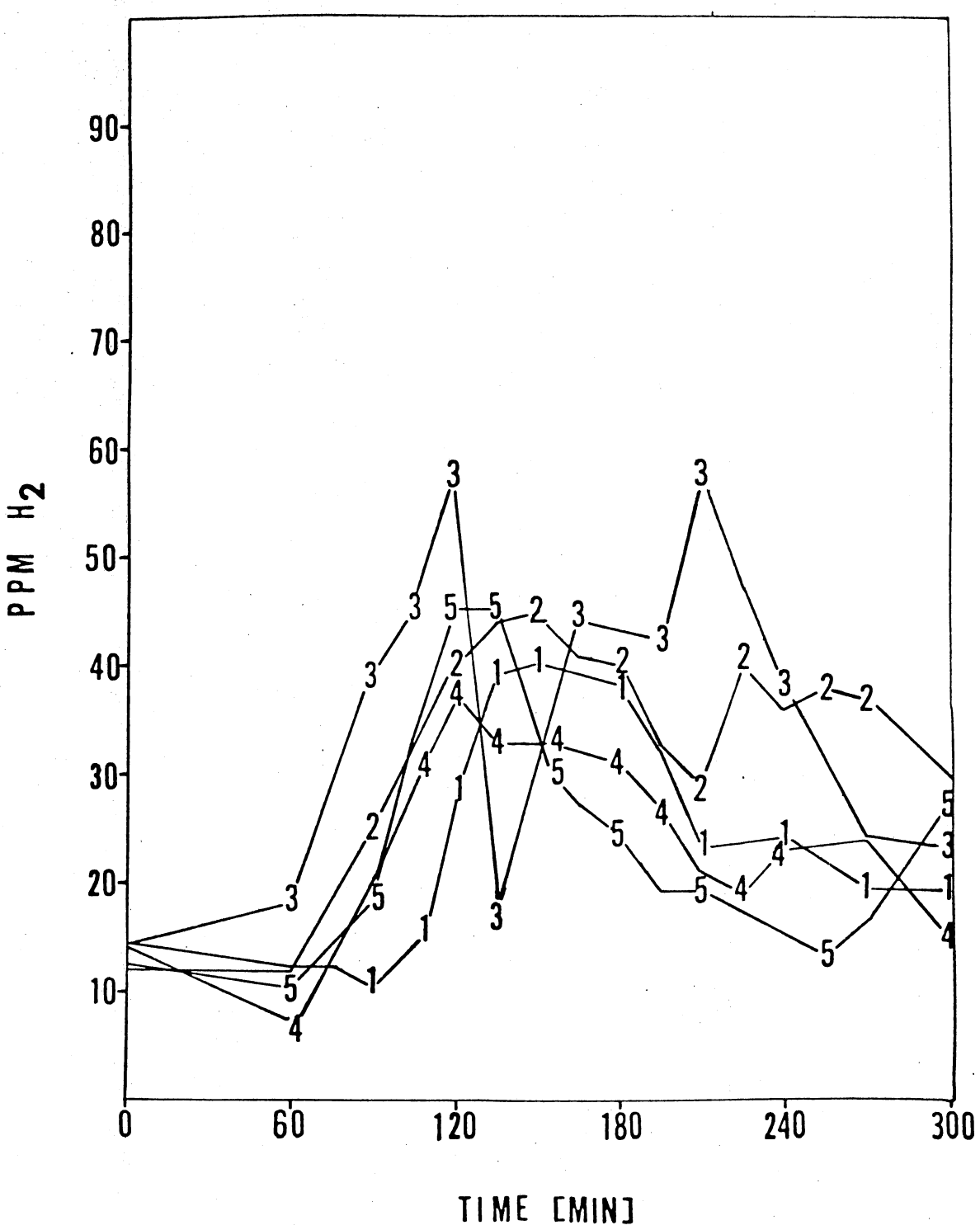

Figure 3. Subject 3--Breath H_2 Concentration Versus Time After Consumption of 5 ml Reconstituted Skim Milk/kg Body Weight (1, Tuesday; 2, Thursday; 3, Saturday; 4, Monday; 5, Wednesday)

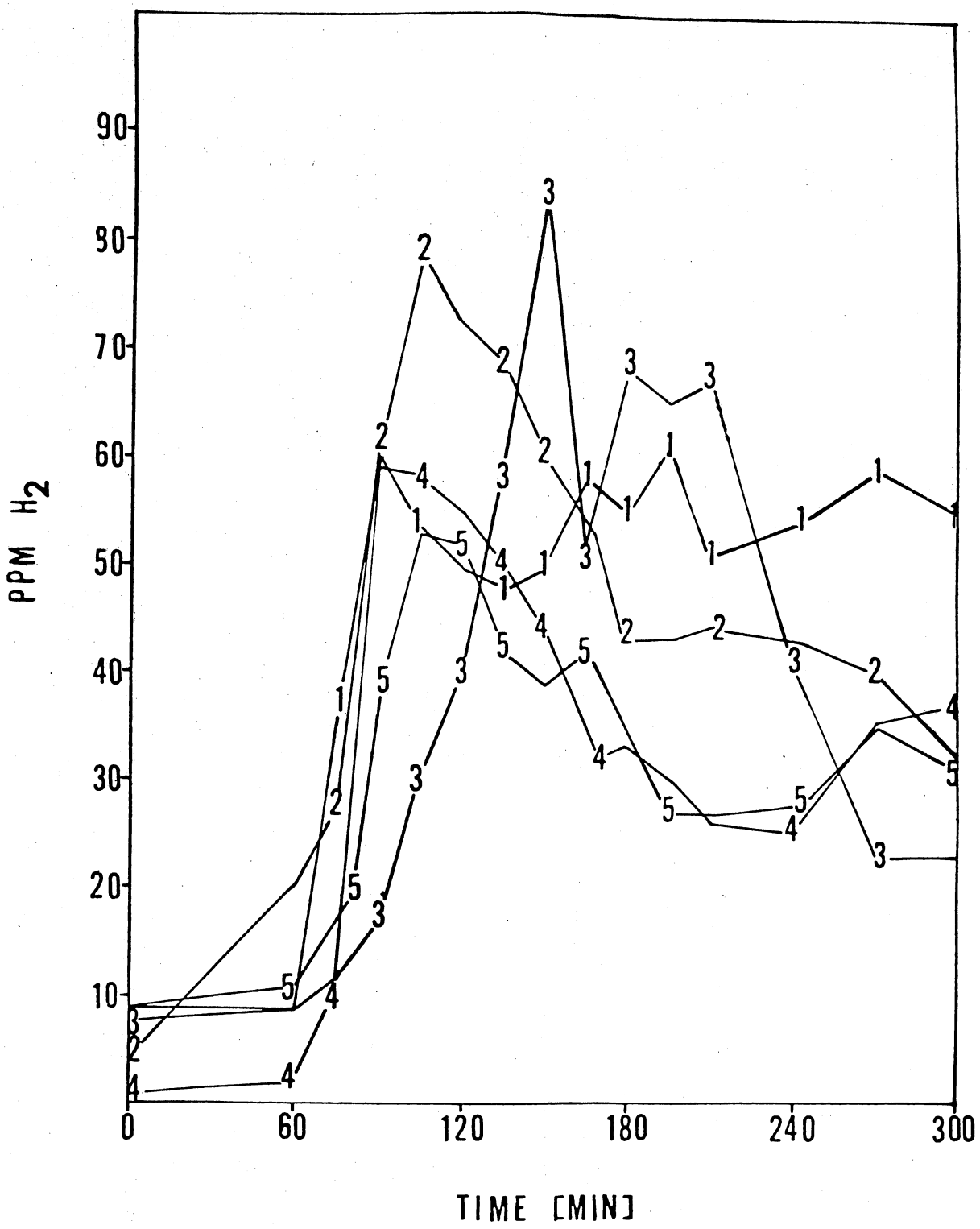

Figure 4. Subject 6--Breath H_2 Concentration Versus Time After Consumption of 5 ml Reconstituted Skim Milk/kg Body Weight (1, Tuesday; 2, Thursday; 3, Saturday; 4, Monday; 5, Wednesday)

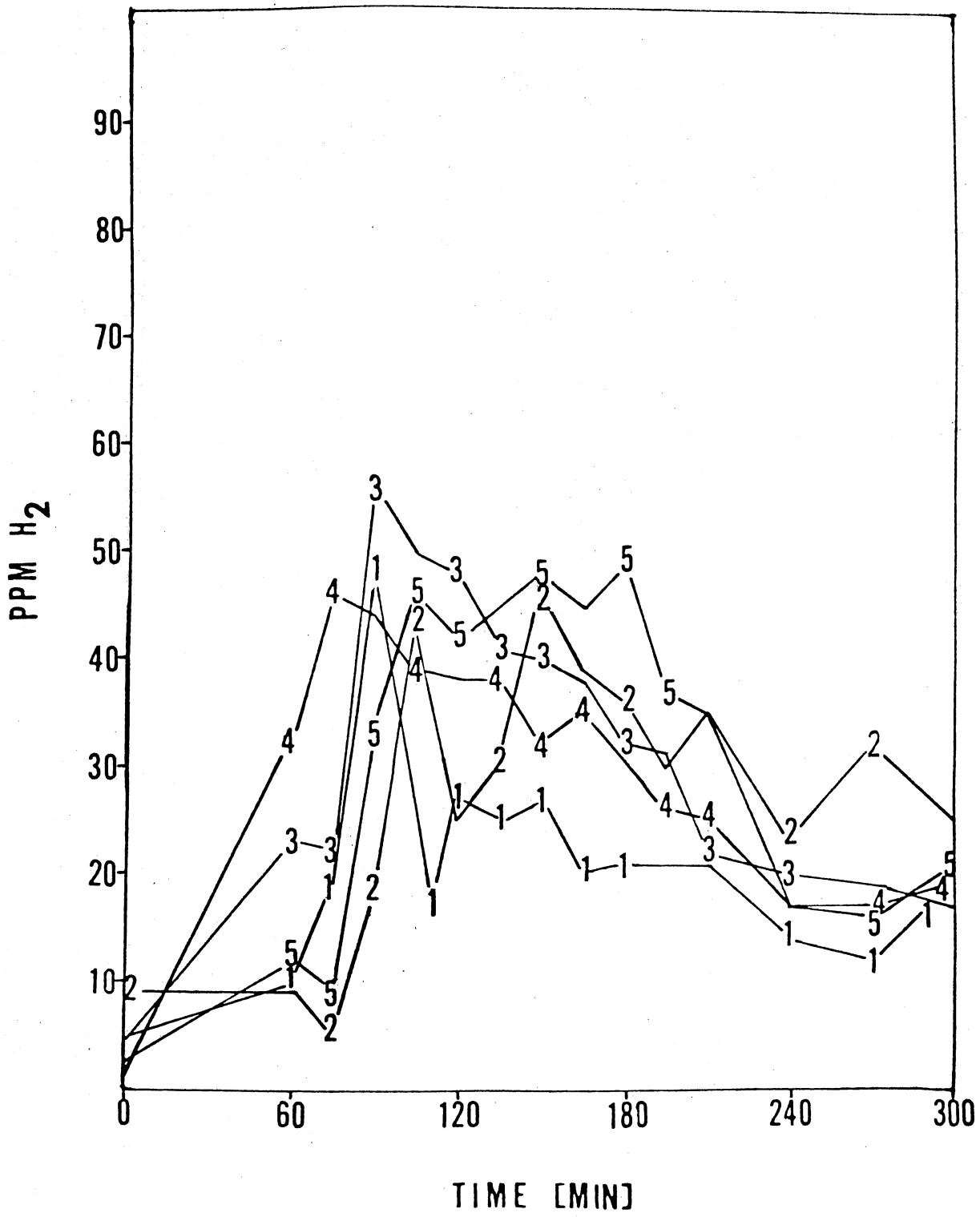

Figure 5. Subject 9--Breath H_2 Concentration Versus Time After Consumption of 5 ml Reconstituted Skim Milk/kg Body Weight (1, Tuesday; 2, Thursday; 3, Saturday; 4, Monday; 5, Wednesday)

VITA

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