Dietary manipulation of postprandial colonic lactose fermentation: II. Addition of exogenous, microbial beta-galactosidases at mealtime[1-3]

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ABSTRACT The feasibility and efficacy of adding microbial beta-galactosidase enzymes directly to milk at the time of consumption was explored in adult lactose-malabsorbers. The hydrogen breath test, and on one occasion, the rise in blood glucose, were used as indices of the completeness of intraintestinal hydrolysis and absorption of milk lactose. When added to 360 ml of cow milk containing 18 g of lactose, empirical dosages of three beta-galactosidases—one from *Kluyveromyces* (yeast) and two from *Aspergillus* (fungal)—had some effectiveness in reducing postprandial H_2 excretion, although no in vivo treatment at the dosages chosen was as effective as pre-incubation of the milk in vitro. The yeast enzyme also reduced symptom frequency as compared to intact milk and enhanced postprandial rises in blood glucose. The replacement therapy with exogenous, food-grade beta-galoactosidases may provide a useful intervention to reduce lactose malabsorption and milk intolerance in individuals with primary lactase deficiency. *Am J Clin Nutr* 1985;41:000–000.

KEY WORDS Lactose, lactase deficiency, milk intolerance, beta-galactosidases, replacement enzyme therapy, milk, hydrogen breath test, blood glucose

Introduction

The problem of primary adult lactase deficiency and milk rejection has been discussed in the accompanying article (1). Therapeutic approaches to relieve lactose intolerance and milk avoidance in that fraction of malabsorbers who experience symptoms with only the usual, dietary amounts of milk-sugar include: reduction of the lactose content of the milk (2–4); addition of psyllium (5); or combination of milk with solid food elements (1). Yet another strategy would be oral enzyme replacement therapy with an exogenous, food-grade, microbial beta-galactosidase, analagous to the approach that has been used for decades in the treatment of human pancreatic insufficiency (6). Such an approach would involve adding enzymes directly to milk *at mealtime*, rather than the conventional pre-incubation of milk for 24 h under refrigeration. In theory, this would obviate certain disadvantages of the in vitro incubation, such as the sweeter taste of the monosaccharide-rich, hydrolyzed milk, the need for forethought and anticipation in the preparation, and the difficulty of consuming the lactose-containing portions of meals taken away from home in restaurants, cafeterias, and the homes of friends and relatives.

Several investigators have reported mealtime addition of beta-galactosidase to milk fed to human subjects. Kobayashi et al (7) found that calcium absorption was enhanced by the immediate addition of an enzyme to a milk-based infant formula, whereas preincubation of the same diet reduced calcium bioavailability. Rand (3) found that enteric-coated capsules of beta-galactosidase reduced

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Received April 12, 1984.
Accepted for publication September 4, 1984.

the symptoms of milk intolerance in lactose-intolerant adults as compared to a placebo. Mizote et al (8) reported a reduction in diarrhea experienced with the ingestion of milk by Japanese postgastrectomy patients when a dose of exogenous beta-galactosidase accompanied its consumption. Both Mizote et al (8) in their postgastrectomy lactose-malabsorbers and Vega-Franco et al (9) in a group of malnourished children between the ages of 5 and 24 months in Mexico City observed a greater increment in the postprandial concentration of glucose when a beta-galactosidase was added directly to the milk at mealtime. Recently, health-food stores have begun to market pills and capsules containing beta-galactosidases and advertised for the purpose of digesting lactose and treating milk intolerance. Despite the aforementioned experience, the physiological bases for such claims have not been evaluated. The notion that exogenous enzymes added to milk should function during intestinal passage has recently been bolstered by the demonstration of "auto-digestion" of the lactose in yogurt by Kolars et al (10). They showed that a bacterial 'lactase' liberated from the culture organisms of yogurt with their disruption in the stomach could assist in the digestion of the intrinsic lactose in commerical, unpasteurized yogurt.

The present paper is an extension of the work described in the accompanying article, and involves many of the same experimental subjects (1). The H_2 breath-analysis test and the change in blood glucose has been used as indices of carbohydrate absorption. We examine in a preliminary fashion the possibility for exogenous, microbial beta-galactosidases to serve in oral enzyme replacement therapy for primary adult lactase deficiency.

Subjects and methods

Subjects

The subjects participating in this study were drawn from the pool of lactose malabsorbers identified in the screening procedure described with our previous study in the companion article (1). They all showed increments of ≥25 ppm in response to a test dose of intact milk (ie 360 ml of unmodified, whole, cow's milk with 18 g of intact lactose). They were free of gastrointestinal illness and of antibiotic usage during the period of study. All subjects agreed to participation after the risks and benefits had been fully explained, and informed consent obtained. The procotol had been approved by the Committee on the Use of Humans as Experimental Subjects of MIT and the Human Rights Committee of INCAP.

Collection of expired air and analysis of hydrogen

The gas collection and gas analysis procedures are described in detail in the accompanying article (1), and only differed insofar as here 30-min collection intervals have been used over a 6-h period. Expired air was collected in rubber anesthesia bags, transferred to plastic syringes fitted with three-way stopcocks, and analyzed on a Microlyzer Model-12 gas chromatograph (Quintron Instruments Co, Milwaukee, WI (11, 12). The machine was calibrated with standard reference gases of known concentration (Scotty Gas II, Supelco, Bellefonte, PA; Linde Gas, Linde Dividion of Union Carbide, N Chicago, IL). H_2 concentrations were expressed in parts per million (ppm).

Blood glucose determinations

In one experiment, the change in blood glucose was used as a complementary index of carbohydrate absorption. One-ml samples of venous blood were collected from an antecubital vein through a 25 gauge stainless-steel needle into a plastic tuberculin syringe and transferred immediately into a small plastic tube containing sodium oxalate and sodium fluoride as anticoagulant and glucose preservative, respectively. Samples were taken in the fasting state, and at 30, 60, 90 and 120 min after the test dose of milk. After centrifugation and separation, the concentration of plasma glucose was determined by an ortho-toludine method (13). Glucose concentration was expressed in mg/dl.

Absorption Tests

Fifteen proven lactose-malabsorbers, including the 13 subjects described in the previous meal-studies (1), had undergone the screening test with 18 g of lactose-contained in 360 ml of intact milk (IM) (La Pradera, Guatemala City, Guatemala). For the purposes of the present protocol, they also underwent two additional tests on two subsequent occasions with the same volume of milk and in a randomized order of presentation. On one occasion, lactose-prehydrolyzed milk (HM), milk incubated for 24 h with 0.25 g of LactAid (LactAid Co, Pleasantville, NJ), a commercial, food-grade beta-galactosidase enzyme preparation derived from the yeast, *Kluyveromyces lactis*, was provivded in a liquid form. This treatment has been shown to effect a >90% hydrolysis of the lactose to its constituent monosaccharides—glucose and galactose (3)—, and this has been confirmed in our laboratory (14). On another occasion, 0.5 g of LactAid was mixed directly into the milk within 5-min of its consumption. Milks mixed with enzymes immediately (5-min) prior to ingestion have been termed "enzyme-added milks" or EM in these studies. The amount of enzyme added was chosen empirically as a simple, two-fold multiple of the manufacturer's recommended dosage for in vitro incubation of this volume of milk. The *K. lactis* enzyme has a pH optimum of 6.8 and a temperature optimum of 37°C. Breath samples were taken before, and at 30-min intervals after the doses for 6 h, and at least 48 h were allowed between successive absorption tests.

Five of these same lactose-malabsorbing subjects were later studied on one further occasion with an identical breath-collection procedure after drinking 360 ml of a 5-min EM prepared by adding 133 mg of Lactase N (GB Fermentation Industries, Kingstree, SC), a commercial, food-grade beta-galactosidase derived from the fungus, *Aspergillus niger*, and provided in a powdered form. This dose was chosen on theoretical grounds, based on kinetic information provided by the manufacturer; 133 mg of Lactase N represents the amount of the preparation that would hydrolyze the 18 g of lactose in the milk in 60 min if incubated at 37°C at its pH optimum in vitro. Lactase N has a pH optimum of 4.4 and a temperature optimum of 60°C, but conserves 70% of its hydrolytic activity at 37°C, body temperature.

Yet a third enzyme preparation, also derived from *A. niger*, was studied; this at multiple dosages but in a single malabsorber (OB). This preparation was Milk Digestant (Malabar Formula, Cypress, CA), a product sold in health-food stores as tablets, each containing 25 mg of 'lactase' and 2 mg of rennin. The label instructions suggested the use of one tablet per 2 oz (60 ml) of milk. The breath collection procedures once again were the same as those in the prior protocols.

To determine whether the existence of in vivo hydrolysis by the *K. lactis* enzyme could be confirmed by an alternative index of carbohydrate absorption (8, 9), a procotol based on blood glucose determination was conducted in three lactose-malabsorbers. The blood glucose method is usually employed with 50 g of lactose in an aqueous solution, but it has been used on occasion with milk, employing modified criteria for the significant blood-glucose rise (15). In order to improve the discrimination, we increased the milk volume to 480 ml to contain 24 g of lactose, or about one-half the conventional dosage used for blood-glucose-based "lactose tolerance tests." In this experiment, we increase the dose of enzyme added 5-min prior to consumption to 3 g of LactAid. HM was produced by incubating 480 ml of milk for 24 h at 4°C with 0.5 g of LactAid. In addition to blood glucose, breath H_2 was collected concurrently, at 30-min intervals, for a period of 6 h.

Finally, in order to determine if solid foods taken with milk interact with the exogenous beta-galactosidase, the efficiency of mealtime addition of enzyme was tested in the presence of a standard meal. The thirteen malabsorbers who had participated in the meal studies in the accompanying paper (1), participated in yet a third experiment. The same basic standard meal—40 g of cornflakes, one hard-boiled egg, and one medium banana—was used. It was consumed along with 360 ml of an EM to which 0.5 g of LactAid was added 5-min prior to the meal, and these data were compared with the breath H_2 response in the meals with IM and HM (1). Breath samples were collected and analyzed before and at *60-min* intervals following the completion of the meals for a total of 6 h.

Symptom response

The subjects were asked at the conclusion of each 6-h collection period by the technician (A-MG) performing the breath test whether they had experienced the *presence* or *absence* of any of the following symptoms: 1) abdominal distension or cramps; 2) flatulence; and 3) diarrhea, since the administration of the test beverage. Diarrhea, in this case, was defined as an urgent, watery defecation. Thus, the subjects were registered as "symptomatic" with respect to gastrointestinal carbohydrate intolerance if he or she had any one or more of the aforementioned symptoms, and "asymptomatic" if not. The tests were not blinded as both the technician and the subject (if he or she chose to inquire) were aware of the composition of the test beverage.

Data Analysis

The excess excretion of breath H_2 was quantified by calculating the area under the discontinuous curve of serial change in breath H_2 concentration by a triangulation method (16), with excess volume of pulmonary H_2 expressed as ppm · hr, an approach analogous to ones used in other laboratories (17, 18). The criterion for significant, incomplete absorption of carbohydrate was a postprandial increase in breath H_2 concentration of ≥20 ppm at one or more collection interval (19). The significance of differences in treatment responses within groups was determined using the Student *t* test for paired data (20). The differential symptoms responses to the treatments of fluid milk containing LactAid at a 360 ml volume were evaluated for significance using Chi square analysis (20).

Results

The effect of mealtime addition of yeast (K. lactis) *beta-galactosidase to fluid milk on breath H_2 concentration and intolerance symptoms*

The individual data for excess excretion of H_2 following 360 ml of IM, HM, and the EM prepared with 0.5 g of LactAid added 5-min prior to consumption for 15 lactose-malabsorbing subjects are shown in **Figures 1 and 2.** The mean (±SEM) excess excretion of H_2 for the three types of fluid were, in ppm · hr: 118 ± 14 (IM); 76 ± 14 (EM) and 15 ± 7 (HM), respectively. All subjects excreted less H_2 with HM than with IM ($p < 0.02$) (Fig 1). Twelve of 15 subjects had a lesser excretion of breath H_2 with the LactAid added 5-min prior to ingestion, as compared to the intact milk ($p < 0.05$) (Fig 2). Specifically for those 12 subjects who showed a decrement in the H_2 excretion with the EM, the average reduction compared to their response to IM was 51% (range: 7 to 86%). With IM, all 15 malabsorbers (by definition) had an increment in breath H_2 concentration of ≥20 ppm, signifying biologically-important carbohydrate malabsorption by contemporary standards. With HM, 4 of 15 subjects (27%) had a positive breath H_2 response. With the

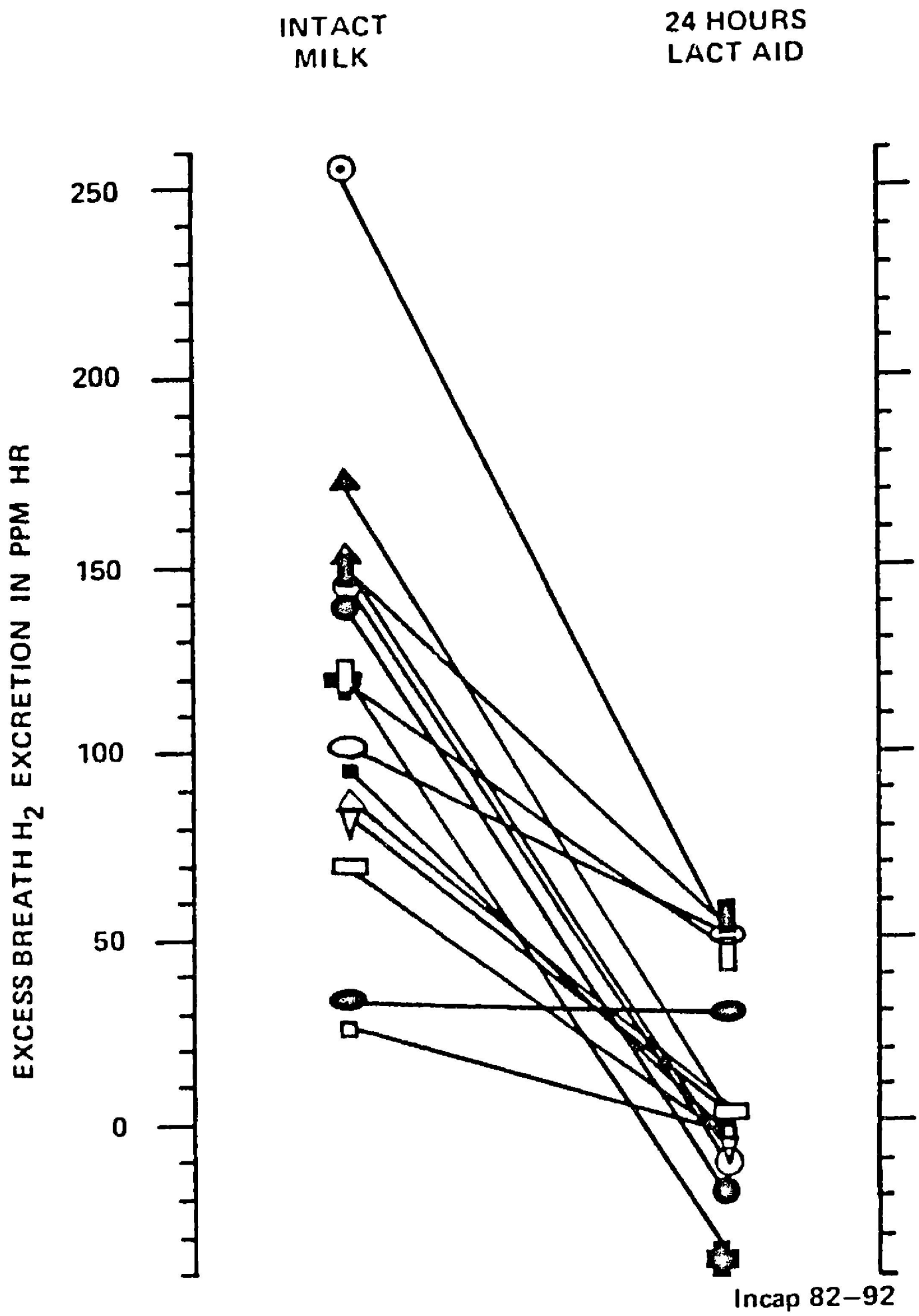

FIG 1. Comparison of the excess volume of breath H_2, expressed in ppm · hr, produced during 6 h following ingestion of 360 ml of intact milk or 360 ml of hydrolyzed milk in 15 lactose-malabsorbers.

EM, 9 of 15 subject (60%) continued to show incomplete absorption of lactose.

The results of the symptom scores for the three treatments are shown in Table 1. Only one subject experienced diarrhea with the 360 ml dose of IM, but 13 of 15 malabsorbers reported some manifestations of intolerance with IM ingestion. With EM, 9 of 15 subjects had symptoms, and with HM, 3 subjects were symptomatic. The symptomatology

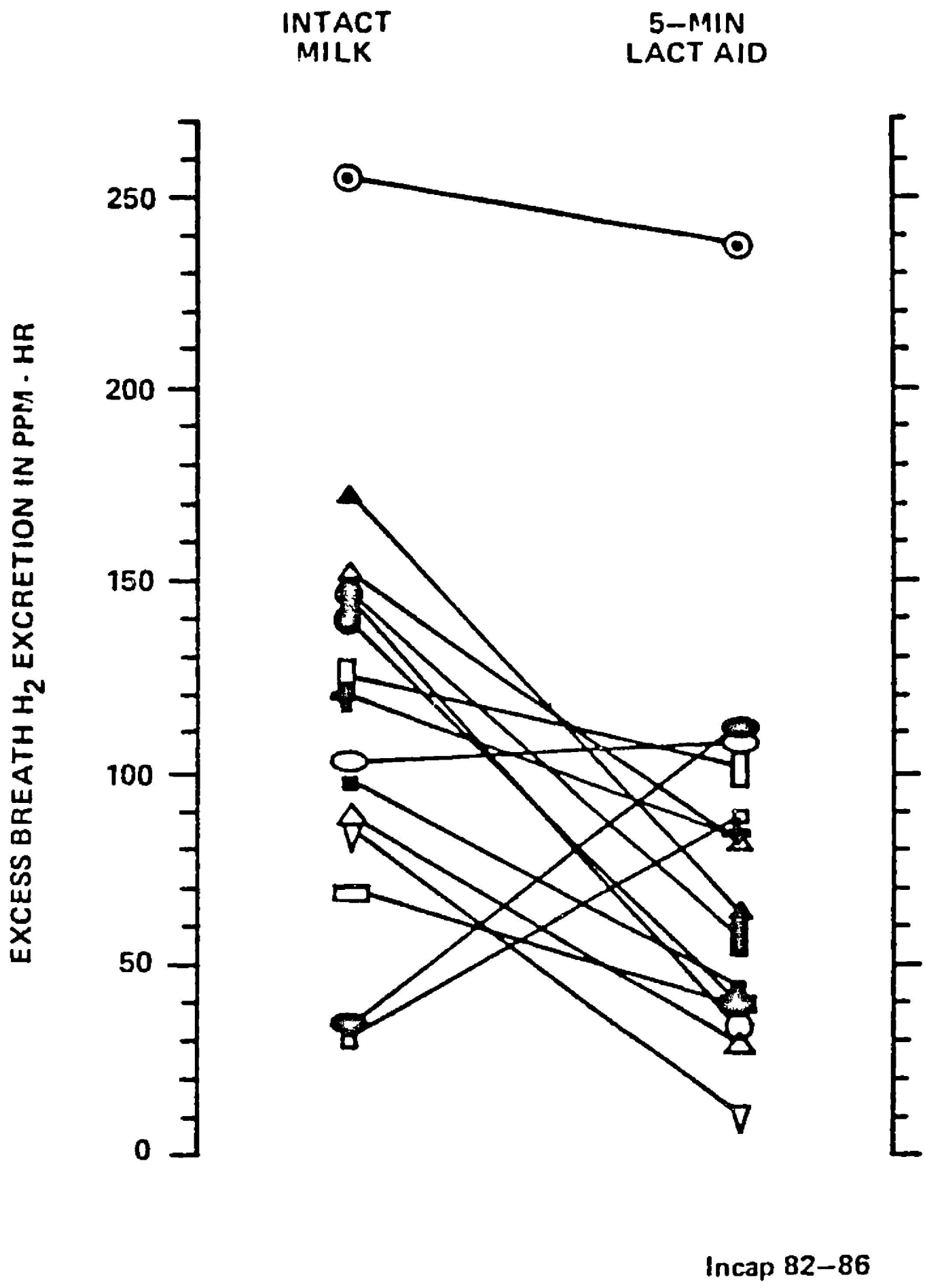

FIG 2. Comparison of the excess volume of breath H_2, expressed in ppm · hr, produced during 6 h following ingestion of 360 ml of intact milk or 360 ml of milk pretreated with 500 mg (10 drops) of LactAid, 5-min prior to consumption in 15 lactose-malabsorbers. The same 15 individuals as in Fig. 1.

produced by hydrolyzed milk was significantly less than that experienced with the other two treatments ($p < 0.05$). The reduction in the number of symptomatic individuals produced with EM was not statistically less than that for IM ($p < 0.1$).

The effect of mealtime addition of yeast (K. lactis) *beta-galactosidase to fluid milk on postprandial blood glucose concentrations*

In order to confirm that the reduction in breath H_2 with the EM was due to true in

TABLE 1
Symptomatic response to the 360 ml dose of milk in 15 lactose-malabsorbers receiving three forms of fluid milk

Subject	Intact milk	5-min treatment	24-hr pretreatment
001	+	+	0
002	+	+	0
003	(+)	+	+
004	+	+	+
005	+	+	0
006	+	0	0
007	0	0	0
008	+	+	+
009	+	+	0
010	0	0	0
011	+	+	0
012	+	0	0
013	+	0	0
014	+	0	0
015	+	+	0

+ = one or more symptoms experienced during the 6 h of the absorption test
(+) = experienced diarrhea
0 = no symptoms experienced during the 6 h of the absorption test

vivo hydrolysis, a complementary index of carbohydrate absorption—blood glucose—was employed in 3 lactose-malabsorbers who had participated in the foregoing experiment. The results of simultaneous determination of breath H_2 concentration over 6 h and of plasma glucose levels over 2 h after *480* ml of milk, containing 24 g of carbohydrate, was given as IM, as HM, and as an EM with *3* g of LactAid added 5-min prior to consumption are illustrated in Figure 3. As with the lower (360 ml) dosage of milk with exogenous enzyme added at the time of consumption (above), a partial reduction in breath H_2 excretion was produced with a 480 ml volume of an EM. The virtual elimination of an H_2 response was produced with 480 ml of HM. The respective mean (±SEM) volumes of H_2 excreted in response to the various treatments of 480 ml of milk were, in ppm · hr: 198 ± 49 (IM); 71 ± 15 (EM); and −2 ± 18 (HM) (Fig 3, upper graphs).

The results for the blood glucose responses for the same experiment are shown in the lower graphs of Figure 5. For each individual, the peak increments in plasma glucose for the HM and EM treatments were virtually identical, but a flat (MC) or concave (AR and EL) glucose response was seen with the milk containing intact lactose (IM). Subject AR had maximum increments of 15 mg/dl with HM, 12 mg/dl with EM and 5 mg/dl with IM. Increments for subject EL were 12 mg/dl (HM), 7 mg/dl (EM) and −2 mg/dl (IM), and for subject MC, 14 mg/dl (HM), 13 mg/dl (EM) and 4 mg/dl (IM). In each instance, the maximum increase in plasma glucose with enzyme-added milk was ≥7 mg/dl, and higher than the respective glucose response to intact milk in the same subject.

The effect of mealtime addition of fungal (A. niger) *beta-galacosidases of fluid milk on breath H_2 excretion*

In 5 lactose-malabsorbers who had previously undergone the screening absorption test with 360 ml of IM, and a test with 360 ml of EM prepared with LactAid (0.5 g), yet another test with 360 ml of an EM prepared with 133 mg of Lactase N, added 5-min prior to consumption, was conducted. As shown in Figure 4, the reduction in the volumes of breath H_2 excretion over 6 h with the Lactase N EM was equal to that for the LactAid EM. The mean excess volumes of excreted H_2 for the three treatments in this subgroup of 5 subjects were, in ppm · hr: 104 ± 12; 54 ± 13 (LactAid EM); and 59 ± 15 (Lactase N EM), respectively.

The commercially-available product (Milk Digestant) which is offered as a treatment for lactose intolerance to be taken with milk at mealtime is comprised of an *A. niger*- derived beta-galactosidase combined with rennin. The pill is not enteric coated. In one of our subjects (OB), we compared the reduction in the H_2 response to 360 ml of intact milk with the full, recommended dosage of tablets for that volume of milk (6 pills), with one-half (3 pills) and with twice this dosage (12 pills). The pattern of postprandial H_2 excretion for each of these treatments along with that for the IM alone is shown in Figure 5. The ascending dosages of Milk Digestant produced a 19, 51, and 46% reduction, respectively, in the 6-h volume of excess breath H_2 excretion with respect to the IM treatment. If the response to 133 mg of Lactase N in this same subject (Figure 4, solid rectangle) is compared to the response to 6 pills (150 mg) of enzyme from Milk Digestant, the

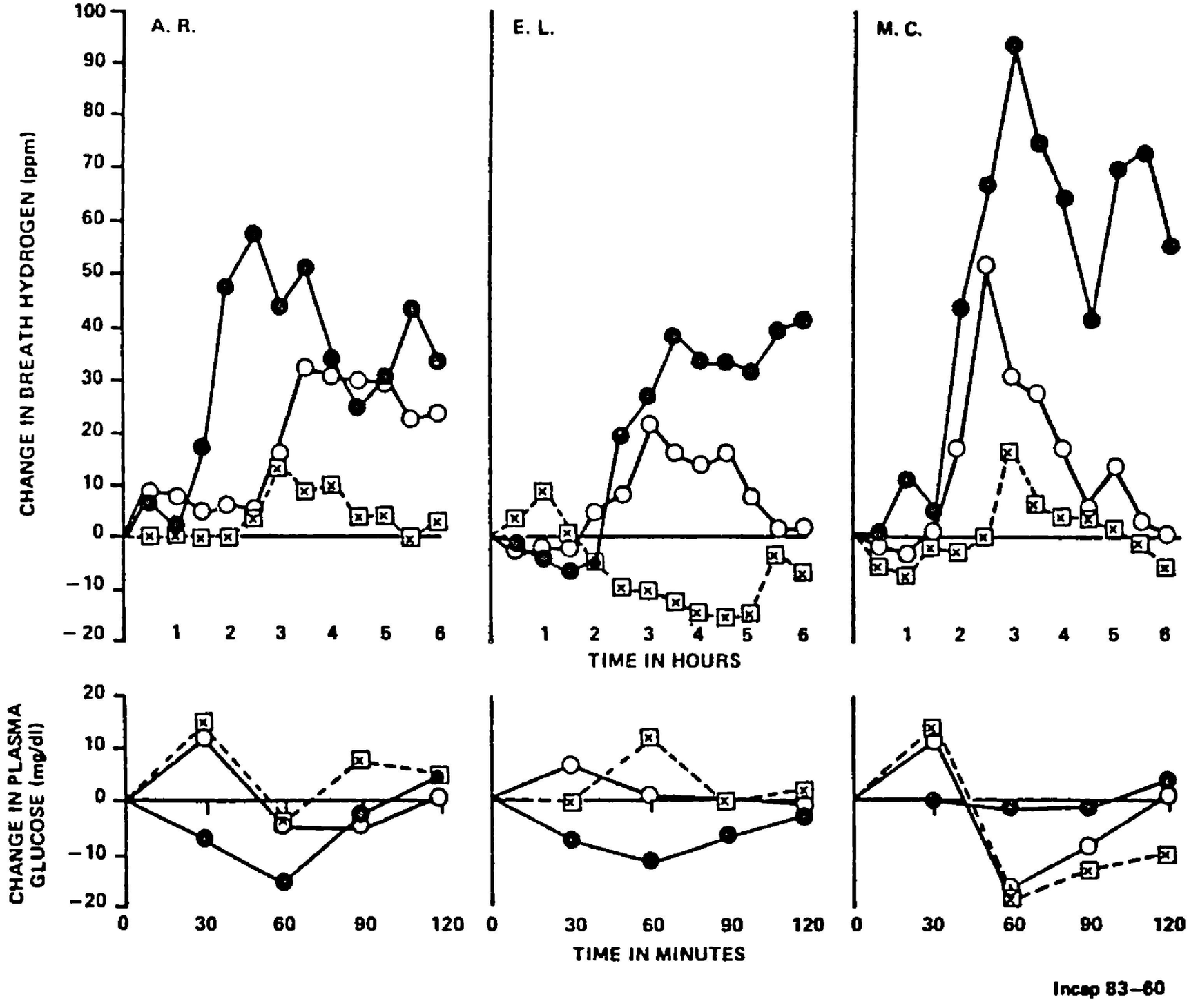

FIG 3. Breath H_2 concentration at 30-min intervals over 6 h in three lactose-malabsorbers after ingesting 480 ml of intact milk alone (●), with 3 g (60 drops) of LactAid added 5-min prior to ingestion (O), and pretreated with 24 h of incubation with LactAid (⊠) (upper graphs). The change in plasma glucose concentration at 30-min intervals over 2 h during the same three treatments in the same three subjects indicated by the same respective symbols (lower graphs).

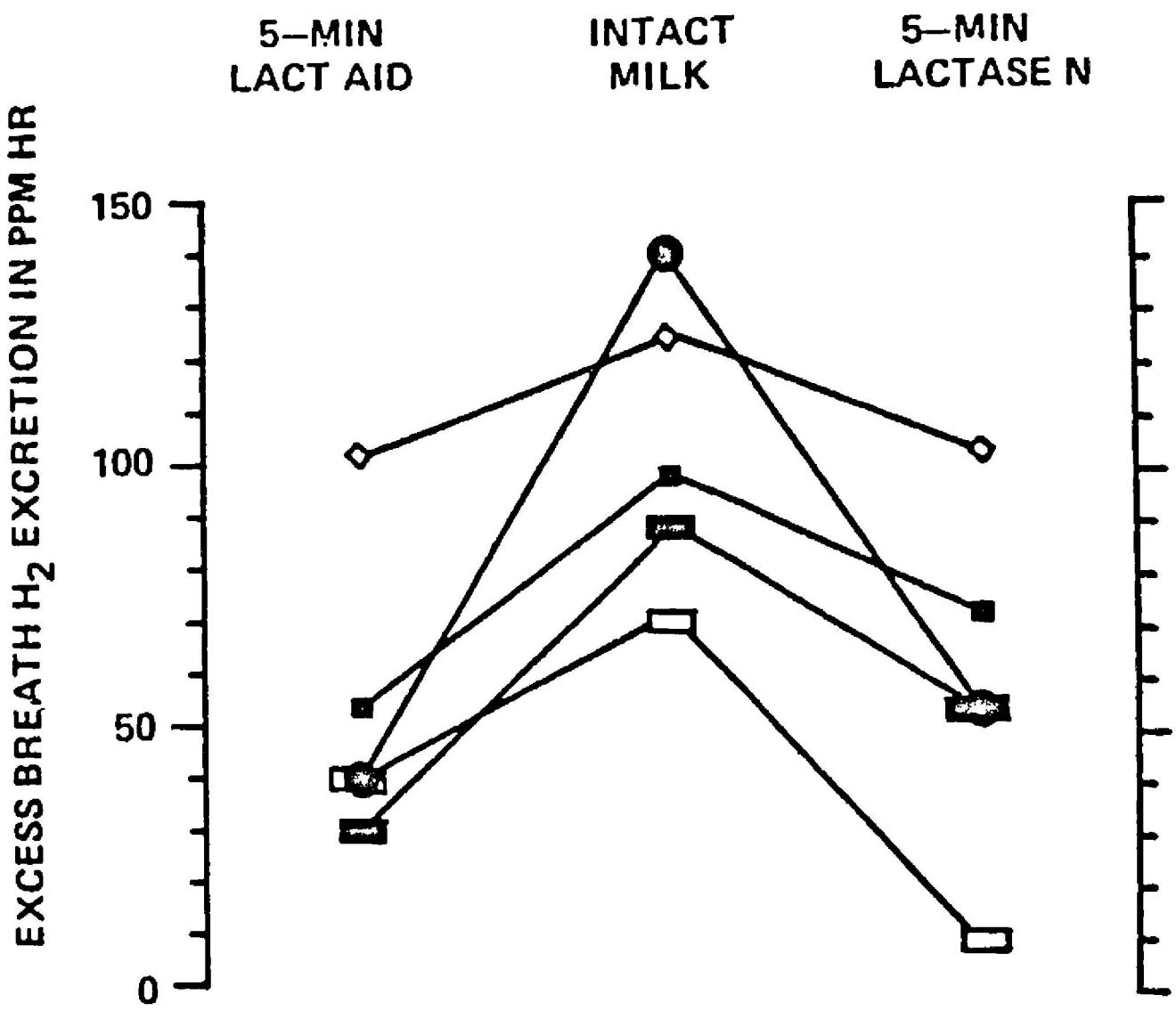

FIG 4. Comparison of the excess volumes of breath H_2 in ppm · hr produced during 6 h following ingestion of 360 ml of milk as intact milk or milk with 10 drops of LactAid or 133 mg of Lactase N added 5-min prior to consumption in 5 lactose malabsorbers.

potency of the enzyme in this over-the-counter product would appear to be about the same as that of the purified powder.

Effect of exogenous yeast (K. lactis) *beta-galactosidase on the production of H_2 in the presence of solid foods*

To determine whether adding solid foods would enhance, inhibit, or exert a nil effect on the in vivo hydrolytic potential of 0.5 g of LactAid added to 360 ml of milk immediately prior to the meal (an EM), the same 13 lactose-malabsorbers who underwent the solid-food studies in the accompanying article (1) also participated in a third round of breath tests with the standard meal plus 360 ml of EM. As shown in Figure 6, the pattern of pulmonary H_2 excretion was virtually identical to, and superimposable on, that produced with the standard meal plus IM, and distinct, and significantly different, from that with the meal plus HM. Thus, essentially no reduction in H_2 production was produced by immediate addition of the *K. lactis* enzyme to milk consumed along with solid foods, as compared with a 51% reduction in H_2 production with the milk taken alone by responders (Fig 2).

Discussion

Individuals with pancreatic insufficiency are commonly treated by adding pancreatic extract directly to meals to replace deficient endogenous digestive enzymes (6). Given the availability of food-grade beta-galactosidases of microbial origin, the concept of "enzyme replacement therapy" for lactase-deficient individuals is a logical consequence. It was

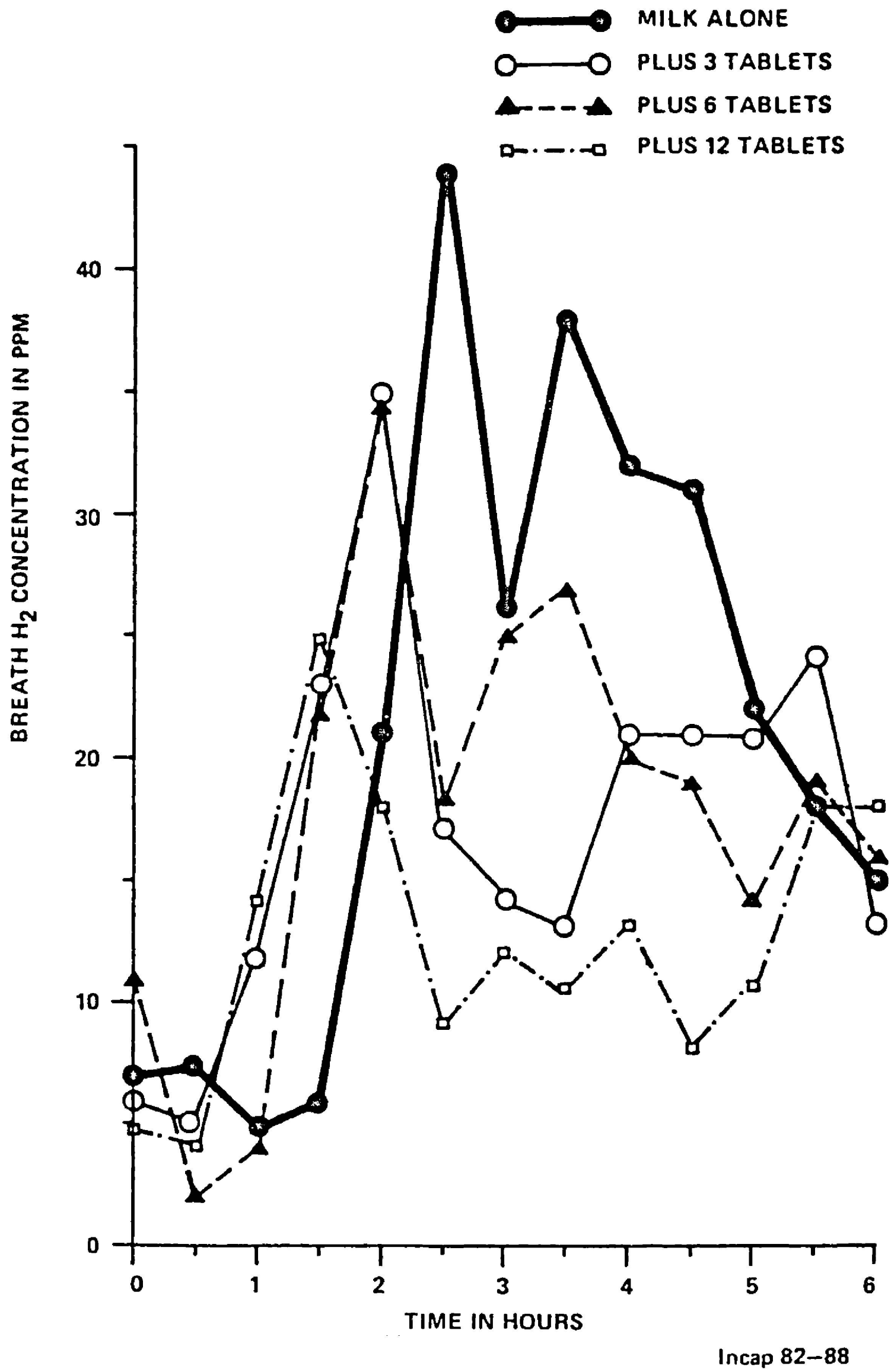

FIG 5. Breath H_2 concentrations in ppm at 30-min intervals over 6 h in a single lactose-malabsorber after ingesting 360 ml of milk as intact milk, alone, or with 3, 6 or 12 tablets of Milk Digestant, containing 75, 150 and 300 mg of an *A. niger* beta-galactosidase, respectively. The manufacturer's recommended dosage for 12 oz of milk is 6 tablets.

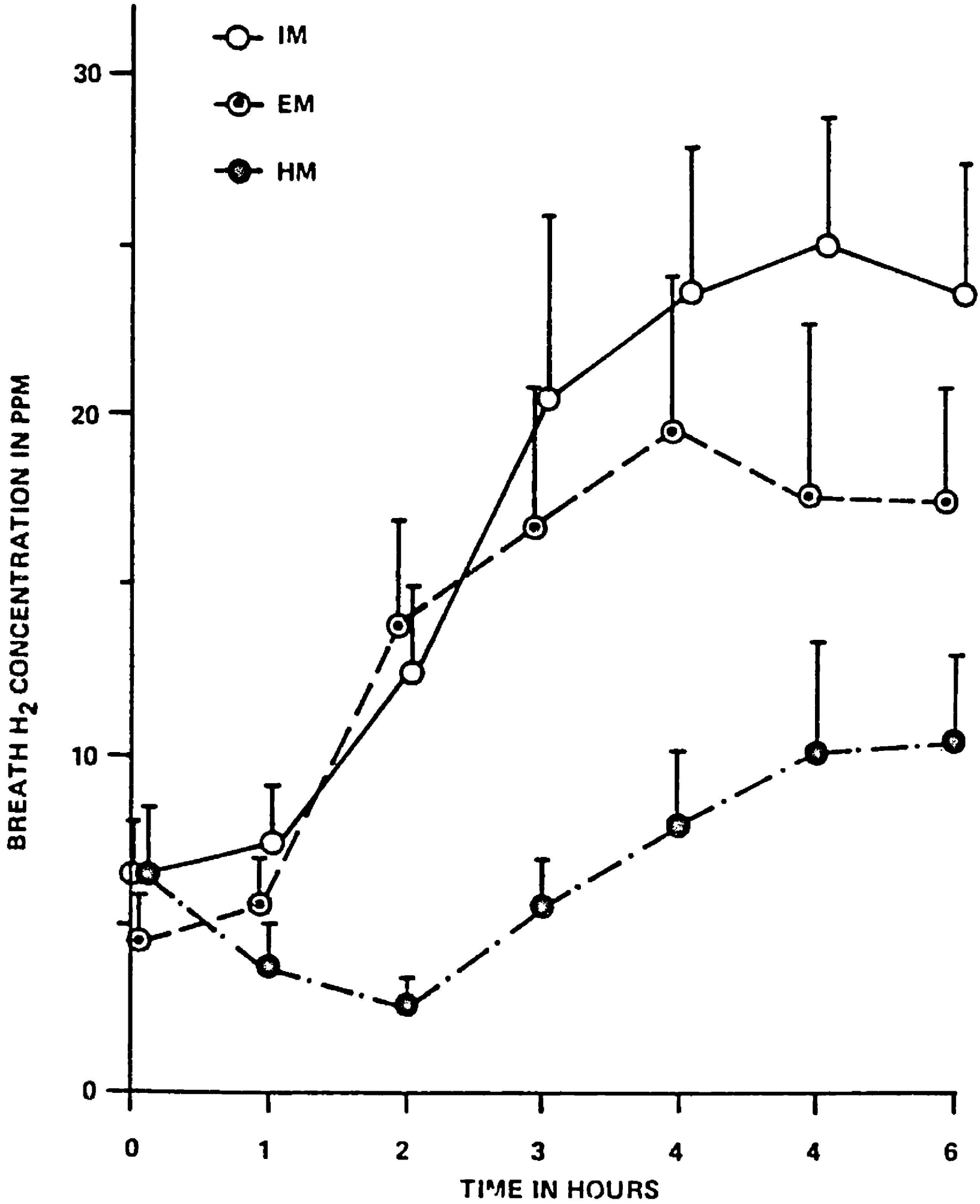

FIG 6. Mean breath H_2 concentration (±SEM) in ppm at 60-min intervals over 6 h in 13 lactose malabsorbers after ingestion of the standard meal with 360 ml of intact milk -O-, prehydrolyzed milk -●-, or milk to which 10 drops of LactAid had been added 5-min prior to consumption -O-.

recently shown that the culture bacteria in yogurt release a functional hydrolase during gastrointestinal passage producing assisted digestion of yogurt-lactose in vivo (10), and, in fact, preparations of 'lactases' expressly designed for oral use with meals have appeared as over-the-counter formulations in health-food stores. Unlike pancreatic enzymes, which normally act in a dispersed form in the intestinal chyme, intestinal lactase is a membrane-bound protein, attached to the brush border of the intestinal mucosa. The pH and temperature optima of microbial enzymes may not correspond to conditions within the intragastic or intraintestinal segments where exogenous beta-galactosidases would have to act to be effective, and there are no a priori assurances that the integrity and enzymatic activity of these microbial proteins would resist acid denaturation or peptic digestion during the transit through the stomach, or resist digestion by pancreatic proteases in the duodenum. Moreover, dietary factors such as food might theoretically influence in vivo efficacy of lactose hydrolysis by exogenous enzymes. Only two scientific reports using 'lactase' replacement provide objective evidence of a true enzymatic effect in vivo (8, 9), and this was exclusively blood glucose curves. Thus, the approach of replacement therapy with beta-galactosidases is conceptually a novel strategy, but it had received only limited investigation. Before the proliferation of products and claims be allowed to reign unchecked, further physiological assessment of the existence and extent of true in vivo hydrolysis of lactose by exogenous enzymes needed to be carried out.

As discussed, the H_2 breath test is an ideal index of colonic fermentation of non-absorbed carbohydrate as it is non-invasive, sensitive and specific, capable of being used with foodstuffs and meals, and able to detect small amounts of carbohydrate that escape small intestinal removal (1, 19). It has proven to be specifically useful in determining which dietary treatments of milk actually produce an effect that reduces lactose malabsorption (21). Using this index, we were able to show the expected virtual elimination of incomplete absorption of lactose when milk was preincubated in vitro with the prescribed amounts of beta-galactosidase as demonstrated consistently by numerous investigators (21–25). The H_2 breath response was also reduced in 80% of lactose-malabsorbers by an average of about 50% when an empirically-chosen dosage of a beta-galactosidase from the yeast, *K. lactis*, was added directly to the milk at the time it was drunk, suggesting that some degree of in vivo hydrolysis of milk lactose was being produced. It is unlikely, although theoretically possible, however, that exogenous enzyme could act specifically on the bacterial flora of the colon to suppress fermentation of carbohydrate and artifactually reduce H_2 production.

To eliminate any doubts about the veracity of our interpretation of the breath H_2 data, we used a far less suitable index of carbohydrate absorption—the blood glucose response—as a complementary indicator. The blood glucose response from whole milk is, admittedly, less sensitive and reliable than that following aqueous glucose, especially if the lactose dosage is less than 50 g (19). However, in the three malabsorbers studied with 480 ml of milk in the form of IM, EM and HM, the rise in glucose was virtually identical for lactose-hydrolyzed milk which contained predominantly preformed glucose and galactose as its carbohydrate fraction, and with milk to which 3 g of LactAid was added at the time of consumption. Admittedly, this is a large dose of enzyme, equivalent to seven times the quantity required to hydrolyze the lactose in the same volume of cow's milk at refrigeration temperatures in 24 h, but the sole purpose of the experiment was to demonstrate the feasibility of in vivo hydrolysis by an exogenous beta-galactosidase. In these subjects, the changes in breath H_2 and in blood glucose with the EM treatment correspond consistently with the interpretation that more lactose had been converted into the monosaccharide constituents and absorbed in the presence of exogenous enzyme than with untreated milk. Thus we confirm previous findings with blood glucose (8, 9).

The enzymatic characteristics of natural microbial enzymes raise the question as to the preferred site of in vivo hydrolysis and of differential efficacy. The fungal enzyme has a pH optimum of 4.4 and conserves 70% of its activity at the human body temperature.

If hydrolysis were to take place in the *stomach*, and *A. niger* preparation would be more logically suited to efficient activity under these conditions. The yeast enzyme has a pH optimum of 6.8 and a temperature optimum of 37°C, precisely the conditions of the human small intestine. If hydrogen were to be primarily *postgastric* in nature, the *K. lactis* enzyme would be more likely to prove more efficient. Since consistent criteria were not used to choose the dosages of yeast and fungal enzyme preparations and they were not matched as to protein content or in vitro enzyme activity units, the comparative treatments are not strictly comparable. What we can appreciate from the various experiments performed with *A. niger* enzymes is that they are also capable of partial—but not complete—elimination of postprandial colonic fermentation of unabsorbed lactose when administered orally at mealtime. Stricter comparisons among beta-galactosidase preparations await more formal dose–response characterization of the various enzyme sources.

Given the physiochemical nature of the *K. lactis* enzyme, two distinct possibilities existed for an interaction with solid foods. On the one hand, the additional buffer capacity of the cereal, egg and banana combined with that of milk, might have *improved* hydrolysis either by protecting the enzyme from acid-peptic destruction or by providing more favorable pH conditions for enzymatic activity within the stomach, or both. On the other hand, a potential for non-specific binding of the beta-galactosidase to food components and segregation of the enzyme to the detriment of its access to the lactose in the meal might impair hydrolysis. Indications from the experiment with solid foods in this study suggest that the latter effect—namely inhibition—is the resultant of the interaction with our 0.5 g dose of LactAid and our standard meal. This has important implications for replacement therapy, since milk is often consumed, not as an isolated beverage, but in the context of a mixed meal. Whether increasing dosages will improve the in vivo efficacy of beta-galactosidases in the presence of solid foods must also be determined.

Relating symptoms experienced during milk challenges to lactose malabsorption is complicated by the complex psychological responses to the sight, smell and taste of milk, and by a background prevalence of gastrointestinal symptoms (25). As these were preliminary physiological trials, symptom recording was not a major component. It was conducted, however, in the trial with various treatments of fluid milk using an EM prepared with 0.5 g of LactAid. Full prehydrolysis had the expected therapeutic results, although three subjects still reported symptoms even with a >90% reduction in lactose content. In only 4 of 13 individuals, however, did the immediate addition convert the subject from symptomatic to symptom-free after 360 ml of milk, not an impressive frequency. Our design, however, was not blinded, and subjects were aware of which treatment was being administered. This reduces the reliability of data based on subjective observations by our participants. The fundamental therapeutic goals of an intervention therapy would be to eliminate lactose intolerance symptoms. Exploration of approapriate dosage levels should incorporate symptom scoring, preferrably in a double-blind design.

The issue of carbohydrate malabsorption due to deficiency of intestinal disaccharidases is complex. Different individuals with primary or secondary lactase deficiency will present different levels of effective hydrolysis, different degrees of symptomatic tolerance to colonic fermentation, and different efficiencies of colonic conservation of the available energy in non-absorbed carbohydrate. The nutritional importance of milk justifies strong efforts to improve the tolerance to and utilization of milk and dairy products by persons with reduced levels of intestinal lactase activity. In the present study, we have demonstrated that addition of food-grade, exogenous microbial beta-galactosidases can reduce the rates of incomplete carbohydrate absorption and colonic H_2 production following the ingestion of milk by lactose-malabsorbers. We feel that the exogenous beta-galactosidases are definitely capable of lactose hydrolysis in vivo. When refined by additional observations, enzyme replacement therapy for human lactase deficiency may prove to be a convenient and effective strategy to permit individuals with greatly diminished endogenous intestinal lactase levels to consume milk and

dairy products without untoward physiological reactions.

We acknowledge support in the donation of product from the LactAid Co, Pleasantville NJ (LactAid) and the GB Fermentation Industries, Kingstree SC (Lactase N). The MicroLyzer Model-12 gas chromatograph was donated by the Quintron Instruments Co, Milwaukee, WI.

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