EFFECT OF A NONFERMENTED MILK INOCULATED WITH *LACTOBACILLUS ACIDOPHILUS* ON LACTOSE MALABSORPTION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

Significance of the Study

Milk is one of mans best sources of a variety of nutrients, especially of calcium, but for many adults the consumption of milk causes illness. The etiology of this ailment is due to a lack or low level of the enzyme lactase (B-galatosidase). This enzyme is found in the microvilli of the small intestine. Without lactase, the disaccharide in milk, lactose is not hydrolyzed and therefore is malabsorbed. Kretchmer (1971) reported:

> Information gathered from a number of ethnic groups has indicated that many of the peoples of the world lack lactase in adult life and if given sufficient milk will show a degree of intolerance, ranging from mild discomfort to fermentative diarrhea and vomiting (p. 809).

Low lactase levels at maturity are prevalent in most people with the exception of those of Scandinavian or northern European descent (Bayless, Paige and Ferry, 1971). Lactose malabsorption is found in a high percentage of Native American Indians, Asians, Orientals, Africans, and other non-Caucasian populations (Caskey, Payne-Bose, Welsh, Gearhart, Nance, and Morrison, 1977; Bose and Welch, 1973; Bayless and Christopher, 1969; Simoons, 1969).

To find a dairy product that these people could consume would be nutritionally advantageous. Better carbohydrate absorption has been reported with lactose-hydrolyzed milk (Paige, Bayless, and Hauang, 1975;

Payne-Bose, Welch, Gearhart, and Morrison, 1977). However this milk has a sweeter flavor and may not be widely accepted. Other dairy products have been reported to be tolerated by lactose malabsorbers. Gallagher, Molleson, and Caldwell (1973) reported:

> Experiences of these investigators indicate that although lactase-deficient individuals are unable to tolerate non-fermented dairy products, such as milk and ice cream, many report they are able to tolerate the fermented dairy products of yogurt, cottage cheese, and buttermilk (p. 418).

Such subjective reports are not conclusive, however, and furthermore many people object to the sour taste of a cultured milk product. More recently another dairy product, a nonfermented milk inoculated with Lactobacillus acidophilus has been reported to be beneficial to lactose malabsorbers in various news articles and newsletters (Ben. organ. of cult. milks and yogurt, Nutrition and the M.D., 1977). However, no data has been taken to support these claimed benefits. Considering the market for Acidophilus food products, Speck (1975) reported:

> Unsolicited testimonials have indicated that the regular consumption of this product can have a number of beneficial effects for consumers, particularly the correction of various types of gastrointestinal disorders (p. 9).

Controlled studies are needed to access the tolerance of different dairy products by the lactose malabsorber. Thus far, most of the evidence of benefit have been testimonials. Therefore scientific evidence of the effect, if any, that a commercial nonfermented milk inoculated with L. acidophilus has on the lactose malabsorber needs to be demonstrated.

Purpose

The purpose of this study was to determine if a commercial non-fermented milk inoculated with L. acidophilus could be consumed by the

person with a low lactase level with less discomfort as compared to regular milk and if the lactose in the inoculated product is absorbed. Since a high percentage of adult Native American Indians are lactose malabsorbers and since Native Amercian Indians represent a large proportion of Oklahom's population, this ethnic group was selected for this study.

Objectives

The objectives of the study were:

(1) To determine if the milk carbohydrate, lactose, is better absorbed by lactose malabsorbers when they consume a milk inoculated with L. acidophilus than when they consume regular milk.

(2) To determine if a milk inoculated with L. acidophilus as compared to regular milk has any effect on symptoms experienced by lactose malabsorbers.

(3) To determine if a milk inoculated with L. acidophilus as compared to regular milk has an immediate and/or an accumulative effect on symptoms or absorption of lactose by lactose malabsorbers.

Assumptions

The assumptions in the study were:

(1) All subjects were in good physical health and relatively free from emotional stress as indicated by a questionnaire and appearance.

(2) All subjects answered the 24-hour recall of food intake accurately.

(3) All subjects gave accurate reports of all symptoms experienced during the testing period.

Limitations

The limitations of the study were:

(1) Only subjects who are at least one-half to full-blooded Native American Indian and who were determined to malabsorb lactose (as determined by breath hydrogen analysis) were used.

(2) The study was limited to eight days of milk consumption and four test days.

Hypotheses

The hypotheses postulated for the study were:

(1) There was no significant difference in breath hydrogen response when the lactose malabsorber consumed a milk inoculated with L. acidophilus as compared to regular milk.

(2) There was no significant difference in symptoms experienced when comparing the two milks consumed by the lactose malabsorber.

Definition of Terms

The following terms need to be uniformly defined and utilized for the study. These are:

(1) Lactose malabsorption--as described by Bayless et al. (1969):

> With disaccharidase deficiency the unsplit disaccharides cannot be absorbed and remain in the

> intestinal lumen. These sugars act as an osmotic load and cause an out-pouring of fluid into the small intestine provoking increased gastrointestinal motility. The resultant symptoms include abdominal cramps, bloating, and frothy diarrhea (p. 181).

(2) Milk inoculated with L. acidophilus--Speck (1975a) defines this milk:

> The product is made using low fat pasteurized milk and a concentrated culture of Lactobacillus acidophilus. The concentrate is added to the cold pasteurized milk in a surge tank, mixed well, packaged, and then maintained at 40°F. The product has the trademark name of Sweet Acidophilus which differentiates it from normal cultured Acidophilus milk; its flavor is the same as the low fat milk used for suspending the culture. Viable and bile resistant cells of Lactobacillus acidophilus are present at a level of several million/ml (p. 9).

(3) Regular milk--in this study refers to pasteurized cow's milk containing one and one-half percent butterfat.

(4) Breath hydrogen analysis--a technique to investigate lactose malabsorption. As explained in Analytical Chemistry by Gearhart, Bose, Smith, Smaller, and Morrison (1976), the method works because:

> . . . the unhydrolyzed lactose on reaching the large intestine is metabolized and endogenous bacteria and hydrogen gas is produced. The H_2 diffuses from the intestine to the blood and then to the lungs (p. 393).

This hydrogen gas is then collected in multilaminar bags and analyzed by a gas chromatograph.

CHAPTER II

REVIEW OF LITERATURE

Until the mid 1960's adult lactose malabsorption had been considered an intestinal disorder rather than a genetic norm for most people of the world. Since that time many developments in detection methods, age of onset, and frequency of occurrence have been studied. Now that the extent of the deficiency is known and easily diagnosed, new developments in consumable dairy products are being investigated. In this review lactose malabsorption, its prevalence and its detection will be discussed as well as Lactobacillus acidophilus and intestinal microflora.

Lactose Malabsorption

Three types of lactose malabsorption are recognized; a rare congenital type occurring shortly after birth, a type caused by damage to the intestinal mucosal, and a type of adult lactose malabsorption prevalent in the majority of adults. Adult lactose malabsorption far exceeds the other two types and it is this type which will be discussed. As mentioned in the introduction, lactase is a hydrolysing enzyme. It must be present to split the disaccharide lactose to glucose and galactose so that absorption through the intestinal mucosal wall can take place. Without this enzyme the sugar continues without absorption through the intestine to the colon where bacteria ferment the sugar.

The malabsorption is accompanied with fermentative diarrhea, abdominal discomfort and gas production.

It has been found that in most populations mucosal lactase decreases rapidly after weaning. Almy (1975, p. 1183) when discussing the evolution of lactase levels said ". . . it appears that in man and virtually every other mammalian species, the capacity of digesting its sugar, lactose, declines sharply beyond the period of infancy." The onset and degree of regressed lactase activity in different populations has been the subject of many studies (Cook and Kajabi, 1966; Huang and Bayless, 1968; Reddy and Pershad, 1972; Simoons, 1973; Bayless, Rothfeld, Massa, Wise, Paige, and Bedine, 1975; Caskey et al., 1977). The degree of intolerance does seem to be age related, increasing as the person matures. The magnitude of the problem is expressed by Bedine and Bayless (1973, p. 739) when they stated "Based on the available studies, it seems reasonable to assume at least 30 million persons in the United States are intolerant of a large lactose load (50 g per M^2)." This estimate, however, only considers whites of Scandinavian and Northern European extraction, Blacks, and the Jewish population in the United States. Not included are Spanish Americans, Mexican Americans, American Indians, Orientals, and those of Mediterranian extraction. Therefore, this is a very conservative estimate.

The high prevalence of lactose malabsorption raised the question of whether or not milk should be given to underdeveloped countries to aid in their nutritional deficiencies. Also extensive investigation of the black population (Bayless et al., 1975; Cautrecasas, Lockwood, and Coldwell, 1965; Bayless and Rosenweig, 1966), especially black children (Huang and Bayless, 1967; Garza and Schrimshaw, 1976; Paige, Bayless,

Mellits, and Davis, 1977), has been under investigation because of the milk offered in the school lunch program throughout the United States.

The study of lactose malabsorption in Native American Indians has been investigated by Caskey et al., 1977; Bose et al., 1973; Leichter and Lee, 1971; Newcomer and Thomas, 1977; and Newcomer, McGill, Thomas, and Hofmann, 1978. Caskey et al., in the study of Oklahoma Native American Indians, reported that the incidence of malabsorption was 20 percent in ages 3 to 5 years, 70 percent at ages 13 to 19 years, and 90 percent at ages 45 to 64 years. In the study (p. 113) they reported "Approximately 82 percent (82.5 %) of subjects who were 13 years and older were lactose malabsorbers. Adolescence appears to be the period in which malabsorption of lactose becomes evident in Native North Americans."

Detection Methods

Much of the determination of lactose malabsorption has been by the lactose tolerance test (LTT) (Huang et al., 1968; Cood et al., 1966; Bayless et al., 1975; Huang et al., 1967; Bayless et al., 1966, 1975; Cuatrecasas et al., 1965). This is an oral carbohydrate tolerance test in which the rise in blood glucose is quantitated as the sugar is absorbed. The test is given after the subject fasts for 8 to 12 hours so that the blood glucose rise would result from the hydrolysis of lactose and absorption of the hydrolysis products, glucose and galactose. The amount of lactose given ranges from 1 to 2 grams per kilogram of body weight or 50 grams per square meter depending on the age of the subject being studied. Capillary blood is analyzed for

glucose and samples are taken generally at 30-minute intervals for two hours after the consumption of the lactose dose. If the rise in blood glucose is less than 26 milligrams per 100 milliliters (Welch, 1966) and if symptoms occur such as abdominal discomfort, flatulence, or diarrhea then the subject is considered to be a lactose malabsorber.

Recent criticism of the lactose tolerance test is that the large sugar dose causes severe symptoms and is unrealistic of milk lactose consumption (Stephenson and Lantham, 1974). Bedine et al. (1973) considers the lactose given for the lactose tolerance test to be a large unphysiological amount and objects to equating the response of symptoms from this large dose to lactose malabsorption. Garza et al. (1976) likewise felt the subject might be able to tolerate lower levels of lactose which would be present in a small amount of milk. They (1976, p. 195) pointed out "the prevalence rate of intolerance to graded amounts of milk, rather than the LTT, and the severity of this intolerance would appear to be more useful in evaluating the significance of these problems."

Lactase deficiency can also be determined by peroral biopsy of the small intestinal mucosa. In this analysis the subject must swallow a biopsy capsule which is attached to a polyethylene tube. The capsule contains a rotating knife which is spring-activated and triggered by suction. The suction draws the mucosa into the capsule and the capsule is held by the polyethylene tube, which also serves to transmit suction and to retrieve the capsule. The capsule's position is followed by fluoroscope until it reaches the jejunum, the site of lactase activity, where sampling takes place. The tissue is then analyzed for lactase activity (Crosby, 1957). Although this procedure is very accurate the

analysis may cause discomfort to the subject and the technique calls for well-trained personnel.

Other methods have been compared such as a radiological test, modified lactose tolerance test, and isotope test (Newcomer, McGill, Thomas, and Hofmann, 1975). Few of these have been used extensively due to expense, complex procedures, or time involved.

The analysis of breath hydrogen by gas chromotography is a very reliable and precise method to determine lactose malabsorption. Also, it is easy to administer and takes a short time to analyze (Levitt and Donaldson, 1970; Bond and Levitt, 1972; Galloway, Murphy, and Bauer, 1973; Gearhart et al., 1976). A highly desirable feature is that the method is agreeable and without risk. Newcomer et al. (1975), when comparing different methods for the detection of lactose deficiency, described the findings as follows:

> The major finding of our study was that measurements of breath hydrogen accurately identifies subjects with normal and those with deficient lactase activity; and the time of sampling was not critical as with the other test. This test is not influenced by gastric emptying or by metabotic factors that offset blood glucose levels. Furthermore, since the breath hydrogen test does not require alchohol isotopes or blood samples, we find it to be the most suitable method for screening large groups for lactase deficiency (p. 1234).

The test requires such a small dose of lactose that severe symptoms are usually avoided and the test is easily administered to children. The breath hydrogen test is a direct test of carbohydrate malabsorption. If lactose is not absorbed it will reach the colon where hydrogen is produced by fermenting bacteria. As Levitt (1972) explained:

> The principal gaseous fermentative products in the intestine are carbon dioxide and hydrogen; both are absorbed into the blood stream and excreted by the lungs. Since intestinal fermentation is the only source of hydrogen in

> the human body, measurements of breath hydrogen gives an approximation of its production in the gut by fermentative activity (p. 487).

If the breath hydrogen rises above 20 parts per million (ppm) after consuming lactose the person is considered to be a malabsorber (Caskey et al., 1977).

Lactobacillus acidophilus and Intestinal Microflora

The interest in bacteria located in the intestinal tract as a therapeutic factor in life was first postulated by Metchnikoff (1908). He attributed the long life of the Balkan peasants to their widespread consumption of milk soured with Lactobacillus bulgaricus. He believed that illness was caused by autotoxins produced by harmful bacteria present in the large intestine of man. He thought that milk inoculated with L. bulgaricus could establish the organism in the intestine and replace objectionable and potentially harmful bacteria.

Although physicians had known for sometime that a sharp alteration of the diet showed many clinical indications of a rapid change in the physiological state of the digestive tract, Herter and Kendall (1909) were the first to attempt to demonstrate a relationship between diet and the bacteria of the intestinal tract. They used two widely different mammalian species, the cat and the monkey, to investigate whether the same alteration in diet would yield the same bacterial change in carnivorous and omnivorous animals. They made an abrupt change in the test animals' diet from one predominantly of protein (meat and eggs) to a diet of milk and sugar. They (1909, p. 216) found that "The chief characteristic of the bacterial change is the gradual but rapid

substitution of an acidophilic non-proteolyzing type of flora for a strongly proteolyzing type." Hull and Rettger (1917) also found that when the diet of white rats consisted of grain feed, milk and lactose that the rats' intestinal flora changed to predominately aciduric bacteria such as L. acidophilus and L. bifidus. Rats on a meat diet did not have predominately aciduric flora unless lactose was added to the diet.

Further work on the theory was developed by Rettgar and Cheplin (1921), but they found that it was not L. bulgaricus but L. acidophilus which establishes itself in the gut. The controversy probably arose due to the lack of tests which would identify those two organisms. More recently, those organisms have been more easily distinguished by the ability to ferment specific sugars (Wheater, 1955; Hawley, Shepherd, and Wheater, 1959).

Work by Kopeloff (1926) reinforced earlier work done by Rettgar et al., which proposed that L. acidophilus could be established and that it is therapeutic, transforming proteolytic flora to aciduric flora in the large intestine. This was noted to benefit those suffering from constipation and diarrhea. Kopeloff (1926) stated:

> From a practical standpoint it has been shown that constipation, diarrhea, and other intestinal disorders may be satisfactorily treated by L. acidophilus. Such treatment is simple and works no hardship on the patient (p. 183).

Kulp (1931) pointed out that in order for milk inoculated with L. acidophilus to be of therapeutic value it must contain live organisms in a large quantity. He suggested that the milk contain at least 100 million viable organisms per cubic centimeter when it reaches the consumer. He (1931, p. 873) stated, "Exponents of acidophilus therapy agree that, regardless of the method of administration, satisfactory

therapeutic effects are dependent upon the use of cultures which contain large numbers of viable organisms." At this time cultures containing large numbers of viable organisms were hard to maintain due to the high acidity of the fermented product.

Maintaining high populations and eliminating objectionable acid taste and flavors motivated Myers (1931) to work on producing a sweet milk containing large numbers of L. acidophilus. He (1931) pointed out:

> To some who would like to take advantage of acidophilus therapy, the ordinary cultured acidophilus milk is distasteful. In view of this and also because the lactic acid in fermented milk has been shown to have little or no value in bringing about a transformation of the intestinal flora, it was thought that a milk product which has the taste of ordinary sweet milk and yet is a means for carrying large numbers of L. acidophilus into the intestinal tract would meet a real need. Such a product has been developed and for lack of a better name has been termed unfermented acidophilus milk (p. 867).

Interest in normal intestinal flora as a protective mechanism in preventing establishment of enteric infections has increased in recent years. Hentges (1970) suggested that there is an interaction between normal flora and enteric pathogens. He (1970, p. 1451) stated, "All this information suggested that the normal intestinal flora represents a major factor that effectively interferes with the establishment of pathogenic bacteria in the intestine." Gillespie, Dimmick, Heuer, and McAteer, 1956) reported of the interest in L. acidophilus to replace objectionable and potentially harmful bacteria in the human intestine. They say that the health and well being of man and animals are influenced by the balance of normal intestinal flora. Speck (1975b) reported that this balance might be aided by the ingestion of L. acidophilus. He (p. 341) stated, "Imbalances in the flora, particularly the presence

of abnormally large numbers of coliform bacteria, can be adjusted desirably by the ingestion of L. acidophilus."

It has been found by Freter (1955, 1956) that after elimination of the enteric flora by administering antibiotics that mice and guinea pigs could be infected with Salmonella, Shigella, and Vibrio cholerae. The wide use of antibiotics has increased the importance of the balance in intestinal microorganisms. Finland and Weinstein (1953) submit that when antibiotics are used to kill susceptible microorganisms in man that resistant and frequently pathogenic organisms may grow. Severe diarrhea may occur after the use of aureomycin, terramycin, and sometimes with chloramphenicol. Finland et al. (1953), when describing the effect of these drugs, reported:

> Some of these diarrheas have been associated with the presence of coagulase-positive and hemolytic strains of Staphylococcus aureus, which have been found in large numbers or even in pure cultures in the stools; these probably represent cases of accute staphylococcol enteritis resulting from the change in the bacterial flora of the bowel produced by the administration of one of these agents (p. 222).

With the expanding use of oral antibiotics the anti-microbial effects of L. acidophilus against enteric pathogens has increased its importance on human health. Kopeloff (1923) first thought that the lactic acid produced the anti-microbial effect. Vincent, Veomett, and Riley (1959) credit L. acidophilus anti-microbial action to the production of an antibiotic, lactocidin. They stated:

> Strains of Lactobacillus acidophilus obtained from mice, rats, rabbits, hamsters, and man were found to produce an anti-microbial agent in cultures grown in liver veal agar. The substance responsible has been called lactocidin (p. 483).

This renewed interest in recent years in L. acidophilus has promoted the food industry to consider new products including the

microorganism. Acidophilus yogurt has been considered, and in the spring of 1975 a new product, Sweet Acidophilus, was introduced (Speck, 1975a). Speck (1975a) the developer of Sweet Acidophilus, believes that milk is the best product for the introduction of L. acidophilus. He pointed out:

> While a number of dietary carriers can be used for Lactobacillus acidophilus, milk still has many of the preferred characteristics for such purposes. Furthermore, the nutritional attributes of milk can be obtained along with the lactobacilli (p. 9).

Recently there has been some speculation that these products might be helpful to lactose intolerant people (Inter. of diet, gut micro., nutr., and health, Dairy Council Digest, 1976; Ben. organ. of cult. milks and yogurt, Nutrition and the M.D., 1977).

CHAPTER III

METHODS AND PROCEDURE

Introduction

The initial design of the study was to consider the effect of a milk inoculated with a culture of Lactobacillus acidophilus (LAM)[1] on lactose malabsorption by breath hydrogen analysis. Originally, one group of five lactose malabsorbers over an eight day period received regular 1½% fat milk (RM)[2]. The second group of five lactose malabsorbers received a nonfermented 1½% fat milk inoculated with L. acidophilus over eight days. The group receiving regular milk was to serve as a control group for the ones receiving the inoculated milk. However, there was wide variance of breath hydrogen response from one group to the other group. Although all subjects malabsorbed lactose, all subjects of one group had much lower responses to regular milk than all subjects in the other group. Therefore, it was decided to set aside the group receiving the RM test meal and use those five receiving LAM along with a sixth person who was originally in the RM group. This new structure then set the experiment in such a manner that each subject served as his own control. Data on the two groups was taken.

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[1]Farm Fresh Dairy, Ponca City, Oklahoma.

[2]Sweet Acidophilus, Page Dairy, Tulsa, Oklahoma.

Subjects

The subjects were six lactose malabsorbers, four males and two females, ranging in age from 18 to 63 years. Five of the subjects were full-blooded Native American Indians and one was one-half Native American Indian. They represented two tribes, Pawnee and Navajo. None of the subjects were related, none were taking drugs other than birth control pills, none had any recent gastrointestinal disturbances, and none had previously consumed a product inoculated with L. acidophilus. The subjects were questioned about health, drug use, and consumption of milk or dairy products by questionnaire (see Appendix A, p. 35). A statement of informed consent to participate in the study was signed by each subject (see Appendix A, p. 36). A description of subjects are given in Table I.

TABLE I

DESCRIPTION OF SUBJECTS

Subject	Native American Heritage	Sex	Age	Ht (cm)	Wt (Kg)
1	full-blooded Pawnee	female	44	155	75
2	full-blooded Pawnee	male	40	193	96
3	½ Pawnee	female	63	170	62
4	full-blooded Navajo	male	18	180	60
5	full-blooded Navajo	male	18	173	69
6	full-blooded Navajo	male	18	178	61

Test Meals

The test meals consisted of either regular 1½% fat milk (RM) or regular nonfermented 1½% fat milk that had been inoculated with a culture of L. acidophilus (LAM). The test dose for either type of milk was five milliliters per kilogram of body weight. The RM was purchased locally, and the LAM, which was not available locally, was purchased from a dairy within the state and transported in an ice chest 1 to 2 days before the study of each subject. Samples of the milk were taken from each carton and analyzed for lactose (Taylor, 1970) and fat (Am. Pub. Health Assoc. Inc., 1965). Additionally, plate counts for lactobacilli were done on the LAM (Gilliland, 1975) before the milk was used and after the last day of use. Nutritional information given on the milk carton by the processing dairy is given (see Appendix A, p. 37).

Experimental Design

The test period for each subject was eight consecutive days. After an overnight 11½ hour fast, breath hydrogen was determined by a method of gas chromatography (Payne-Bose, Tsegaye, Morrison, and Waller, 1977). The subject was instructed to eat or drink nothing except water after 9:00 p.m. the night before breath hydrogen was to be determined and testing began at approximately 8:30 a.m. the next morning. On day one, a basal breath hydrogen sample was taken before the consumption of the RM test meal and breath hydrogen excretion was determined at 15-minute intervals for 180 minutes. Information of milliliters of milk consumed and the time of sampling were recorded on a data sheet (see Appendix A, p. 38). After an overnight 11½ hour fast, the testing

procedure for the second day was identical to the first day, except that subjects consumed the LAM test meal. Each morning on the third through sixth test days, subjects consumed the LAM test meals but were not required to fast and no breath hydrogen analyses were carried out during this period. Subjects consumed the milk in the presence of the technician, however, on all eight days. On the seventh test day, after an overnight 11½ hour fast, all subjects consumed LAM and breath hydrogen analysis was again determined as described. On day eight, after the 11½ hour overnight fast the subject received the RM test meal and breath hydrogen analysis was repeated. Test meal sequence and breath hydrogen test sequence are given in Table II.

TABLE II

SEQUENCE OF TEST MEALS AND BREATH HYDROGEN (H_2) TEST

	Day							
	1	2	3	4	5	6	7	8
Test Meal	RM	LAM	LAM	LAM	LAM	LAM	LAM	RM
H_2 Test	X	X					X	X

Other than overnight fasting before the breath hydrogen test, subjects' dietary intake was not restricted beyond instruction to avoid common gas-producing foods such as beans. Daily records of food intake were kept for the day before and throughout the eight day test period.

Also any symptoms such as diarrhea, stomach cramps, stomach growling, or gas were recorded (see Appendix A, p. 39).

Statistical Analysis

The statistical design of the study was a randomized block in which the subject was considered a block and the four treatments were considered days. The treatment effects (days) were broken into two contrast: (1) RM response versus LAM response and (2) between days within milk types.

CHAPTER IV

RESULTS AND DISCUSSION

The mean value of the breath hydrogen response of each day of the six subjects on the LAM test meal and of each of the five subjects in the initial RM test meal are given in Table III. As can be seen in Table III, there was a much higher overall breath hydrogen response by the subjects receiving the RM test meal as compared to the group receiving the LAM test meal. Also, within the groups there is variation of breath hydrogen response, as explained previously this is why each subject should be used as his (her) own control.

Graphic representation of the mean values of the breath hydrogen response for each subject on the LAM test meal is given in Figure 1 through 6 with the overall mean of the six subjects on the LAM test meal given in Figure 7. As shown by the graphs subjects one, three, four, and five had breath hydrogen responses on the LAM test meal relatively the same on all four test days. Subject two showed a drop in breath hydrogen response on days two and eight. While subject six showed a drop in breath hydrogen response after day one and never again rose above basal level. The overall breath hydrogen response for the six subjects showed little change as seen in Figure 7. The three consecutive values used for the six subjects on the LAM test meal are given in Table IV.

TABLE III

MEAN BREATH HYDROGEN RESPONSES OF THE THREE CONSECUTIVE VALUES THAT INCLUDED THE HIGHEST PEAK

		LAM Test Meal						RM Test Meal			
Subject	Milk Day	RM 1	LAM 2	LAM 7	RM 8	Subject	Milk Day	RM 1	RM 2	RM 7	RM 8
*1		74	110	106	124	*1		54	65	86	102
2		41	8	39	13	2		87	79	136	124
3		66	135	109	86	3		136	155	151	149
4		83	86	68	77	4		66	159	85	115
5		45	47	28	52	5		50	57	44	21
6		41	18	16	7						
	Mean	58	67	61	60		Mean	79	103	100	102

*Subject received RM test meal and LAM test meal. Test meals were administered three months apart

TABLE IV

THREE CONSECUTIVE BREATH HYDROGEN VALUES USED FOR THE SIX SUBJECTS ON THE LAM TEST MEAL

Subject	Day 1 (ppm)	Day 2 (ppm)	Day 7 (ppm)	Day 8 (ppm)
	85	105	106	119
1	69	104	126	147
	70	122	87	106
	55	14	34	18
2	39	5	40	10
	30	5	43	12
	90	145	99	98
3	61	136	107	73
	48	125	121	88
	91	115	77	99
4	89	67	75	63
	68	75	53	70
	60	55	32	59
5	39	46	28	53
	37	40	23	45
	53	27	27	9
6	37	12	14	6
	34	16	6	5

The statistical analyses of the breath hydrogen responses of subjects on the LAM test meal are given in Table V. The difference in response due to the two types of milk showed no significant difference. No significant difference in response was found between test days. There was, however, considerable variation between the responses of individuals. Individual breath hydrogen readings of all subjects on both meal types are given in Figures 8 through 51. Based on the average of the three highest consecutive values the coefficient of variation (C.V.) was 31.5% which is in agreement with previous work done with lactose malabsorbers by the breath hydrogen method.

TABLE V

ANALYSIS OF VARIANCE FOR BREATH HYDROGEN RESPONSES OF THE SIX SUBJECTS ON THE LAM TEST MEAL

Source	df	Mean Square	F
Total	71		
Subject	5	16387.0	14.28
Day	3		
(LAM) vs (RM)	1	435.1	<1.0
Days in Milk Type	2	195.6	<1.0
Subject * Day (exp. error)	15	1147.1	
Time (day) (sampling error)	48	167.0	

C.V. = 54.8% (based on individual observation)
C.V. = 31.6% (based on the average of 3 highest readings)

Plate counts showed viable organisms were present in the nonfermented inoculated milk. The initial plate counts on the cartons used ranged from 2.1×10^6 - 4.1×10^6 Lactobacillus per milliliter of milk and final counts showed viable organisms still present.

The lactose analysis showed that the amount of lactose was approximately the same for the regular milk and the nonfermented inoculated milk. The regular 1½% fat milk used contained from 4.9% to 5.5% lactose with a mean lactose concentration of 5.4%. The nonfermented 1½% milk inoculated with L. acidophilus contained lactose ranging from 5.2% to 5.6% with a mean of 5.3% lactose. The fat content was very close in both milks ranging from 1.1% to 1.9%. The mean fat content for the regular 1½% fat milk was 1.4% and the mean fat content for the nonfermented inoculated milk was 1.5%.[1]

Some of the subjects experienced typical symptoms of lactose malabsorption, such as diarrhea, stomach growling, and/or gas. Subject symptoms recorded on the LAM test meal are listed in Table VI.

This study demonstrated that in six malabsorbing subjects there was no significant difference between hydrogen response whether the subject consumed regular 1½% fat milk or a nonfermented 1½% fat milk inoculated with L. acidophilus based on the groups' mean breath hydrogen response.

Viable organisms were present and lactose and fat content varied little between milks. Since lactose variation was small, it appears that little or none of the lactose could have been hydrolyzed by the L. acidophilus in the LAM milk and the lactose in both milks was

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[1]Lactose and fat determinations and plate counts were done under supervision of Dr. Stanley Gilliland in the Dairy Science laboratories.

malabsorbed. Also, no effect on lactose absorption was noticed after the subjects consumed the milk over a period of time.

TABLE VI

SYMPTOMS RECORDED DURING LAM TEST MEAL

Subject	Milk Day	RM 1	LAM 2	LAM 3	LAM 4	LAM 5	LAM 6	LAM 7	RM 8
1			bc	abc	bc	bc	bc	bc	bc
2			b						
3			ac	ac	c	ac	c	ac	ac
4					a	b			b
5									
6			b						

a diarrhea
b growling stomach
c gas

There was little difference in symptoms of diarrhea, stomach growling, or gas. If the subject experienced one or more of the symptoms he (she) would experience them throughout the test meal. Some subjects experienced little or no symptoms throughout the test meal, but there was no change noticed in either case.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Ten lactose malabsorbing subjects were picked at random to participate in this study. Initially, one group of five was to be used as a control group and was to consume a regular 1½% fat milk for eight days. This group was to be compared to another group of five lactose malabsorbers having a test meal with a nonfermented 1½% fat milk inoculated with L. acidophilus. Due to the wide variation of individual responses from one group to the other the study was restructured so that each subject served as his (her) own control. Six lactose malabsorbers each serving as their own control were used to determine if there was a difference in lactose absorption when they consumed a nonfermented 1½% fat milk inoculated with L. acidophilus as compared to regular 1½% fat milk.

The subjects were on an eight day study and breath hydrogen analyses were done on days one, two, seven, and eight. Testing was done on these days to see if there was an immediate and/or a long term effect. The subjects consumed regular 1½% fat milk on days one and eight and a nonfermented 1½% fat milk inoculated with L. acidophilus on days two through seven.

The data was analyzed as a randomized block design considering the subject as a block and the four treatments as days. Symptoms during the eight days were recorded daily as well as food consumption.

No significant difference was found in breath hydrogen response when comparing the milks. Differences in symptoms were not noticed.

As the subject had to undergo an 11½ hour fast before each breath hydrogen analysis, there was some concern as to whether the double fasting, one day after the other, might have an effect on the intestinal tract. It is, therefore, suggested that this double fast be eliminated in future studies.

Also, the subject should always be used as his (her) own control because of large individual differences in breath hydrogen response. Increasing the number of L. acidophilus per milliliter of milk consumed by the subject and also controlling the diet might be investigated. Since this was a commercial product, the age of the culture was not known but viable organisms were present. There is no data yet on the metabolitic rate of lactose by the organism as compared to age.

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APPENDICES

APPENDIX A

FORMS USED FOR DATA COLLECTION

AND MILK INFORMATION

QUESTIONNAIRE

BREATH H_2 TEST

NAME:_____

ID #:_____

DATE:_____

PHONE:_____

Have you ever consumed Sweet Acidophilus or Nutrish Milk? yes__ no__

Have you had anything to eat or drink in the last 11½ hrs? yes__ no__

Are you a diabetic? yes__ no__

Have you taken any of these drugs in the last ten days?

Antibiotics: yes__ no__

Sulfa drugs: yes__ no__

Other medication: yes__ no__

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

Diarrhea: yes__ no__

Constipation yes__ no__

Other: yes__ no__

Do you like milk or dairy products: yes__ no__

Comments:_____

_____

_____

STATEMENT OF INFORMED CONSENT

Procedure:

The subject will arrive in the morning after having a good night of sleep and no food or drink (except water), since 9:00 p.m. the previous evening. Testing will start soon after the arrival in the laboratory. The subject should become familiar with the surroundings and feel relaxed and comfortable in the lab and lounge area. Please feel free to ask the technician any questions that may concern you.

The study will start by collecting a breath sample. This is done by blowing your breath through a plastic tube which is connected to the breath bag. Then you will drink a milk or dairy product. Breath samples will be taken every 15 minutes for the next three hours.

The subject will need to remain in the lounge area during the entire testing period unless other arrangements have been made previously.

Discomforts:

There should be few if any discomforts experienced. If the subject is not absorbing the milk sugar in the intestine he (she) may experience mild stomach cramps, intestinal gas, diarrhea, and a growling stomach. These discomforts, if occurring, should last only a short time, 15 minutes to 2 hours. The subject will be given a small amount of the lactose sugar, approximately what is found in 1½ to 2 cups of milk; therefore, one should experience few or possibly no symptoms.

I have been given an opportunity to ask and receive answers to any questions concerning procedures. 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. I hereby give my informed consent to participate in the research study.

Signature:_____

Date:_____

MILK INFORMATION ON CARTON OF REGULAR MILK

Lowfat milk
Farm Fresh Dairy, Inc. Ponca City, OK 74601
1½% Milkfat
Grade A Pasteurized Homogenized
Vitamin A Plamitate And Vitamin D added
Nutrition Information per serving

Serving Size	One cup
Servings per container	8
Calories	110
Protein	8 grams
Carbohydrates	11 grams
Fat	4 grams

Percentage of U.S. Recommended Daily Allowances (U.S. RDA)

Protein	20
Vitamin A	10
Vitamin C	4
Thiamine	6
Riboflavin	25
Niacin	*
Calcium	30
Iron	*
Vitamin D	25

* Contains less that 2% of the U.S. RDA of these Nutrients

MILK INFORMATION ON CARTON OF SWEET ACIDOPHILUS™ MILK

Lowfat Milk
Page Dairy, Tulsa, OK
1½% Milkfat
Grade A-Homogenized-Pasteurized
Vitamin A and D added
Lowfat milk with Vitamin D_3, Vitamin A Palmitate and viable Lactobacillus Acidophilus added
Nutrition Information per serving

Serving size	One cup
Servings per container	8
Calories	110
Protein	8 grams
Carbohydrate	11 grams
Fat	4 grams

Percentage of U.S. Recommended Daily Allowances (U.S. RDA)

Protein	20	Vitamin D	25
Vitamin A	10	Vitamin B_6	4
Vitamin C	4	Vitamin B_{12}	15
Thiamine	6	Phosphorus	20
Riboflavin	25	Magnesium	8
Niacin	**	Zinc	4
Calcium	30	Pantothenic Acid	6

**Contains less than 2% of the U.S. RDA of these Nutrients

BREATH H_2 TEST

DATA SHEET

NAME:_____ PHONE #:_____

DATE:_____ AGE:_____

ID #:_____ RACE:_____

AMOUNT:_____ml FOOD:_____day_____ STUDY:_____

DOSAGE:_____ml/Kg of body weight_____ SEX:_____

WEIGHT:_____Kg HEIGHT:_____cm BAG COLOR:_____

BAG#	O'clock	MINUTES into test	ppm H_2	SYMPTOMS	COMMENTS
		0			
		15			
		30			
		45			
		60			
		75			
		90			
		105			
		120			
		135			
		150			
		165			
		180			

Symptoms: No:____ Yes:____

QUESTIONNAIRE
24 HOUR FOOD RECALL

NAME_____

DATE_____

What did you eat for Breakfast?

Drink Approximate Serving Size

Food

What did you have to eat for a morning snack?

Drink Approximate Serving Size

Food

What did you have to eat for lunch?

Drink Approximate Serving Size

Food

What did you have to eat for an afternoon snack?

Drink Approximate Serving Size

QUESTIONNAIRE
CONTINUED

Food Approximate Serving Size

What did you have to eat for dinner:

Drink Approximate Serving Size

Food

What did you have for a bedtime snack?

Drink Approximate Serving Size

Food

---

24 HOUR RECALL OF SYMPTOMS

Did you experience any of the following?

Diarrhea: _____

Stomach Cramps: _____

Growling Stomach: _____

Gas: _____

APPENDIX B

FIGURES

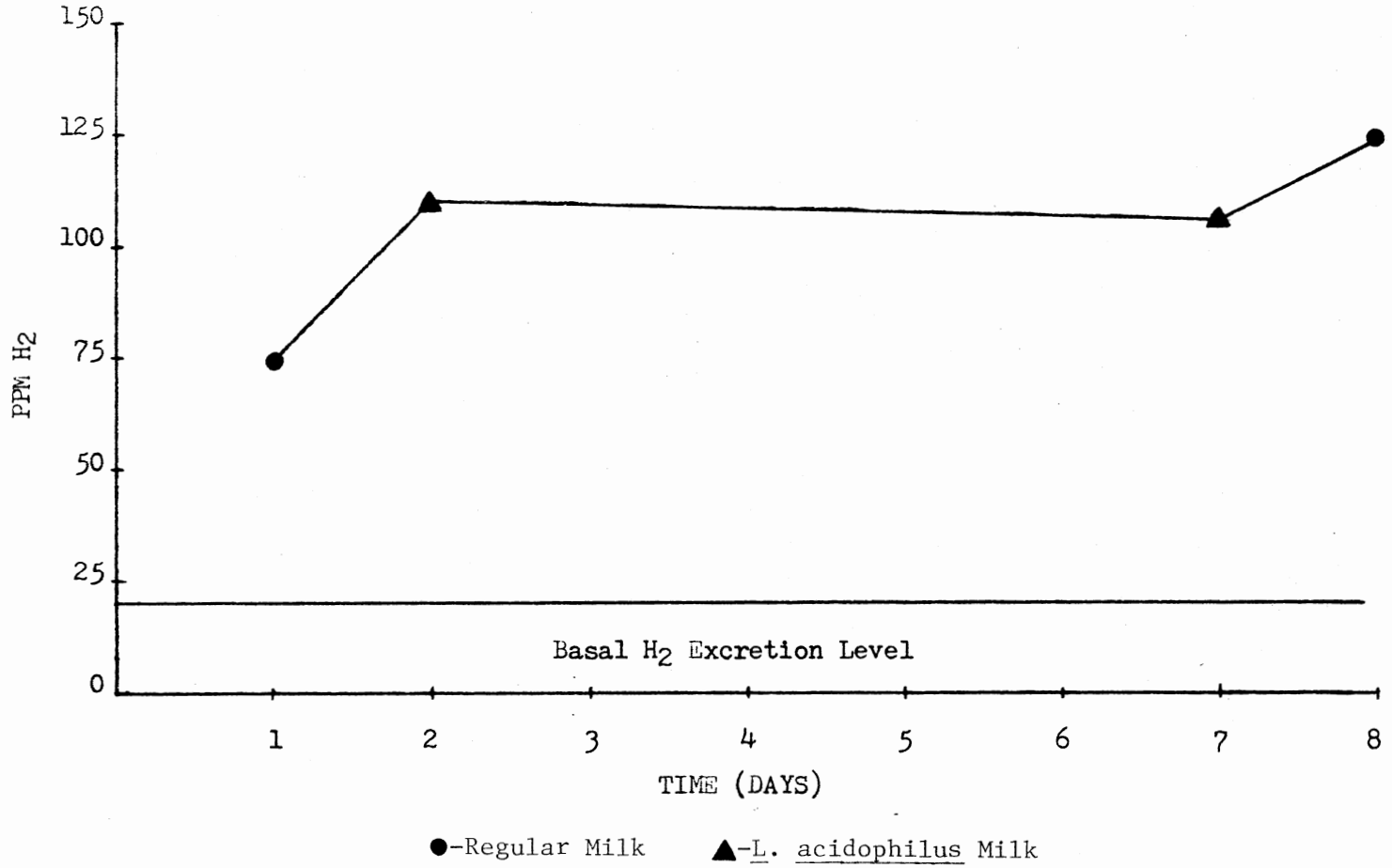

Figure 1. Subject One--Breath Hydrogen Response on the LAM Test Meal

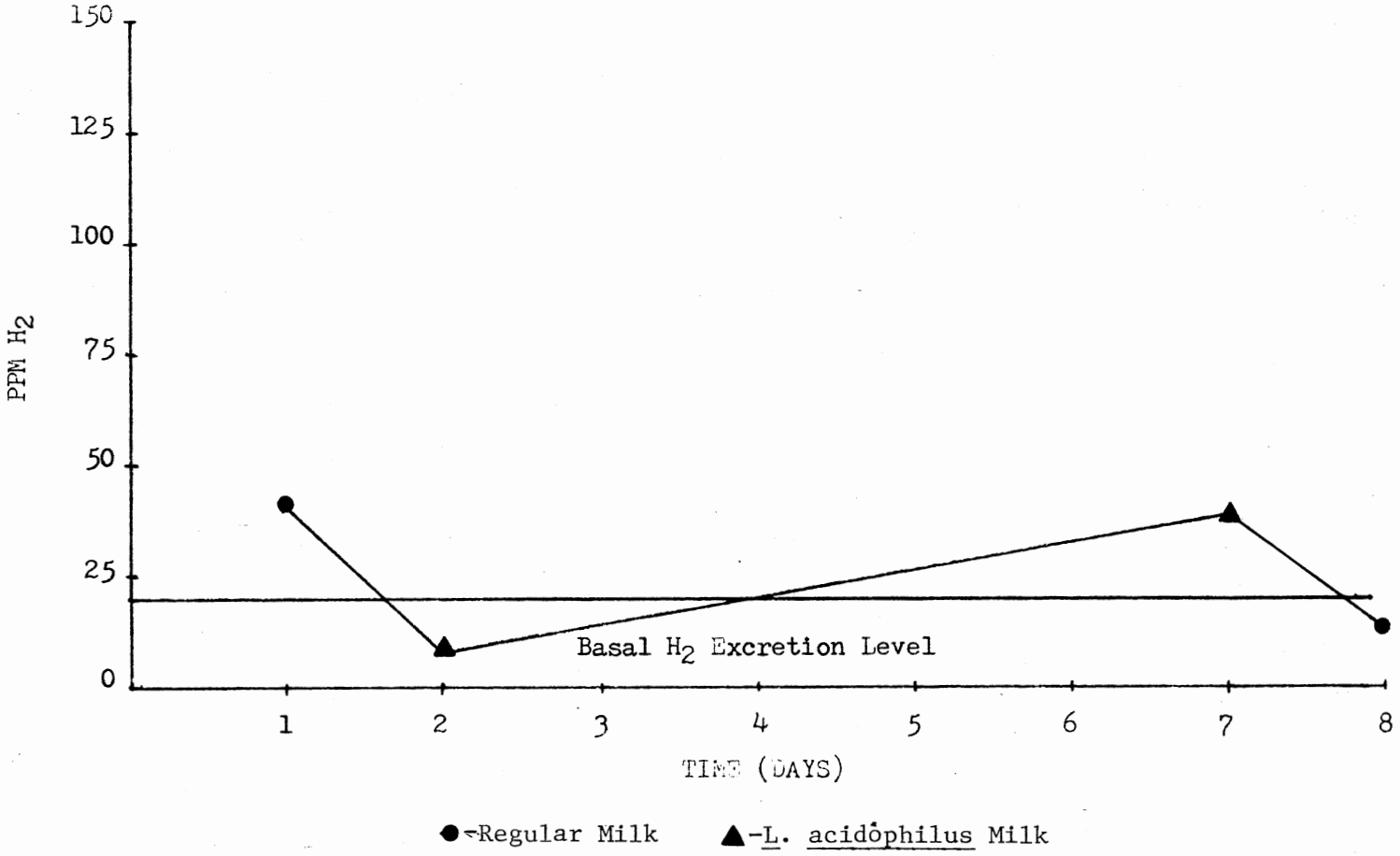

Figure 2. Subject Two--Breath Hydrogen Response on the LAM Test Meal

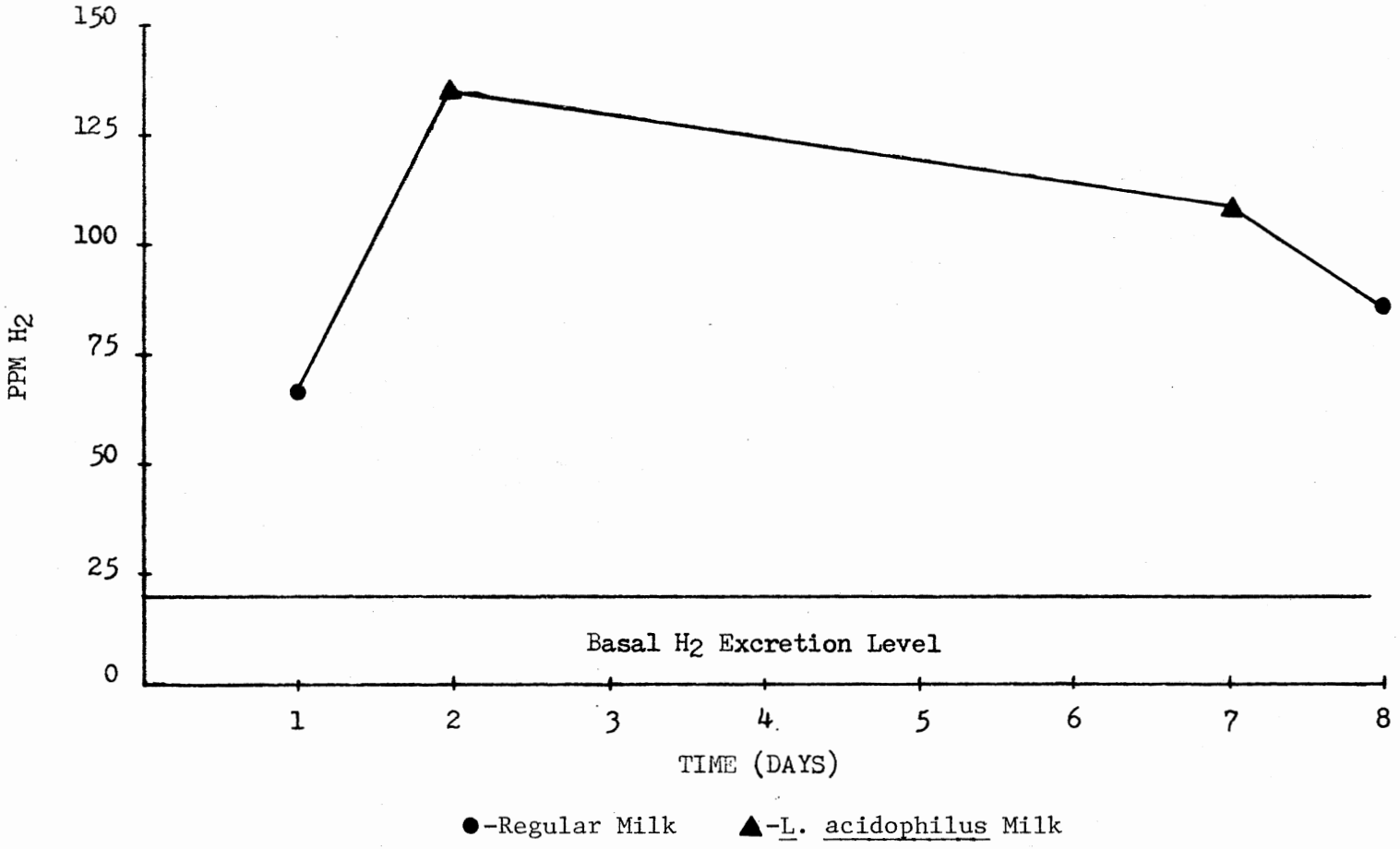

Figure 3. Subject Three-Breath Hydrogen Response on the LAM Test Meal

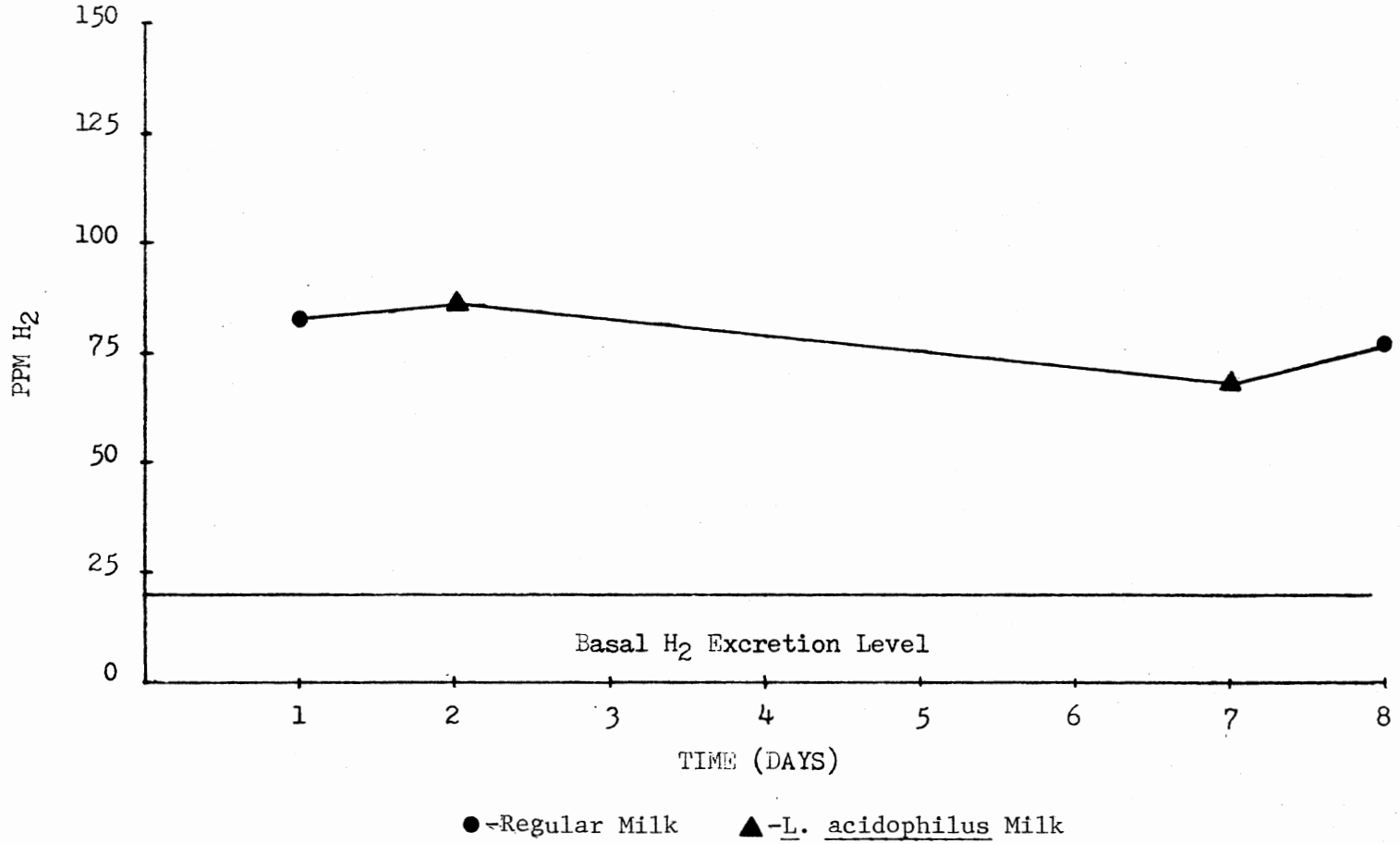

Figure 4. Subject Four--Breath Hydrogen Response on the LAM Test Meal

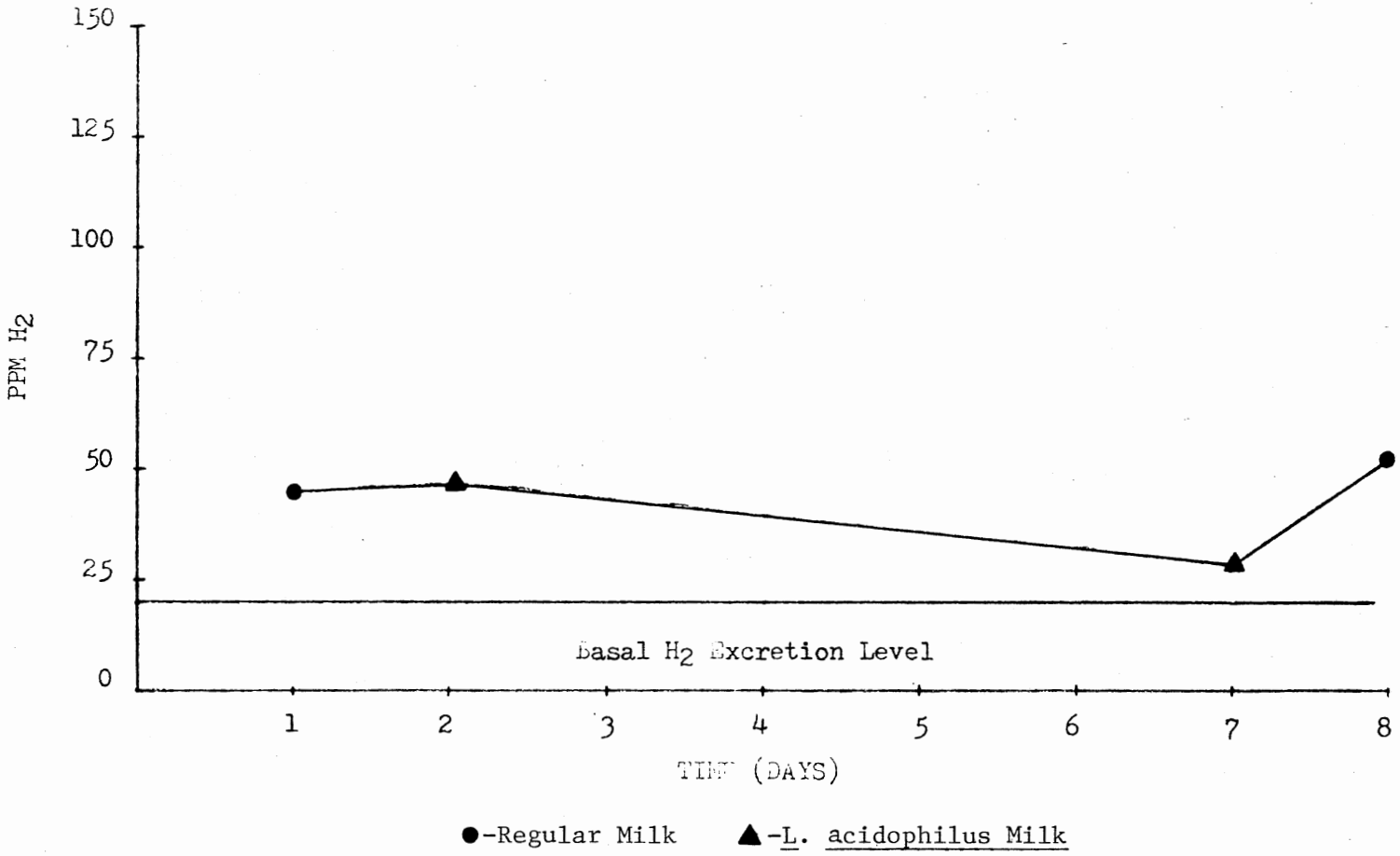

Figure 5. Subject Five--Breath Hydrogen Response on the LAM Test Meal

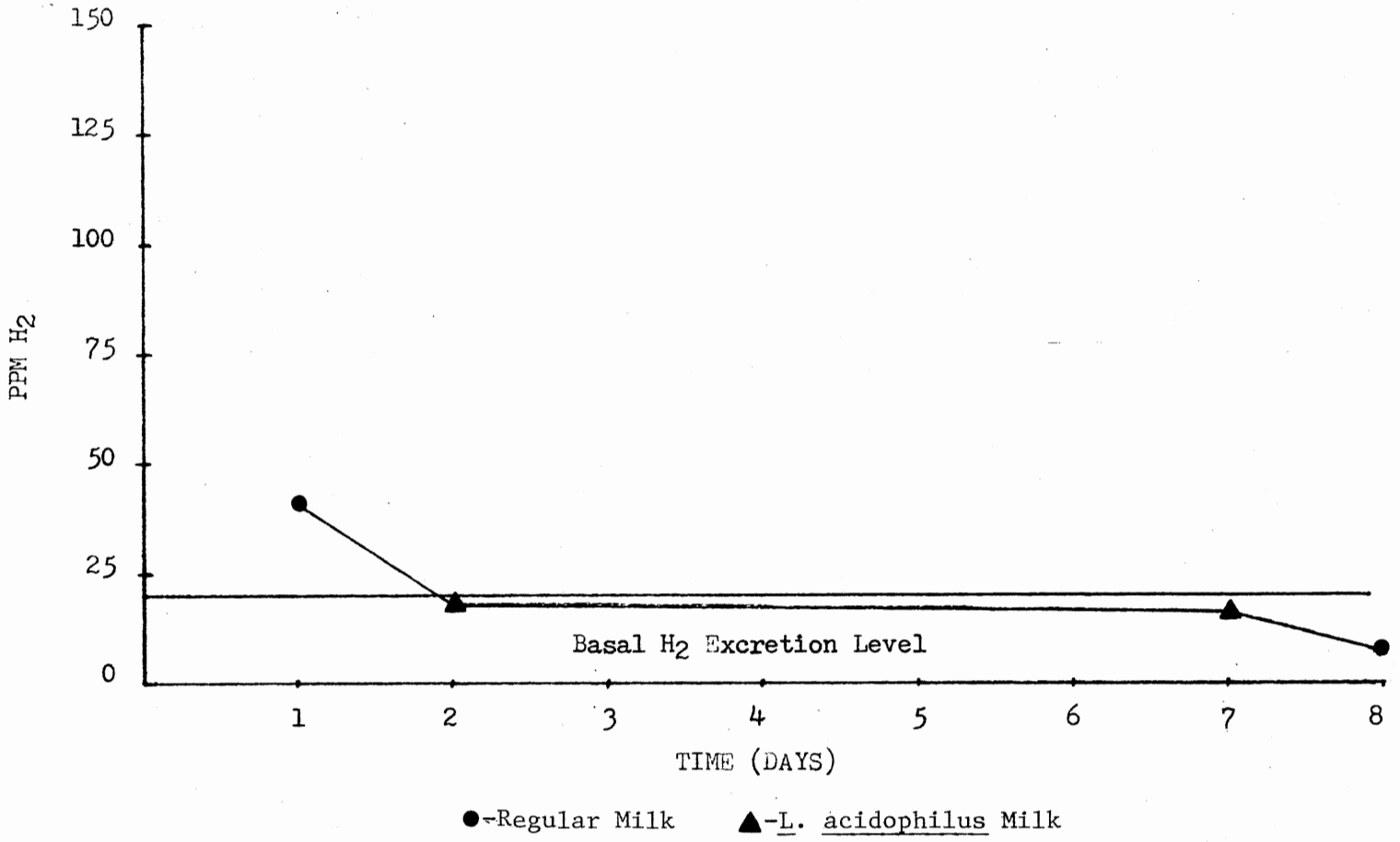

Figure 6. Subject Six--Breath Hydrogen Response on the LAM Test Meal

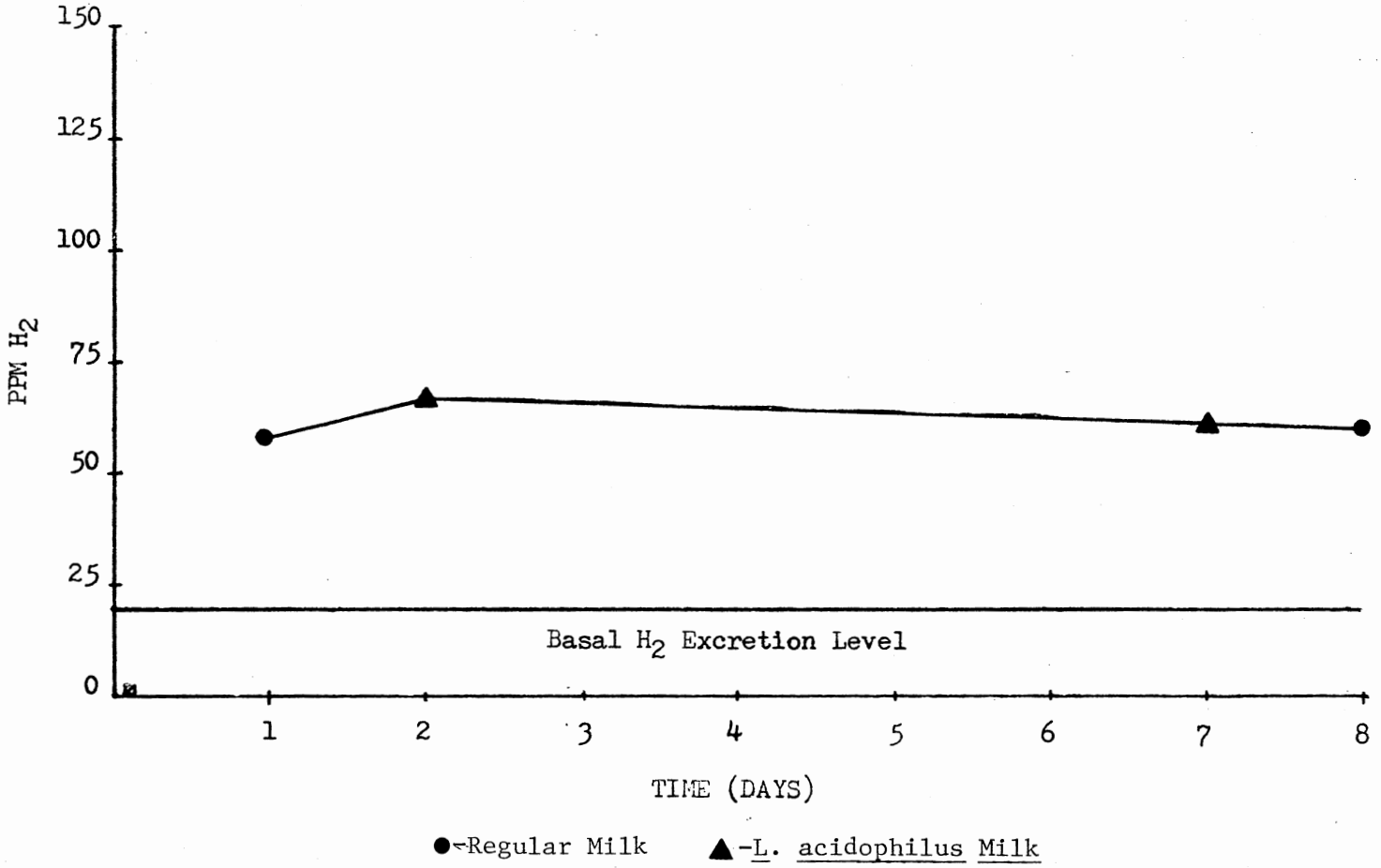

Figure 7. Mean Breath Hydrogen Response for the Six Subjects on the LAM Test Meal

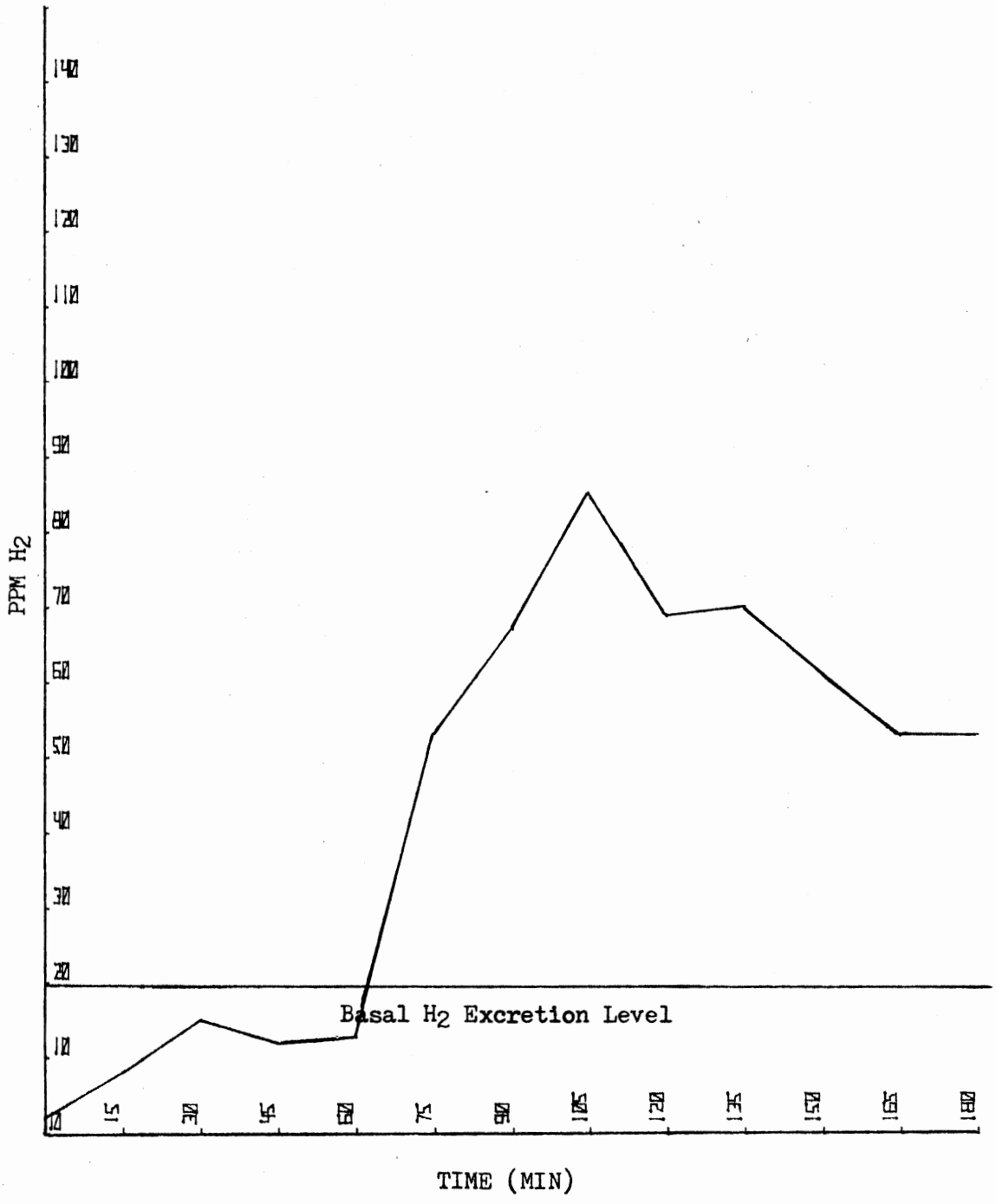

Figure 8. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the LAM Test Meal

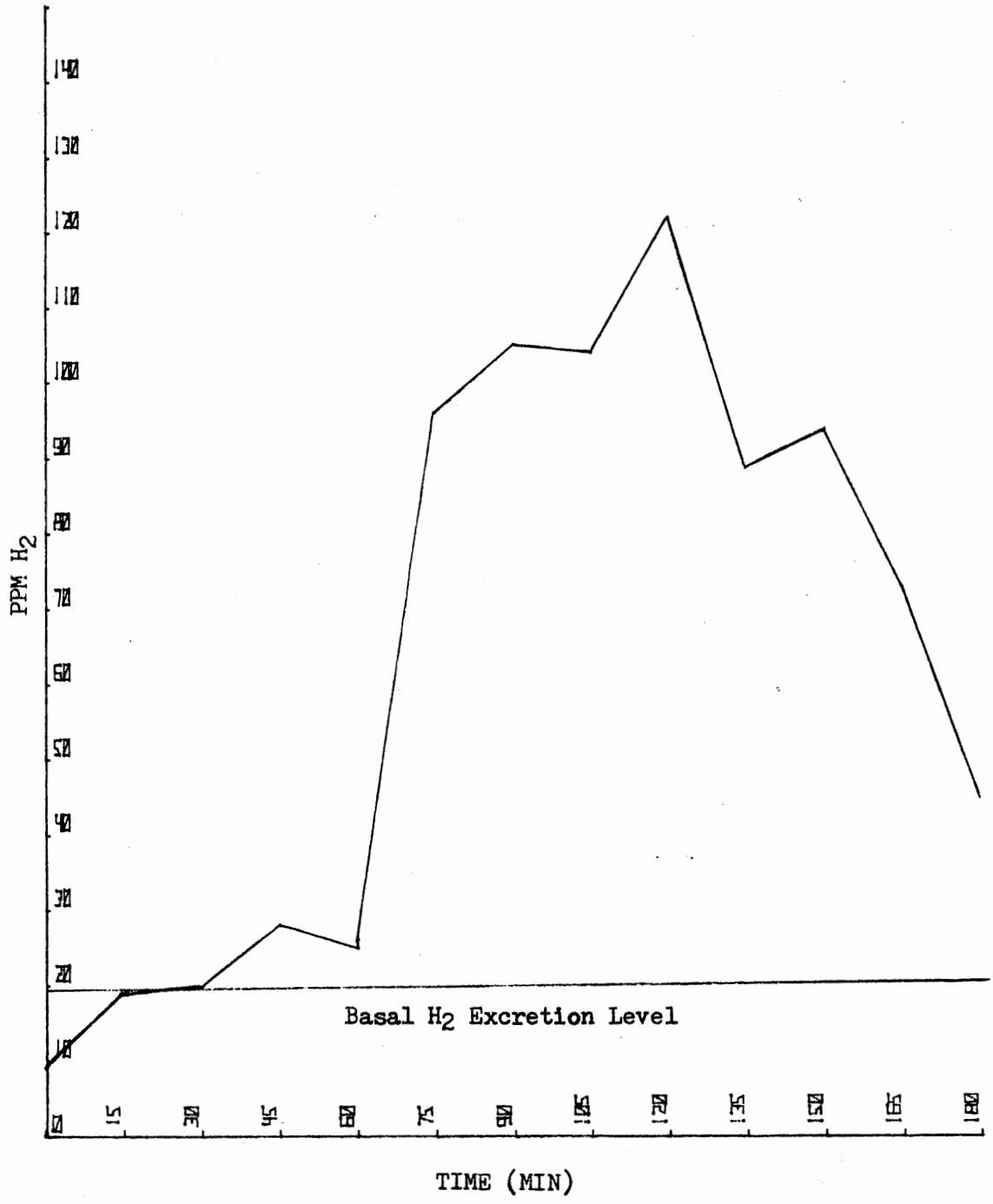

Figure 9. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Two of the LAM Test Meal

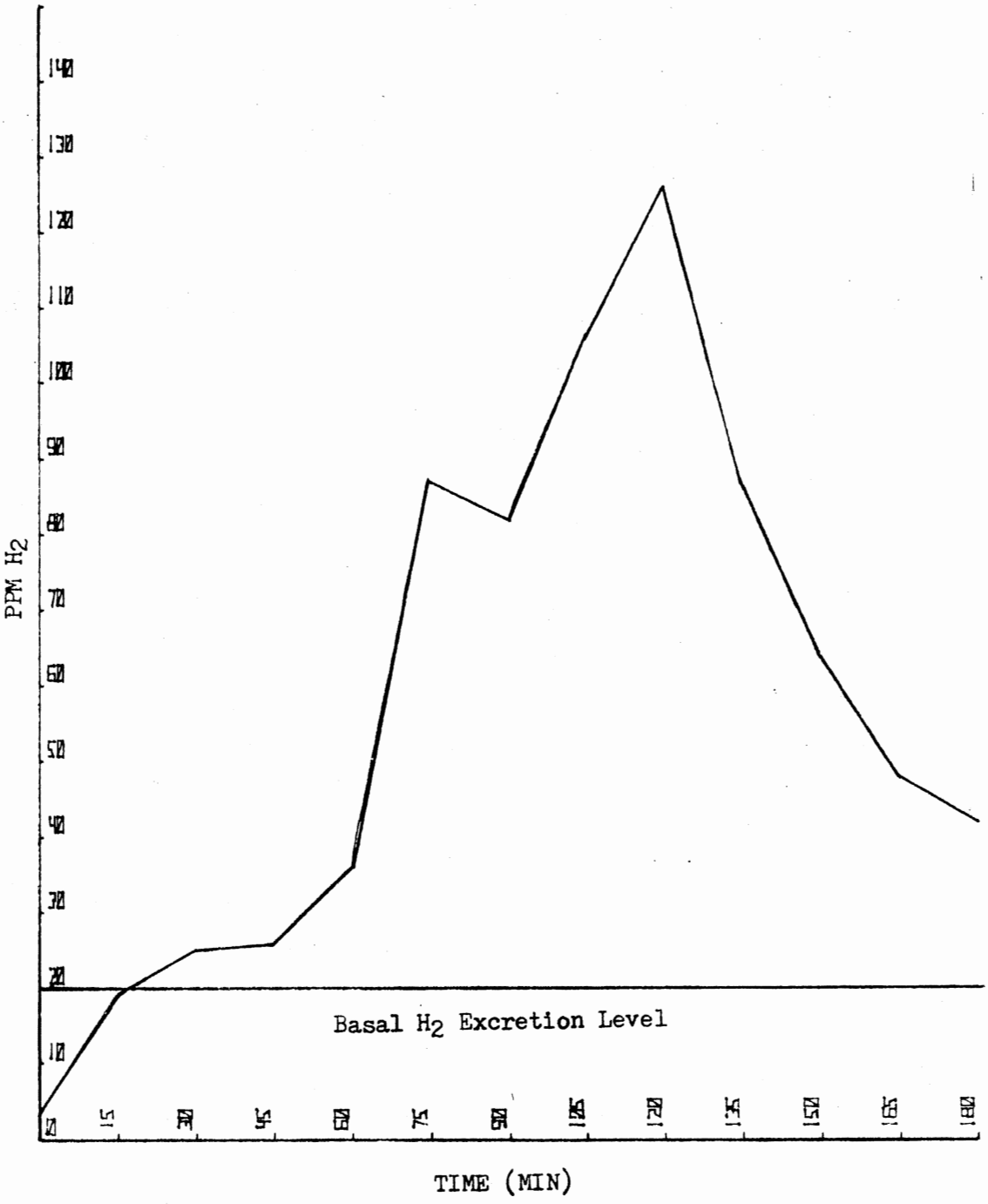

Figure 10. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Seven of the LAM Test Meal

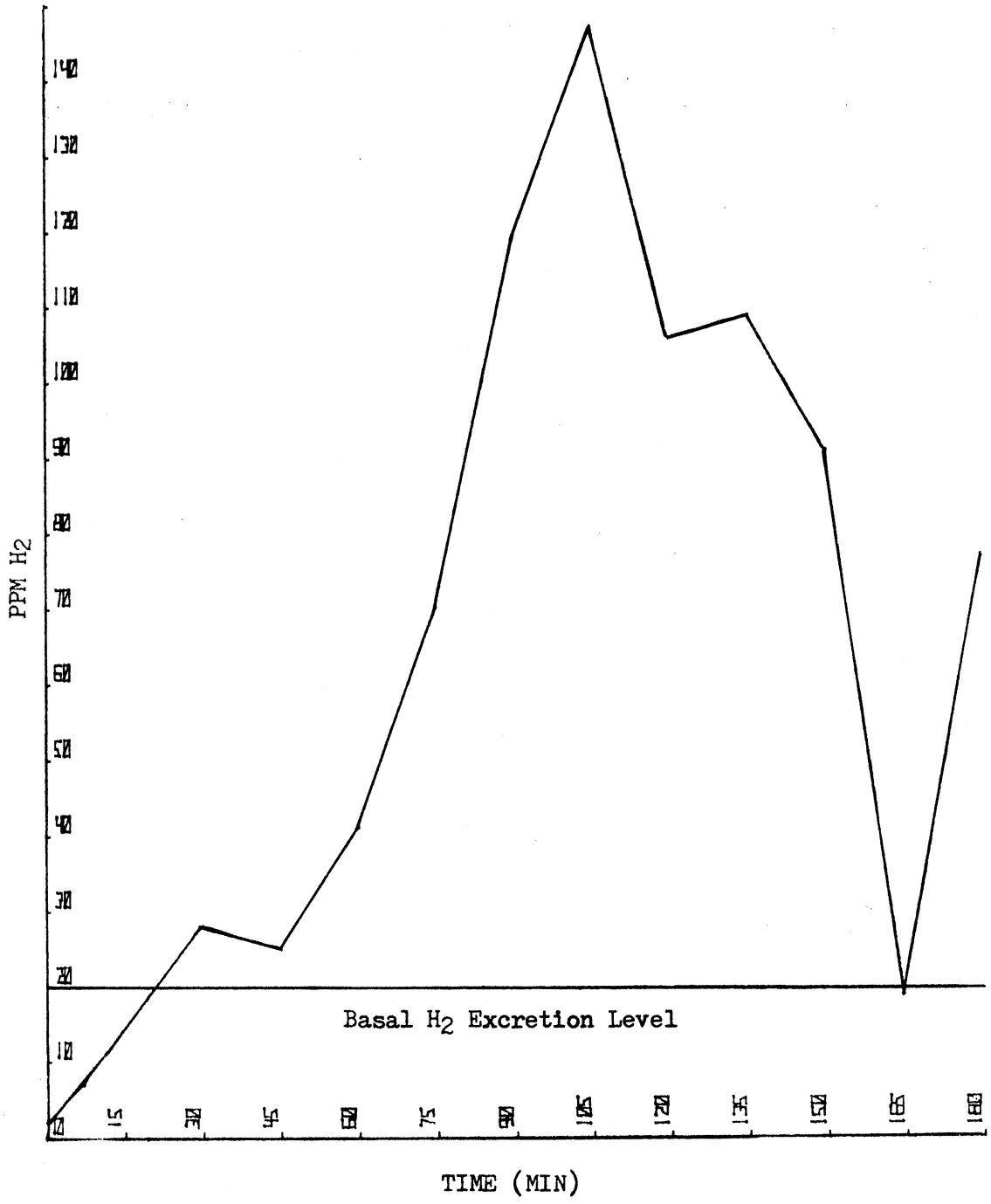

Figure 11. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the LAM Test Meal

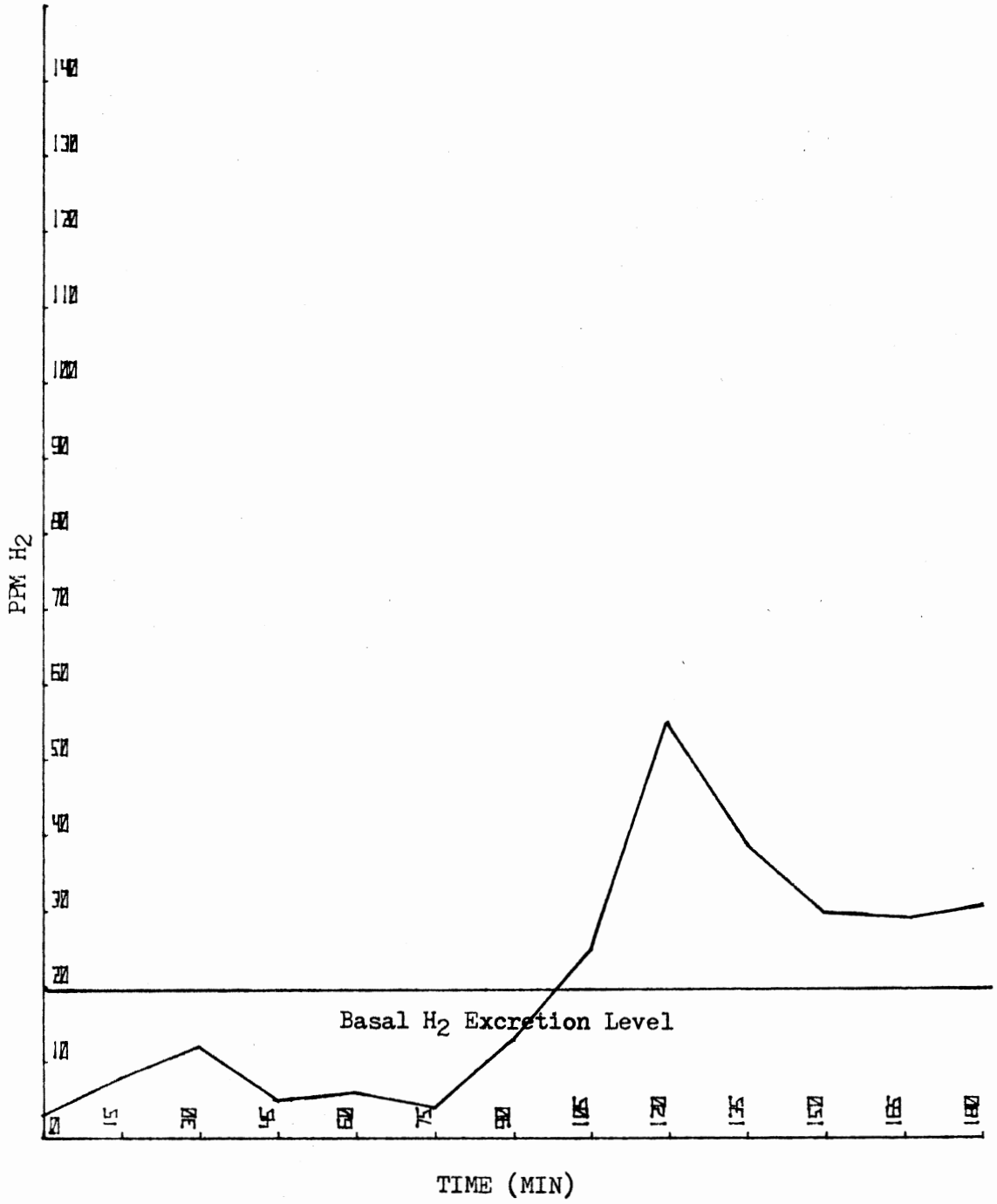

Figure 12. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the LAM Test Meal

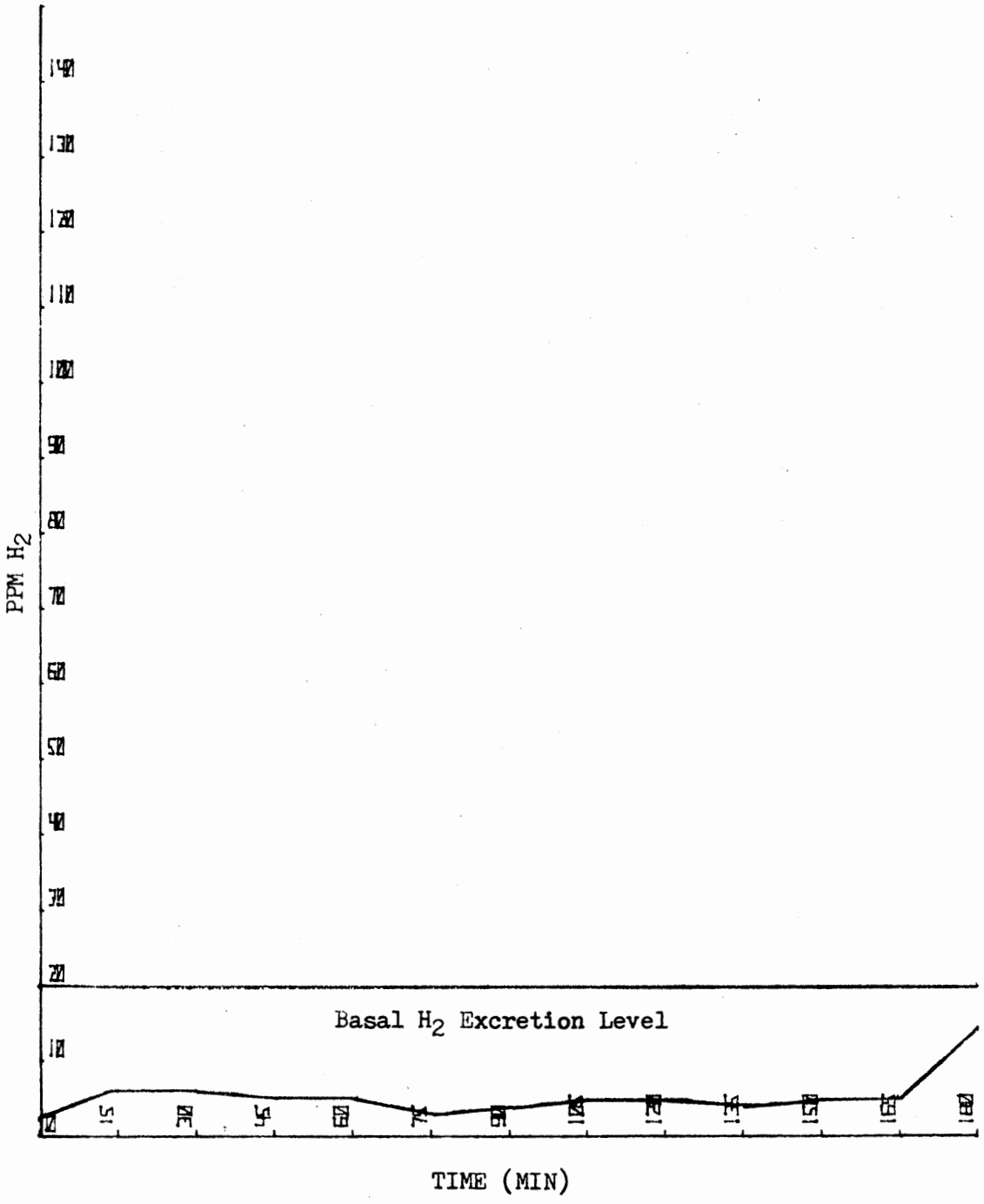

Figure 13. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Two of the LAM Test Meal

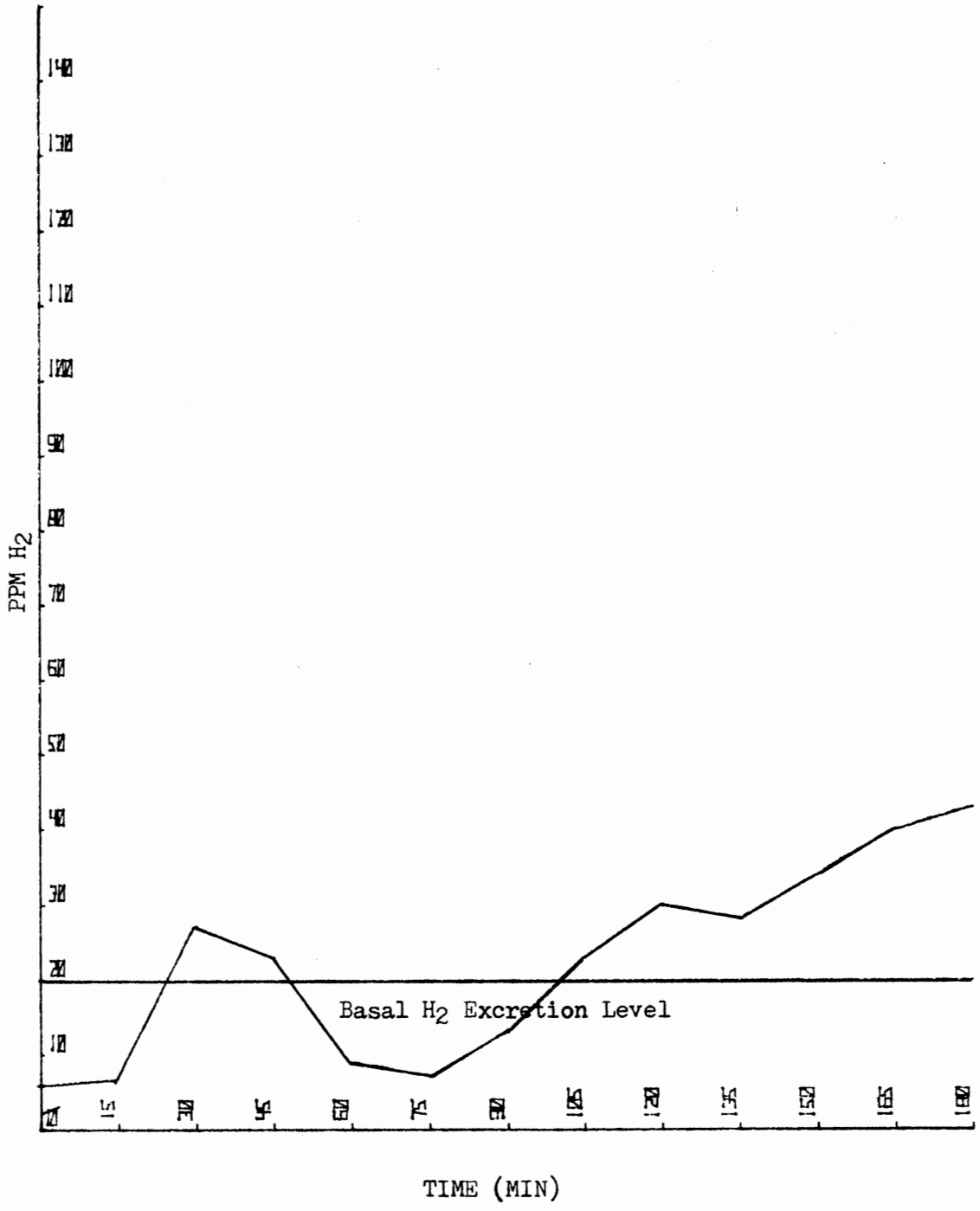

Figure 14. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Seven of the LAM Test Meal

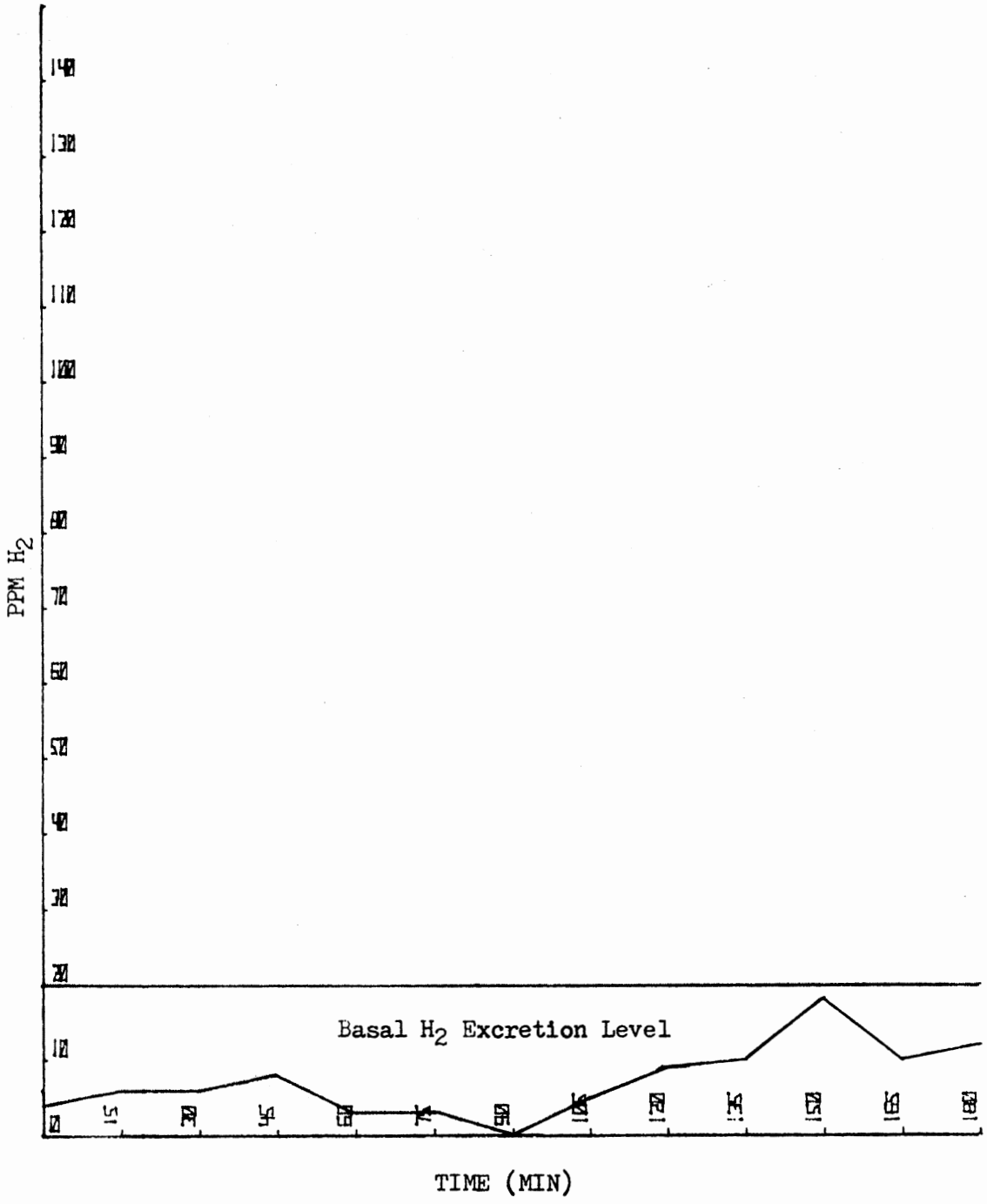

Figure 15. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the LAM Test Meal

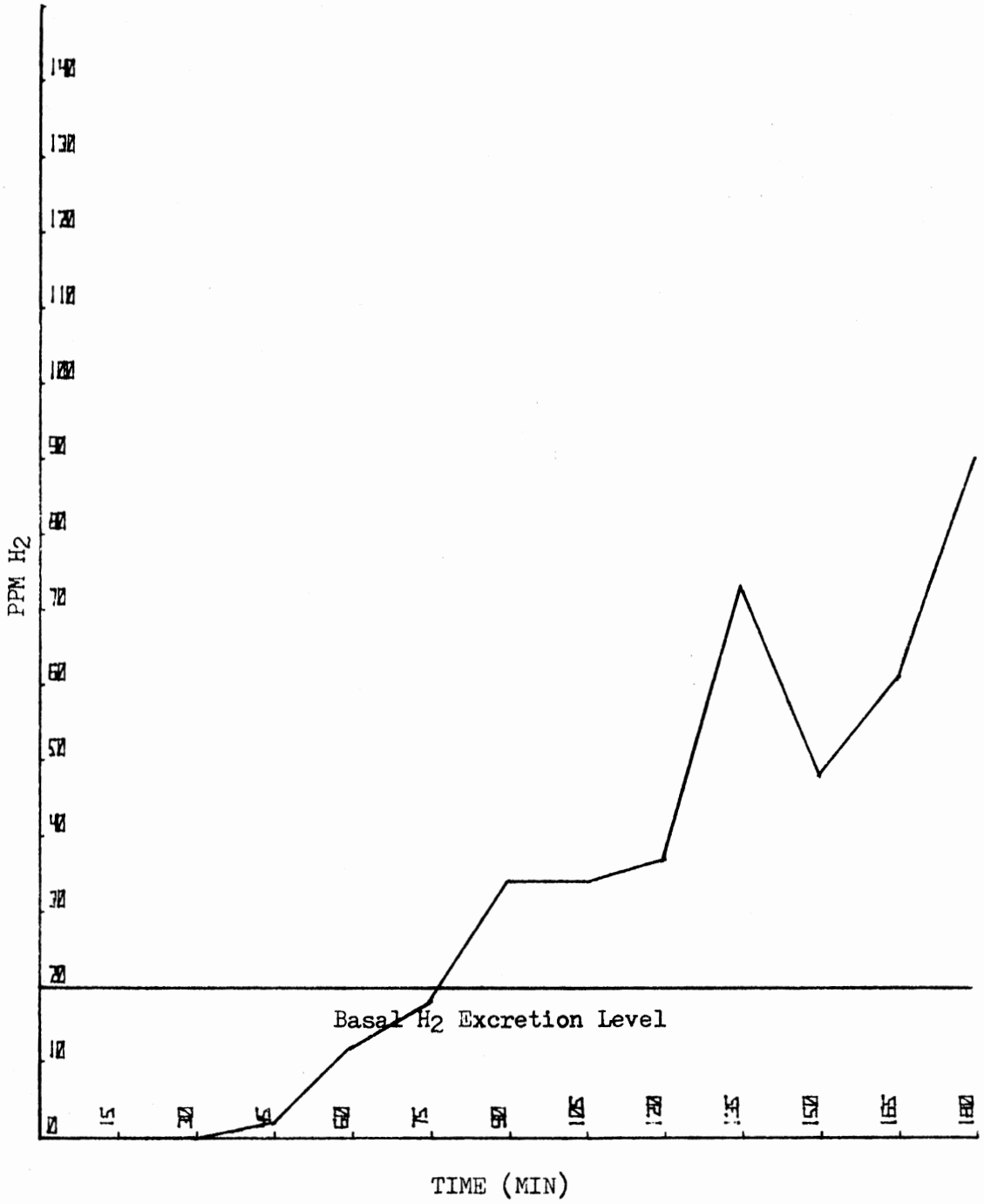

Figure 16. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the LAM Test Meal

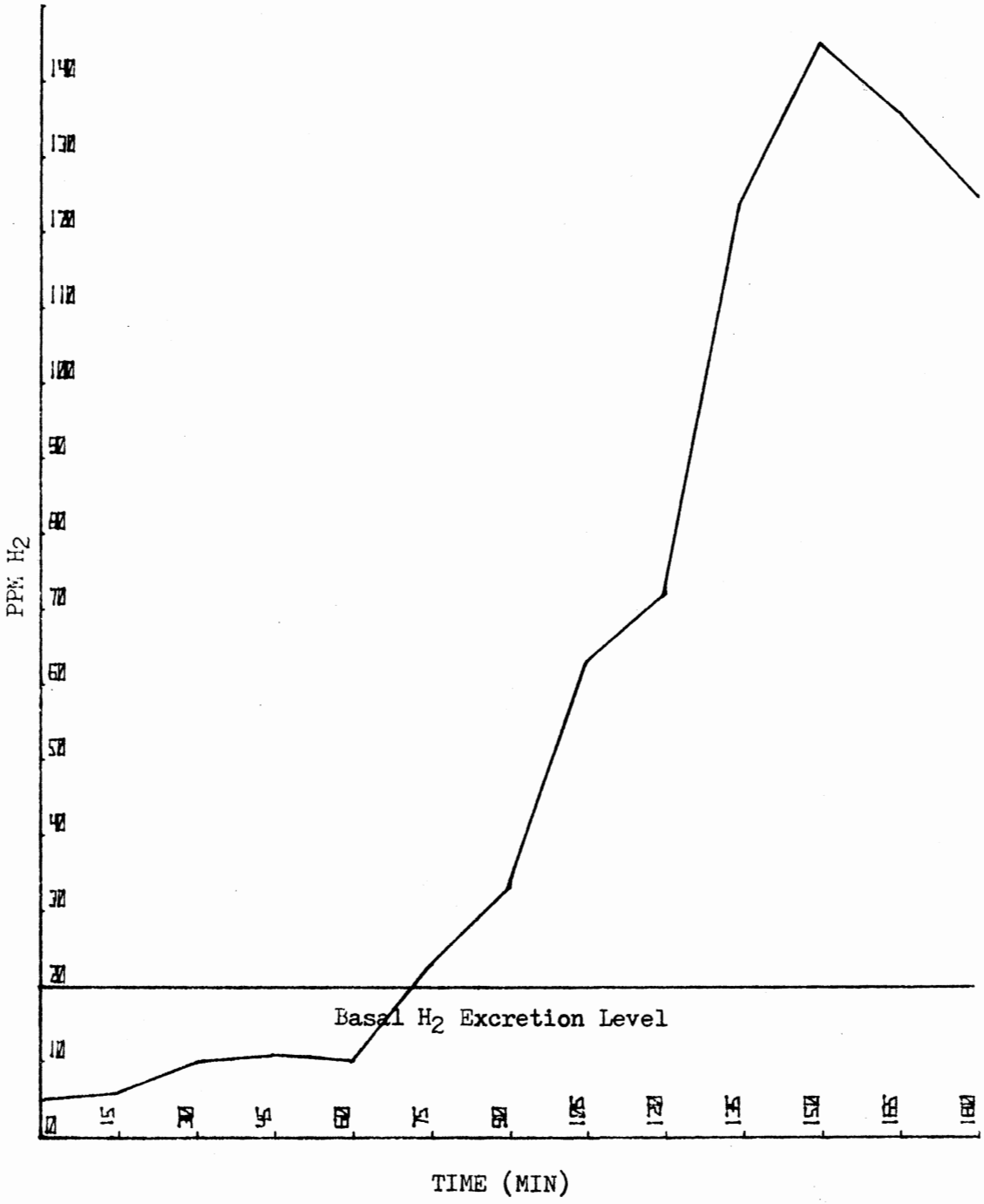

Figure 17. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Two of the LAM Test Meal

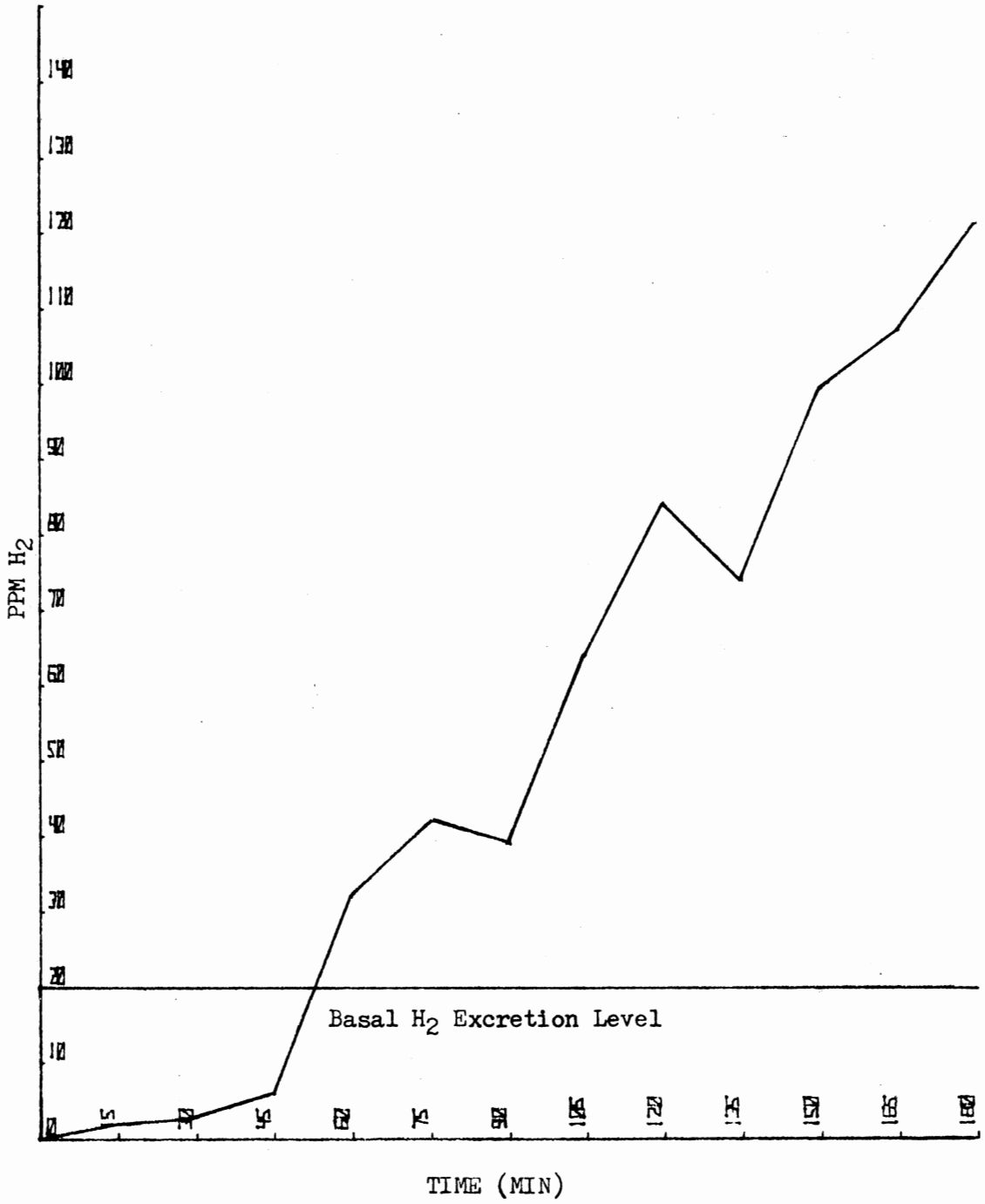

Figure 18. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Seven of the LAM Test Meal

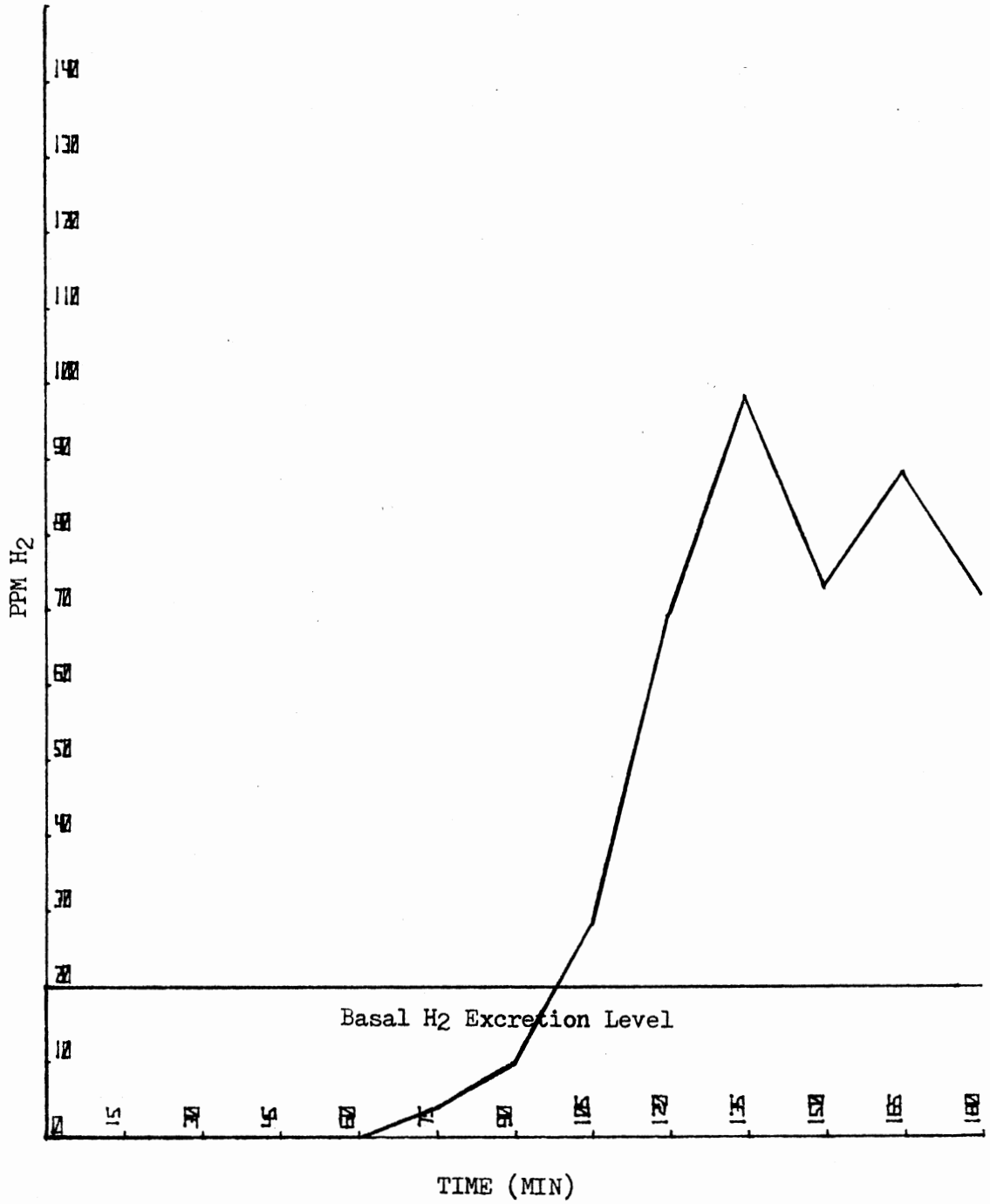

Figure 19. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the LAM Test Meal

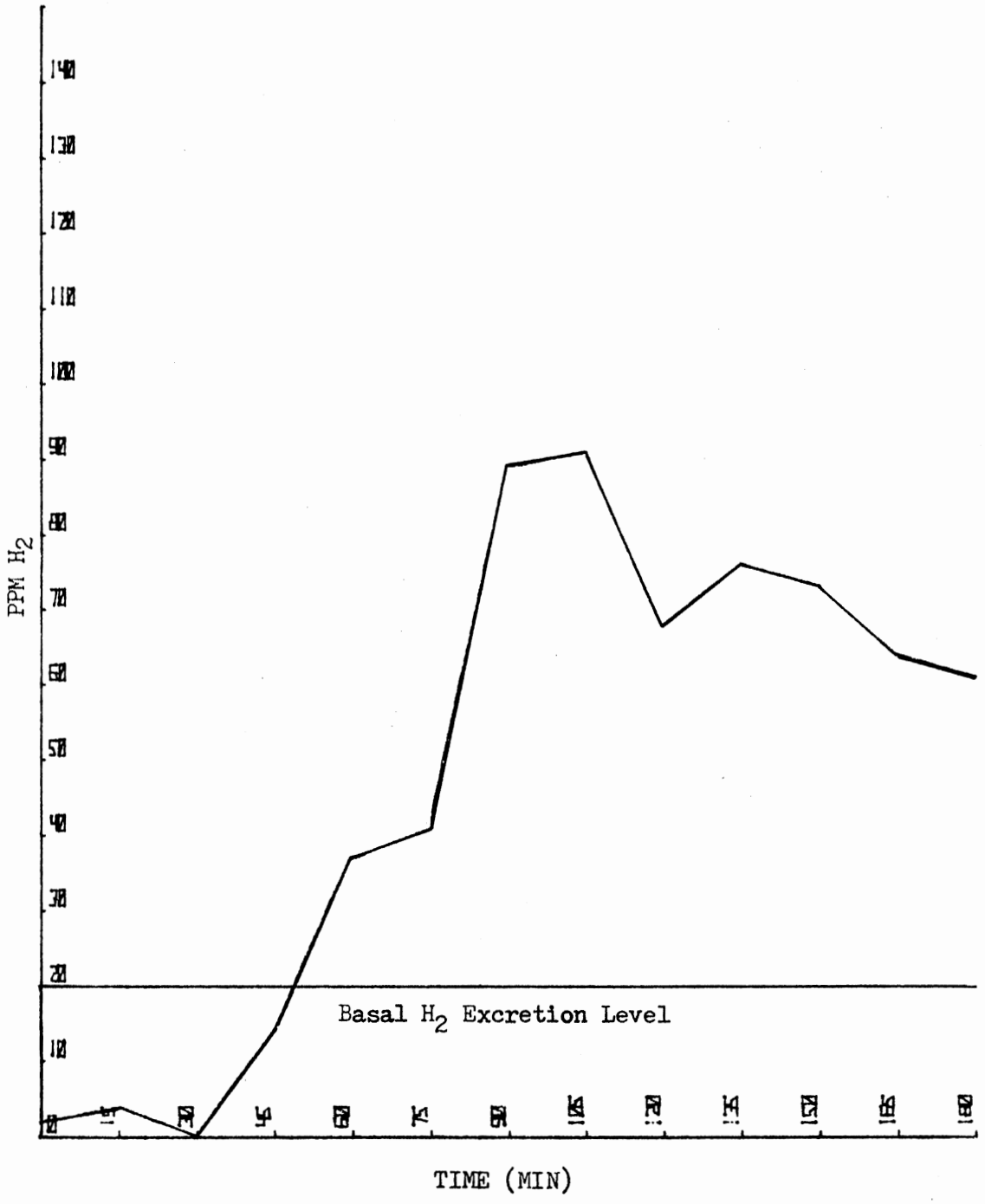

Figure 20. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the LAM Test Meal

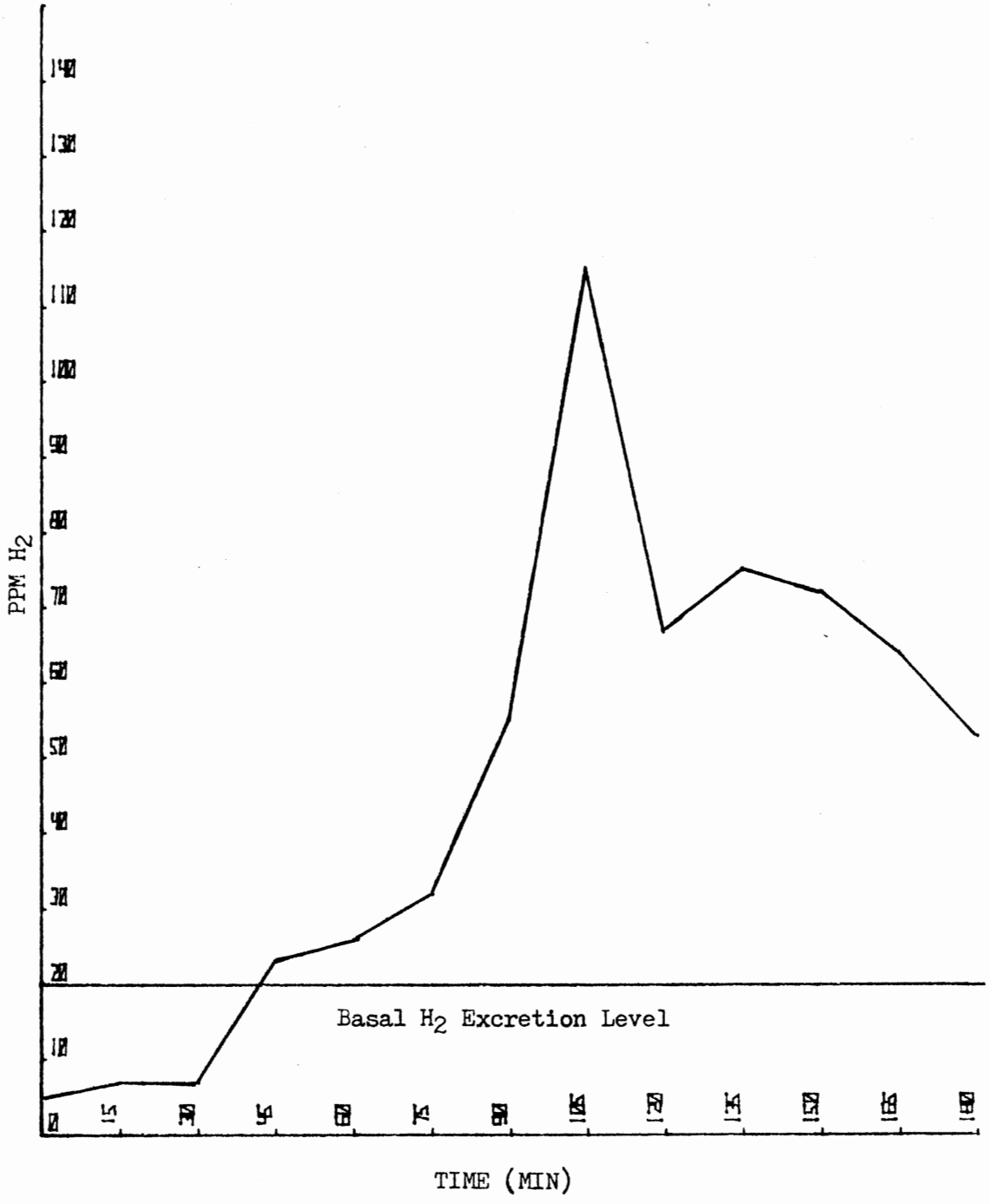

Figure 21. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Two of the LAM Test Meal

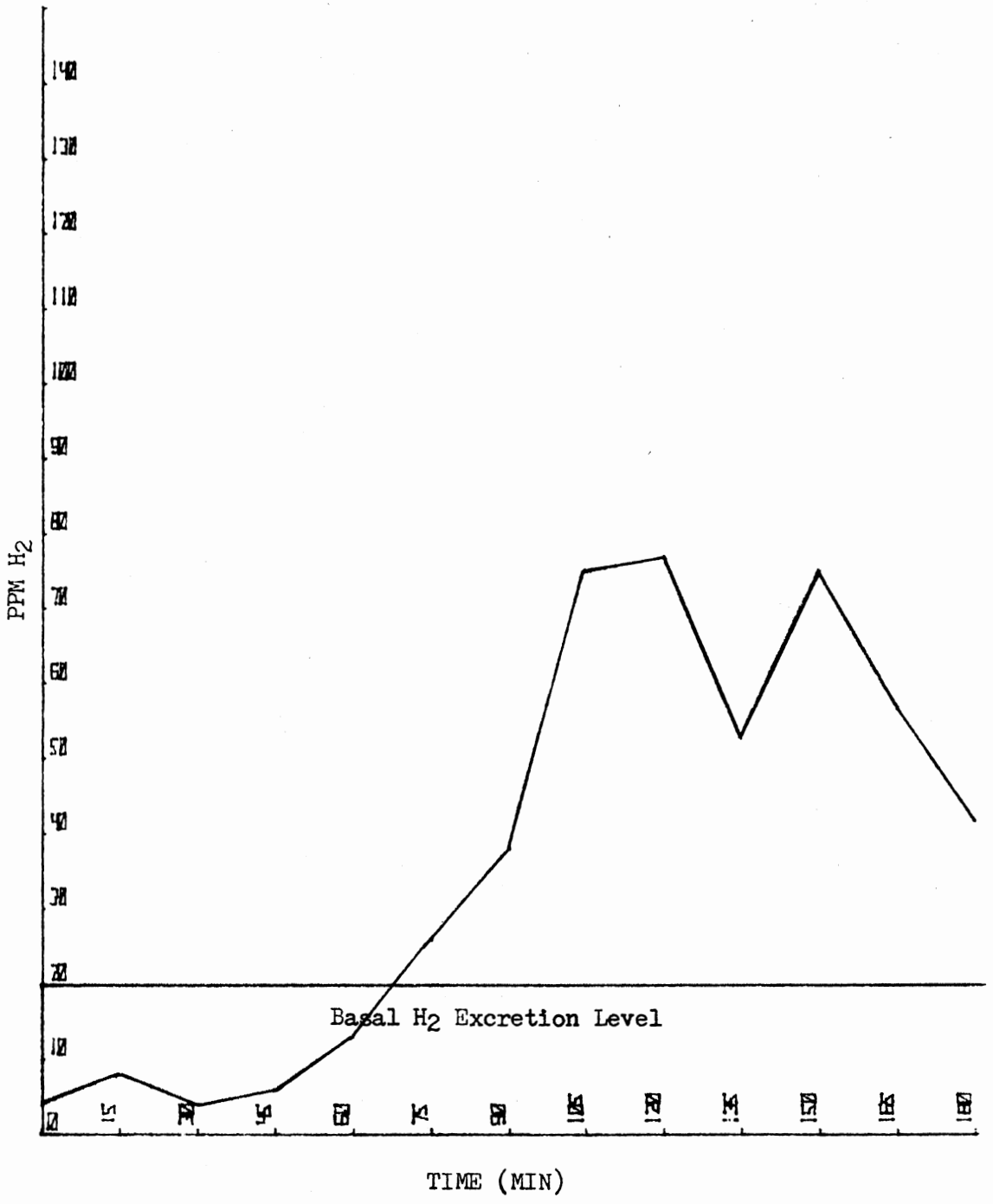

Figure 22. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Seven of the LAM Test Meal

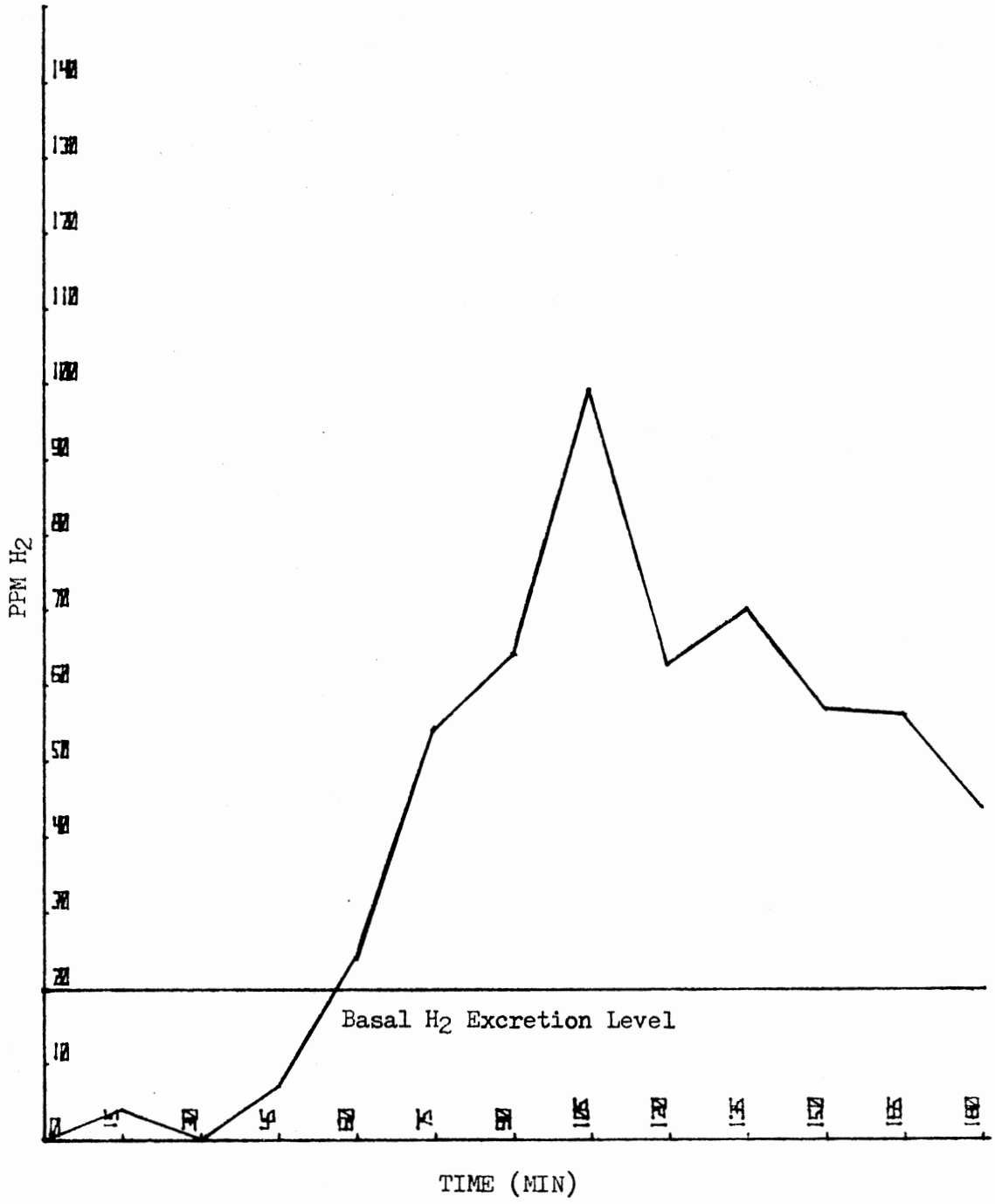

Figure 23. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the LAM Test Meal

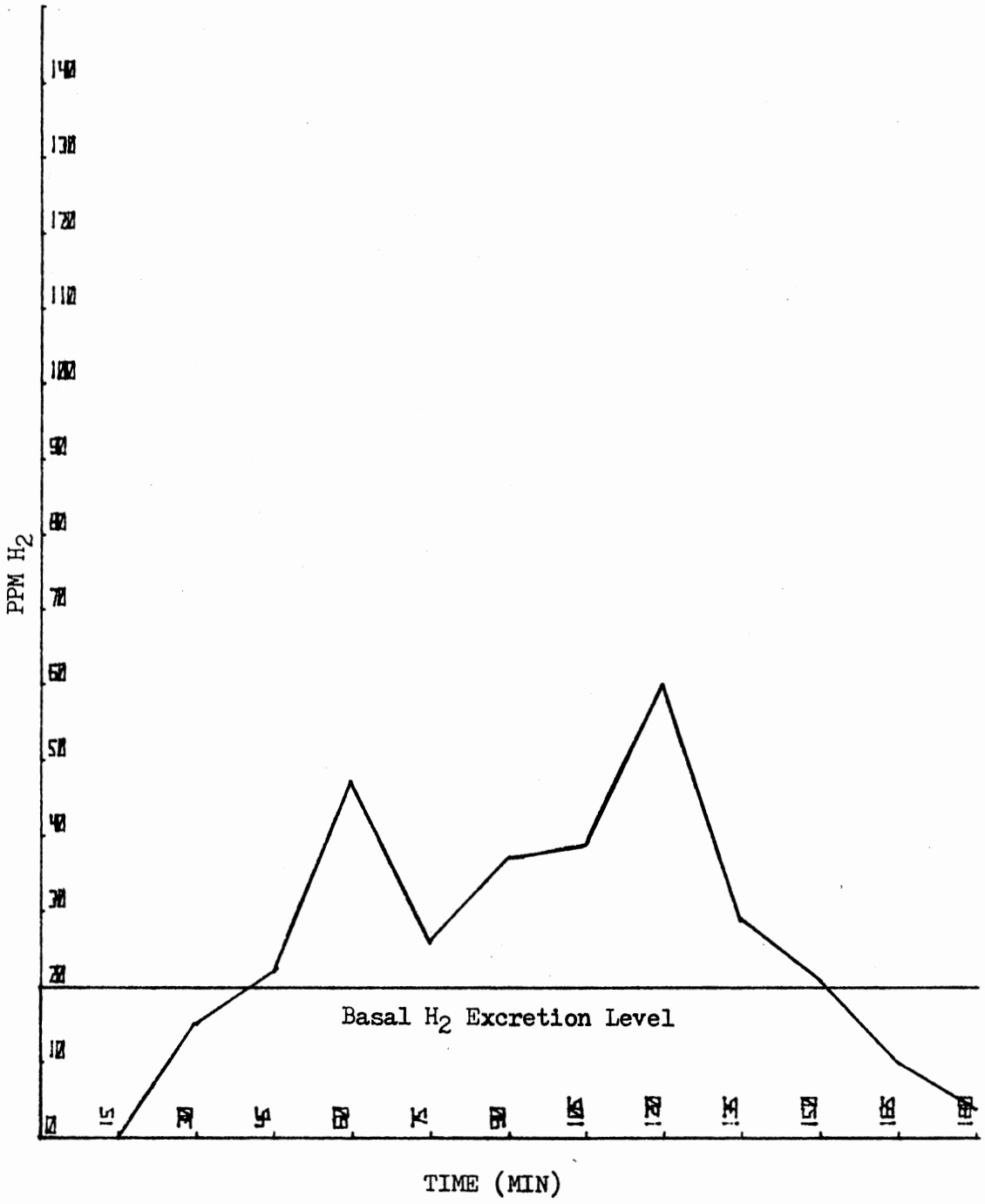

Figure 24. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the LAM Test Meal

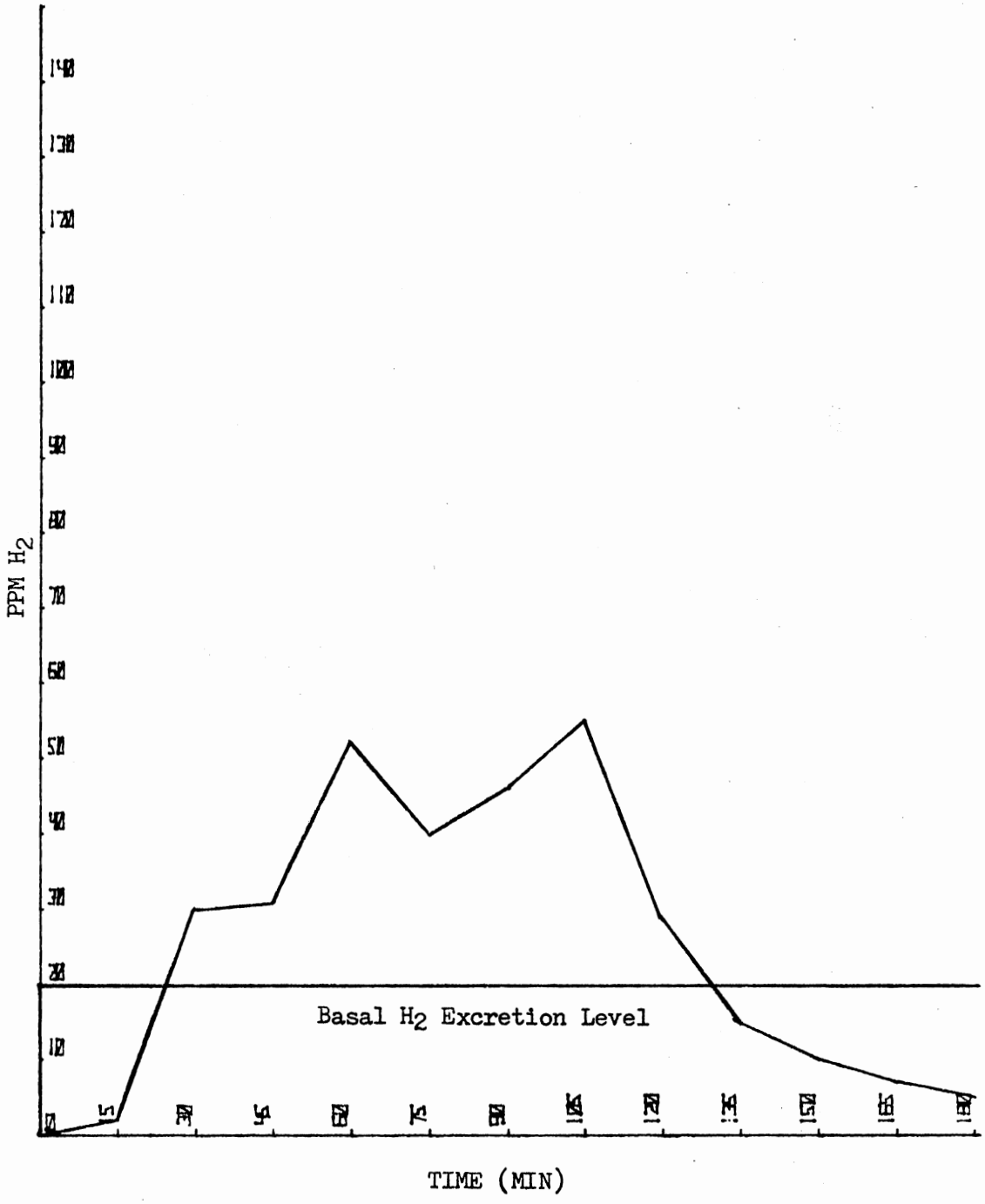

Figure 25. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Two of the LAM Test Meal

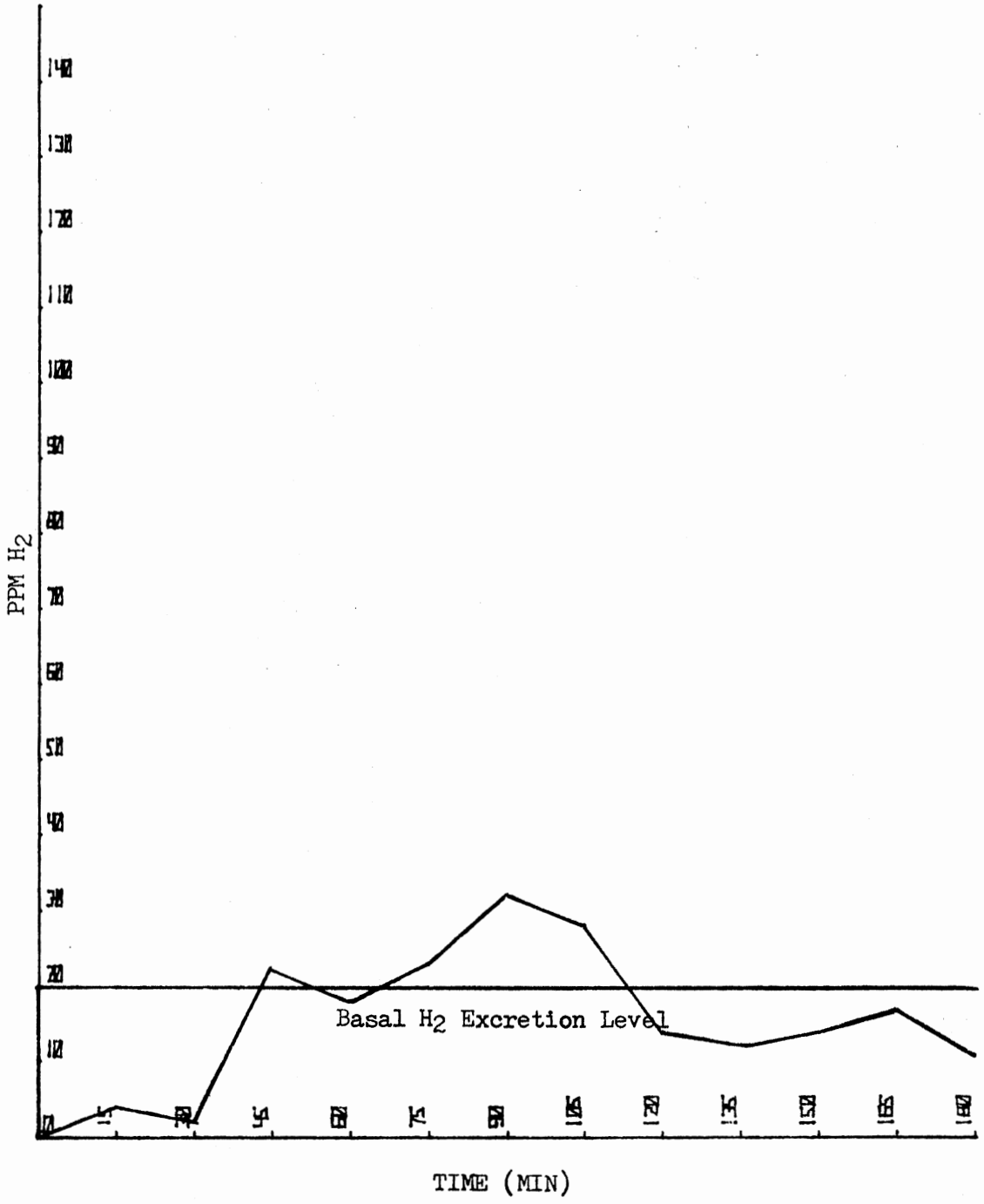

Figure 26. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Seven of the LAM Test Meal

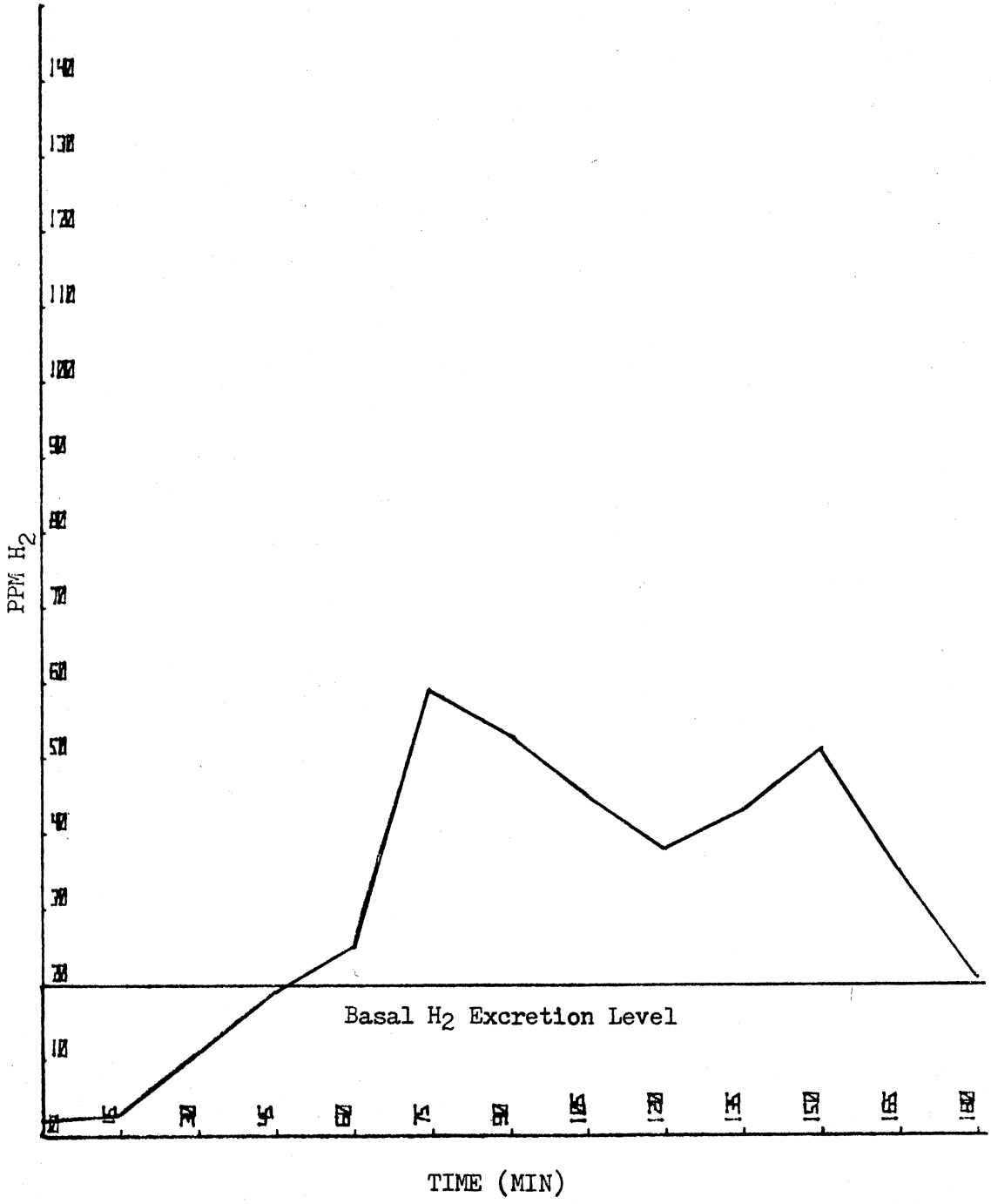

Figure 27. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the LAM Test Meal

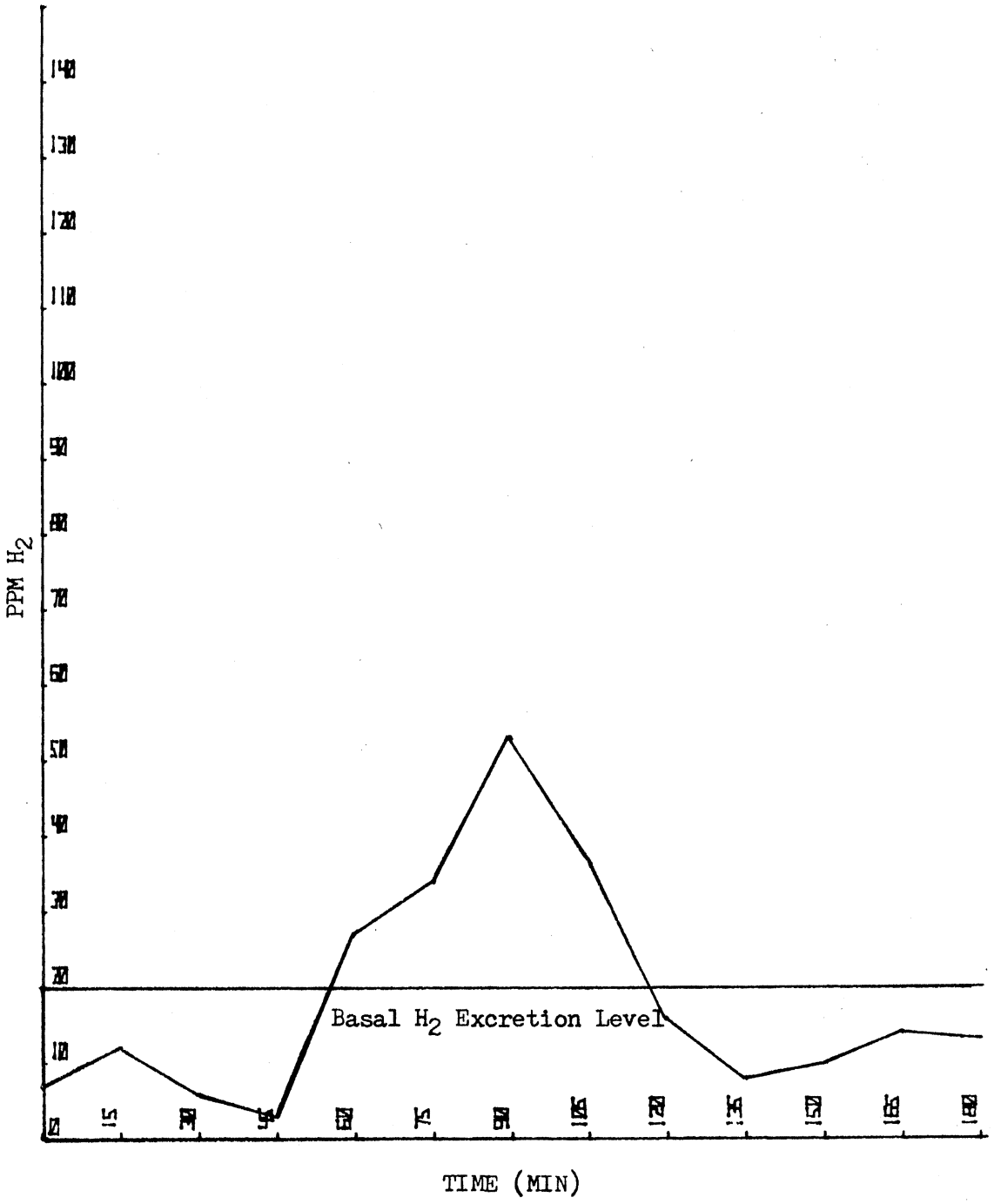

Figure 28. Subject 6--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the LAM Test Meal

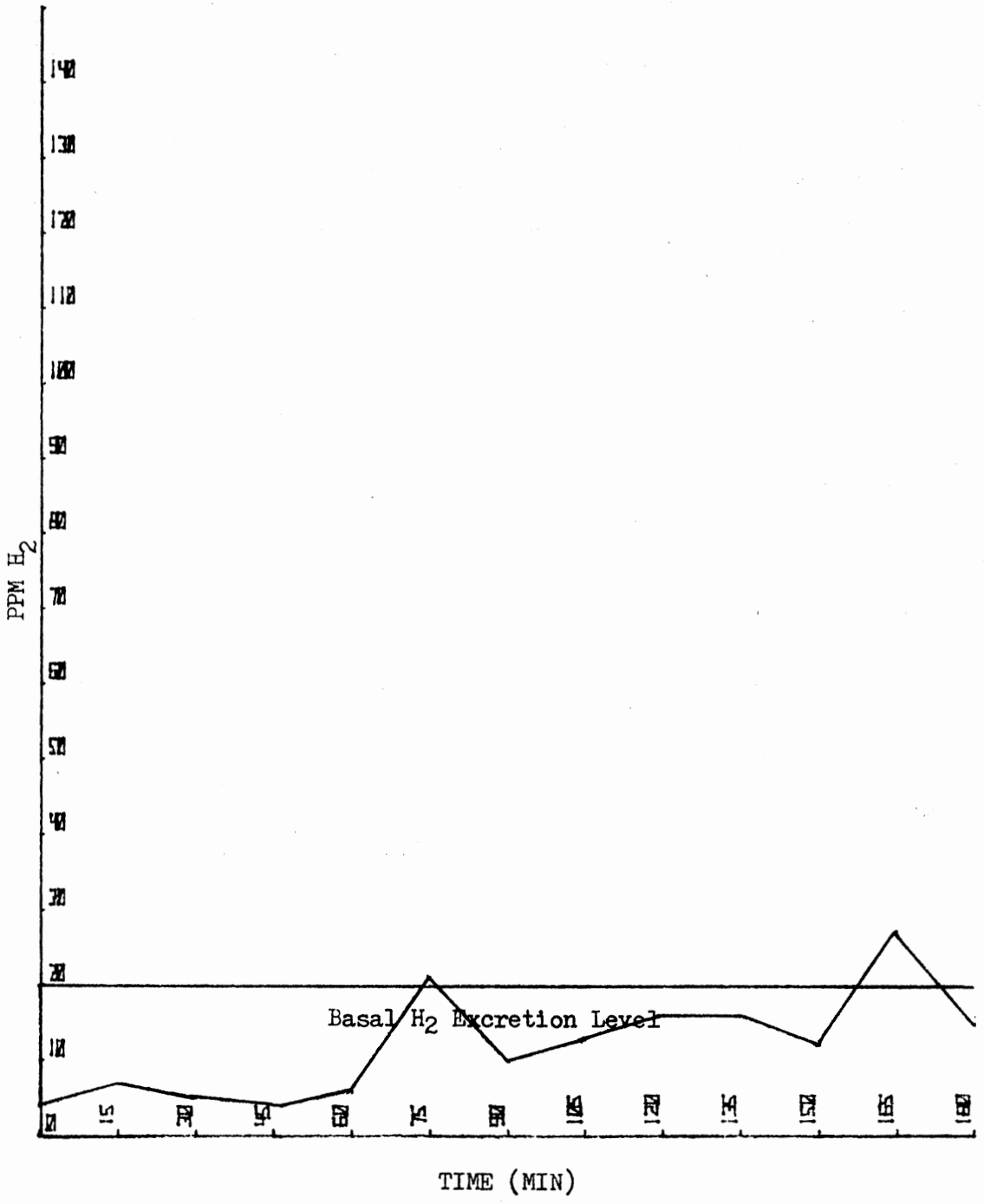

Figure 29. Subject 6--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Two of the LAM Test Meal

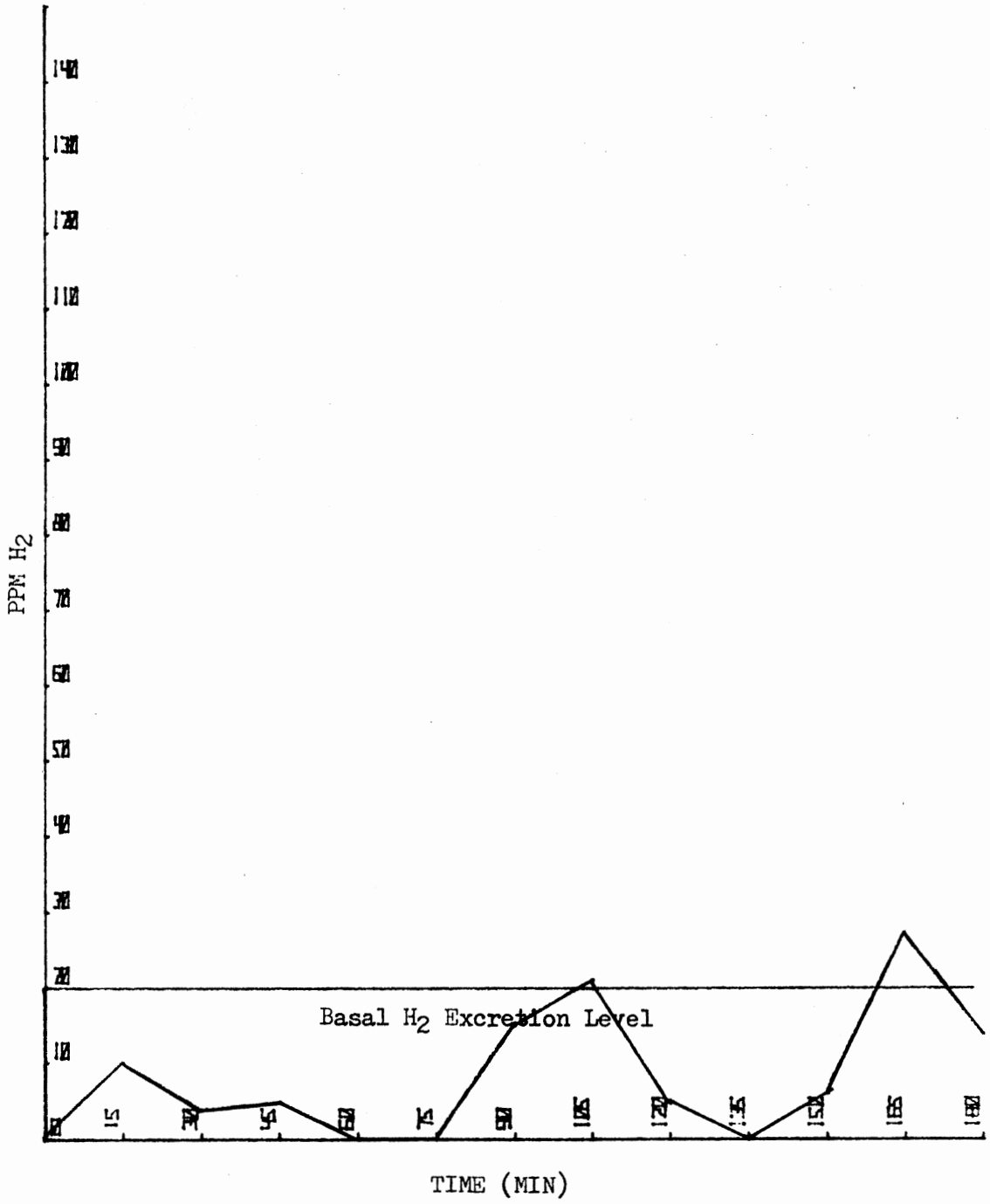

Figure 30. Subject 6--Breath H_2 Concentration Versus Time After Consumption of Milk Inoculated With L. acidophilus on Test Day Seven of the LAM Test Meal

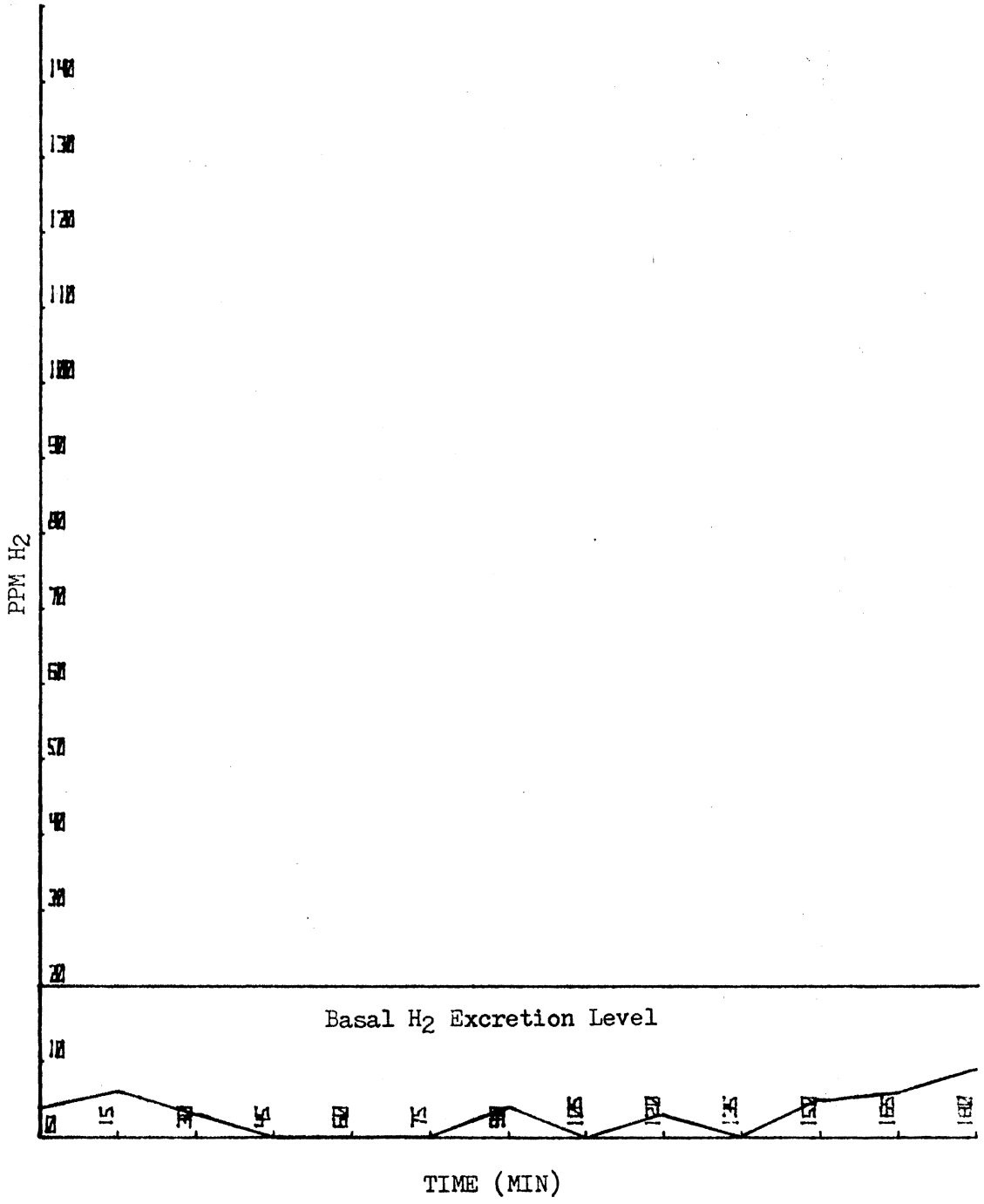

Figure 31. Subject 6--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the LAM Test Meal

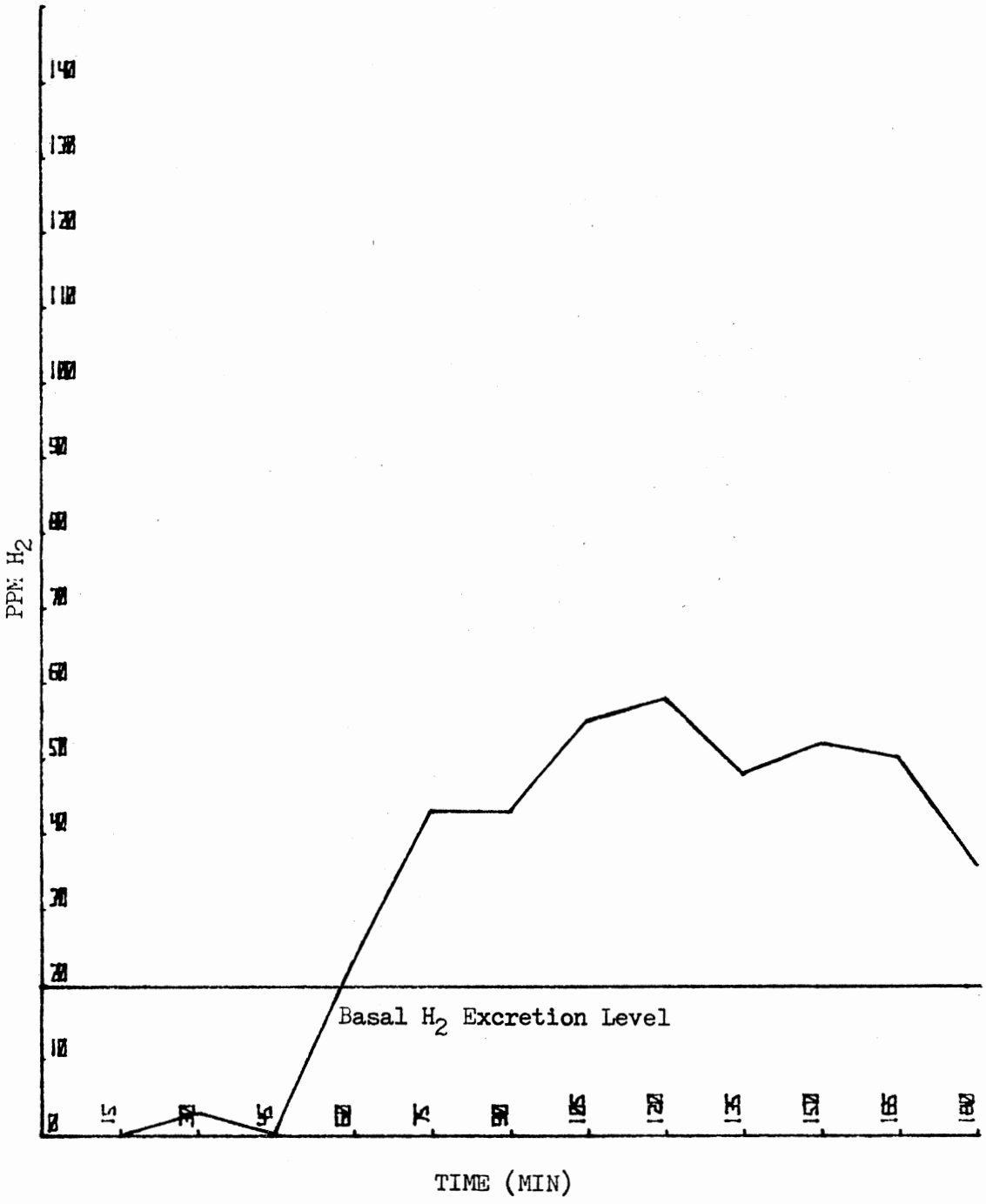

Figure 32. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the RM Test Meal

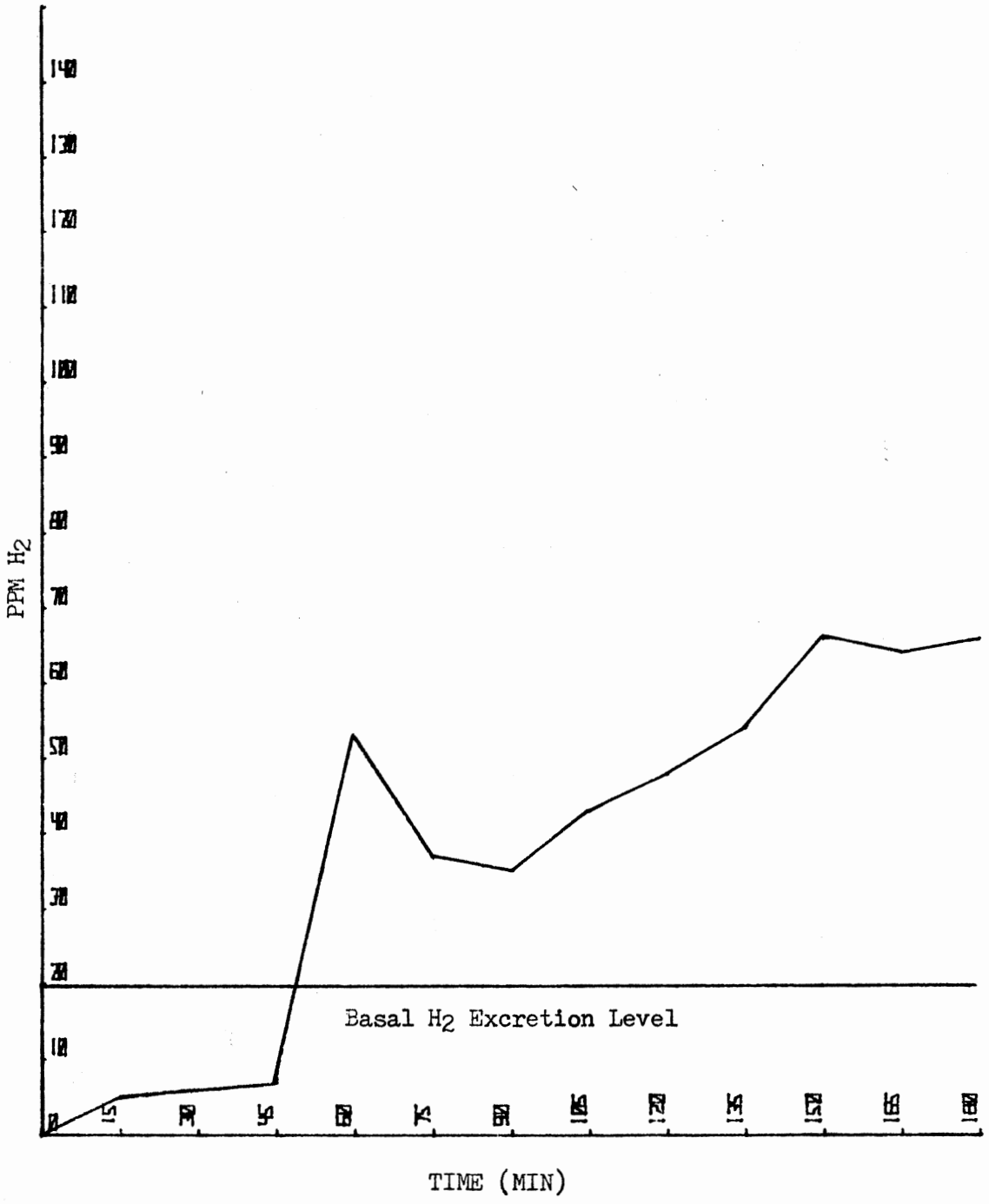

Figure 33. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Two of the RM Test Meal

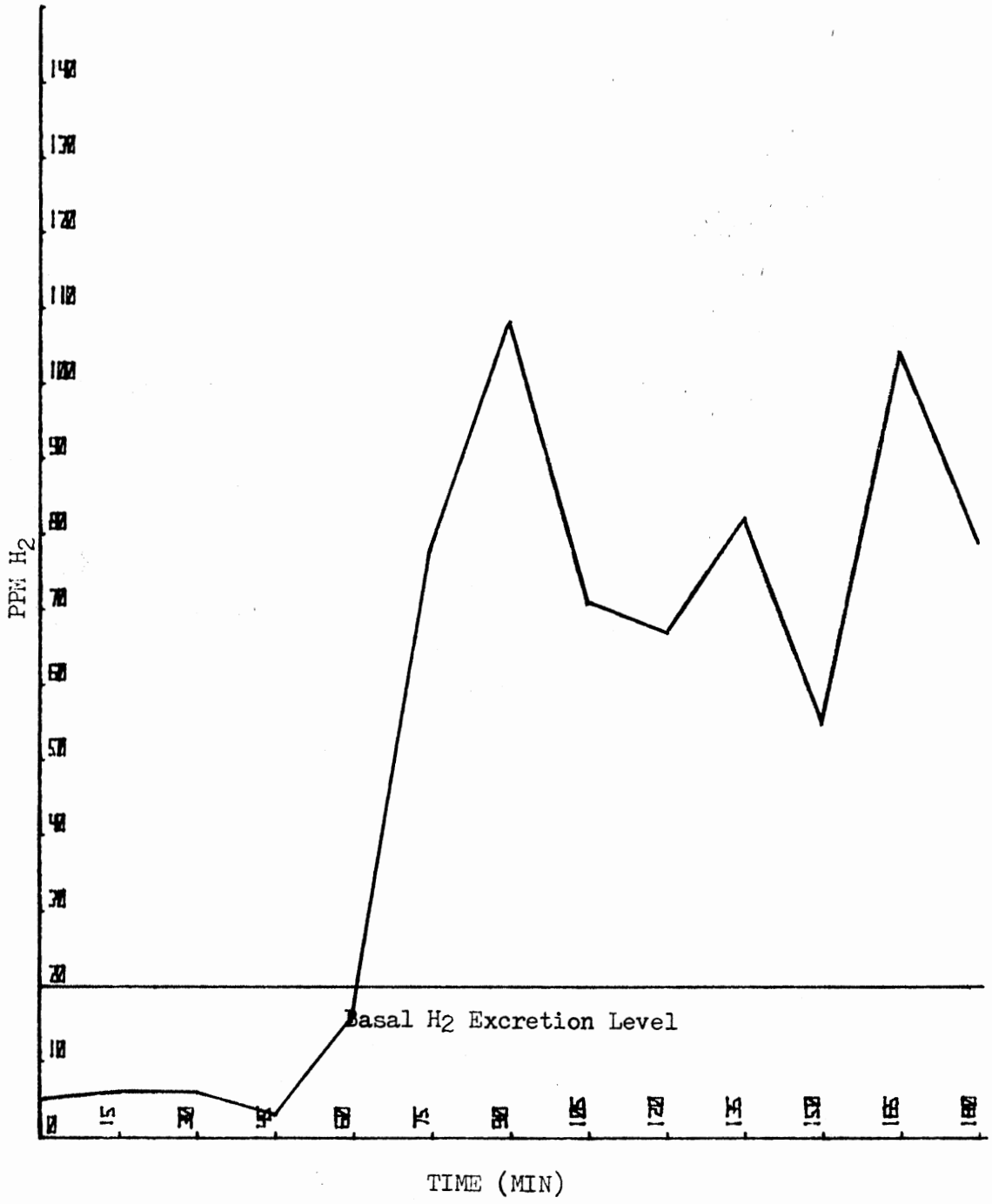

Figure 34. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Seven of the RM Test Meal

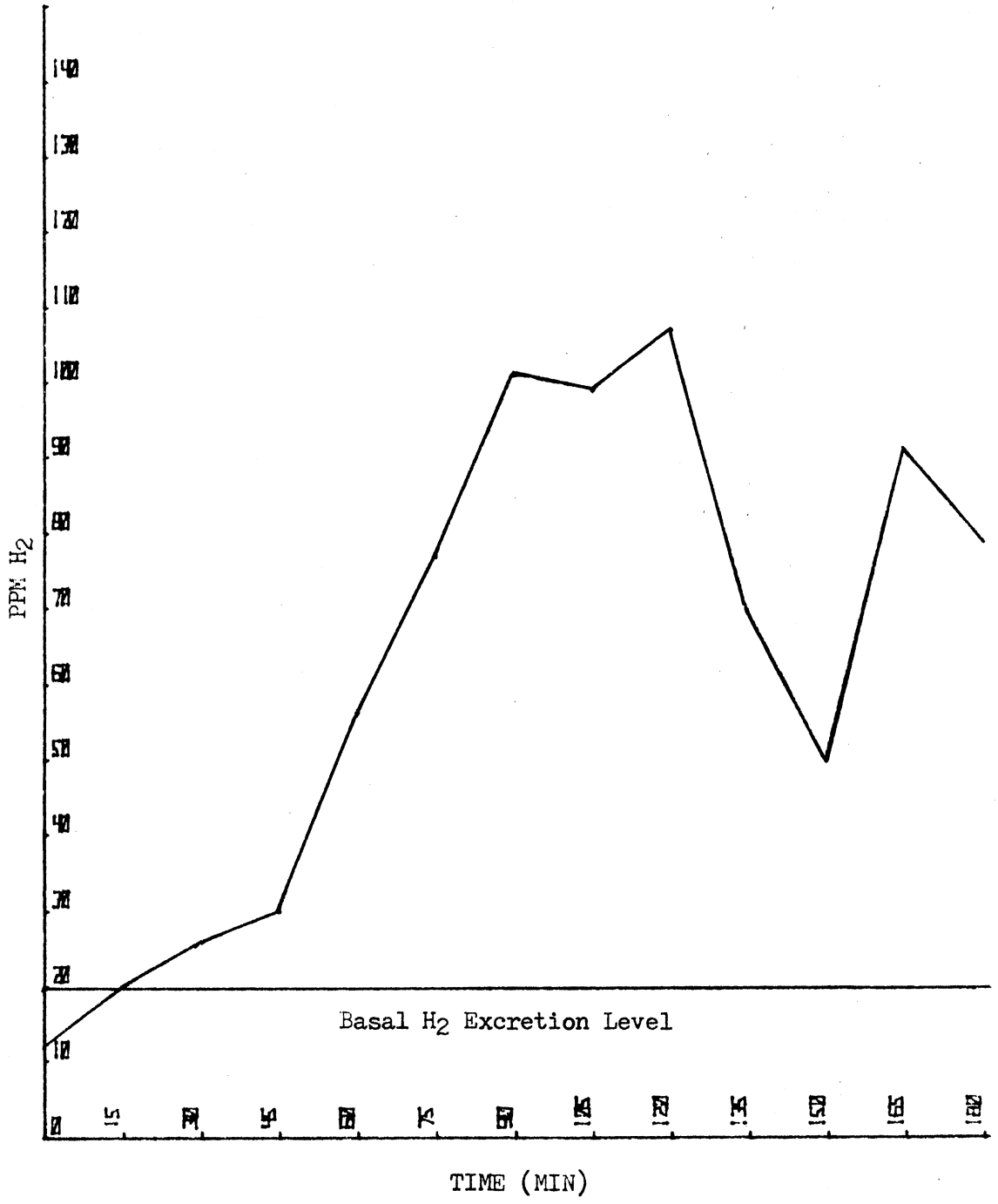

Figure 35. Subject 1--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the RM Test Meal

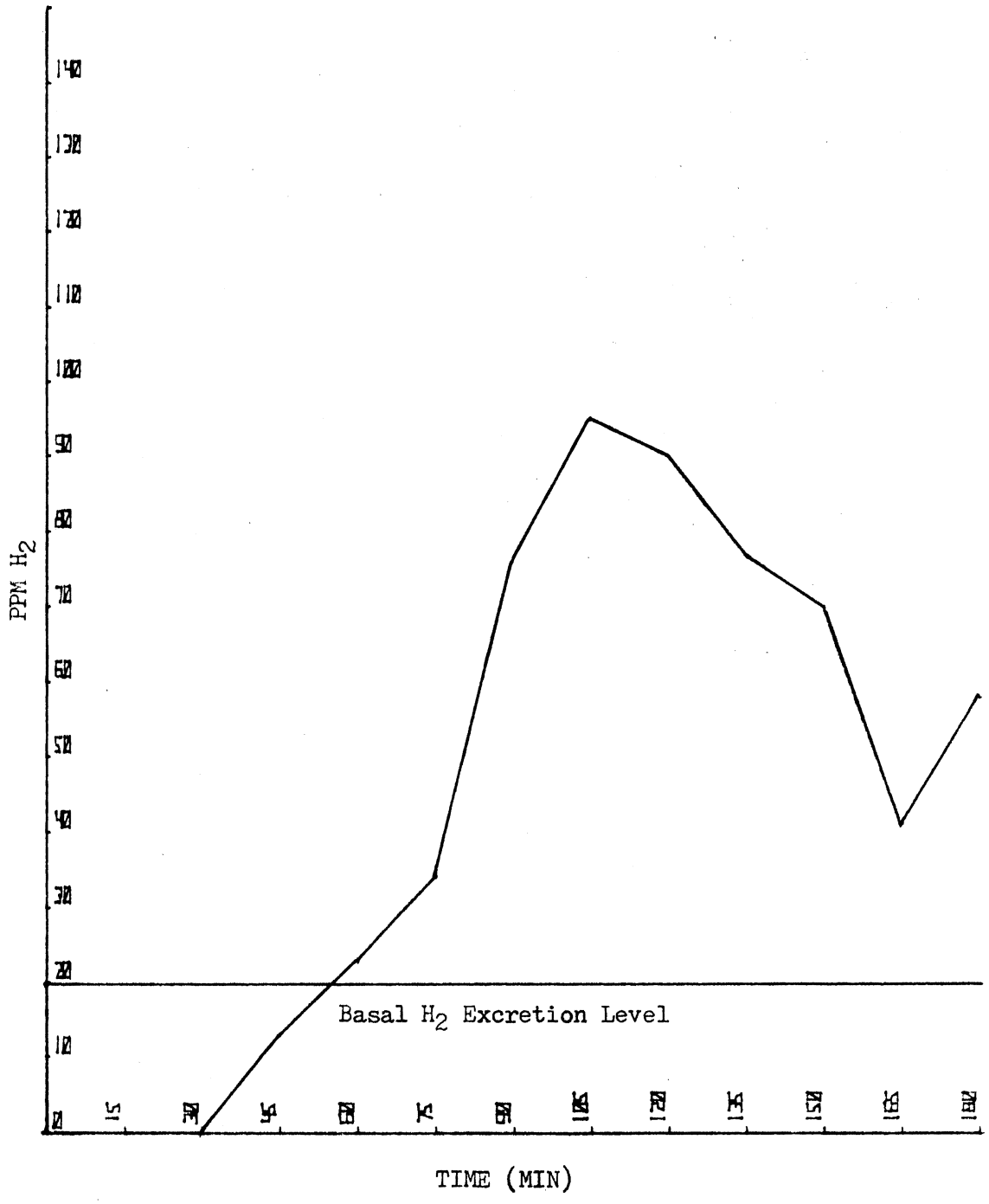

Figure 36. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the RM Test Meal

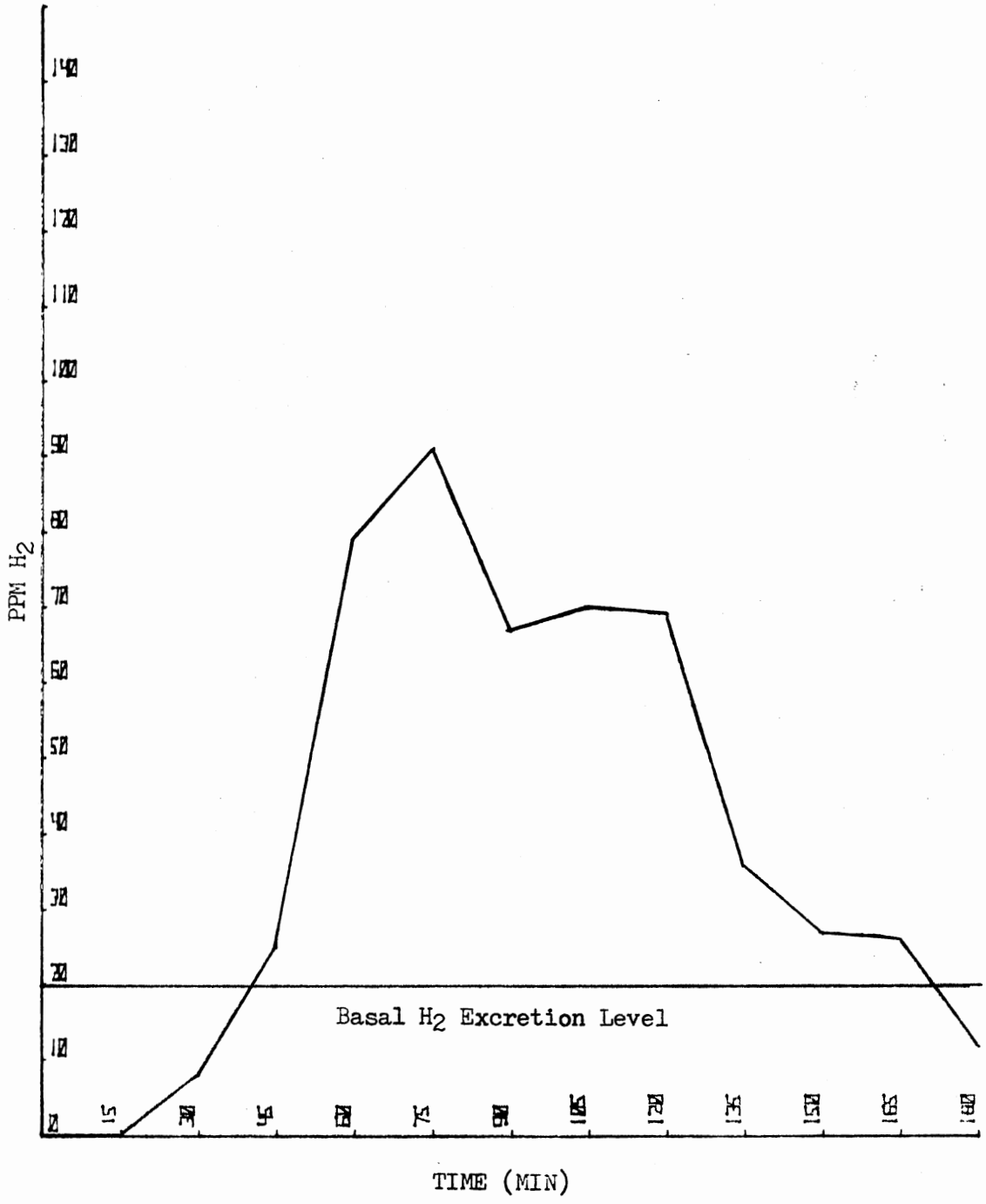

Figure 37. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Two of the RM Test Meal

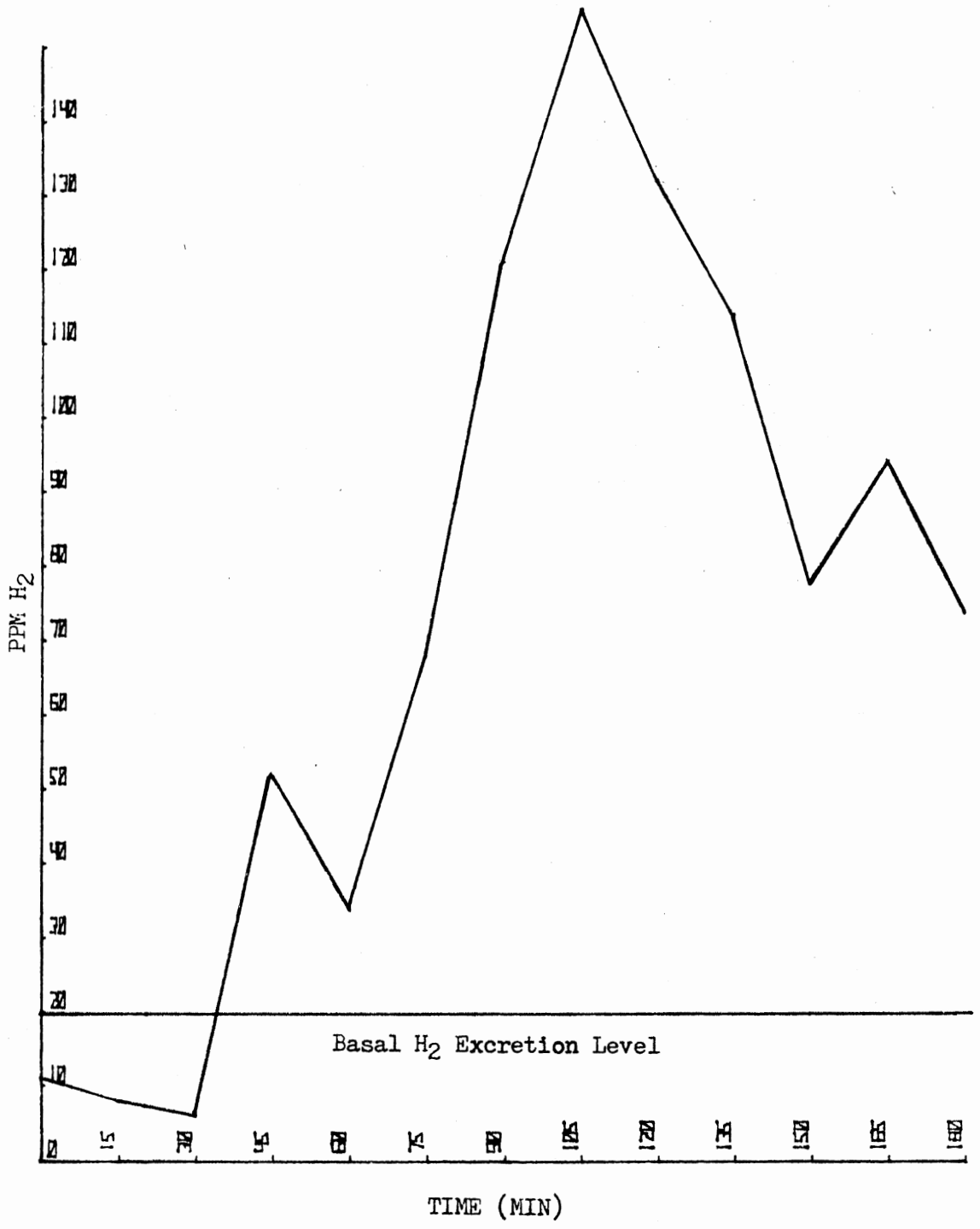

Figure 38. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Seven of the RM Test Meal

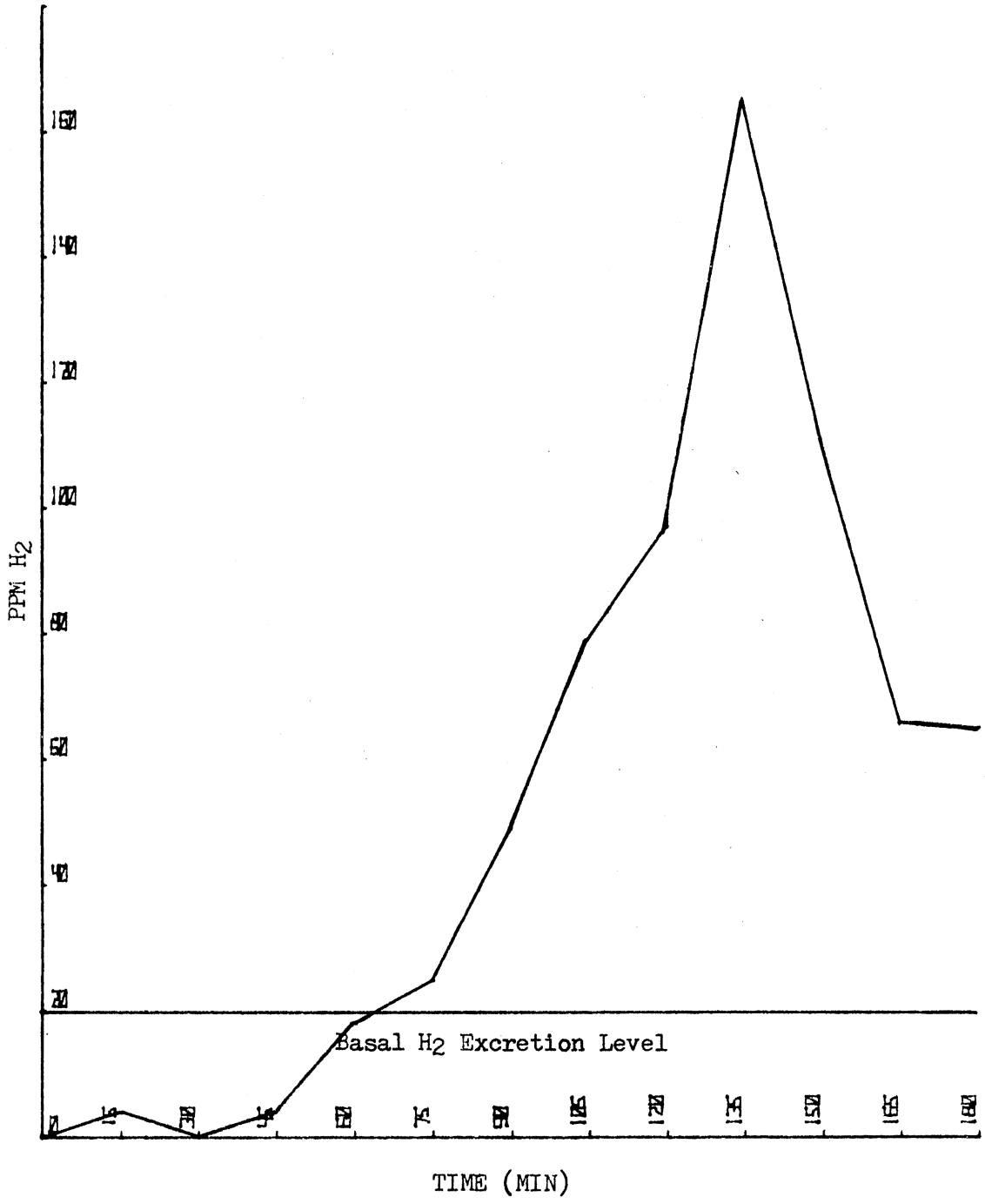

Figure 39. Subject 2--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the RM Test Meal

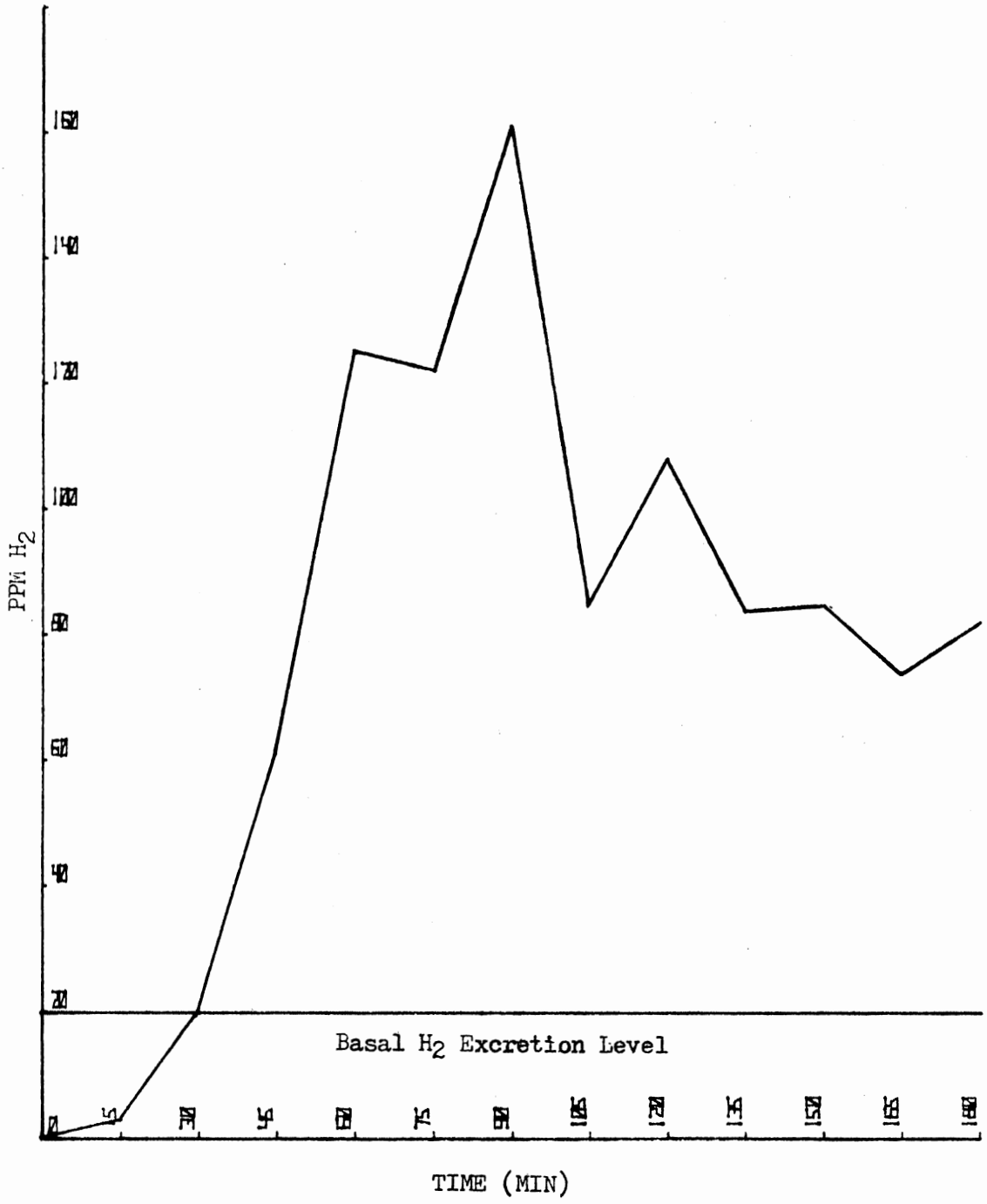

Figure 40. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the RM Test Meal

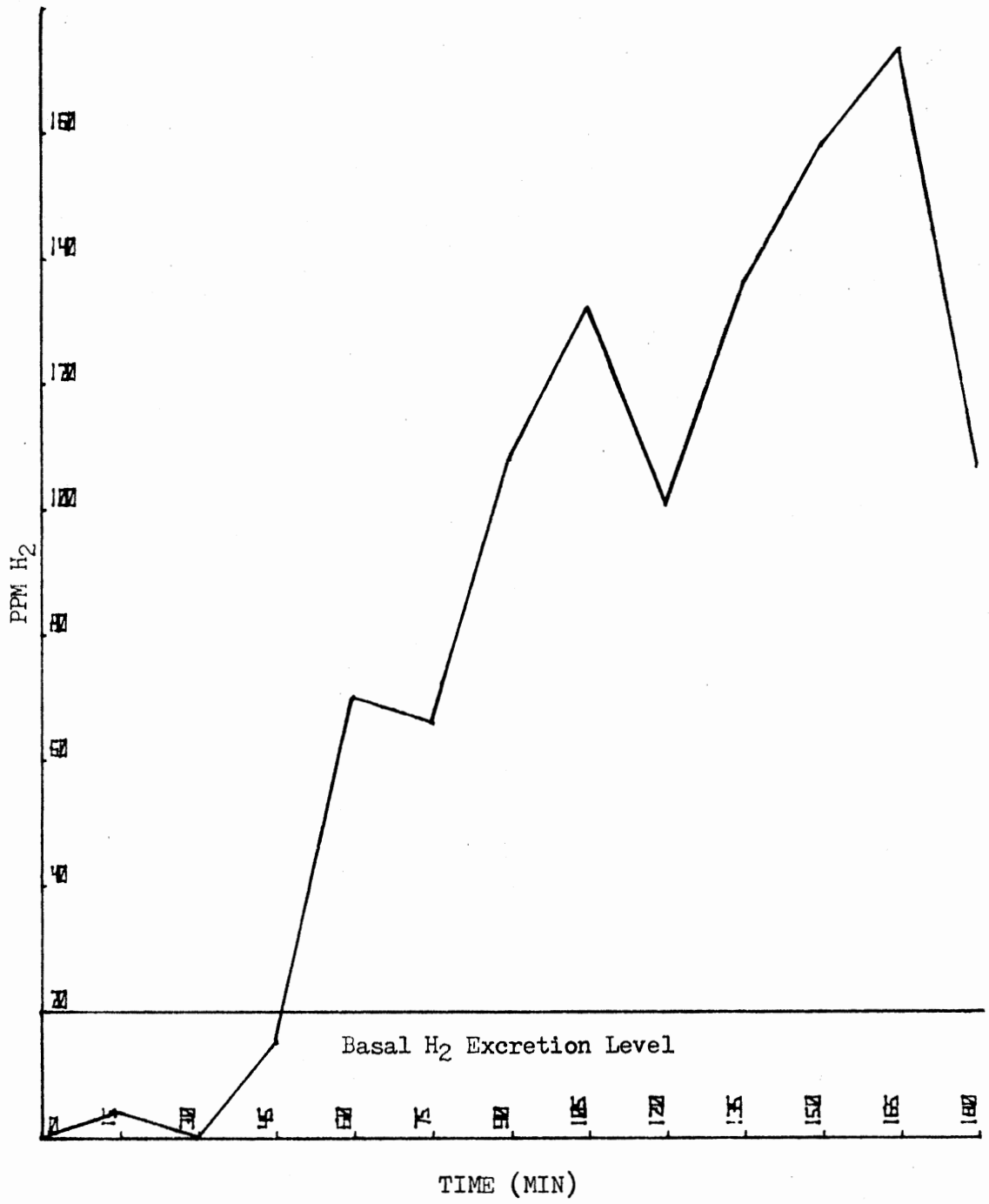

Figure 41. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Two of the RM Test Meal

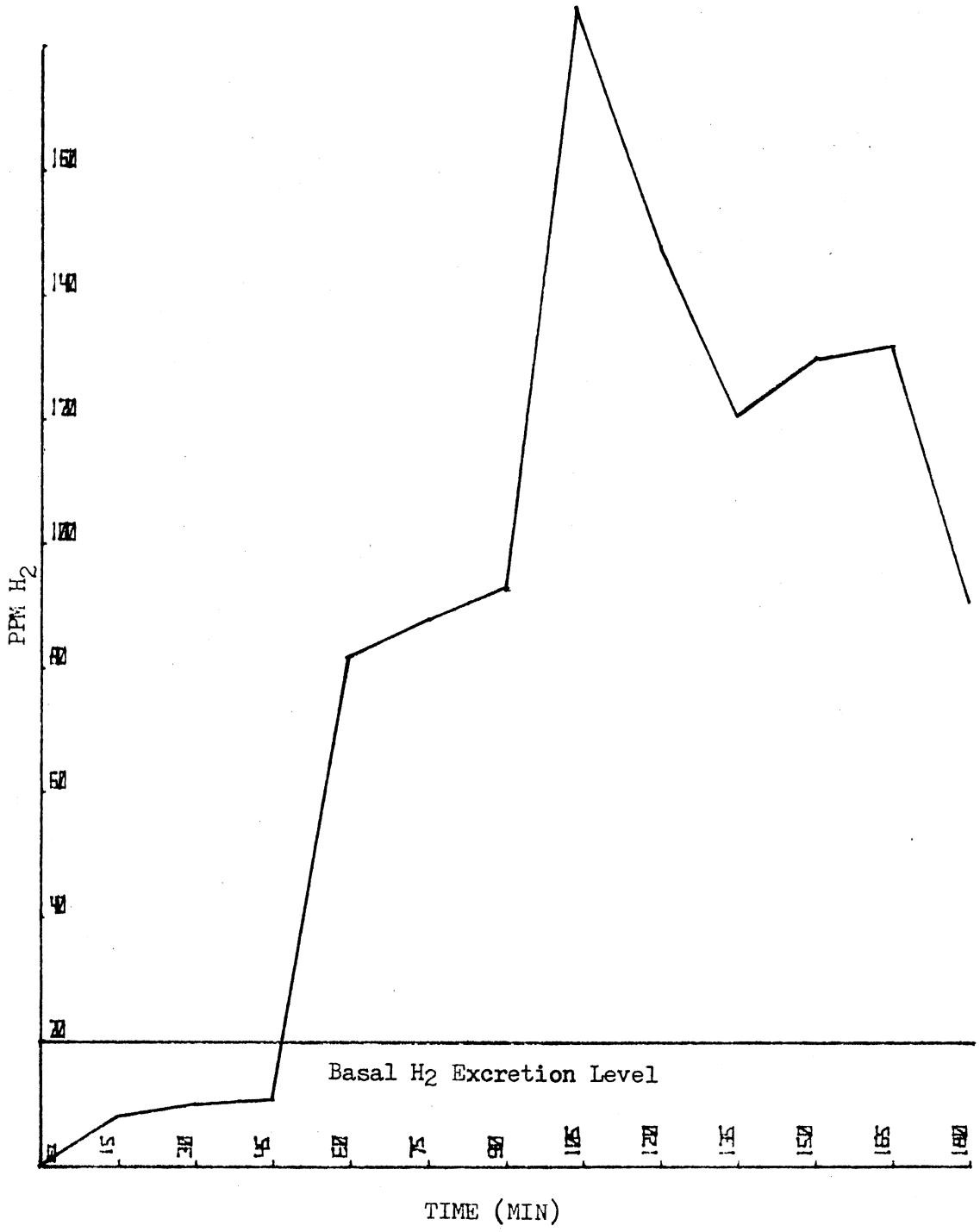

Figure 42. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Seven of the RM Test Meal

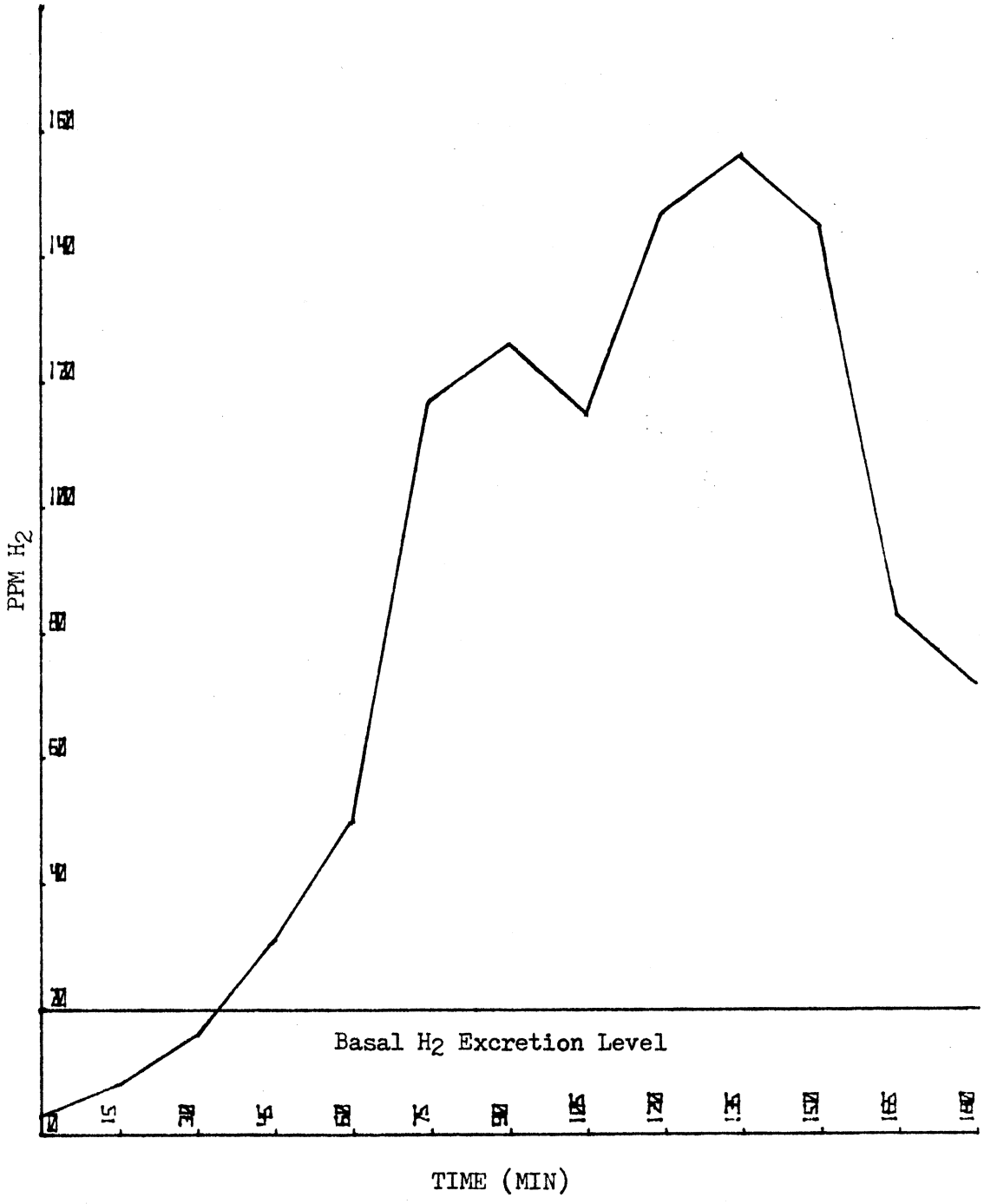

Figure 43. Subject 3--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the RM Test Meal

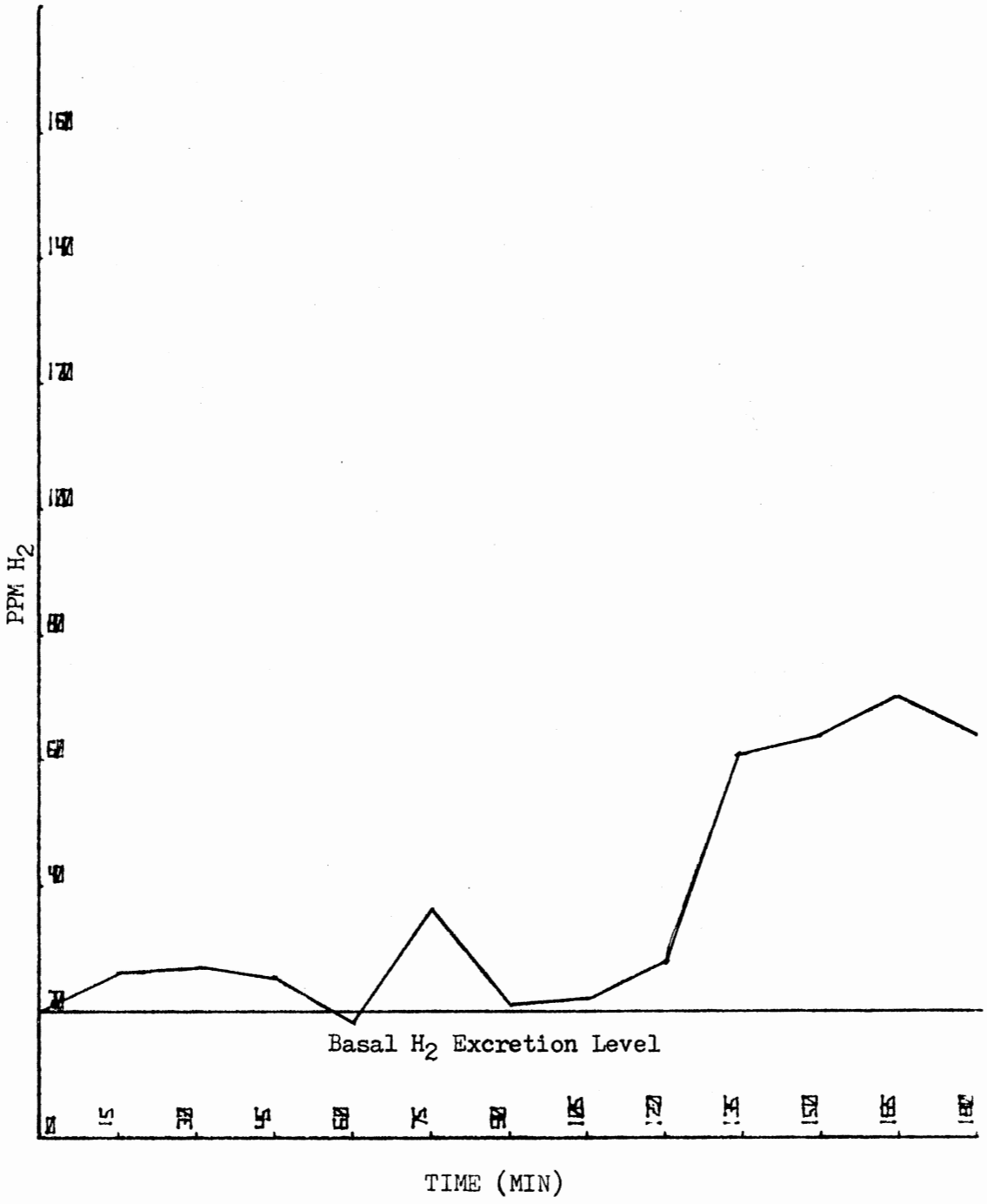

Figure 44. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the RM Test Meal

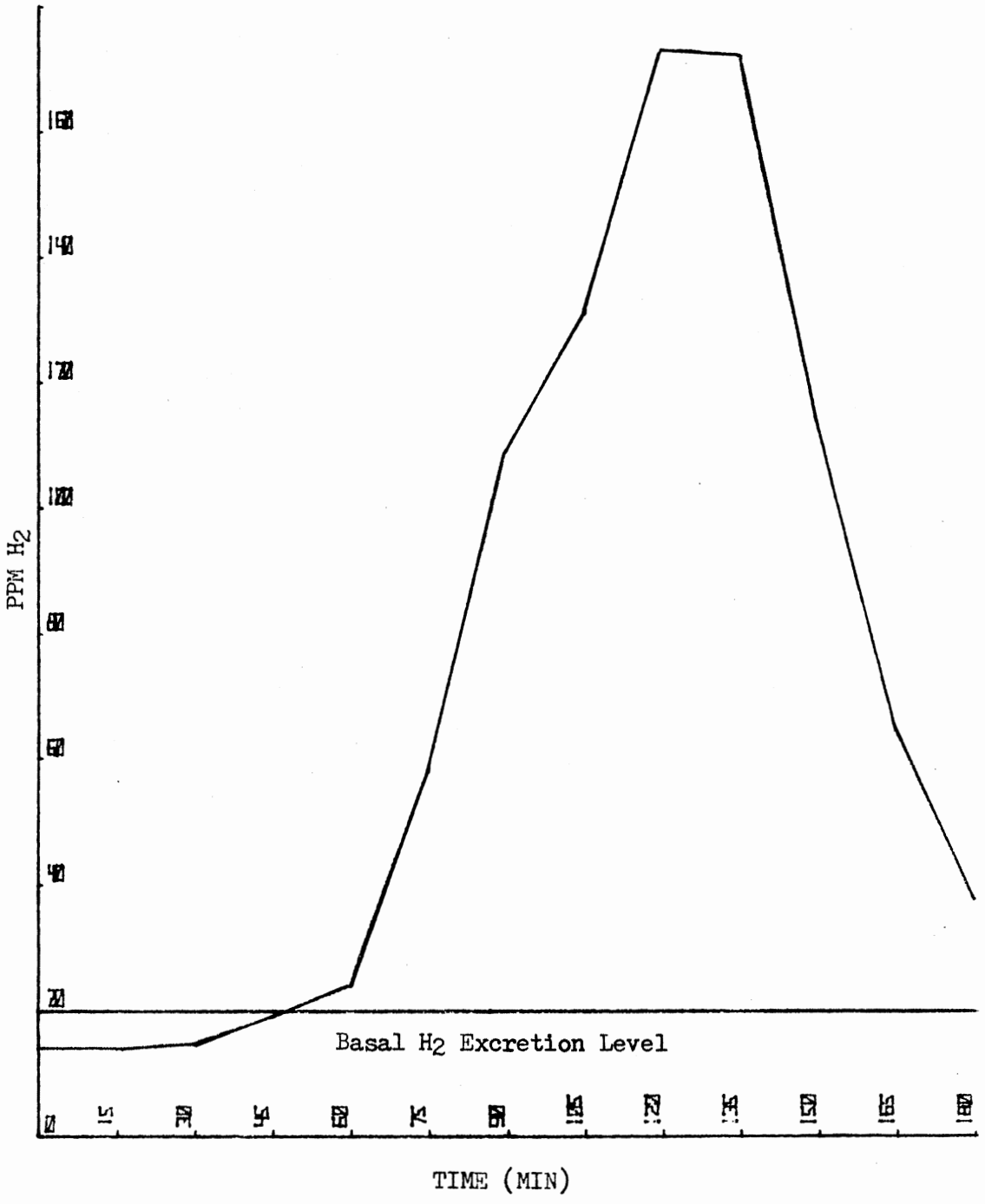

Figure 45. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Two of the RM Test Meal

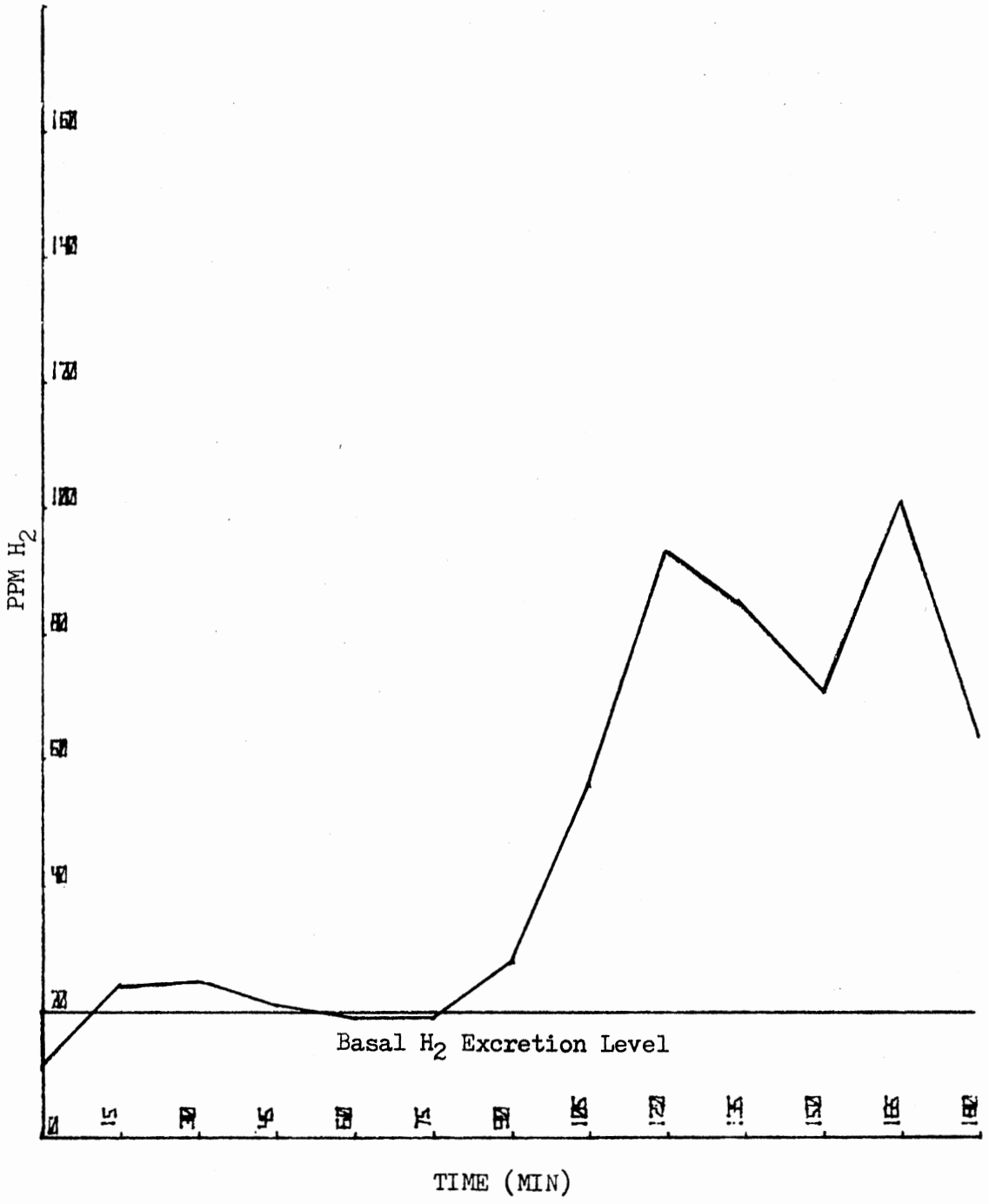

Figure 46. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Seven of the RM Test Meal

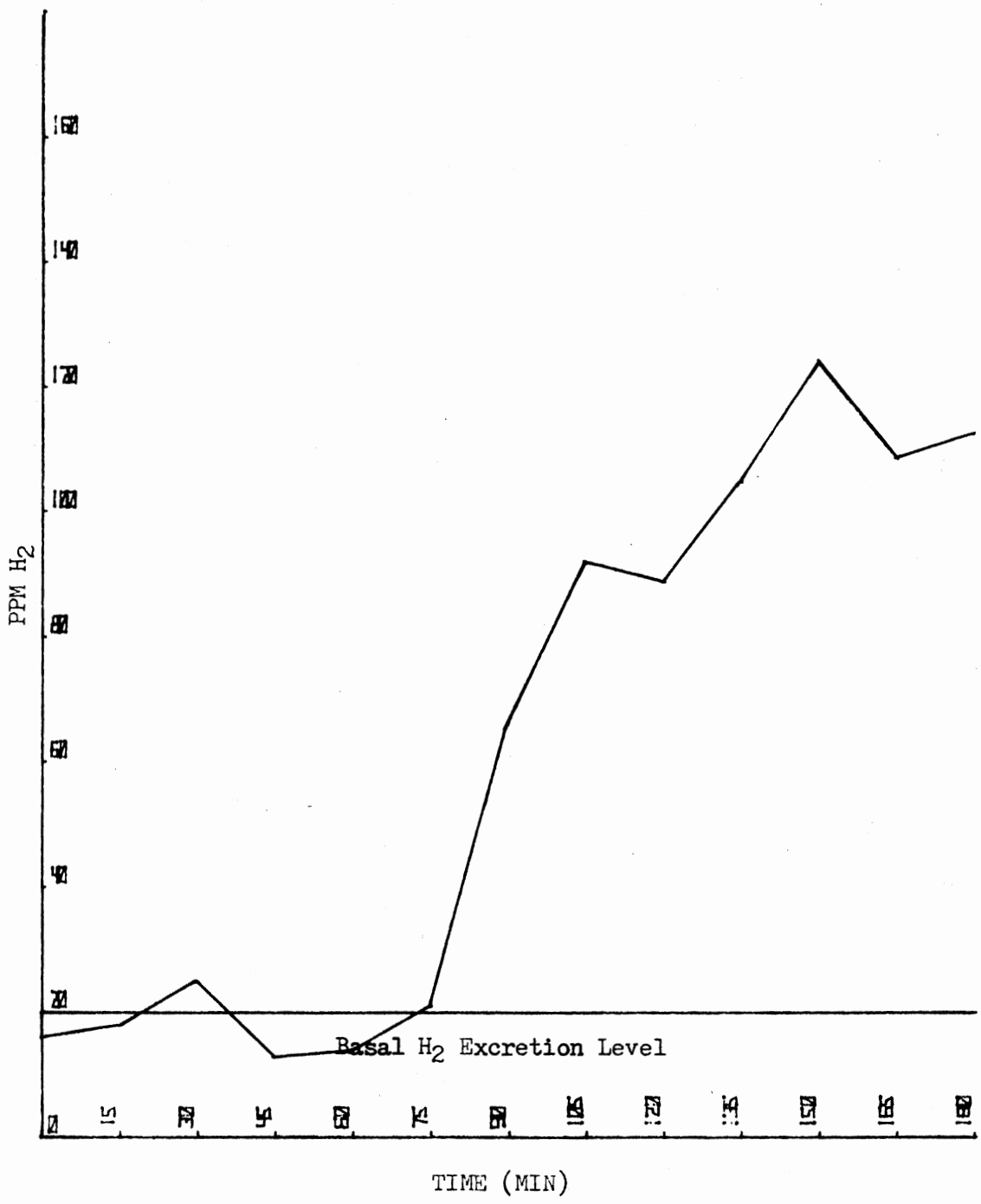

Figure 47. Subject 4--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the RM Test Meal

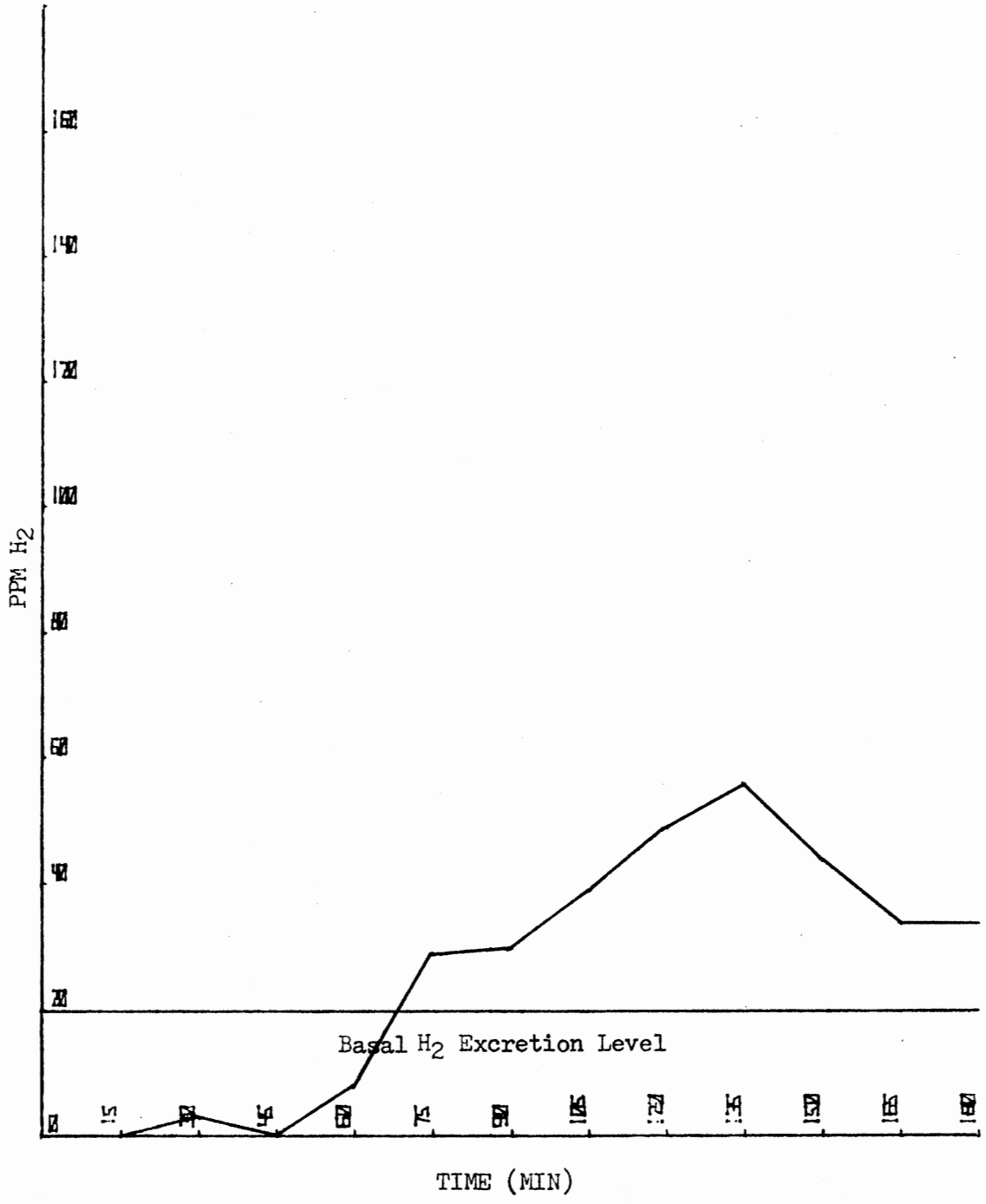

Figure 48. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day One of the RM Test Meal

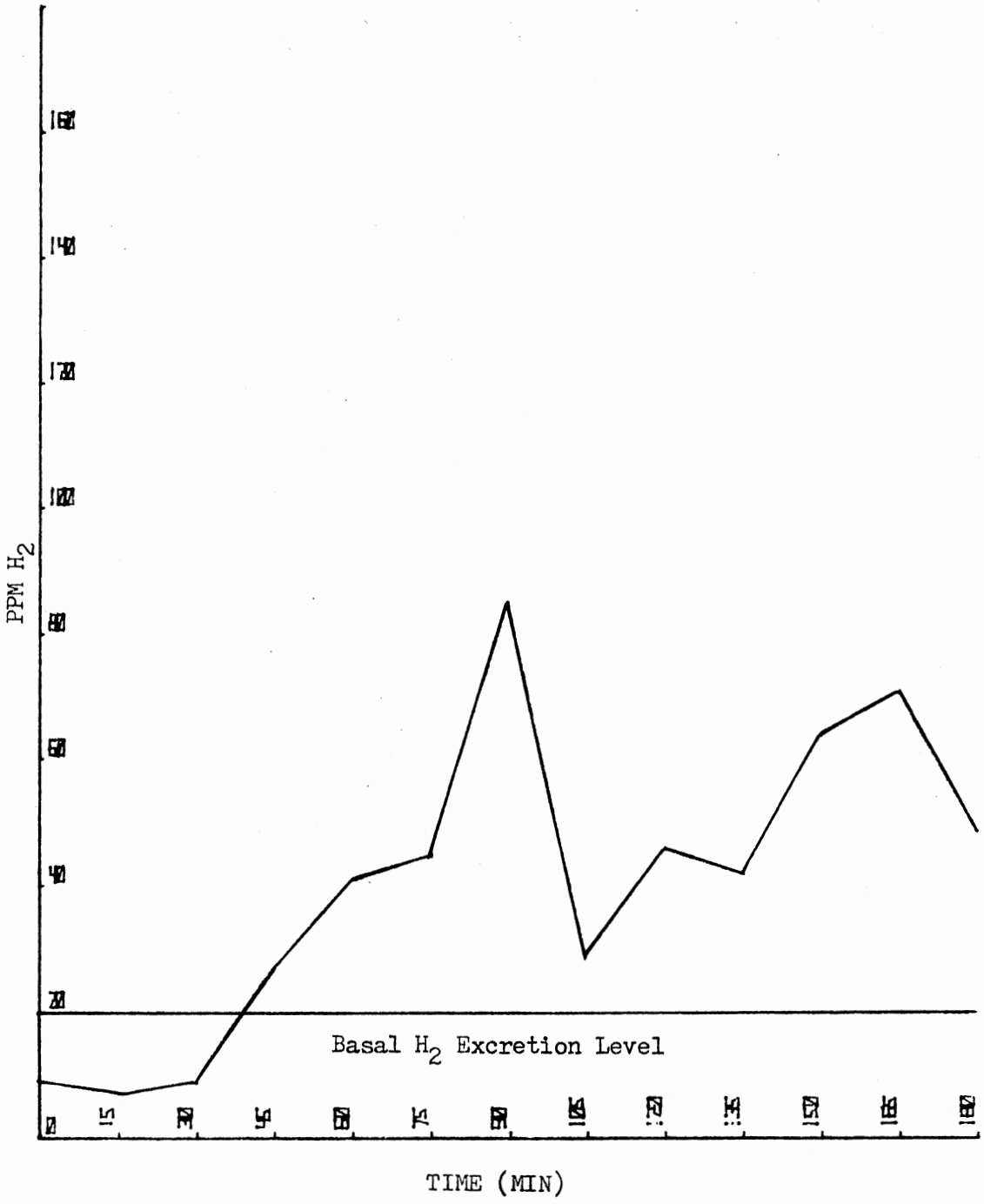

Figure 49. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Two of the RM Test Meal

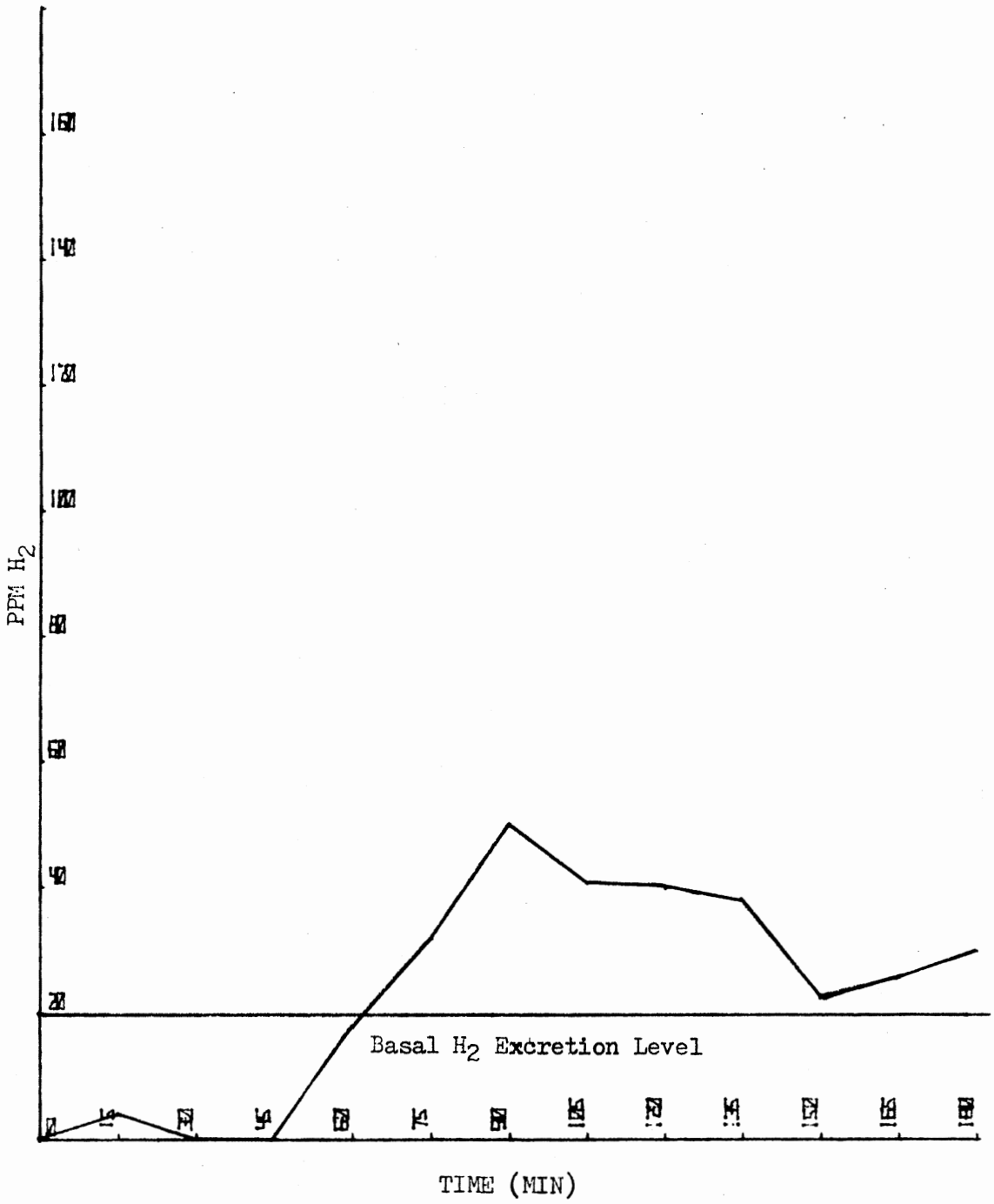

Figure 50. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Seven of the RM Test Meal

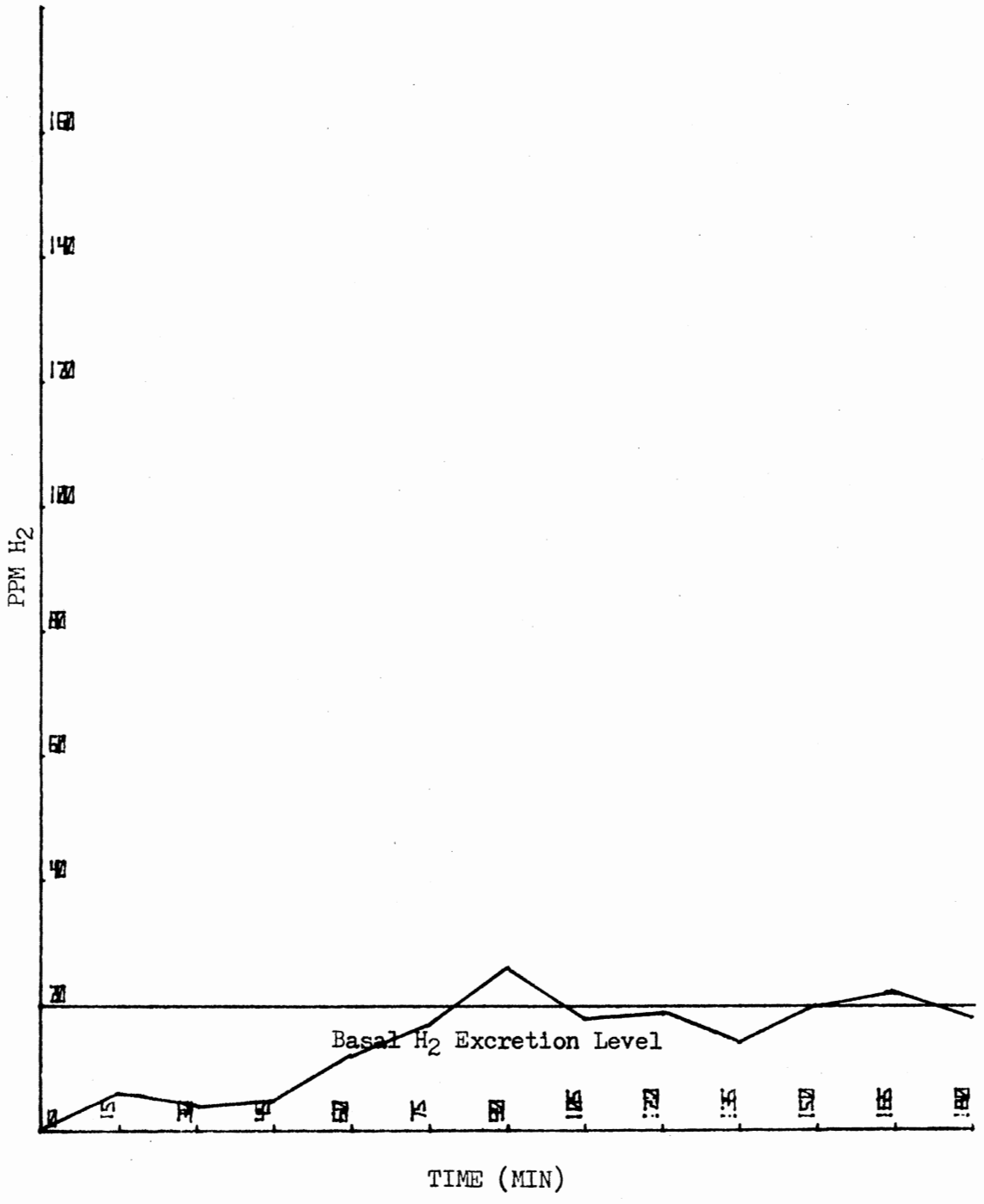

Figure 51. Subject 5--Breath H_2 Concentration Versus Time After Consumption of Regular Milk on Test Day Eight of the RM Test Meal

VITA

Mary Ann Nichols

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF A NONFERMENTED MILK INOCULATED WITH LACTOBACILLUS ACIDOPHILUS ON LACTOSE MALABSORPTION

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Professional Organizations: Institute of Food Technologists, Omicron Nu.