

Allergenicity of enzyme detergents in guinea pigs exposed to simulated home-use conditions

To the Editor:

The addition of a proteolytic enzyme preparation derived from mutant strains of *B. subtilis* to household laundry detergents has become a widespread commercial practice. Initially this widespread use was not without medical complications, and several reports¹⁻⁴ have noted that factory personnel exposed to the enzyme material had become sensitized, necessitating factories to adopt special measures to eliminate this risk to workers.⁵ Questions have arisen as to whether the householder may become sensitized to enzyme detergents following normal use, particularly from chronic exposure. Griffith and co-workers⁶ have carried out extensive tests for the safety evaluation of enzyme detergents using both animal models and man. Although their human studies were restricted to skin irritation tests and patch tests, their results are frequently cited as demonstrating the absence of an allergenic potential in enzyme detergents following normal home exposures. Unfortunately, their experimental design was restricted to measuring the delayed type of hypersensitivity and could not evaluate other types of hypersensitivity reactions.

Experiments were performed in this laboratory to establish whether guinea pigs could demonstrate immediate hypersensitivity reactions under simulated home-use conditions to a household laundry detergent containing enzymes. The criteria of response were the classical Schultz-Dale reaction, using guinea pig jejunum for evidence of sensitization, and the passive cutaneous anaphylaxis (PCA) technique for detection of sensitizing serum antibodies. A solution of the crude enzyme was used as the challenging antigen. In the Schultz-Dale reaction, the tissue preparations were tested before and after antigen exposure to standard histamine preparations, as well as to increasing doses of the antigen (10^{-8} , 10^{-7} , 10^{-5} , and 10^{-4} Gm. per milliliter). Only those test animals whose sections of isolated gut were responsive to histamine before and after the antigen exposure are included in the study.

Devising suitable experiments presented difficulties, since many of the household detergent preparations contained a blend of two or more enzyme powders or did not contain the same crude enzyme material in every batch. However, one widely used laundry detergent was known to contain the same crude enzyme material in all lots offered for sale, and a sample was obtained from a local supermarket. A supply of the crude enzyme used in its preparation was also obtained from the manufacturer.

In our initial studies, aqueous solutions of two different *B. subtilis* preparations were investigated (Maxatase and Alcalase). Guinea pigs received intraperitoneal injections of weak enzyme solutions (1 mg. in 0.5 ml. + 0.5 ml. of Freund's complete adjuvant) once a week for 3 weeks. The animals were examined for sensitization from 3 to 9 weeks after the final injection. As might be expected, strongly positive responses (4 of 4 animals) were obtained by both the Schultz-Dale and the PCA techniques for each enzyme preparation. Similar positive responses (4 of 4) were obtained following the intraperitoneal injection of the laundry detergent (5 mg.)—this constituted a positive control. Untreated guinea pigs gave consistently negative results in Schultz-Dale and PCA tests.

For the simulated home exposure tests, the experimental animals (groups of 6) were female albino guinea pigs weighing approximately 300 grams. They were housed in standard accommodations and fed a commercial guinea pig ration and lettuce. Three routes of exposure were investigated: inhalation, topical application to intact or abraded skin, and oral ingestion. In the inhalation studies, the guinea pigs were transferred to a cardboard box 2 feet $\times$ one foot $\times$ one foot and the top closed. They were kept in a small plastic mouse cage at one end of the box, and a cupful of detergent (250 ml.) was poured through a powder funnel through a hole at the other end approximately 18 inches away. The hole in the box was covered for 30 minutes. A transparent polyethylene plastic window permitted observation of the animals. This procedure was repeated two times a day, 5 days each week, excluding Saturday and Sunday. The topical application consisted of applying a 0.1 per cent aqueous solution of the detergent to the right shaved flanks of the guinea pigs with a one-inch paint brush once daily,

TABLE I. Sensitization of guinea pigs exposed to enzyme detergents by various routes using the Schultz-Dale response

Inhalation		Intact skin		Abraded skin		Oral ingestion	
Exposure (days)	Response*	Exposure (days)	Response	Exposure (days)	Response	Exposure (days)	Response
38	—	46	—	38	+(10 ⁻⁸)	46	—
39	—	50	—	39	—	50	+(10 ⁻⁷)
52	—	53	—	53	—	52	—
58	+(10 ⁻⁵)	59	+(10 ⁻⁵)	59	—	58	+(10 ⁻⁷)
60	+(10 ⁻⁸)			65	+(10 ⁻⁸)	64	+(10 ⁻⁸)
60	+(10 ⁻⁸)			65	+(10 ⁻⁸)	64	—

*Figures in parentheses indicate lowest concentration (grams per milliliter) of antigen eliciting a positive response; — = negative response.

5 days a week. In one group, the skin was abraded every Monday with fine sandpaper. In the oral ingestion studies, 2 ml. of a 5 per cent solution was sprinkled over the lettuce each day, 5 days each week. These treatments were continued for a minimum of 5 weeks and a maximum of 9 weeks.

Guinea pigs, after prolonged exposure to the household enzyme detergent, became specifically sensitized to this enzyme material for all routes of exposure. The results of the Schultz-Dale test (Table I) indicated that there was a critical exposure time of approximately 50 to 60 days. The PCA test gave equivocal results with the simulated home exposures, although it was strongly positive (titer > 40) in the positive controls. Possibly the oral, dermal, and inhalation routes did not permit adequate levels of the antigen to be available systemically for production of sufficiently high titers of serum-sensitizing antibodies to elicit the PCA response. However, preliminary studies using the rat mast cell technique of Perelmutter and Khara⁷ indicated the presence of circulating antibodies in the sera for all routes of exposure. Three of the positive results were noted as early as the twenty-third day of treatment.

The cupful of detergent was frequently returned to the original container and reused. It is possible that this repeated use may have broken the "detergent capsule"—and increased the level of airborne enzyme. Standard industrial practice is to encapsulate the enzyme detergent within the detergent builder and to treat the granules with a nonionic surfactant. This treatment, it is claimed, strongly reduces the dusting properties of the detergents.⁶ However, these considerations do not apply to the other routes of exposure.

There was no overt evidence of asthmatic-like episodes in the guinea pigs in the inhalation studies. However, a hyperemia of the ears (erythema and vasodilation) suggested that some histamine was being released in the blood. Similarly, no evidence of skin irritation was noted following topical application. Indeed, the laundry detergent appeared to hasten the healing of the abraded skin.

The extrapolation of these results to man is problematic. The presence of circulating antibodies in the guinea pig does not imply that prolonged human use will be accompanied by allergic manifestations. Nevertheless, since our experiments were initiated several reports have appeared indicating that specific sensitization to enzyme detergents may occur in the home.⁸⁻¹² One of these reports¹¹ has incriminated the IgE type of circulating skin-sensitizing antibody.

There has been little evidence so far of large numbers of householders exhibiting pulmonary allergic manifestations to the enzyme detergents. On the other hand, we are not aware of any attempt by allergists to explore the possibility of linking asthmatic-like episodes to chronic exposure to antigens in laundry detergent enzymes, other than that of Bernstein.¹⁰ The crude enzyme is not a single homogenous protease but rather an ill-characterized material containing a variety of proteins and protein fragments, many of which could possess antigenic properties. Accordingly, atopic individuals may be well advised to use only enzyme-free household detergents, particularly since some investigators¹⁻⁴ have noted a higher in-

cidence of sensitization to the material in allergy-prone workers exposed to the enzyme dust.

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