

WHEAT BRAN PARTICLE SIZE AND
GASTROINTESTINAL FUNCTION

BY

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Bachelor of Arts

Bethany College

Lindsborg, Kansas

1975

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1981

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PREFACE

This study concerns the effects of coarse and fine particle size wheat bran on gastrointestinal transit time. The grinding of wheat bran to a fine particle state affects many of its physical characteristics. Water-holding capacity is one such feature that is directly involved in the performance of bran in the gut. Variations in the degree of grinding affect the ability of bran to absorb water, which in turn reduces the effectiveness of bran as a bulking agent and decreases gut motility. Gas solid chromatography was employed to measure transit time.

I wish to express my sincerest appreciation to my major adviser, Dr. Donna L. Payne, for her careful direction of this study, her enthusiasm for the project, and her kind friendship. I wish also to thank Dr. E. C. Short, Dr. P. L. Claypool, and Dr. R. D. Morrison for their invaluable assistance in the preparation of the final manuscript. I am also deeply grateful to Dr. Asegash Tsegaye for her expert technical assistance with the gas chromatograph and other aspects of the study. A special friendship developed from our association. Appreciation is also expressed to Marlece Ebbesen, who helped initiate the study, and to my mother and my friends, who participated as subjects. I wish also to thank Mr. and Mrs. Jack Razook for their support and encouragement.

I wish to dedicate this thesis to my dear husband, editor, and typist, Steven, and to my mother, Kathleen. Their continued confidence in me, their understanding, and their love will never be forgotten.

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CHAPTER I

INTRODUCTION

In recent years the topic of dietary fiber has received considerable attention. It has been inconclusively associated with several and various "diseases of Western civilization," including colonic polyposis, inflammation of the colon, diverticular disease, and tumors of the colon (Burkitt, Walker, and Painter, 1974; Painter and Burkitt, 1971).

Epidemiological evidence shows that colonic disorders are of a low incidence in rural areas of Africa and other regions of the world in which high fiber diets are regularly consumed. Conversely, the occurrence of tumors of the large intestine, diverticular disease, and other disorders of the large intestine are prevalent in Western Europe and in North America where the typical intake of food is of a lower fiber content (Burkitt, 1975). Such epidemiologic evidence does not prove a cause and effect relationship, however.

Dietary fiber has been recognized for some time to act as a laxative. Modifications in the fat and protein content of the diet do not alter intestinal transit time or the characteristics of fecal material to any great degree. The fiber content of the diet, however, has been shown to produce significant changes in composition and weight of the stool and to alter total intestinal transit time. An inverse relationship exists between intestinal transit time and the daily weight of the

stool. As a result, according to Burkitt (1975),

not only do transit times increase and stool weights diminish with a reduction of dietary-fiber, but the prevalence of bowel cancer and its associated diseases is related to stool bulk and consistency, to intestinal transit time (p. 143).

Although Burkitt, a surgeon in South Africa, has brought much attention to the role of dietary fiber, many of his theories are untested and his claims unproven.

It has been shown that the resultant bulk from high fiber diets relieves symptoms of diverticular disease (Brobribb, 1977). Studies also show that when wheat bran is added to a refined diet, the transit time through the intestines of persons with slow times is decreased but is increased in persons with accelerated transit times (Harvey, Pomare, and Heaton, 1973). This phenomenon is unexplained, however.

The various types of dietary fiber have been known for some time to possess a water-holding capacity (Bastedo, 1935). This characteristic is associated with the laxative effect of fiber and its fecal bulking properties (Cummings, Hill, Jenkins, Pearson, and Wiggins, 1976). Wheat bran, however, which only holds about 3g of water per gram of fiber, has a greater effect on stool weight than other dietary fibers that retain much more water (McConnell, Eastwood, and Mitchell, 1974).

Wheat bran is a rather dense material and holds less water than does dried vegetable fiber, yet many studies showed that the addition of 30 to 40g of dry wheat bran to the daily human diet will increase fecal wet weights by about 60 percent (Mendeloff, 1976, pp. 396-397).

The effect of different particle sizes on the water-holding capacity of various fibers has also been examined (Stephen and Cummings, 1979). Heller, Hackler, Roe, Lewis, and Robertson (1980) recently compared the mean transit times for a coarse bran diet and a fine bran

diet ingested by young adult males. The researchers concluded that "the ingestion of coarse bran results in a faster rate of passage of digesta through the gastrointestinal tract than does eating an equal amount of fine bran" (p. 1739).

Several methods have been used to measure intestinal transit time. Among these is the use of polyethylene glycol and barium impregnated radio-opaque pellets as markers. The low recovery rate of the markers because of their absorption to bran fiber and of their incomplete ingestion may cause obstacles in evaluating results (Heller et al., 1980).

Another measuring tool, the breath hydrogen (H_2) test, has been shown to be a useful device in diagnosing lactose or sucrose malabsorption (Bose and Welsh, 1977; Metz, Jenkins, Peters, Newman, and Blendis, 1975; Calloway, Murphy, and Bauer, 1969; Metz, Jenkins, Newman, and Blendis, 1976). Levitt (1969) reported that the colon is the major site of H_2 production in man. Bacteria normal to the gut ferment unabsorbed carbohydrate when it reaches the colon. Hydrogen, a product that results from this fermentation, is absorbed through the intestinal mucosa, then passes into the blood stream, and finally is excreted in the expired air (Levitt, 1969).

Levitt and Donaldson (1970) measured respiratory H_2 excretion in which a rebreathing technique was employed to detect carbohydrate malabsorption. Later, Payne-Bose, Tsegaye, Morrison, and Waller (1978) demonstrated that carbohydrate malabsorption can be assessed when the hydrogen content of whole human breath is determined by gas chromatographic analysis using a thermal conductivity detector and an electronic peak integrator.

Various researchers have expressed the need for greater research to characterize the effects of fiber particle size on bowel function and to explore the use of the breath H₂ test as an accurate index of these effects on mouth-to-cecum transit time. In this regard Albert I. Mendeloff (1978) recently acknowledged that "the importance of following the metabolism of fiber in man by use of breath testing for hydrogen and other gases has not yet been assessed" (p. S147). He further noted that

it appears valuable to establish the time required for known ingested fiber to reach the colon, and to indicate, in general terms, which fibers seem to be metabolized by colonic bacteria. Further work in this area needs to be encouraged (p. S147).

Justification of Thesis Study

The purpose of this study was to determine whether particle size of wheat bran has an effect on intestinal transit time to the cecum. Grinding of wheat bran to a fine particle size affects many of its physical characteristics. Water-holding capacity is one such feature that is directly involved in the performance of bran in the gut. Variations in the degree of grinding may affect the ability of bran to adsorb water, which in turn reduces the effectiveness of bran as a bulking agent and decreases gut motility.

Hypothesis

The study tested the hypothesis that the processing of wheat bran by grinding does have a significant effect on mouth-to-cecum transit time as defined by breath hydrogen response.

Justification of Method

The following theories have been thoroughly researched. They form the basis of this study.

1. Wheat bran is a fermentable substrate for colonic bacteria (Vercellotti, Salyers, and Wilkins, 1978).
2. Lactulose is a fermentable, but completely non-absorbable, carbohydrate (Gryboski and Boehm, 1965; Dahlqvist and Gryboski, 1965; Müller, Walker-Smith, Shmerling, Curtius, and Prader, 1969).
3. Hydrogen is a product of carbohydrate fermentation by colonic bacteria, and respiratory hydrogen concentration is directly related to colonic hydrogen concentration (Levitt, 1969).
4. There is a minimal period of time between production of colonic hydrogen from fermentation and the appearance of hydrogen in the breath; therefore, intestinal transit time to the cecum can be measured through the use of the breath hydrogen test (Bond and Levitt, 1978; Bond and Levitt, 1972).

In this study a comparison is made between the mouth-to-cecum transit time of coarse bran and finely ground bran. The breath hydrogen test, which has been shown to be effective in indicating carbohydrate malabsorption, is used to determine when the bolus of undigested fiber reaches the cecum and is fermented by bacteria in that portion of the intestine. Lactulose is combined with bran in the first series of tests as an unabsorbable marker to aid in raising the breath hydrogen response to levels that are clearly indicative of the transit time. Lactulose is omitted from the second series of tests to show the breath

hydrogen response for only bran meals.

Assumptions

The following assumptions applied to the study.

1. Test meals were measured accurately and baked, frozen, and reheated identically to minimize any variations due to preparation.
2. The components of the test meals (i.e., bran and lactulose) moved through the gastrointestinal tract at the same time.
3. The subjects followed the researcher's instructions for fasting and for excluding foods that would influence colonic H₂ production.
4. Any rise in breath hydrogen concentration during testing was not influenced by previous meals eaten by subjects.
5. The minimal activities of the subjects before and during testing did not influence the breath hydrogen response.

Definition of Terms

The following definitions were significant to the study.

1. Breath hydrogen test: "The hydrogen content of whole human breath (non-concentrated or end-expiratory samples), . . . can be determined accurately by gas chromatographic analysis" (Payne-Bose et al., 1978, p. 659).
2. Dietary fiber: Unavailable carbohydrates that are resistant to animal digestion and that pass to the lower intestine where they are subject to fermentation by the intestinal microflora (Van Soest, 1978).

3. Lactulose: "a galactose-fructose disaccharide (4-~~O~~- β -D-galactopyranosyl-D-fructose), cannot be hydrolyzed by the human intestinal mucosa" (Müller et al., 1969, p. 45).
4. Unprocessed wheat bran: "The bran layer of the wheat kernel which has not undergone bleaching, drying, pressing, or other processing" (Smith, 1978, p. 4).

CHAPTER II

REVIEW OF LITERATURE

Since early times it has been observed that fiber, and in particular wheat bran fiber, acts as a laxative in altering bowel function. Hippocrates, the ancient Greek physician, was well aware of the value of increased fiber content in the diet for the treatment of constipation (Boorde, 1567). In his writings on medicine and health, Hippocrates discussed the benefits of a high fiber regimen in the diet:

To the human body it makes a great difference whether the bread be fine or coarse; of wheat with or without the hull, whether mixed with much or little water. . . . For, by everyone of these things a man is affected and changed this way or that . . . (Runes and Kiernan, eds., 1964, p. 9).

Trowell (1978) briefly outlined a history of the fluctuating interest since the age of Hippocrates in the merits of dietary fiber. He noted that during the past 150 years much research on the topic of wheat bran fiber has been undertaken. The stimulus for the research is attributed to the advent in eighteenth century France of a high-grinding system of milling that produced refined low-fiber white bread (McCance and Widdowson, 1956).

Kent (1966) held that the purpose of such a system of white flour milling was

to make as completely as possible a separation of the endosperm from the bran and germ, so that the flour shall be free from bran specks and of good colour, and so that the palatability and digestibility of the product shall be

improved and its storage life lengthened (p. 114).

Although the merits of refined low-fiber white wheat flour are debatable, medical opinion in the eighteenth and nineteenth centuries generally endorsed the refinement of white wheat flour. However, a few recognized authorities, including Graham (1839) and Allinson (1885), advocated a healthful reduction in the use of refined foods. In 1921 a Michigan physician and surgeon, J. H. Kellogg, and his colleague, university professor M. V. O'Shea (1921), extolled the value of "grain foods" as "bone-building material" (p. 154). Since that time much additional research has been undertaken to assess the role of the high-fiber diet in the treatment of various disease states. J. H. Cummings (1978) noted that sufficient preliminary evidence was at hand "to suggest that fiber has important nutritional implications in addition to its already well-known gastroenterological effects" (p. S27). A year earlier, the United States Senate Select Committee Report on Dietary Goals (1977) recommended that starchy foods from high-fiber whole grain products be increased as a source of carbohydrate in the diet. The report by Senator George McGovern's committee (1977) stated that

an increase in fiber consumption, preferably natural fiber rather than fiber added to refined products such as white bread, will markedly reduce the incidence of bowel cancer and other diseases, primarily those of the intestine (p. 15).

Although it does not appear to be scientifically based, the latter statement does demonstrate the interest focused on dietary fiber.

Dietary Fiber in the Gastrointestinal Tract
and Its Relationship to Certain
Non-infective Diseases

Until recently the importance of dietary fiber as a nutritionally valuable component of food has received scant attention. The topic of fiber as a dietary constituent has been virtually overlooked or ignored because of its seemingly minimal contribution to nutrition, such as minute levels of absorbable energy (Southgate, 1973), and its role in providing "merely bulk to the stool and laxation to the bowels" (Trowell, 1976, p. 418). Burkitt, Walker, and Painter (1974) suggested that "its nature has been misunderstood and its important role in maintaining gastrointestinal function has not been appreciated" (p. 1070). J. H. Cummings (1978) further stated:

In addition to the increasingly well documented effects fiber has on colonic function, it is now clear that fiber is a nutrient of importance in its own right. The amount and type of fiber included in the diet may have significant nutritional implications (p. S21).

The authors cited above and elsewhere in this review are well recognized for their research on dietary fiber. It should be remembered, however, that some of the benefits and effects that they attribute to dietary fiber are premature and are not supported by research. It does appear that printed space devoted to theorization is abundant in the area of dietary fiber and its physiological benefits.

Van Soest (1978) briefly outlined those qualities of dietary fiber that are of principle importance to gastrointestinal function. He wrote that "the properties of fiber that are of nutritional significance include bulk density, hydration capacity, binding properties, and fermentability" (p. S16).

It is commonly recognized that the inclusion of fiber in the diet has the pronounced effect of increasing fecal weight (Connell, 1975; Mendeloff, 1976). Taylor and Duthie (1976) showed a significant increase in the weight of the stool when patients were fed bulk laxative and bran tablets. A three-fold increase in fecal weight was demonstrated by Cummings et al. (1976) when they increased the daily fiber intake of subjects from 17g fiber in a "Western" diet to 45g with the addition of Allinson's "Bran Plus" and substituted whole meal and bran products. The researchers attributed the increase in fecal weight to water. Other investigators, including Burkitt, Walker, and Painter (1974), similarly demonstrated that dietary fiber has an effect on fecal bulk. Differences in fecal weight measurements may be due to methods used to measure fecal materials, test diets, and individual responses.

As well as its stool bulking properties, "there is little doubt that the water content of human stools can be increased and that transit time can be decreased by the addition of certain natural fibers to the diet" (Connell, 1978, p. S152). It is generally held that the ability of fiber to retain water leads to an increase in fecal bulk (McConnell, Eastwood, and Mitchell, 1974). Eastwood (1978) presented a description of the water-holding capacity of fiber and its resultant bulk formation.

A tentative logic for the behavior of fiber can be determined by regarding it as a physical agent. Water is held in the fiber as surface water, and interstitial and free water are present. If bran or cellulose is added to a normal diet at 16g./day, stool weight will nearly double because of water being held in the stool (p. S31).

Mitchell and Eastwood (1976) offered an explanation of the mechanism by which water is held in the fibrous network.

Although vegetable and cereal fibers are known to absorb water, the constituents of fiber responsible for this have not been conclusively identified. It is probable that the water

holding property of fiber is due to the polysaccharide content. These compounds also have a cation-exchange capacity and will therefore adsorb sodium, potassium, calcium, and all positively charged elements. It is possible therefore that water will be adsorbed directly to the fiber and also indirectly as a result of the adsorption of sodium to the various vegetable dietary fibers (p. 190).

Mendeloff (1976) further elaborated on the subject.

The swelling of fibers with water creates networks trapping electrolytes and organic acids in the right colon. Presumably the fiber network creates electrical charges which influence further imbibition of water (p. 396).

Burkitt, Walker, and Painter (1972) found that an inverse relationship exists between stool weights and transit time and fiber intake and transit time. Various studies have shown that fiber decreases total gastrointestinal transit time (Bond and Levitt, 1978; Kirwin, Smith, McConnell, Mitchell, and Eastwood, 1974; Findley, Smith, Mitchell, Anderson, and Eastwood, 1974).

It is believed that dietary fiber may alter the specific gravity of colonic contents, thus affecting transit time. The addition of fiber to the diet and its resultant uptake of water tends to minimize the streaming effect of particles and of water passing through the gastrointestinal tract at different rates of time (Kirwin and Smith, 1974; Findley et al., 1974). The reduction of streaming and the production of a more bulky and watery fecal material enables the muscles of the colon to eliminate the feces much easier and with less straining (Mitchell and Eastwood, 1976).

Mitchell and Eastwood (1976) also mentioned that "the bulking action of dietary fiber will have the added effect of diluting colonic contents" (p. 190). In a similar study Cummings et al. (1976) researched the effect of wheat fiber on colonic function and concluded.

The increase in fecal bulk suggests that colonic

contents are diluted by fiber. The concentration of specific substances in colonic contents is difficult to measure but marker concentration in the stool in this study was significantly diluted by fiber. At equilibrium during the control period there were 19.0 ± 1.5 markers per 100g. of stool while during the fiber period 6.6 ± 0.6 a dilution of 3-fold. How far this reflects dilution of actual colonic contents is uncertain but it seems likely that at least the contents of the left colon are diluted by fiber. . . .

In this study wheat fiber has been shown to affect colonic function in a way that might be important in protecting individuals against colon cancer. Fecal output was increased and an inert marker was significantly diluted by the feces. Transit time was shortened and there was a diluting effect on bile acids although overall output was increased (p. 1472).

Kritchevsky, Tepper, and Story (1975) suggested a mechanism by which fiber is involved in the binding and excretion of bile acids. Eastwood (1978) also reported that unconjugated secondary bile acids found in the colon can be adsorbed onto dietary fiber. It is believed that dietary fiber can affect colonic function and "the colonic ecosystem" by carrying fecal components and gut secretions and by diluting the products of metabolism by gut microflora (e.g., volatile fatty acids) (Spiller, 1978).

Van Soest (1978) held that the most significant function fiber performs in the gastrointestinal tract is its ability to act as a substrate for fermentation by gut microflora. Salyers, Palmer, and Balascio reported in 1979 that

many of the bacteria in the colon require a fermentable carbohydrate for growth. Since mono- and di-saccharides are absorbed efficiently from any material that passes through the small intestine, it is likely that saccharolytic colon bacteria are forced to obtain carbon and energy from polysaccharides (p. 195).

The environment of the colon is thus established by the microbial fermentation of polysaccharides and the resultant products of this fermentation (Van Soest, 1978).

Eastwood (1975) stated that a complex interrelationship exists between fiber, gut microflora, and bile. "Bacteria metabolize both fiber and bile, and bile influences bacterial metabolism" (p. 22). Such an interrelationship could potentially influence water distribution, transit time, fecal concentration, and a realm of factors related to bowel function.

It is evident that much of the information obtained from research on the function of fiber in the gastrointestinal tract remains in the embryonic stage. This is also the case for current information concerning the relief high fiber diets provide in various non-infective disease states. Much of the evidence of a relationship between dietary fiber and the colonic and metabolic diseases of Western civilization is epidemiologic and comes primarily from data accumulated in regions of Africa and, more recently, from the Middle East, India, and Pakistan. Burkitt suggested a sequence of events that lead to development of such colonic disorders as constipation, diverticular disease, and hemorrhoids and metabolic diseases, including diabetes mellitus, obesity, ischemic heart disease, and gallstones (Burkitt and Trowell, eds., 1975).

In direct contrast to the high fiber diets of Africa and the Middle East, the foregoing diseases abound in affluent regions of the industrialized world where diets of refined foods with a lower fiber content are regularly consumed. In the first stage of the evolution of non-infective diseases, according to Burkitt, the aforementioned diseases rarely occur because the diet in a primitive, undeveloped area mainly consists of plant food that contains significant amounts of unprocessed carbohydrate. The second stage commences with the westernization of the diets. Obesity and diabetes begin to appear among the more prosperous

classes that can afford expensive, highly-processed foods. In the third period of the process, as the effects of Western industrialization spread, more of the local population purchase refined grains and cereals. As a result of these moderately-westernized diets, constipation, hemorrhoids, varicose veins, and appendicitis begin to appear as common clinical ailments. Finally, in the fourth stage, that of an advanced westernization of local diets, hiatus hernia, ischemic heart disease, diverticular disease, and cancer of the colon become prevalent conditions (Burkitt and Trowell, eds., 1975).

Trowell listed 40 Western-risk diseases in 1978 that may be linked to fiber-depleted diets. In an earlier study (1960) he noted that among these non-infective diseases are eight colonic disorders for which fiber might serve as a protective factor: constipation, diverticular disease, irritable colon, hemorrhoids, ulcerative colitis, appendicitis, polyps, and cancer of the large bowel.

Of these afflictions diverticular disease of the colon is the only non-infective disorder known to be directly related to fiber-depleted diets. It is "a deficiency disease caused by a lack of fiber in the diet, the increased consumption of refined carbohydrate foods" and, moreover, results in small firm stools and prolonged transit times (Burkitt, Walker, and Painter, 1974, p. 1071). The passage of such stools along the colon produces increased luminal pressures that in turn cause areas of weakness in the colon to protrude and form small pockets of diverticula. The authors appear to imply that lack of dietary fiber is the only cause of diverticular disease, which is certainly a debatable assumption.

Low residue diets have recently been contraindicated in patients

suffering from diverticular disease (Painter, 1975; Plumly and Francis, 1973). Treatment of the ailment with a high fiber diet has been shown to provide symptomatic relief, which increases in effectiveness with time (Painter, Almeida, and Colebourne, 1972; Plumly and Francis, 1973; Brodribb and Humphreys, 1976; Brodribb, 1977). Brodribb and Humphreys (1976) in their study of the treatment of diverticular disease stated that

treatment with cereal fibre not only provided good symptomatic relief in patients with complicated diverticular disease but also improved colonic function by increasing stool weight, altering transit time, and reducing the abnormally high intraluminal pressure within the bowel (p. 427).

They further concluded that "increasing the fibre intake does have a genuine physiological action in slowing fast transit times and accelerating slow transit times in patients with diverticular disease" (p. 427).

Although diverticular disease is well-known, it is not unique clinical manifestation of colonic muscle spasm. Other diseases associated with extreme contraction of the smooth muscle of the colon and high luminal pressures include irritable bowel syndrome and ulcerative colitis (Grimes, 1976). Gall bladder disease and hiatus hernia are also commonly associated with diverticular disease.

Various studies have also indicated that an association exists between diverticular disease and varicose veins. Burkitt, Walker, and Painter (1974) suggested that "some causative factor is common to each" (p. 1072). Moreover, they believed that "a possible mechanism is that intra-abdominal pressure, raised unnaturally when straining at the stool, could well damage the proximal valves of the leg veins" (pp. 1072-1073). Of course it should also have been suggested that the

causative factor may not be related to dietary fiber intake. Other studies have also shown that patients suffering from diverticular disease are predisposed to venous disorders (Painter, Almeida, and Colebourne, 1972; Latto, Wilkinson, and Gilmore, 1973).

Diseases of the bowel and varicosities are often associated with obesity, diabetes mellitus, and coronary heart disease. Burkitt, Walker, and Painter considered that "fiber-deficiency associated with excess refined cereal and sugar consumption may be the primary cause of this trend and some other Western ailments" (p. 1073).

In 1978 Van Itallie summarized his personal observations and those of Davis and Collins (1978) in his discussion of the merits of sufficient fiber in the diet for the prevention of excessive food intake and obesity.

Obesity is uncommon among the populations of countries where a high proportion of dietary calories is consumed as starchy vegetables virtually or wholly undepleted of their natural fiber content. This fact (and related epidemiological observations) has given rise to the hypothesis that diets with a high proportion of fiber-intact vegetable foods contribute significantly to the prevention of obesity. Attributes of dietary fiber that might serve as physiological obstacles to excessive energy intake include the following: 1) fiber reduces the caloric density of the diet; 2) it slows the rate at which calories can be ingested; 3) it decreases slightly the efficiency of absorption of dietary energy; 4) by virtue of the extra volume that it adds to the post-prandial gastrointestinal contents, dietary fiber may promote satiety (p. S252).

Various relationships have also been shown to exist between dietary fiber, plasma insulin, and diabetes mellitus. Albrink reported in 1978 that high-carbohydrate, high-fiber meals caused insulin to rise less than one-half that of high-carbohydrate, low-fiber meals. From his epidemiological studies, Trowell (1978) formulated the hypothesis that fiber-depleted diets are diabetogenic and high-fiber foods serve as a

protective factor.

In 1954 Walker and Arvidson became the first researchers to equate the low incidence of heart disease among South African Bantu with their high daily fiber intake. Since 1960 Trowell and his coworker, Burkitt, have pursued the epidemiological relationship that exists between high-fiber intake and low incidence of heart disease. In a study published in 1976, Trowell suggested that high-fiber diets may serve as a protective factor in ischemic heart disease by lowering serum cholesterol levels. In restating the British Department of Health and Social Security nutritional guidelines, Trowell (1976) wrote that

IHD (ischemic heart disease) mortality is considerably lower in populations who eat a diet containing a large portion of energy derived from starchy carbohydrate foods--cereals, pulses, starchy roots, and fruits--than those who eat a Western-type diet. These foods are usually undepleted or only lightly depleted of dietary fiber (p. 423).

In reviewing the influence of dietary fiber on the metabolism of lipids and arteriosclerosis, Kritchevsky (1978) suggested that "one possible mechanism of hypolipidemic action of fiber involves the binding of bile acids, which would result in reduced absorption of cholesterol, resulting in lower levels of serum cholesterol" (p. S65).

In a related study Cummings (1978) showed that fiber alters lipid absorption. Fecal fatty acid excretion rose when subjects in Cummings' research were fed controlled diets to which whole wheat products were added. Cummings noted that

this increase in fecal fat is not one that would be considered significant in terms of overall digestibility and absorption of fat, nor does it represent a pathological rise in fecal fat excretion in gastroenterological terms. It is, however, a well-described phenomenon (p. S21).

He concluded that

the overall balance of fat absorption is not seriously

altered by fiber but that small intestinal events leading to lipid absorption are affected. In addition, some fatty acids may be associated with fiber in such a way as to lead to their malabsorption (p. S22).

Hardinge and Stare (1954) and Hardinge, Chambers, Crooks, and Stare (1958) conducted somewhat related research involving the relationship between fiber and cholesterol. Their studies showed that vegetarians who ingest higher quantities of fiber than those who subsist on mixed diets exhibit lower cholesterol levels.

The role that fiber plays in acting as a protective factor in heart disease as a hypolipidemic agent possibly can be extended to the etiology of colonic-rectal cancer. The observation has been made that the products of bile salt degradation are proportionally greater in people in low-risk regions (MacLennan, Jensen, Mosheck, and Viori, 1978; Reddy and Wynder, 1973).

Pomare and Heaton (1973) demonstrated that bacterial degradation of bile salts to potentially carcinogenic substances, such as deoxycholate, is inhibited when fiber in the form of bran is added to the diet. In a later study Heaton (1975) showed that bran reduces the amount of deoxycholate, a secondary or colonic bile salt, in bile to one-half its previous level.¹ Fiber may perform a second function by its production of a bulky stool, which dilutes potential carcinogens and negates fecal stasis, thus reducing the amount of time noxious substances are in contact with intestinal mucosa.

Burkitt (1978) stated that "colonic rectal cancer is the only

¹Deoxycholate exerts feedback inhibition on chenodeoxycholate, a primary bile salt that recent findings suggest suppresses secretion of cholesterol. The regulation of cholesterol secretion into bile thus is determined by the amount of chenodeoxycholate in the bile salt pool, which is ultimately determined by the amount of fiber in the diet.

human malignancy in which a low-fiber intake has been specifically implicated as a causative factor" (p. S58). He also wrote,

The hypothesis that fiber-depleted diets are a major cause of the high prevalence of colonic-rectal cancer in Western countries is consistent with all epidemiological and other evidence. The hypothesis blaming increased fat consumption is consistent with most but not all situations (p. S62).

Conversely, Mitchell and Eastwood (1976) and Mendeloff (1976) are not convinced that the fiber-depleted diet is a cause of colonic-rectal cancer. Their studies, which appeared two years prior to that of Burkitt's, outline the dilemma that envelops the production of colonic carcinogens and the role fiber may play as a potential protective factor. Lyon and Sorenson (1978), in their research into colon cancer in Utah, also questioned the effect of a high-fiber diet on colonic-rectal cancer.

Although research findings support both points of view, it is obvious that researchers must necessarily continue their efforts to untangle and decipher the possible role of fiber in the etiology of colonic-rectal cancer. In this regard Spiller (1978) concluded that

the study of the effect of dietary fiber on prevention of cancer, specifically of colonic cancer, cannot be carried on without considering that: 1) various types of dietary fiber have different metabolic effects; 2) other components of the diet (e.g. amount of fat may influence the way dietary fiber affects the colonic environment; 3) the overall effect of dietary fiber may be the sum of the effects on fecal bulk colonic microflora pattern and metabolic dilution of carcinogens, colonic pH, transit time, alteration of nutrient absorption, and postprandial hormonal function (p. S231).

Dietary fiber has been shown to increase fecal bulk and stool weight, to reduce transit time through the gastrointestinal tract, and to modify the liquid and solid phases of gastrointestinal contents. It also has been proven to reduce colonic pressure and provide symptomatic

relief to patients suffering from diverticular disease. By virtue of its bulking action, fecal constituents such as bile acids are diluted. It is believed that this mechanism may serve as a protective factor in the prevention of ischemic heart disease and colonic-rectal cancer. Researchers have demonstrated effectively the positive results of an increase in dietary fiber intake. The processes by which fiber causes these effects, however, are not well comprehended and will require more exhaustive examination.

Wheat Bran as a Source of Dietary Fiber

Current research has shown that it is not presently practical to set a nutritional allowance for fiber to protect against the various non-infective diseases. Trowell and Burkitt recommended in 1975, however, the addition of sufficient amounts of cereal fiber to food to ensure the daily passage of at least one soft stool. Three years later, Trowell stated that "adding bran to a Western-type diet will remedy constipation, ease hemorrhoids, alleviate the symptoms of diverticular disease, and relieve a proportion, but only a proportion of irritable bowel syndrome" (p. S9). He cautioned, however, that the increased fiber intake "will only slightly improve carbohydrate tolerance and it will not cure diabetes. It will not reduce hyperlipidimia or improve atherosclerosis" (p. S9). In a much earlier study, Cowgill and Anderson (1932) examined the laxative effects of wheat bran and "acid washed wheat bran." One year later, Cowgill and Sullivan further demonstrated the efficiency of commercial wheat bran over fruits and vegetables in correcting constipation. In a related later study, Payler (1973) found

that the replacement of white bread with whole meal and the addition of bran to the diet of school-age males reduced their intestinal transit times by one-third and increased their stool weights by one-fourth. Other researchers, including Painter, Almeida, and Colebourne (1972), Harvey, Pomare, and Heaton (1973), and Findley et al. (1974), using bran as a source of dietary fiber have obtained similar results.

Bran has been incorporated into test meal diets in various forms. Plumley and Francis (1973) found that bran crispbread gave good symptomatic relief to patients suffering from diverticular disease, and Taylor and Duthie (1976) showed that bran tablets proved to be more effective in increasing daily stool weight and in decreasing intestinal transit time than either a high roughage diet or bulk laxative. Bran tablets, which are currently marketed, may be in such concentrated form that they are undigestible, causing them only to begin swelling and absorbing water upon entering the intestine. Bulk laxative, on the other hand, swells with water before it reaches the intestine.

Wheat bran recently has been adopted in several potentially marketable test meals. Vratana and Zabik (1980) showed that oatmeal cookies in which wheat bran had replaced 50 percent of the normal ingredient of flour were a palatable and acceptable source of dietary fiber. Wheat bran and a flour-milling by-product, middling, also have been substituted for flour in cakes (Rajchel, Zabik, and Everson, 1975; Brockmole and Zabik, 1976) and in bread (Pomeranz, Shogren, Finney, and Bechtel, 1977).

It has been well documented that the addition of bran to the diet reduces intestinal transit time and increases stool weight. The effectiveness of bran in relieving the symptoms of diverticular disease also

has been demonstrated. In addition it has become economically more reasonable and feasible to incorporate cereal fibers into the production of processed foods than to use more expensive natural foods, such as fresh fruits and vegetables. In general the more economical foods are those that have been processed for storage and transportation (Scala, 1974). Wheat bran may well serve as an important source of dietary fiber when it is successfully used in processed food products.

Particle Size of Wheat Bran

Worldwide public interest in the fortification of processed foods with wheat bran as protection against some colonic disorders, including diverticulosis, has prompted food manufacturers to supply a variety of high-fiber products that are palatable and appealing to the consumer. The production of these foods involves the grinding of wheat bran to a fine particle size in order to eliminate undesirable textures and to obtain quality results (Pomeranz et al., 1977). This alteration in the bran's major physical characteristic by grinding in turn may alter the action of fiber in gut function. Heller et al. (1980) reported,

Since patients with diverticular disease and consumers looking for a high-fiber diet will be seeking out high-fiber foods, it is important to understand how the grinding of wheat bran may affect its role in colonic function. No definitive papers have been published to show the effect of bran particle size on gastrointestinal function (p. 1735).

In 1933 Cowgill and Sullivan observed, "the smaller size of the fiber particle in the processed bran product is a factor tending to decrease slightly its laxative value" (p. 801). Wyman, Heaton, Manning, and Wicks (1976) noted that the addition of 16g of unprocessed bran fiber effectively accelerated intestinal transit time. Particle size was found to have no effect, however, on stool weight when bran was fed

to subjects on an uncontrolled diet (Fantus, Hirschberg, and Frankl, 1941). Moreover, finer bran was determined to have less effect on cholesterol absorption. A decrease in particle size had no effect on fecal mass when bran of three different mesh sizes was fed to hyperlipidemic rats (Ranhotra, Loewe, and Puyat, 1977).

Kirwin and his coworkers (1974) may have found some clue to the unanswered question of whether particle size of wheat bran actually influences gastrointestinal rate and output. They drew the conclusion that the water-holding capacity of bran in the feces is a function of particle size. Because of a decrease in interstitial space available for water uptake when bran is ground, wheat bran of fine particle size was reported to have a lower in vitro water-holding capacity than coarse bran (Kirwin et al., 1974; Eastwood and Mitchell, 1976).

The bulking property of fiber is in turn a function of its water-holding capacity. Kirwin et al. (1974) were able to determine the water-holding capacity of various particle sizes of bran using a centrifugation technique. Conversely, Stephen and Cummings (1979), using a method that measured water uptake in dialysis bags, reported that the water-holding capacity of finely-ground fiber is increased. It appears evident that the effect of particle size on water-holding capacity depends on the method of measurement employed.

Some researchers, including Heller, Rivers, and Hackler (1977), Lewis (1978), and Ward, Shellenberger, and Wetzal (1979), have explored the various aspects of the importance of particle size to the physiochemical properties of fiber. Much controversy still surrounds the subject of the relationship of wheat bran particle size to bowel function, however. Numerous statements in dietary literature indicate the need

for continued research to define the effects of particle size on gut function.

Lactulose

Lactulose (4-~~O~~- β -D-galactopyranosyl-D-fructose) has been defined previously as a ketoanalogue of lactose (Dahlqvist and Gryboski, 1965).

It is a

synthetic disaccharide containing fructose and galactose and is formed from lactose by conversion of the glucose radical to the keto-sugar fructose. Such a conversion occurs during the heat processing of milk or in the presence of an alkaline medium (Gryboski and Boehm, 1965, p. 341).

Gryboski, Lillis, and Ma (1964) further elaborated on the formation of lactulose,

This sugar, lactulose, is a synthetic disaccharide formed from lactose during the processing of milk. The chemical reaction is that of enolization during which the glucose constituent of the lactose molecule in the presence of calcium hydroxide or alkali, is converted into fructose (p. 36).

Lactulose has been identified in commercial formula preparations and in evaporated milk. Modified milk products used for feeding infants contain 1 to 5 percent lactulose as a portion of the total carbohydrate (Bernhart, Gagliardi, Tomarelli, and Stribley, 1965). Breast milk and fresh homogenized cow's milk do not contain lactulose (Gryboski and Boehm, 1965).

Studies show that infants excrete lactulose in their stools after they have consumed formulas containing the sugar (Gryboski and Boehm, 1965). Gryboski, Lillis, and Ma (1964) reported,

The presence of lactulose and the absence of lactose in most infant stools suggest that human intestinal - galactosidase has greater specificity for the naturally

occurring disaccharide than for its synthetic derivative. An hypothesis of impaired hydrolysis of lactulose is further supported by the presence of this disaccharide in the urine of normal infants who are taking processed milk formulas. The concentration of lactulose in milk is less than half that of lactose, and if this were merely due to overloading or inadequacy of the enzyme system, one would expect to find both disaccharides present in the urine and the stool (p. 30).

Dahlqvist and Gryboski (1965) studied the hydrolysis of lactulose by human small-intestinal lactase. The in vitro research utilized extracts of human mucosa of the small intestine incubated with lactulose. They determined that "all the mucosal preparations failed to hydrolyze lactulose, in spite of their strong lactase activity. . . . The small intestinal lactase cannot bind lactulose to its substrate-binding groups" (p. 635). Bond and Levitt demonstrated additional positive evidence of the reliability and usefulness of lactulose as a standard unabsorbable carbohydrate in their quantitative study conducted in 1972.

Breath Hydrogen

Little research has been completed on the use of the breath hydrogen (H_2) test as a means of expressing the effect of wheat bran on mouth-to-cecum transit time and fiber fermentability in the gut. Bond and Levitt (1978) used the breath H_2 test to indicate the influence of dietary fiber on colonic gas production and small bowel transit time. They based their technique on a previous observation that the amount of colonic H_2 produced by bacterial metabolism of a fermentable substrate can be affected accurately by pulmonary H_2 excretion (Levitt, French, and Donaldson, 1968; Levitt, 1969).

Levitt suggested in 1969 that

measurement of breath excretion might be used for investigation of carbohydrate malabsorption. Malabsorption of

carbohydrate should result in the delivery of substrate to the colon bacteria with a resultant increase in H₂ production and excretion. Since H₂ is not produced in the small bowel, there should be no increase in H₂ excretion if carbohydrate is completely absorbed. Preliminary studies suggest that H₂ excretion may be a useful indicator of carbohydrate malabsorption (pp. 126-127).

Levitt and Donaldson (1970) conducted further studies on the use of the breath H₂ test to detect carbohydrate malabsorption. Finally, in 1972, Bond and Levitt made use of the breath H₂ test to quantify carbohydrate absorption.

Since these preliminary studies occurred, the use of the breath H₂ test in the diagnosis of disaccharide malabsorption has become well established (Galloway, Murphy, and Bauer, 1969; Bose and Welsh, 1973; Metz et al., 1975; Metz et al., 1976; Bond and Levitt, 1976; Maffei, Metz, and Jenkins, 1976; Caskey, Payne-Bose, Welsh, Gearhart, Nance, and Morrison, 1977). Metz et al. (1975) described the advantages of the breath H₂ test,

End expiratory sampling of breath H₂ would seem to be a simple, non-invasive and accurate method of diagnosing hypolactasia, which is also very acceptable to patients. This should make it a valuable tool both in diagnostic gastroenterology and in epidemiological surveys (p. 1155).

Breath H₂ and methane excretion have also been used to study intestinal microflora (Levitt, French, and Donaldson, 1968). Studies such as this indicate that

excretion of hydrogen and methane (1) accurately reflects bacterial production of these gases, (2) is relatively stable under standard conditions, and (3) promptly reflects changes in bacterial metabolism resulting from dietary or antibiotic manipulation (p. 989).

It is further suggested that "measurement of gas production may provide a useful means of studying the in situ metabolism of the intestinal flora" (Levitt, French, and Donaldson, 1968, p. 989).

Gas Chromatography

Gas chromatography was previously defined as

an analytical method of separation in which two substances to be analyzed are distributed between two phases. One of these phases is stationary; the other, the mobile phase, is a fluid flowing past the stationary phase (Heftmann, 1967, p. 182).

Historians attribute the development of chromatographic experiments, or fractional diffusion procedures, to the genius of two late nineteenth and early twentieth century scientists, an American, David Talbot Day, and a Russian, Mikhail Tswett (Heftmann, 1967). Day's experimentation with petroleum products and Tswett's chromatographic exploration of chloroplast pigments initiated the development of the modern branches of the science, including gas chromatography. Heftmann (1967) credited Tswett with providing scientists and researchers with "a remarkably useful tool for the investigation of chemical substances" (p. 11).

Gas chromatography, introduced in 1952 by A. T. James and A. Martin, had been used extensively in biochemical studies (Morris and Morris, 1976). Ambrose (1971) explained the features of the method.

In gas chromatography the sample to be analyzed is introduced into the moving gas stream and carried by it down a column. This column contains . . . a liquid of low volatility held upon an inert support (gas-liquid chromatography); . . . the involatile fluid forms the stationary phase. The constituents of the sample distribute themselves between the 2 phases. In general, their absorptions or solubilities differ and they are therefore carried along the column at different rates, emerging at the end in distinct zones (peaks) separated by the substantially pure carrier gas. On emerging, the vapour in the carrier gas must be detected by suitable means, now generally by instrumental methods which are adapted to automatic recording (p. 2).

Morris and Morris (1976) elaborated on the chromatographic

procedure:

The carrier gas which constitutes the mobile phase is usually obtained from a gas cylinder. . . . It then passes through a flow controller and regulator. . . . The gas stream then passes through the device which provides for the introduction of the sample. . . . The carrier gas and the sample then pass into the chromatographic column, where the actual separation takes place. . . .

The separated components of the initial solute mixture emerge from the column and are monitored by the detector, and their zone profiles displayed on the recorder (p. 473).

Carrier gases are stored in gas cylinders from which they can be readily obtained for use. Helium is the choice carrier gas for use with the thermal conductivity detector (Morris and Morris, 1976). Ambrose (1971) reported that "hydrogen and helium have much higher thermal conductivities than any other materials and all peaks will be in the same direction" (p. 55).

The thermal conductivity detector or katharometer has been used with a high degree of accuracy for the detection of changes in gas composition. In explaining the principles of the thermoconductivity detector, Ettore and Zlatikis (1967) stated that

(1) Each gas has its own individual thermal energy transfer characteristic or transmission factor and (2) metal filaments and thermistors (semiconductors of fused metal oxides) have fixed resistance temperature relationships (p. 242).

The same researchers elaborated,

The cell usually consists of a metallic wire or thermistor mounted coaxially within a metal or glass cylinder through which a gas flows. Due to the flowing gas, the rate of heat loss from the sensing element, heated by the application of a constant electric current, is determined by measuring the sensor's resistance which can be then converted to a specific temperature value. When a foreign substance is introduced into the gas stream, the resistance of the sensor and consequently the temperature change. If the detection device is incorporated into some form of a wheatstone bridge circuit, an 'out-of-balance' of the bridge is noted, this signal being a measure of the

rate of heat loss (pp. 242-243).

Chromatography is an invaluable technique for the separation and description of substances. Heftmann (1967) claimed that

the importance of chromatography lies primarily in its use as an analytical tool. It serves as a means for the resolution of mixtures and for the isolation and partial description of the separated substances (p. 13).

Gas chromatography provides a sensitive method "capable of very rapid analytical separations down to the sub-nanogram level" (Morris and Morris, 1976, p. 526). Moreover, the gas chromatographic technique provides a useful method for the analysis of blood and respiratory gases (Porter, 1969).

CHAPTER III

METHODS AND PROCEDURES

Five caucasian females, ranging in age from 24 to 56 years, volunteered for the study. Each subject submitted a written informed consent (Appendix A) before participating in the research. The proposal for this study was submitted on February 5, 1980, to the Oklahoma State Institutional Review Board for the Protection of the Rights of Human Subjects and was subsequently approved by the board.

The five volunteers were in apparent good physical health at the time of the study. They neither suffered from any symptoms associated with gastrointestinal disorders or diabetes mellitus, nor had they ingested any antibiotics during the two weeks prior to the testing. Tests for each individual were not less than two days apart.

The three test meals, which were administered in random order to each subject after a twelve-hour overnight fast, were:

CB+L: A loaf containing 30g coarse wheat bran with 10g lactulose syrup added.

FB+L: A loaf containing 30g finely ground wheat bran with 10g lactulose syrup added.

L: 10g lactulose syrup in 200ml water.

Later, two other test meals were administered in random order to the same volunteers. The final two test meals were:

CB: A loaf containing 30g coarse wheat bran.

FB: A loaf containing 30g finely ground wheat bran.

The bran loaf test meals were prepared prior to testing and stored in freezer-proof bags at 0°F. Loaves were defrosted and warmed in a microwave oven the morning of the test.

Certified soft white wheat bran was purchased from the American Association of Cereal Chemists.¹ The bran was sieved as purchased on a 2.0mm screen, United States Standard Sieve Series #10, to obtain the coarse wheat bran. The fine wheat bran was prepared by grinding a portion of coarse bran through a 1.0mm screen using a Cyclone Sample Mill.²

In addition to soft white wheat bran, the test meal loaves contained 40g white flour, 15g baking powder, 83.3ml water, and 15g whole beaten egg. CB+L and CB test meals contained the previously described ingredients, plus 30g coarse bran. Fine bran was added to the aforementioned mixture to obtain FB+L and FB test meals.

The Cephalac brand³ of lactulose syrup used in this study was stored at room temperature. On the morning of each test, 15ml lactulose syrup, which is equivalent to 10g lactulose, was poured on the bran loaves for the CB+L and FB+L test meals. Fifteen milliliters lactulose syrup was combined with 200ml water for the L test meal.

Subjects drank 200ml water with the CB+L, FB+L, CB, and FB test meals. An unlimited amount of water in 200ml measured portions was allowed each subject throughout each test period.

¹Certified soft white wheat bran, AACC Headquarters, 3340 Pilot Knob Road, Saint Paul, Minnesota, 55121.

²Cyclone Sample Mill, UD Corporation, Boulder, Colorado, 80302.

³Cephalac, Merrell-National Laboratories Division, Richardson-Merrell, Incorporated, 2110 East Galbraith Road, Cincinnati, Ohio, 45215.

Test Procedure

After a subject arrived at the laboratory, a fasting breath sample was collected, using previously described procedures (Payne-Bose et al., 1978). The prescribed test meal was eaten, and another breath sample was collected immediately following completion of the entire meal. The subject's breath was sampled every ten minutes thereafter. The test period for the CB+L, FB+L, CB, and FB meals were five hours in length. A three-hour sampling period was required for the L test meal.

Analysis of Breath Samples

The hydrogen content of the breath sample was determined by gas solid chromatographic analysis as described previously (Payne-Bose et al., 1978). A Hewlett-Packard 5834-A gas chromatograph with a thermal conductivity detector and electronic peak integrator was used for the analysis of breath samples. The carrier gas was argon with a flow rate of 13ml per minute. An oven temperature of 60°C was maintained throughout the experiment. The temperature of the thermal conductivity detector was maintained at 165°C. A 5-A molecular sieve column, six feet long with a one-eighth-inch diameter, was used for the analysis.

Breath samples were analyzed within two days following their collection. The samples were analyzed in informal random order. Hydrogen concentration of each breath sample was recorded in parts per million.

Statistical Analysis

The study was designed as a randomized complete block pattern in which subjects were considered as blocks and test meals as treatments. Each subject served as her own control. Small intestine transit time

was determined by the subjective observation of the first obvious rise in breath H_2 concentration following the 0-time breath sample. The analysis of variance (ANOVA) procedure for the randomized block design given by Steele and Torrie (1980) was used to test for differences in mean transit times due to test meals. When differences were declared, the least significant difference (LSD) was used to determine which means differ from the others.

CHAPTER IV

RESULTS

As previously stated a total of five test meals were administered to each of the five subjects. A sixth woman, subject 3, participated in only Series I of the study. She was unable to eat the total amount of any of the test meals. The results of her three tests were therefore not analyzed. Only one test meal was consumed on a given day by the five participants. The first three meals given in random order consisted of fine or coarse bran and a lactulose marker or only lactulose. Later, two more meals of coarse or fine bran were eaten. Appendix B shows the consecutive order of both series of test meals.

Figures 1-5 illustrate the breath H_2 response of subjects to the meals. The graphs show hydrogen concentration measured in parts per million (ppm) in 10-minute increments. The results for each subject are separated into three graphs for ease of viewing and to limit confusion.

The breath H_2 response for the 0-time sample is used as a starting point in each of the graphs. A 0-time sample was taken immediately after a subject finished eating the test meal. A discussion of the fasting breath samples, which were taken just before the subject began to eat a test meal, are presented in a later chapter.

Generally, only one analysis of each breath bag was made; periodically, however, the researcher did analyze a breath bag more than once as a repeatability check. In most cases the repeated analyses were

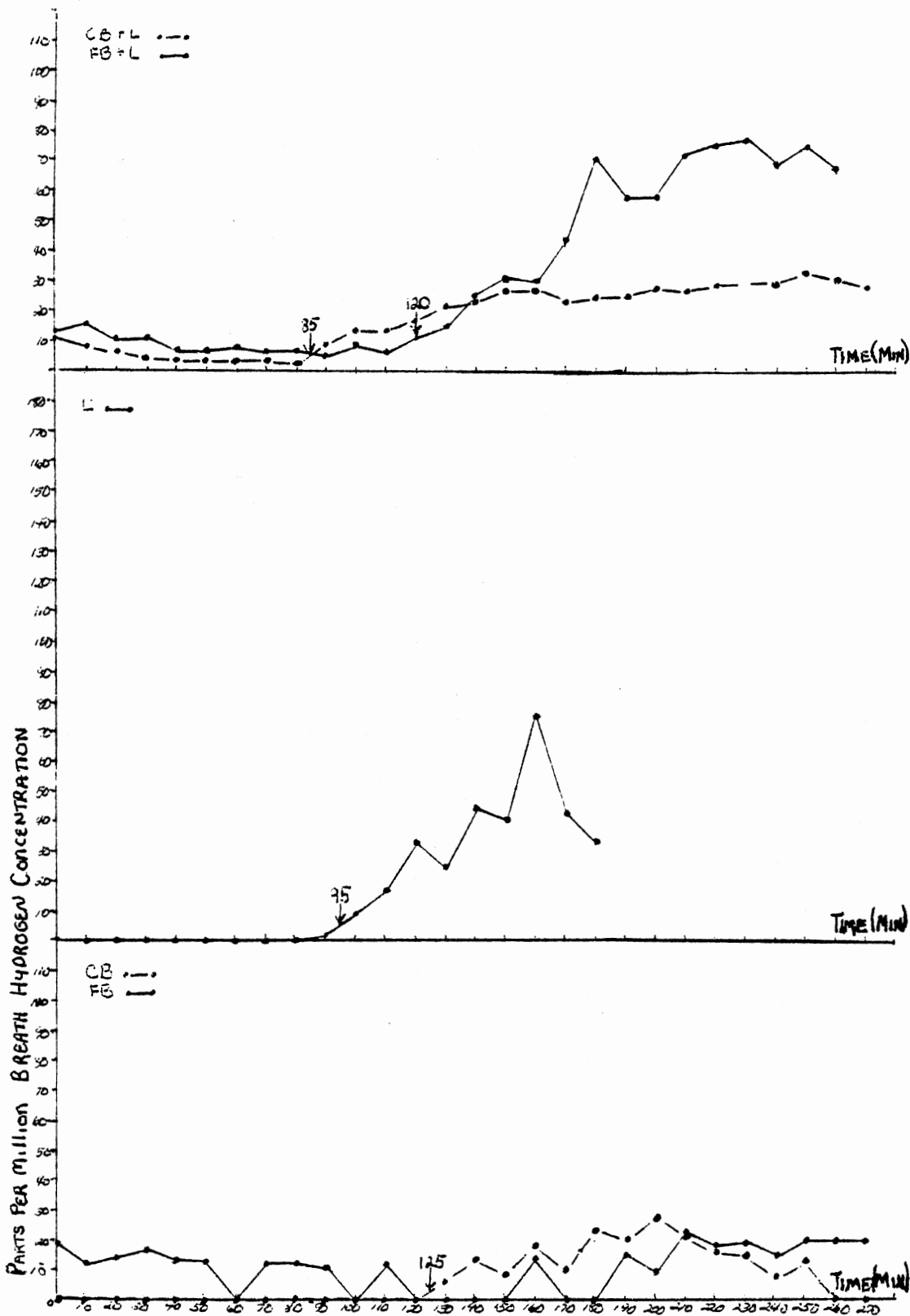


Figure 1. Breath H₂ Response: Subject 1

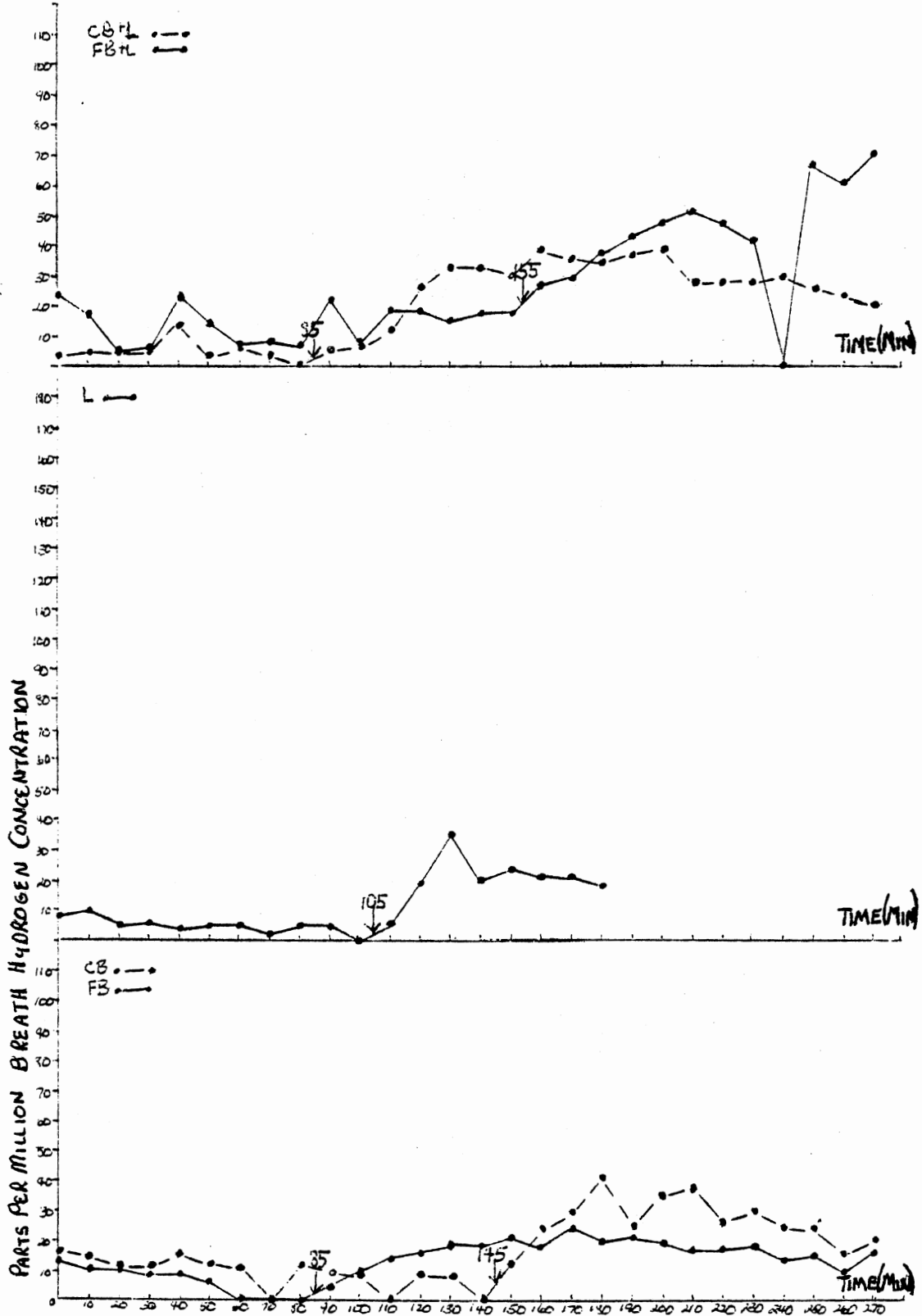


Figure 2. Breath H₂ Response: Subject 2

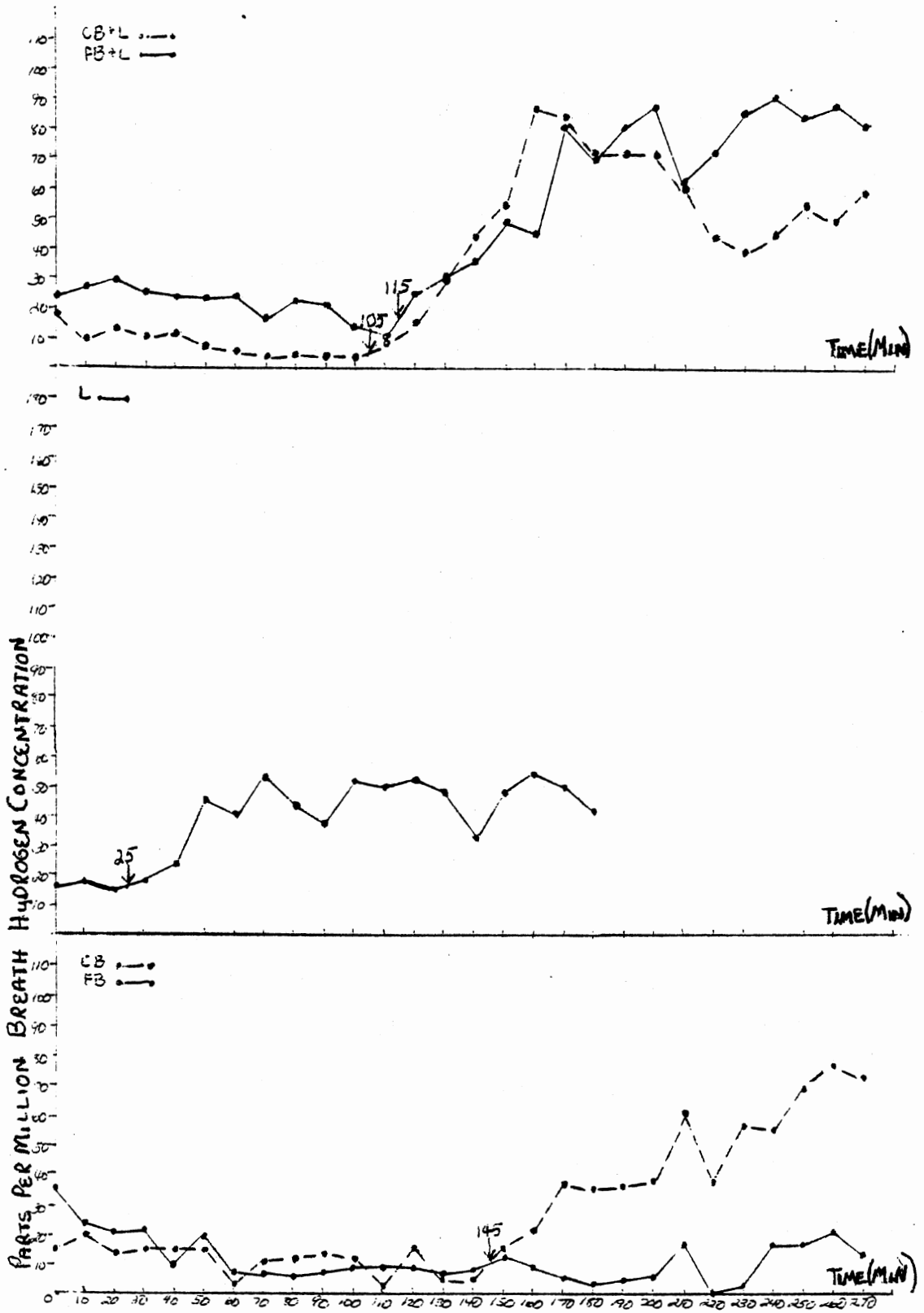


Figure 3. Breath H₂ Response: Subject 4

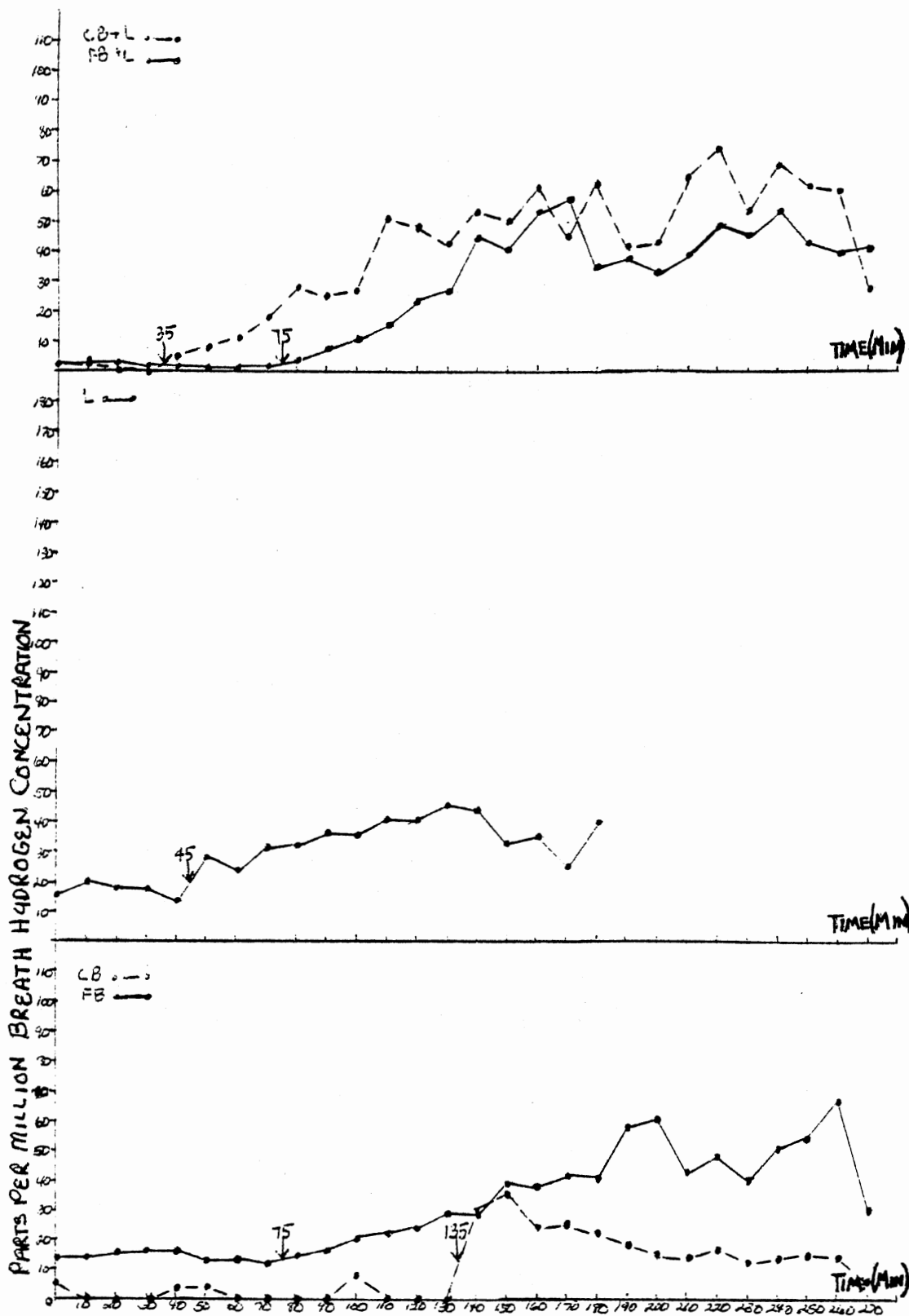


Figure 4. Breath H₂ Response: Subject 5

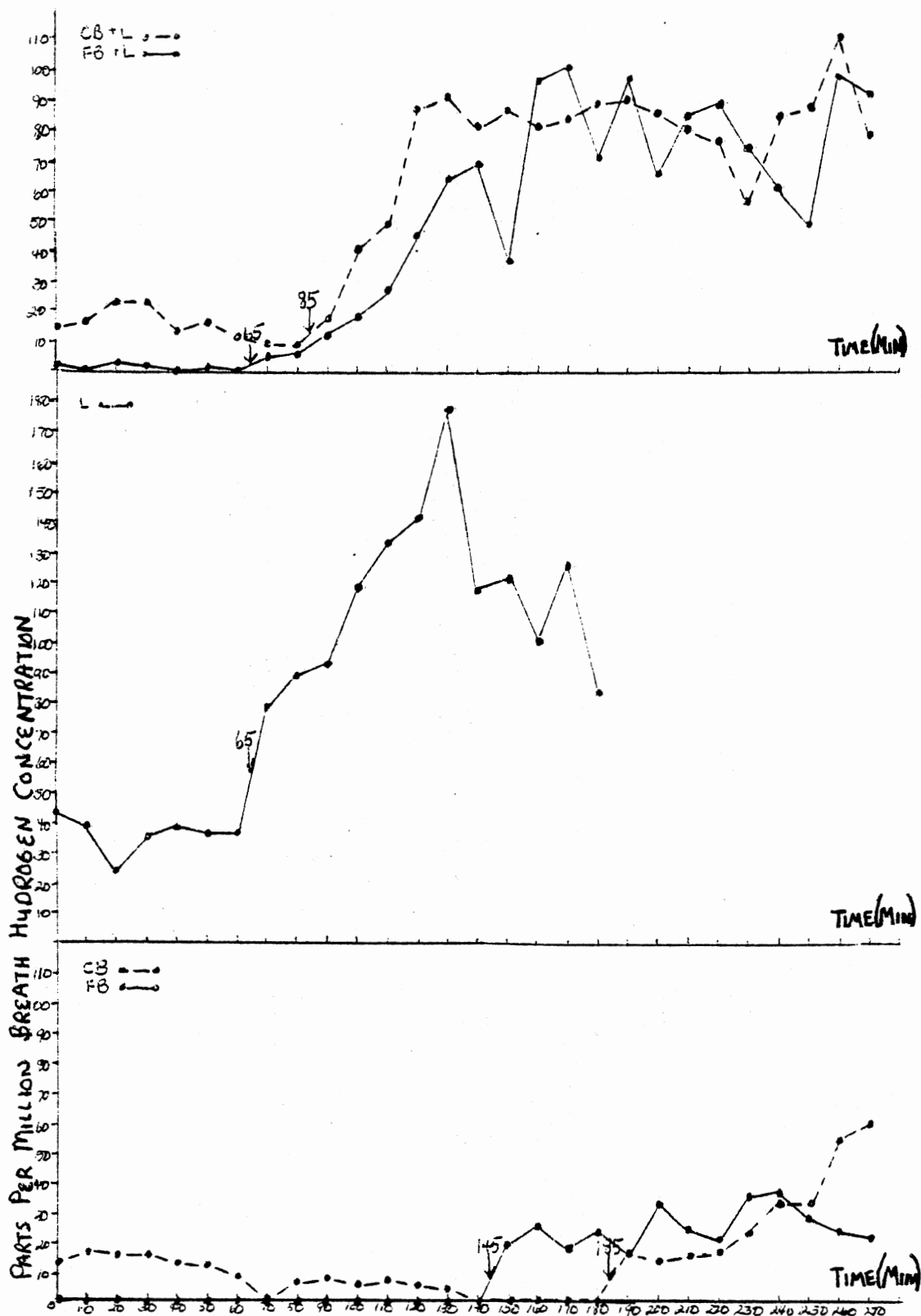


Figure 5. Breath H₂ Response: Subject 6

within 3ppm of each other. The average of the repeated analyses was recorded as the breath H_2 response for that particular sample.

For each of the CB+L, FB+L, CB, and FB test meals, the final breath bag was taken at 270 minutes. The testing period for the L meal was 180 minutes. The L test meal period was shortened to 180 minutes because subjects complained that a liquid drink could not sustain them from hunger as long as the more filling bran meal.

Transit times for each of the test meals are indicated by arrows in the graphs. The first obvious rise in breath H_2 concentration following 0-time sampling was subjectively determined by the researcher as the intestinal transit time. Transit times for the FB test meal eaten by subject 1 and subject 4 could not be determined because of a lack of response and the varying base line values in the chromatogram.

Breath H_2 responses for the test meals consumed by subject 3 have been omitted from the results section. Subject 3 was unable to eat the entire test meal. The results of her tests could not be compared with those of other subjects. Further discussion of this subject's results follow in Chapter 5.

Transit times for the series of test meals, CB+L, FB+L, and L, are presented in Table I. The transit times listed in the table are the same as those indicated in the foregoing graphs. Means for subjects and meals have also been calculated and included in the table.

Transit times of the second series of test meals, CB and FB, are found in Table II. As previously mentioned, the transit time of the FB test meals could not be determined for subjects 1 and 4. The order of subjects has been rearranged in the table for ease of determining means that have been calculated where possible. Means for meals and subjects

have only been determined for subjects 2, 5, and 6.

TABLE I
TRANSIT TIME OF SERIES I TEST MEALS

<u>Subject</u>	<u>Meals</u>			<u>Mean</u>
	<u>CB+L</u>	<u>FB+L</u>	<u>L</u>	
1	85	120	95	100
2	85	155	105	115
4	105	115	25	81.67
5	35	75	45	51.67
6	85	65	65	71.67
Mean:	79	106	67	

TABLE II
TRANSIT TIME OF SERIES II TEST MEALS

<u>Meal</u>	<u>Subject</u>					<u>Mean*</u>
	<u>1</u>	<u>4</u>	<u>2</u>	<u>5</u>	<u>6</u>	
FB	---	---	85	75	145	101.67
CB	125	185	145	135	185	155.00
Mean*:	---	---	115	105	165	

*Subjects 2, 5, and 6 only

The analysis of variance of Series I test meals is shown in Table III. The statistical analysis of the final two tests is not included with that of the first three tests because the former were administered at a later date. The size of the sample for the Series II meals has been reduced to three subjects because of missing data; therefore, an ANOVA is not offered. Such results would not be indicative of a larger sample's response to these test meals.

TABLE III
ANALYSIS OF VARIANCE--
SERIES I TEST MEALS

Source of Variation	Degrees of Freedom (d.f.)	Sum of Squares (S.S.)	Mean Square (M.S.)	Variance Ratio (F)
Total	14	16510		
Subject	4	7260	1815	2.76
Meal	2	3990	1995	3.03
Error	8	5260	657.5	

The analysis of variance (Table III) did not show a significant difference in transit time as a result of test meals at any level less than .085. Differences in transit time due to individual subjects were also not shown to be significant at any level less than .103. Because

no significant differences were seen in the ANOVA, the LSD procedure was not used to make pair wise comparisons of the test meals.

CHAPTER V

DISCUSSION

The breath H_2 test, which previously has been used as an effective indicator of carbohydrate malabsorption, was adapted to this study as a method of determining mouth-to-cecum transit time when wheat bran and, in some cases, a non-absorbable carbohydrate, lactulose, were consumed. The purpose of the study was to show whether the particle size of wheat bran has an effect on mouth-to-cecum transit time. Results of the study show that there were no significant differences in transit time due to test meals of individual subjects. As prescribed by Steele and Torrie (1980), when there are no significant results in the ANOVA, a LSD procedure is not applied to the data.

The small size of the test sample may have resulted in failure to show significant differences in transit time for coarse and fine bran. Only six female subjects participated in the study. Because subject 3 could not consume the test meals, her results were omitted from the statistical analysis, thus leaving five measurable subjects. A study with a larger sample, perhaps 12 or 24 subjects with test meals repeated, might reveal differences in the influence of coarse and fine bran on transit time.

The ages of the subjects also may have been a factor that affected their responses to the test meals. Five of the women were between 24 and 29 years of age. One person, subject 2, was 55. She did not

suffer from diverticular disease or other gastrointestinal afflictions. The ages of the women may have played a role when the menses are taken into consideration. The effect of the menstrual cycle on the rate of flow of digesta through the gastrointestinal tract has not been researched thoroughly. However, because women between puberty and menopause often complain of constipation just prior to periods of menstruating, it seems likely that changes accompanying menses do affect transit times. No records were obtained from subjects in this study concerning their menstrual cycles. Subject 2 did indicate, however, that she had already experienced menopause.

Variations in the individual weights of the five subjects used in the statistical analyzes would have had a limited effect on the outcome of the study. The range of their weights was between 51.5kg and 62.7kg, with the average being 54.4kg. The table in Appendix C shows the age and weight of each of the six subjects for the first series of test meals.

The weight of subject 3 did have a significant bearing on her performance during the testing. Subject 3 weighed almost 10kg less than the average of the other five subjects. Her height, 5' 7", was not related to her low weight, although she was of slight bone structure. The subject indicated that she normally consumed very small meals of one-fourth-cup portions four times daily and rarely ate breakfast. She was unable to complete the entire bran test meal without becoming ill. Only 81 percent of the first test meal, CB+L, was consumed. Thereafter, she was fed test meals that consisted of 81 percent of the total weight of the prescribed meal. Other problems also arose because the subject took considerable time to finish eating the test meals. On the first day of

testing she ate her reduced test meal portion of CB+L in 70 minutes. With the coaxing of the researcher, she was able to finish the FB+L meal in 35 minutes. The L test meal presented no problem as she drank this meal in 15 minutes. Because the subject took so long to consume the bran meals, it is possible that the digesta from the meals passed through the tract at different rates of speed. Breath bags either were not taken until the subject finished eating or late into her period of consuming the meal. It is likely, therefore, that any rises in breath H_2 concentration were not recorded, especially during the 70-minute eating period. Her fasting breath samples, which were within the acceptable ranges at the time of the study, posed no problem. After completing the Series I test meals, subject 3 was released from the study.

The size of the bran test meals presented a similar problem for the other subjects. They also found them too large to consume quickly. None of the subjects were able to eat the entire meal in less than five minutes, and some took as long as 15 to 20 minutes to finish the bran loaves. Thus, any portions of the digesta from the meals may have passed immediately into the intestine while the subject was still eating. The rise in breath H_2 that occurred when their bolus reached the intestine was thus lost because breath bags were not administered until after a subject had finished eating. Future studies in this field should use a more palatable test meal and possibly be administered on a gram per kilogram weight basis.

As the graphs of breath H_2 response to Chapter III indicate, the subjects usually did not return to Oppm H_2 concentration after the 0-time for fasting samples. A comparison of the curves of breath H_2

response in the lactose malabsorption studies of Bose and Welsh (1973) and Payne-Bose et al. (1977) show that the breath H_2 concentration was invariably almost 0 or 0ppm at some point following the fasting sample. This return to a 0-level of concentration makes the subjective determination of the rise in concentration that indicates transit time much easier to discern. Subjects may have actually returned to a 0-level, but a sample was not taken at that time because the women were still eating.

On the other hand, the length of the sampling period may not have been long enough. The time for the major portion of the bolus of food to reach the intestine might have occurred much later, perhaps after the termination of the 270 minute test period for the bran loaves or the 180 minute test period for the lactulose. Rises in H_2 concentration may have been indicative of small portions of the meal that were dumped into the intestine. The bumpy appearance of the graphs may in fact be due to a dumping of the stomach contents at different times in the testing period.

The H_2 concentration also may have been a response to meals consumed before subjects arrived in the laboratory. Although the subjects were instructed to fast for at least 10 to 12 hours and to avoid gas-forming foods, they were not monitored before their arrival at the testing center. Their fasting breath samples were frequently high, although within acceptable limits at the time of testing. Table IV lists the breath H_2 concentration of the fasting breath samples for each of the subjects.

High fasting breath samples often mean that a subject has not fasted long enough or has consumed gas-forming foods prior to the

testing. Table IV shows that several of the subjects had fasting breath H_2 concentrations above 30ppm. These subjects should have been sent home, and the test rescheduled for another day. A better procedure for this study may have been to reduce the limits of acceptable fasting breath H_2 concentration and to monitor more closely subjects on the day prior to the test period.

TABLE IV
FASTING BREATH H_2 CONCENTRATION IN PPM

<u>Subject</u>	<u>Test Meal</u>				
	<u>CB+L</u>	<u>FB+L</u>	<u>L</u>	<u>CB</u>	<u>FB</u>
1	8	12	0	0	17
2	4	23	12	15	13
3	11	27	12	--	--
4	14	39	14	19	22
5	5	3	10	4	15
6	12	3	36	17	0

The results of this study are intriguing. The researcher had expected to see a difference in transit time due to particle size of bran.

Failure to detect a difference may have been due to several factors:

I) the test sample may have been insufficient to demonstrate differences; II) there may have been enough of a difference in particle size between the fine and the coarse bran; or III) perhaps, in some cases the transit time actually occurred after the testing period had ended.

Other factors, including those mentioned previously in this discussion, may have also have affected the outcome of the study. More research is needed to determine the effects of wheat bran particle size on gastrointestinal function and to devise an accurate and reliable method for determining transit time.

CHAPTER VI

CONCLUSION

The public increasingly has become aware of the importance of fiber in the human diet because of its possible connection to various non-infective diseases. Food manufacturers have catered to this rising public interest by marketing a variety of high-fiber products. Among the fiber-rich foods available to the consumer are those containing wheat bran, an important source of dietary fiber. Individuals in the food industry believe, however, that various physical characteristics of wheat bran, such as particle size, must be altered to produce a better textured and more palatable product that will be acceptable to the average consumer.

Researchers have recently raised the question of whether changes in particle size of bran alter intestinal transit time. Few studies in this field of inquiry have been conducted, however. Another area that is virtually unexplored is the use of the breath H_2 test as a non-invasive method of determining intestinal transit time of wheat bran.

This study was undertaken to assess whether there is a difference in intestinal transit time between coarse and fine textured bran as indicated by a rise in breath H_2 concentration. To make that determination, the breath H_2 test using gas solid chromatography was employed.

This study showed that particle size of wheat bran has no effect on mouth-to-cecum transit time. The multitude of variables involved in

the research and the less-than-perfect conditions under which it was pursued leave sufficient room for further investigation, however. This study should provide a point of departure for that future research.

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APPENDIX A

STATEMENT OF INFORMED CONSENT

Procedure

The subject will arrive in the morning after a good night of sleep and no food or drink (except water) since 10:00 p.m. the previous evening. Testing will start soon after the subject's arrival in the laboratory. The subject should become familiar with the surroundings and feel relaxed and comfortable in the lab area. Please feel free to ask the technician any questions that may concern you. This project has been reviewed by the Institutional Review Board and has been evaluated as non-risk.

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 consume one of the following two test meals: a loaf containing 30 g. coarsely ground wheat bran and 15 ml. lactulose, a loaf containing 30 g. finely ground wheat bran and 15 ml. lactulose, or a drink containing 15 ml. lactulose syrup in 300 ml. water. Breath samples will be taken every 10 minutes for the next five hours.

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

Discomforts

There should be few if any discomforts experienced. The subject

may experience distension, intestinal gas, and mild diarrhea. These discomforts, if occurring, should last only a short time. The subject will be given half the usual dosage of Cephulac brand lactulose syrup. A physician's consent to administer lactulose has been obtained. The subject will also be given 30 g. of standard wheat bran which has been prepared for human studies by the American Association of Cereal Chemists.

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 convince 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: _____

APPENDIX B

RANDOM ORDER OF TEST MEALS

TABLE V
RANDOM ORDER OF TEST MEALS

	Days-Series I			Days-Series II	
	<u>1</u>	<u>2</u>	<u>3</u>	<u>1</u>	<u>2</u>
<u>Subject</u>					
1	FB+L	CB+L	L	CB	FB
2	CB+L	L	FB+L	CB	FB
4	FB+L	L	CB+L	CB	FB
5	FB+L	CB+L	L	FB	CB
6	FB+L	CB+L	L	FB	CB

APPENDIX C

AGE AND WEIGHT OF SUBJECTS DURING
FIRST THREE TEST MEALS

TABLE VI
AGE AND WEIGHT OF SUBJECTS DURING
FIRST THREE TEST MEALS

<u>Subject</u>	<u>Age</u>	<u>CB+L</u>	<u>Weight*</u>	
			<u>FB+L</u>	<u>L</u>
1	26	52.3	52.3	51.4
2	55	59.1	57.7	59.5
3	29	56.4	57.3	56.8
4	29	45.0	44.3	44.3
5	29	60.5	61.4	62.7
6	24	52.3	52.3	53.6

*in kilograms

VITA

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