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Trypsin Inhibitor in Guar Meal. (31461)

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Cyamopsis tetragonoloba (Guar) is a drought-resistant annual legume which is adapted to a wide range of soil types. The seed has long been used as a source of gum. The residue remaining after extraction of the gum contains 36-45% protein. Attempts to feed this protein concentrate to animals have met with varying degrees of success. Guar meal has been reported to contain toxic factors which have caused diarrhea, a decrease in growth rate, anorexia, and increased mortality(1,2). Guar gum has been reported to decrease growth and increase mortality in studies with chicks(1,2). The meal contains a trypsin inhibitor which could be destroyed by heat, according to preliminary reports from this laboratory(3,4). It was reported earlier (5) that the nutritive value of Guar meal was not improved by autoclaving. The oil or fat of the Guar meal has been reported not to contain a toxic factor(6).

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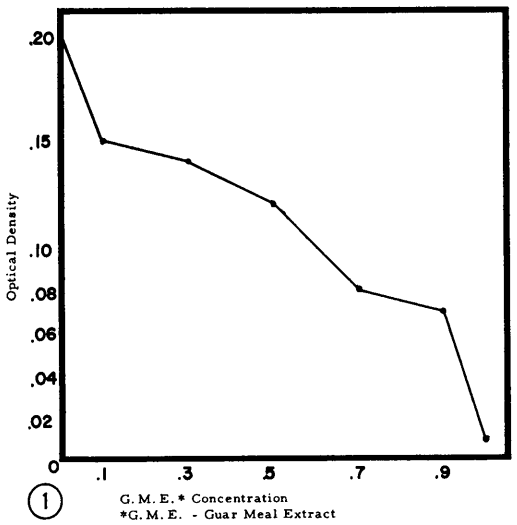
The present report is concerned with the trypsin inhibitor found in Guar meal.

Experimental. An extract of Guar meal was made by placing 20 g of Guar meal in a 250 ml Erlenmeyer flask containing 100 ml of phosphate buffer (pH 7.6, 0.02 M). The flask was shaken gently and allowed to stand in a cold room at 5°C for 6 to 8 hours with intermittent shaking. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was used for assay of the toxic factor.

Worthington lyophilized, salt free hemoglobin was prepared according to the method of Anson(7). The procedure of Northrop *et al*(8) was used for the trypsin assay. Tryptic activity was determined at 37°C with the urea-denatured hemoglobin substrate. The extent of proteolysis was determined by reading the absorbance of the 5% trichloroacetic acid soluble degradation products at 280 mμ with a Beckman model DB spectrophotometer.



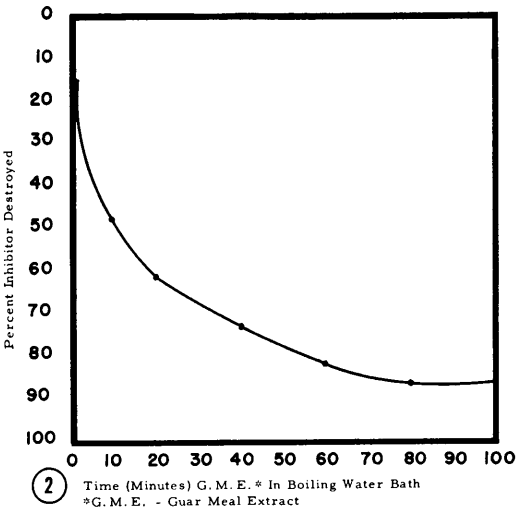


FIG. 1. Effect of G.M.E.* concentration on trypsin inhibitor activity.
FIG. 2. Effect of heat on destruction of G.M.E.* trypsin inhibitor.

For the enzyme assay, 5 ml of the hemoglobin substrate were incubated with 1 ml of trypsin solution for 5 minutes at 37°C. The addition of 10 ml of 5 percent trichloroacetic acid served both to terminate the enzyme action and to precipitate the undigested protein, which was removed by filtration through Whatman No. 3 filter paper. Ten ml of distilled water were added to the 5 ml trichloroacetic acid filtrate. Appropriate blanks were prepared by adding trichloroacetic acid to the substrate prior to addition of enzyme. Substrate and enzyme were equilibrated to the temperature of the water bath (37°C) before being mixed.

In order that the extent of protein hydrolysis would be proportional to the enzyme concentration, the enzyme solution was adjusted to a dilution which would give zero order kinetics during a 5-minute incubation period. For studies on the trypsin inhibitor extracted from Guar meal, cyrstalline trypsin (Armour) solution was added to the Guar meal extract, the mixture was allowed to stand for half an hour, and then assayed for proteolytic activity according to the procedure used for trypsin-hemoglobin preparation. Activity of Guar meal extract was compared with standard trypsin-hemoglobin results to determine the extent of proteolytic activity.

Results and discussion. Guar meal contains a trypsin inhibitor and the level of trypsin inhibition is concentration dependent (Fig. 1). Such has been demonstrated by adding varying levels of the Guar meal extract in the standard trypsin assay procedure of Northrop *et al*(8).

The trypsin inhibitor in Guar meal is destroyed by heating (Fig. 2). When the Guar meal extract was heated for a period of 10 minutes, 50% of the trypsin inhibitor was destroyed. Heating the extract for 60 minutes destroyed 80% of the activity. A continuation of the heating for a period of 80 minutes resulted in some further destruction. It is possible that a more highly purified preparation of the trypsin inhibitor would result in a destruction more nearly approaching 100 %. The fact that the trypsin inhibitor in Guar meal is destroyed by heating contradicts the earlier report(5), and suggests that the trypsin inhibitor is protein in nature.

Dialysis of the Guar meal extract against Sorensens' phosphate buffer (pH 7.6 at 5°C) for periods up to 24 hours and subsequent testing of the dialysate showed that the inhibitor was not dialyzable under these experimental conditions. This also indicated that the inhibitor is a macromolecule.

Summary. The presence of trypsin inhibitor in Guar meal has been demonstrated by the trypsin-hemoglobin digestion procedure. The

level of inhibition is concentration dependent. The inhibitor is destroyed by heating and is apparently not dialyzable, which may be indicative of a macromolecular nature.

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Termination of Immunologic Tolerance in Mice by Antigen-Antibody Complexes.* (31462)

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The theory of antibody formation of Eisen and Karush(1) proposes an extracellular control of tolerance and immunity exerted by circulating antibodies. According to Eisen and Karush, the ratio of the concentrations of antigen and antibody (natural or immune) determines whether the outcome of the exposure of an animal to an antigen will be immunity or tolerance. Thus, it should be possible to terminate immunologic tolerance by manipulations intended to shift the ratio of antigen to antibody from one giving rise to complexes of 2 molecules of antigen with one of antibody (Ag_2Ab) assumed by the model to result in tolerance, to one favoring the formation of complexes of one molecule of antigen with one of antibody (AgAb) which, according to the model, will induce immunity. Since the Ag_2Ab complexes are assumed to be unable to gain entrance into the immunologically competent cells, a specific prediction of this model is that tolerant animals should contain cells immunologically competent to respond to the tolerated antigen. Because other theories of antibody formation explain tolerance by the absence or the repression of immunologically competent cells genetically capable of synthesizing antibody specific to the tolerated antigen, the demonstration of the existence of inducible cells in lymphoid tissues of a tolerant animal, and their induction by bimolecular complexes of antigen and antibody, would provide strong support for an extracellular control of tolerance and immunity of the type postulated by Eisen and Karush. Successful attempts to terminate tolerance by the means outlined are described here.

Materials and methods. Fifty-four NIH strain white mice‡ received 3 subcutaneous injections of 20 mg of crystallized human serum albumin (HSA)§ each on the day of birth and at 2 and 3 weeks of age. A fourth injection of 20 mg HSA was given intraperitoneally at 6 weeks of age to insure maintenance of the tolerant state. At 6 weeks of age the mice also received 20 μg of radioiodinated human serum albumin (I^{131} HSA).‖

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‡ Adult mice were obtained from Belair Acres Laboratory Animals, Danville, Ind., and bred at the authors' laboratory.

§ Pentex, Inc., Kankakee, Ill.

‖ Abbott Laboratories, North Chicago, Ill. I^{131} HSA had a specific activity of approximately 10 μc/mg and was used within 48 hours of receipt.