widths of the methylene, olefin, and steroid absorptions observed in the protein-free lipids in D_2O are not broadened in the native lipoproteins. We conclude that in the lipoproteins, unlike the lysolecithin–serum albumin system, the lipid hydrocarbons are not constrained in motion by association with protein. In addition, unlike the lysolecithin–serum albumin system, the methylene protons in the lipoproteins show no upfield chemical shift. As with the lipoprotein lipids dispersed in D_2O, the cholesterol molecules appear to be mobile and are consequently solubilized by inclusion in the phospholipid micelles.

Since only a small fraction of the lipid molecules in the lipoproteins may be sufficiently unconstrained to produce motional narrowing of resonance lines, proton counting by comparison of peak areas in the lipoproteins and the protein-free lipid is essential. This operation was carried out for both classes of lipoproteins by integration of the total area under the peaks assigned to steroids and fatty acid hydrocarbons. In both the high- and low-density lipoproteins, about 95 percent of the proton absorption in the hydrocarbon region can be accounted for by lipids. Because of inaccuracies inherent in the integration procedure and because protein would contribute a small amount of absorption in the region integrated, a small fraction of the lipid molecules, possibly 5 or 10 percent, may be constrained in motion, and hence bound by apolar association. However, apolar binding does not appear to be the major mode of lipid-protein association.

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11. Supported by PHS grant GM 14696.

21 June 1968; revised 9 September 1968

Boll Weevil Found in Pre-Columbian Cotton from Mexico

Abstract. *A well-preserved, teneral adult female boll weevil,* Anthonomus grandis *Boheman (broad sense), was discovered in fragments of a cultivated cotton boll found in Guila Nacquitz Cave, Level A, dated about A.D. 900, near Mitla, Oaxaca, Mexico. This find antedates any previously known association of the boll weevil with cultivated cotton by about 900 years and negates the contention that this association began in the 18th century. The specimen is intermediate in form between* Anthonomus grandis grandis *Boheman and the thurberia weevil,* Anthonomus grandis thurberiae *Pierce.*

Archeological excavations made under the direction of Flannery (*1*) in the Oaxaca Valley of Mexico furnished us with numerous samples of cotton fragments including boll segments, seeds, and fiber. Although size of the boll is small, the fragments can be readily identified as *Gossypium hirsutum* L. All fragments studied to date are from Guila Nacquitz Cave (near Mitla, Oaxaca), Level A, which is dated about A.D. 900 (Monte Alban IV cultural horizon).

While dissecting an intact lock, Stephens found a well-preserved adult boll weevil, *Anthonomus grandis* Boheman (broad sense), that had failed to emerge from the pupal chamber within the cotton seed (*2*). This lock included five seeds, two of them empty shells. Also the boll wall immediately adjacent to the pupal chamber containing the weevil had a small (2.0-mm diameter) circular perforation, apparently the emergence hole of another weevil or beetle that had occupied that pupal chamber. A piece of cast skin with a setiferous tubercle was found in a second chamber in the lock (Fig. 1).

As currently understood, the species *Anthonomus grandis* Boheman (*3*) includes three infraspecific forms, two treated as subspecies, *Anthonomus grandis grandis* Boheman, the economically important common boll weevil and *Anthonomus grandis thurberiae* Pierce, which is normally associated with the wild cotton *Gossypium thurberi* Todaro and is not of significant economic importance. The third form includes intergrades between the two subspecies. Such intergrades have been collected in cultivated cotton and in wild cotton other than *G. thurberi.*

Three taxonomic characters (*3*) are used by taxonomists to distinguish adults of infraspecific forms of *Anthonomus grandis,* namely, the curvature of the setae of the pronotum, the sculpture of the metepisternum, and the shape and sculpture of the scutellum. By resort to these characters, it is possi-

Fig. 1. Dissected cotton lock in which boll weevil was found. Specimen is shown at right beneath millimeter scale.

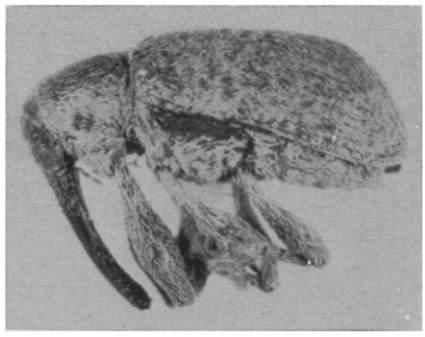

Fig. 2. Specimen from Guila Nacquitz Cave, near Mitla, Oaxaca, Mexico; lateral view, with metepisterum denuded to show smooth surface characteristic of *Anthonomous grandis thurberiae*.

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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2 August 1968; revised 16 September 1968

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-