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SUMMARY

In order to meet the cellular demands of adequate nutrients, there must be a good selection of the foods containing the nutrients and also a good digestion/absorption within the gastrointestinal tract. This awareness to ensure good bioavailability of nutrients is gaining more interest. As a result, the administration of supplemental digestive enzymes has increased. Also, the growing incidences of digestive disorders resulting mostly from malabsorption have led to an increase in the supply and oral administration of digestive enzymes. It should also be noted that supplemental digestive enzymes have also been used successfully as adjunct to various forms of therapies.

Materials: various fungal enzymes (protease 4.5, acid protease, lipase, lactase, amylase, cellulase, and glucoamylase). Other enzymes used were bromelain and papain. Simulated gastric fluid (pH=2 with 3.2 mg/ml pepsin - Sigma), and simulated intestinal fluid with pancreatic enzymes. In order to address the objective, specific enzymes were exposed to a simulated gastric fluid (U. S. Pharmacopeia) formulation. The specific enzymes include various proteolytic enzymes (fungal proteases, bromelain, papain) as well as glucoamylase, lactase, lipase, and cellulase. Each of the commercial enzyme products was incubated at 37°C in a solution of simulated gastric fluid (USP) at pH=2 and containing 3.2 mg/ml of pepsin (Sigma cat# P-7000).

The data in this study indicated the enzymes used could sustain the simulated gastric fluid (low pH and presence of the enzyme pepsin).

The conditions of these experiments, i.e., direct contact of the enzymes with the harsh simulated gastric fluid do not occur under normal conditions.

This set of data indicated that even without the benefits therein mentioned of the ingested foods, i.e., protective shield and buffering, the supplemental enzymes used in this study could sustain the stomach environment, as can be deduced from the simulated gastric fluid exposure.

KEY WORDS

ENZYMES, GASTRIC STABILITY, NUTRITION

GASTRIC STABILITY STUDY OF SUPPLEMENTAL DIGESTIVE ENZYMES

INTRODUCTION

Wellness is fundamentally based on the presence and proper utilization of nutrients within the body. Any impairment in the proper acquisition and utilization of nutrients by the cells will lead to their inability to maintain homeostasis, fight diseases, reproduce, detect and eliminate cancer cells for instance. Additionally, the body response to any therapy depends greatly on the availability of nutrients to control inflammation, integrate metabolic reactions, repair damage and enhance the pharmacology and pharmacokinetics of therapeutic agents such as homeopathic remedies, medications, and various botanicals.

Thus, providing nutrients to the cells not only helps maintain health but also helps with any therapeutic intervention. The intake of foods should not be just to satisfy hunger and "fill the belly", but rather as a judicious mean to provide needed nutrients to the cells of the body. Most human foods are presented in macromolecular forms, i.e., in complex structures that cannot be absorbed directly into the cells of the gastrointestinal (GI) tract. Under normal conditions, most of human foods are hydrolyzed (broken down) into smaller molecules to allow their absorption. It is often assumed that the process of digesting and absorbing food nutrients is straightforward. However, the GI tract is a very complex system and many factors such

as prenatal and postnatal conditions, types and modes of preparation of the foods, medications, stress, eating habits, and exposure to environmental pollutants could impair the digestion and absorption processes.

Thus, in order to meet the cellular demands of adequate nutrients, there must be a good selection of the foods containing the nutrients and also a good digestion/absorption within the GI tract. This awareness to ensure good bioavailability of nutrients is gaining more interest.

As a result, **the administration of supplemental digestive enzymes has increased.** Also, the growing incidences of digestive disorders resulting mostly from malabsorption have led to an increase in the supply and oral administration of digestive enzymes.

It should also be noted that supplemental digestive enzymes have also been used successfully as adjunct to various forms of therapies.

- Most digestive enzymes used therapeutically to alleviate pancreatic insufficiencies and malabsorption are derived from porcine or bovine pancreatic preparations. The use of animal derived enzymes has recently been the subject of several controversies because of the difficulties associated with animal health issues, as well as gastric stability of those enzymes following oral administration.

Recent aseptic biofermentation processes have led to the extraction and production of **effective and pharmaceutical grade fungal enzymes with high activity in hydrolyzing various macromolecules in human foods**.

These advances in biofermentation have provided alternative sources of dietary enzymes that are gastric acid stable and do not have some of the problems associated with pancreas-derived enzymes for oral administration.

Enzymes, as proteins, are normally susceptible to pH change as well as hydrolysis and denaturation by various factors. A common argument against the oral administration of supplemental digestive enzymes has been their susceptibility to denaturation and hydrolysis by the gastric secretions.

In the case of animal-derived enzymes, there is a need to enterically coat the enzymes to prevent their inactivation or hydrolysis by the gastric acid. In terms of the fungal and plant derived enzymes, some may need to be protected from the gastric acid. The addition of enteric coating agents is subject to various concerns within the holistic healthcare industry. Thus, there is a need to establish quality parameters and specifications of dietary enzymes that could be used without inactivation in the stomach. Several enzymes are on the market, yet few of these products have been shown to sustain the harsh

gastric environment. The enzymes in this study are specifically used in the GUNA digestive enzyme formulation.

OBJECTIVE

This study was conducted to specifically address the effectiveness and stability of specific commercial dietary enzymes in conditions simulating the gastric environment. More specifically, this study would determine the stability and level of activity of selected enzymes used as dietary supplements.

MATERIALS AND METHODS

Materials: various fungal enzymes (protease 4.5, acid protease, lipase, lactase, amylase, cellulase, and glucoamylase). Other enzymes used were bromelain and papain.

Simulated gastric fluid (pH=2 with 3.2 mg/ml pepsin - Sigma), and simulated intestinal fluid with pancreatic enzymes.

In order to address the objective, specific enzymes were exposed to a simulated gastric fluid (U. S. Pharmacopeia) formulation. The specific enzymes include various proteolytic enzymes (fungal proteases, bromelain, papain) as well as glucoamylase, lactase, lipase, and cellulase. Each of the commercial

enzyme products was incubated at 37°C in a solution of simulated gastric fluid (USP) at pH=2 and containing 3.2 mg/ml of pepsin (Sigma cat# P-7000). At specific time intervals, aliquots were taken, and reacted with the appropriate substrate. Depending on the specific enzyme of interest, the appropriate assay was conducted to determine level of activity. All the assays were performed according to the compendial methods in Food Chemical Codex, supplement IV. In some cases, after the incubation time in the simulated gastric fluid, the enzyme sample was taken and placed into a simulated intestinal fluid (USP) at pH 6.8 and containing Pancreatin (Sigma cat# P-8096).

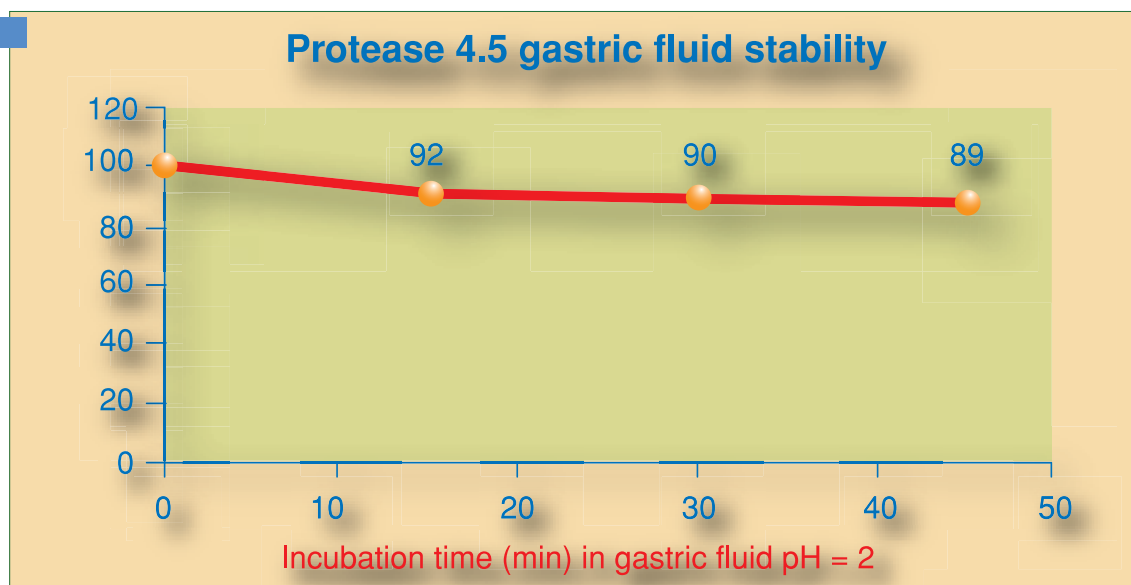
Prior to addition of the commercial enzyme sample to the intestinal fluid containing pancreatin, the intestinal fluid was heated to denature the pancreatic enzymes in pancreatin after a control baseline was determined reflecting any activity by the enzymes in the pancreatin preparation.

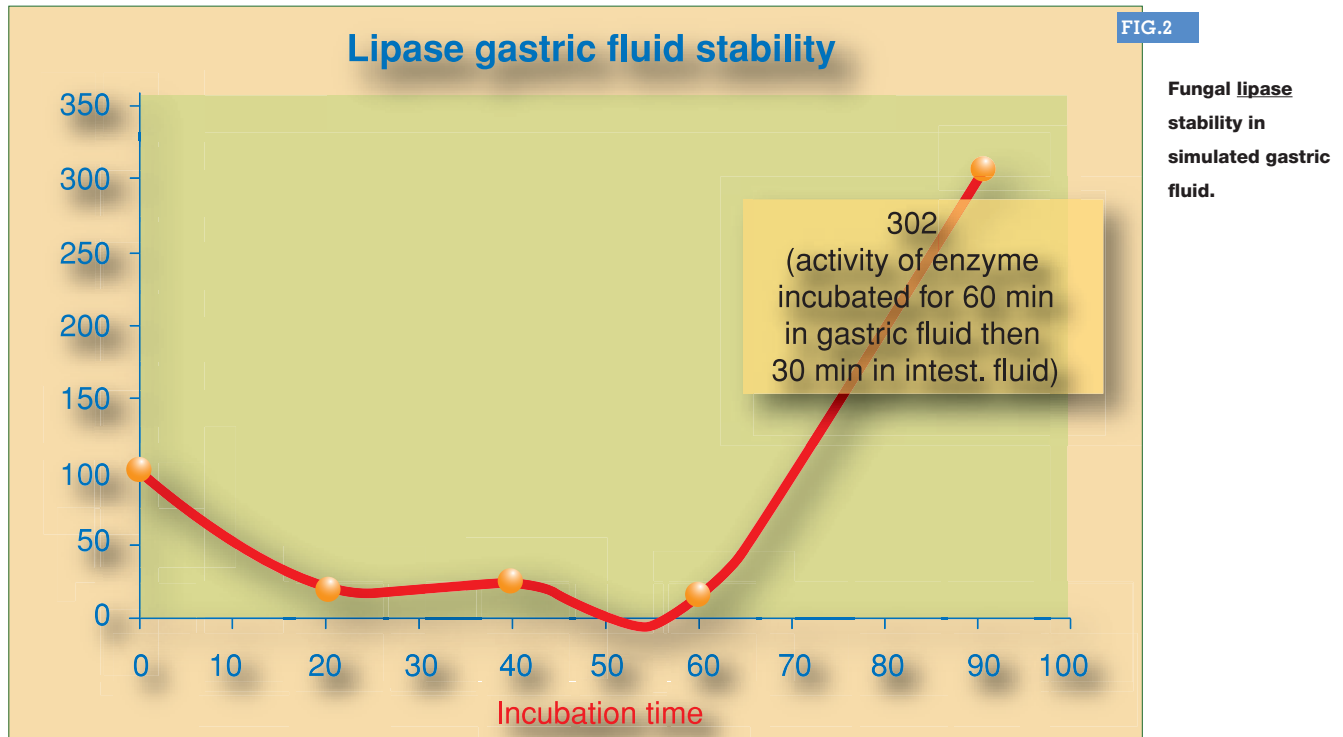
RESULTS

In all cases, the enzymes assayed proved to sustain the simulated gastric fluid, albeit with varying degrees of stability. In cases where some enzymes appeared to have considerably lower activity, a sample was further incubated

FIG.1

Fungal (*A. niger*)
protease 4.5
stability in
simulated gastric
fluid.





in a simulated intestinal fluid that was previously heated to inactivate the endogenous enzymes of Pancreatin. Upon assay, the sample that was incubated in gastric fluid and then transferred in intestinal fluid recovered its activity and moreover had a higher enzyme activity than even the control that has not been subjected to any gastric fluid treatment. This indicated that the gastric fluid did not irreversibly denature that enzyme and, interestingly, there appeared to be some activity enhancement resulting from the treatments.

► After 45 minutes of exposure to high acid condition, this **Protease** maintains almost 90% of its activity indicating its stability in gastric environment (FIG. 1). This protease is very stable and is used in digestive formulation. It helps hydrolyze proteins and improve bioavailability of various amino acids.

► **Lipase** is an enzyme involved in catalyzing the hydrolysis of triglycerides to provide free fatty acids that could be taken within the cells of the enterocytes. A deficit in lipase activity within the gut creates conditions such as steatorrhea, deficiency in lipid soluble vitamins, inadequate transit time of foods in the gut, and

overall malabsorption.

When the lipase in this study was incubated in the gastric fluid for 60 minutes, the enzyme activity dropped.

However, upon re-exposure of the sample to the simulated intestinal fluid (as will occur in the human GI tract during transition from the stomach to the duodenum), the lipase activity level was increased (FIG. 2). This indicates that the gastric acid does not irreversibly inactivate the lipase used in this formulation, because its activity surges when placed in neutral pH conditions. Moreover, because the pancreatin lipase was heat denatured before reintroducing the experimental lipase and assaying it, it could be concluded that the activity observed is due to the experimental lipase used. The incubation in the simulated intestinal fluid appeared to enhance the activity of this lipase.

► **Lactase** is an enzyme that breaks down lactose, the main sugar in milk.

The lack of lactase activity in the small intestine leads to lactose intolerance that is characterized by diarrhea, bloating, and other digestive disorders. This condition is observed in many adults who fail to properly digest lactose and thus avoid milk and other dairy products. This supplemental enzyme is incorporated in formu-

lations to help digest lactose and alleviate lactose intolerance problems. When this enzyme was investigated as to its gastric stability, the data showed that although it loses some of its activity due to the gastric acid, there remains over 40% activity under harsh acid conditions (FIG. 3).

It should be noted that the conditions in this *in-vitro* study of stability are harsher than in the normal GI tract conditions where there will be various molecules acting as buffers over a period of time before the enzymes are completely affected by the gastric acid.

► **Amylase** is an enzyme responsible for breaking down starch.

Starch is the main ingredient in many human foods. It is found in potatoes, bread, rice, and various other grains, and plant materials. The lack of proper starch digestion creates flatulence, bloating, and impairs the body's ability to receive needed energy molecules in the form of glucose and other carbohydrates.

In the present stability study, it was found that although this fungal amylase is initially affected by the exposure to the gastric acid, it regained about 50% of its activity upon re-exposure to simulated intestinal fluid (FIG. 4).

This indicates that this amylase is not irre-

FIG.3

Fungal lactase
stability in
simulated gastric
fluid.

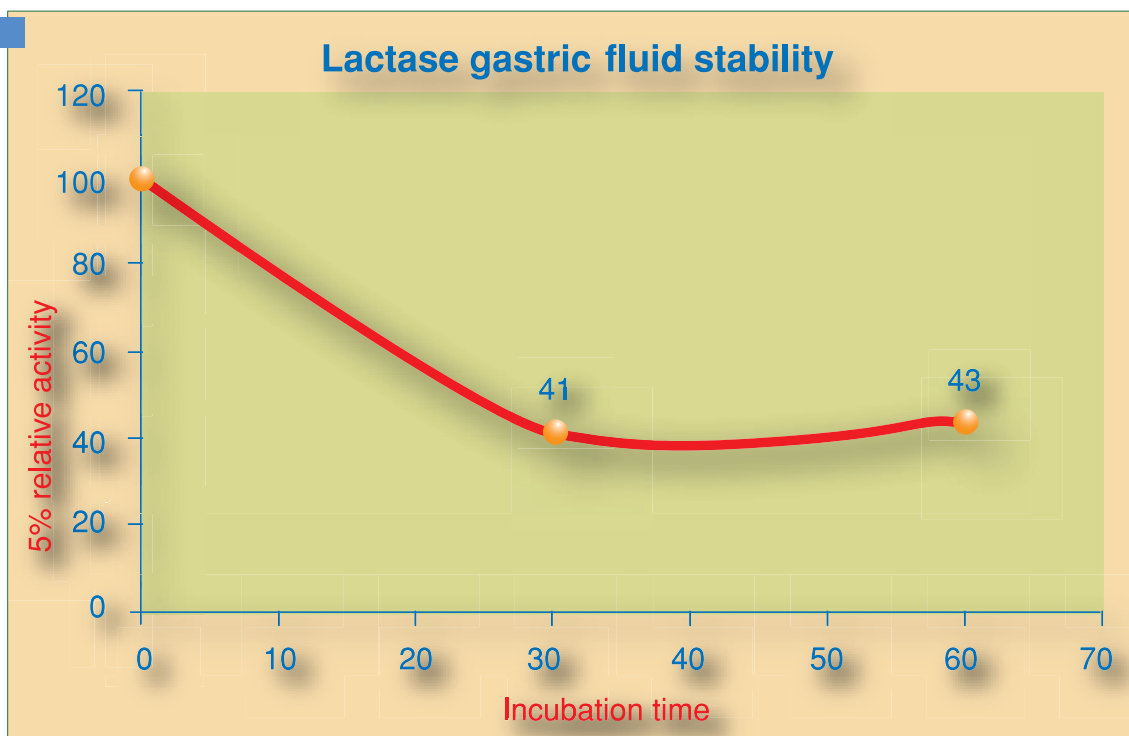
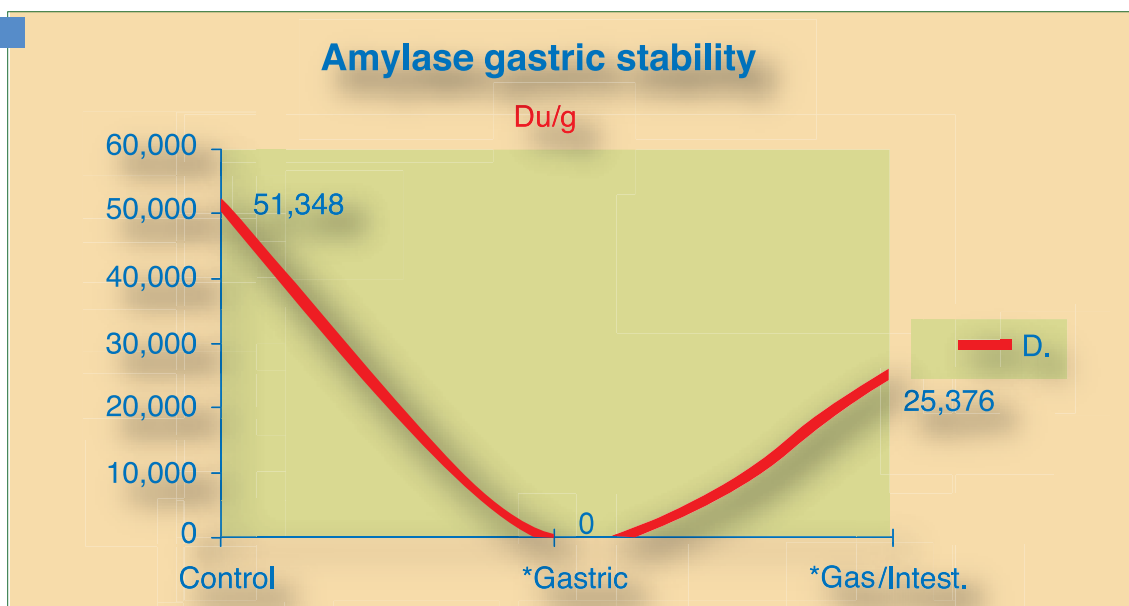


FIG.4

Fungal amylase
gastric stability.



versibly inactivated by the simulated gastric acid in the experiment.

The addition of this amylase in digestive formulations contributes greatly in supplementing the pancreatic amylase, enhances bioavailability of nutrients, and helps avoid various digestive disorders.

► **Cellulase** is an enzyme responsible for digesting cellulose, the main component in vegetables and plant materials. It is used in formulations to break down the cellulosic fibers, thus enhancing their water hol-

ding capacity and thus creating conditions favorable for stool formation and bowel regularity. Additionally, cellulose can help bind various toxins and facilitate their removal.

In this gastric stability study, it can be seen that after 60 minutes exposure to high acid condition of the simulated gastric fluid, the fungal cellulase in the formulation maintained over 60% of its activity (FIG. 5).

Other enzymes that have been shown to have gastric stability are **Invertase** and **Malt-diastase**. These are disaccharidases

and are involved in hydrolyzing sucrose and maltose respectively.

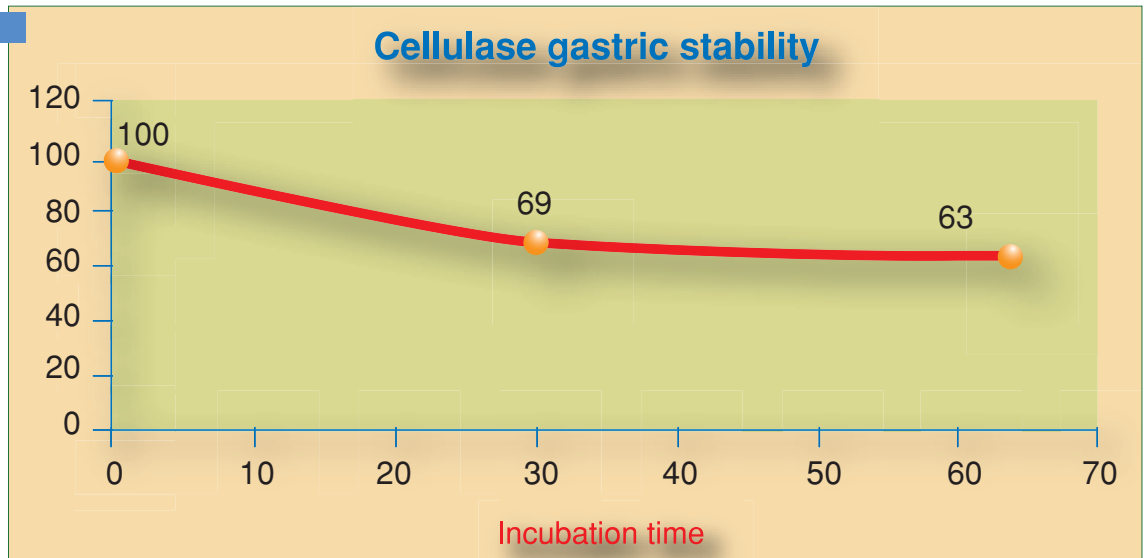
They are also found in the GUNA-ENZYMES formulation.

DISCUSSION

The data in this study indicate that the enzymes used could sustain the simulated gastric fluid (low pH and presence of the enzyme pepsin). The conditions of these experiments, i.e., direct

FIG.5

Fungal cellulase
gastric stability.



contact of the enzymes with the harsh simulated gastric fluid do not occur under normal conditions. Upon taking supplemental digestive enzymes with meals, various macromolecules act as a “protective shield”, protecting the direct exposure of the enzymes to the low pH, and some foods actually contribute some buffering of the gastric content during the initial phases of eating. During that time, supplemental enzymes within the food bolus continue their hydrolytic action.

- This set of data indicated that even without the benefits therein mentioned of the ingested foods, i.e., protective shield and buffering, **the supplemental enzymes used in this study could sustain the stomach environment**, as can be deduced from the simulated gastric fluid exposure. Furthermore, the relative activity of the enzymes after exposure to the various secretions indicates effectiveness in hydrolyzing the various food substrates, thus enhancing absorption. Under some conditions, the enzymes appeared to temporarily lose their activity until they reached a more favorable environment in the small intestine (see Figures).

It should be mentioned that the simulated gastric fluid, although similar in pH, is not the same as the normal human gastric fluid.

However, under this in-vitro condition, an argument could be made as to the

stability or preservation of some enzyme activity in low pH condition similar to the human gastric environment.

CONCLUSION

This study indicated that enzymes used do not need enteric coating as other enzymes on the market.

Some consumers are scrutinizing the incorporation of some enteric coating agents. Thus, this set of enzymes studied here helps provide an alternative for the dietary supplement market. Furthermore, this study proved that some digestive enzymes are stable to low pH conditions and could benefit the digestive system when taken orally. ■

Key Reference

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National Academy of Sciences
National Academy Press
Washington D.C.

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