Antigenicity of Various Gluten Fractions

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The harmful effect of gluten in patients with nontropical sprue has been attributed either to its antigenic properties\(^1\)\(^-\)\(^2\) or to an inherent enzymatic defect at the level of the intestinal mucosal cell. It has been postulated that, as a consequence of this latter defect, gluten is incompletely degraded to amino acids. The passage of incompletely degraded products of gluten beyond the intestinal lumen has been thought to be of pathogenetic importance.\(^3\)\(^-\)\(^5\)

That gluten and some of its fractions are antigenic has been conclusively demonstrated,\(^6\)\(^-\)\(^8\) and antibodies to a gluten fraction have been shown to be present in patients with nontropical sprue who are consuming gluten in their diets.\(^9\)

The deleterious properties of gluten are known to reside in gliadin, a fraction insoluble in water but soluble in ethanol.\(^9\) This harmful effect remains present if gluten or gliadin is subjected to peptic-tryptic digestion\(^7\),\(^10\),\(^11\) or autoclaved,\(^7\) but disappears when the substance is incubated with pig's intestinal mucosa,\(^12\) or treated by acid hydrolysis,\(^11\),\(^12\) or digested by papain.\(^11\) The antigenic properties of the various derivates resulting from treatment of gluten by these procedures have not been studied. However, Frazer and his co-workers\(^7\) found data suggesting that autoclaving of a peptic-tryptic digest of gluten may alter its antigenic properties without changing its harmful potential. The existence of such a dichotomy between the deleterious and the antigenic properties of the various gluten derivates would mean that the antigenicity of gluten plays no significant role in the pathogenesis of nontropical sprue.

The purpose of this study was to determine the antigenicity of various gluten fractions, both harmful and nonharmful, and to see whether their antigenicity is correlated with their reported deleterious effects or innocuousness in patients with nontropical sprue.

**MATERIALS AND METHODS**

Male albino rabbits were sensitized with gluten fractions by foot-pad injections of 0.5 ml. of a suspension of each of five fractions in complete

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Freund's adjuvant (Difco). The suspension was injected daily for 5 days. This was followed on the sixth day by an intraperitoneal injection of 2 ml. of the respective gluten fraction.

Two rabbits were injected with each of the gluten fractions. The fractions were as follows:

A. Saturated solution of commercial wheat gliadin* in 95% ethanol

B. A peptic-tryptic digest of commercial gluten* (Fraction III), prepared as described by Frazer and co-workers: Lyophilized Fraction III was dissolved in sterile saline in a concentration of 67 mg/ml. (Clinical studies with this fraction have conclusively demonstrated its harmful effect in sprue patients.7-10, 11)

C. Fraction III (prepared as in B, above) adjusted to pH 7.8 and autoclaved at 120°C for 20 min. (Fraction IIIA) (It has been found that such autoclaving of Fraction III does not alter its harmful properties.7)

D. Fraction III incubated for 4 hr. at 37°C with fresh pig's intestinal mucosa (Fraction VII): After incubation it was filtered with Whatman No. 40 paper, lyophilized, and redissolved in sterile saline in a concentration of 67 mg/ml. (Treatment with pig's intestinal mucosa has been found to render gluten harmless.12)

E. Fraction III subjected to intense hydrolysis by boiling 1 gm. in 500 ml. of 6 N hydrochloric acid until the solution was reduced to 10% of its original volume: Nine volumes of buffered saline at pH 7.2 was then added, and the solution dried in vacuo. The residue was redissolved in 15 ml. of normal saline, and the pH of the resulting solution adjusted to 7. (Acid hydrolysis of gluten and of its various fractions is known to render them harmless.10-12)

Three and six weeks after injection of these fractions into the respective animals, blood was drawn for antibody studies. Antibodies were investigated by means of the double gel-diffusion technic of Ouchterlony, performed in Petri dishes with 1% agar. The results were recorded in photographs and drawings. The serum of each rabbit was tested for antibodies to each of the gluten fractions studied. Sera previously absorbed with excess normal rabbit serum and with each of the gluten fractions were studied comparatively. Absorption studies with inactivated trypsin were also conducted because of possible contamination with this enzyme during preparation of Fraction III.

RESULTS

Identical results were obtained with the sera of the 2 rabbits injected with each fraction. No antibodies were present in the sera before sensi-

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