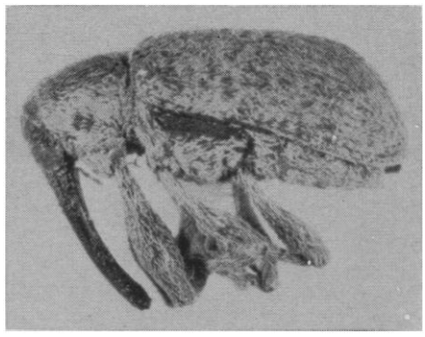

Fig. 2. Specimen from Guila Nacquitz Cave, near Mitla, Oaxaca, Mexico; lateral view, with metepisterum denuded to show smooth surface characteristic of *Anthonomous grandis thurberiae*.

ble to plot the geographic distribution of *A. g. grandis*, *A. g. thurberiae*, and the intergrading forms termed intermediate.

Unfortunately no "recent" specimens of *A. g. grandis* from the state of Oaxaca, Mexico, have been available for study, but representatives from the neighboring areas (Veracruz, Guerrero, and Chiapas) are of the intermediate form. Such intermediates are now concentrated in an area extending from southern Baja California and Nayarit southward to Costa Rica (*3*); they do, however, extend northward to southern Arizona and western Texas and are found in the Greater Antilles. *Anthonomus g. grandis* is found in the southeastern United States to western Texas, southward to the state of Durango, and in northern Colombia and Venezuela; *A. g. thurberiae* is found in southern Arizona southward along the east coast of the Gulf of California to Sinaloa.

The weevil, which is more than 1000 years old is an almost perfect, well-preserved teneral adult female (*4*) with dustlike particles adhering to various sections of the body. The apex of the scape and funicle of the left antenna, the fifth, sixth, seventh segments, the club of the funicle of the right antenna, and the third and fourth tarsal segments of the right anterior tarsus are missing. The elytra are slightly separated, exposing a small part of the metathoracic wings. The body is light yellowish-brown, and the vestiture is of coarse setae which are golden yellow dorsally and pale, lighter yellow ventrally and on the legs. The weevil has the structural peculiarities of the form referred to as intermediate (Fig. 2).

Thus, the discovery of this weevil makes it evident that an intermediate form lived in a region of Mexico that today contains similar intermediate forms of the boll weevil. Its association with cultivated cotton of the same age is of special significance since recency of the association of the boll weevil with cotton is a matter of concern to both plant scientists and entomologists.

In 1966, Warner (*3*) said the intermediate form existed 100 years ago and was not of recent origin. This view is confirmed. More recently Fryxell and Lukefahr (*5*) reported finding a severe infestation of *Anthonomus grandis* in the male buds of *Hampea* sp. (*6*). This flowering tree was observed to be part of the natural vegetation in a number of areas in Veracruz, which happens also to be the type locality of *Anthonomus grandis* described by Boheman in 1843 from a specimen collected in 1841. The host of Boheman's specimen is not known, and the species was not reported to occur on cotton until 1880. Fryxell and Lukefahr thought that the weevil might have transferred from *Hampea* to *Gossypium* during the 18th century. However, the association of the weevil with the cotton in the Guila Nacquitz Cave clearly indicates that if *Hampea* were the weevil's original host, the transfer to cotton must have occurred no later than A.D. 900.

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6. The plants referred to here constitute an as yet undescribed species whose range is from northern Veracruz to western Tobasco at low elevations. This species will be described and named in a forthcoming revision of *Hampea* by P. A. Fryxell (M. J. Lukefahr, personal communication).
7. Supported in part by NSF grant No. GS-1616. We thank J. L. Lorenzo for permission to borrow specimens.
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Ribonuclease Activity in Commercial Crystalline Trypsin and a Method for Removal

Abstract. *Several preparations of crystalline trypsin hydrolyze RNA because of contaminating ribonuclease activity. Filtration of these materials through Sephadex G-50 yields a trypsin devoid of ribonuclease activity and having a proteolytic specific activity about 70 percent of the starting material.*

Trypsin has been useful as a proteolytic enzyme in biological studies of structure of subcellular particles (such as ribosomes), of the release of fragments from cell surfaces, and of release of cells from glass in tissue culture (*1*). Also, attempts have been made to identify certain molecules as proteins on the basis of a loss of biological activity after treatment with this enzyme (*2*).

As a result of examining crystalline trypsin for absence of ribonuclease activity before its use as a protease, I found that some commercial preparations (from a usual source) contained enough ribonuclease even at low concentrations of enzyme protein (10 μg/ml) to degrade RNA extensively to acid-soluble fragments.

Three different preparations were subjected to this analysis. They were labeled with the manufacturer's lot numbers. Two (designated here as A and B) were also labeled "twice crystallized." The third (designated here as C) was labeled "once crystallized." Samples of these preparations were incubated at 37°C with a preparation of fully characterized RNA obtained from Ehrlich ascites cells by extraction with phenol (*3*). Generally the orcinol method of Dische (*4*) was applied to the supernatants resulting from precipitations with 5 percent trichloroacetic acid after incubation with the enzyme to determine ribonuclease activity. Of the three preparations, the once-crystallized trypsin was most active in releasing RNA fragments; but both twice-crystallized preparations produced the same amount of solubilization after a short period of time. Activity toward RNA was also measured by the hyperchromic shift at 260 nm produced by incubation of RNA with trypsin at room temperature.

The following results were obtained with the sample of trypsin which con-

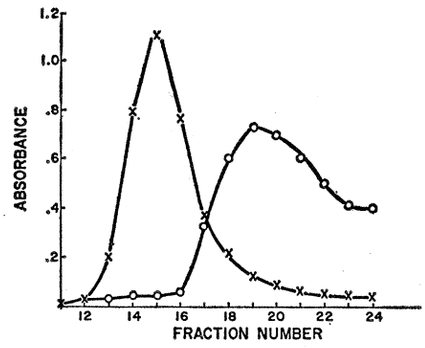

Fig. 1. Elution pattern of crystalline trypsin by gel filtration on Sephadex G-50. Void volume was 20 ml with blue dextran. Ribonuclease was determined by the orcinol method, and optical densities were recorded at 670 nm; X, 280 nm; ○, 670 nm (ribonuclease activity).

tained an amount of activity intermediate between the three samples. At a trypsin concentration of 100 μg/ml, 50 percent of the RNA, incubated at a concentration of 1 mg/ml, was solubilized within 30 minutes. Even at a concentration of trypsin of 10 μg/ml, 17 percent of the RNA was degraded in this time. Longer incubations with 10 μg/ml produced further RNA breakdown.

Since certain experiments require that trypsin be entirely devoid of ribonuclease activity, a procedure was devised which yields trypsin that contains no ribonuclease. The optimum purification was obtained with a column (45 by 1.5 cm) of Sephadex G-50 in 0.15*M* NaCl. One milliliter of solution containing 10 mg of trypsin in 0.15*M* NaCl was filtered at room temperature through the column at a flow rate of approximately 2 ml in 5 minutes. The effluent was collected in 2-ml fractions immediately after the column was loaded. Protein was determined from absorbance measurements at 280 nm, and ribonuclease activity was assayed. In several experiments with different trypsin preparations, the protein peak preceded the peak of ribonuclease activity by four tubes (Fig. 1). The tubes containing protein devoid of ribonuclease activity were pooled, dialyzed in the cold against distilled water, and lyophilized.

The recovered trypsin was compared to the original trypsin with respect to hydrolysis of RNA and the protein azocasein (*5*). Each sample of trypsin was diluted to the same concentration of protein as determined by absorption at 280 nm. Assays with RNA of the trypsin recovered from the column showed a lack of ribonuclease activity. Even at 2 hours of incubation when the original trypsin solubilized over 60 percent of the RNA, less than 5 percent degradation resulted. The activity of the protease was decreased somewhat in the process of removal of the contaminant ribonuclease. The rates of splitting azocasein were reduced by 30 percent in one experiment and 40 percent in a second experiment with a different trypsin preparation. However, the loss in specific activity can be compensated for by doubling the amount of trypsin without introducing significant amounts of the contaminant into the incubation mixture.

Trypsin incubated overnight at *p*H 8.5 lost most of its tryptic activity, but there was no decrease in the ability of the solution to hydrolyze RNA. This ribonuclease is thus similar to ribonuclease A in that neither enzymatic activity is abolished by incubation with trypsin (*6*). Therefore, if large amounts of trypsin of low proteolytic activity are used, large quantities of ribonuclease will be introduced into the incubation mixture.

Tests of all of the samples of trypsin for deoxyribonuclease activity by the diphenylamine method of Dische (*4*) revealed that this substance was not present.

In view of this finding, certain observations in complex biological systems and their interpretations which are based on the proteolytic activity of trypsin may have to be reexamined with respect to the possibility that ribonuclease action contributed significantly to the results.

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Diabetes Mellitus: Induction in Mice by Encephalomyocarditis Virus

Abstract. *Hyperglycemia and lesions of the pancreatic islets of Langerhans developed in some, but not all, adult mice infected with a variant of the encephalomyocarditis virus. Large amounts of virus were recovered from the pancreas during acute stages of infection. At this time blood glucose concentrations were markedly elevated and the islets of Langerhans exhibited focal necrosis and degranulation of beta cells. Evidence of abnormal glucose metabolism persisted for varying periods after recovery from the infection. The islets of Langerhans of chronically hyperglycemic mice were distorted and decreased in size, and the beta cells were degranulated. Encephalomyocarditis virus appears to cause diabetes mellitus by reducing the mass of functional beta cells of the islets of Langerhans.*

Diabetes mellitus can be induced in animals with endocrine hormones and with chemicals such as alloxan. It occurs spontaneously in selected lines of inbred rodents and in mice fed high caloric diets. An infectious etiology has never been established, although cases have been described in man after mumps and in cattle after foot-and-mouth disease (*1*). We report here the induction of diabetes mellitus in mice with a variant of encephalomyocarditis (EMC) virus which causes lesions in the islets of Langerhans.

The source of the M variant of EMC has been recorded (*2*). Pools were prepared from both myocardial tissue of infected mice and cultures of L cells inoculated with virus that had been purified by plaque selection. Adult male mice, free of pathogens (*3*), were given 10 to 100 plaque-forming units subcutaneously and maintained in individual cages with Purina mouse chow and water freely available. All were tested routinely before inoculation to exclude glycosuria and hyperglycemia (fasting blood glucose $\geq$ 140 mg/100 ml); glucose determinations were made on blood from the orbital sinus (*4*). Glucose tolerance tests were carried out by bleeding animals (fasted for 15 hours) at intervals after the intraperitoneal injection of 1 mg of glucose per gram of body weight. Urine was tested by the glucose oxidase method.

Evidence of abnormal carbohydrate