
Pierce's Disease Bacterium: Mechanism of Transmission by Leafhopper Vectors

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tained from each subject after an explanation of the purpose, methods, and potential benefits and risks of the study.

Liquid formula diets provided 45 percent energy from carbohydrate, 40 percent from fat, 15 percent from protein, and 300 mg of cholesterol daily. Casec (Mead Johnson Laboratories), a lactose-free, defatted casein preparation, supplied 95 percent of dietary protein; the remainder was provided in an egg yolk mixture. Dietary fat was provided as a mixture of peanut oil, cocoa butter, and egg yolk. The proportions of these components were adjusted to maintain a constant iodine number of 80 to 85 and a ratio of polyunsaturated to saturated fat of 0.7 for each dietary period.

Only the carbohydrate composition of the diets varied. Both diets provided 50 percent of the carbohydrate from cornstarch. The remaining 50 percent was provided as sucrose in one diet and as an equimolar mixture of fructose and glucose in the other.

The timing of ingestion of the test diet and the proportion of calories consumed at each meal varied between subjects on the basis of personal preferences, but remained consistent for each subject during all study periods. The mean pattern provided 28 percent of calories at 7:30 a.m., 4 percent at 10:00 a.m., 29 percent at noon, 29 percent at 5:00 p.m., and 10 percent at 9:00 p.m. The evening snack varied between subjects more than any other meal, providing 0 to 28 percent of total calories and being taken between 8:00 and 10:00 p.m.

The mean plasma concentrations of triglyceride obtained after a 12-hour fast were 64 ± 9.9 mg/dl (mean \pm standard error) after the sucrose diet and 58 ± 9.9 mg/dl after the ingestion of glucose and fructose as monosaccharides; the difference is not statistically significant. In contrast, the mean 24-hour triglyceride concentration of 97 ± 13.8 mg/dl during ingestion of the sucrose diet was significantly higher ($P < .02$) than the mean of 77 ± 9.6 mg/dl during ingestion of the glucose and fructose diet.

The integrated concentration represents the arithmetic mean of the 48 individual integrated samples collected over the 24-hour period. The higher triglyceride integrated concentration observed during ingestion of sucrose resulted from a marked difference in triglyceride concentration between approximately 10:00 a.m. and 5:00 p.m., as seen in Fig. 1.

These results indicate that the disaccharide effect originally described by Michaelis and co-workers (2) occurs in human subjects. Under the conditions of

the study, consumption of a formula diet containing sucrose by normal adult males results in significantly higher integrated concentrations of triglyceride than does consumption of equivalent amounts of fructose and glucose provided as monosaccharides. The mechanism for the difference in the integrated concentration and the diurnal pattern of triglyceride remains undefined. The possibility that a difference in insulin response to the two diets could explain the observed difference in triglyceride concentrations was suggested in earlier animal studies (4). However, the mean insulin concentrations after an overnight fast, the mean integrated concentrations of insulin, and the diurnal pattern of insulin concentrations did not vary between the two diets evaluated in this study. Thus, insulin does not explain the disaccharide effect in humans.

These results indicate that dietary carbohydrate may influence prandial and postprandial triglyceride concentrations without resulting in similar changes in plasma triglyceride concentrations after an overnight fast. The higher triglyceride concentrations observed between 10:00 a.m. and 5:00 p.m. can only be ascribed to the different sources of dietary carbohydrate, because the amount of carbohydrate, fat, and protein and the source of fat and protein were identical in the two diets. This discrepancy between the triglyceride integrated concen-

trations and the triglyceride concentrations in the fasting state (Fig. 1) suggests that future studies of dietary influences on triglyceride concentrations should include evaluation of the concentrations after ingestion of the test diets as well as after an overnight fast.

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Pierce's Disease Bacterium:

Mechanism of Transmission by Leafhopper Vectors

Abstract. *The bacterium that causes Pierce's disease of grapevines is isolated most consistently from the foregut of its leafhopper vector Graphocephala atropunctata. As seen in light and scanning electron microscopy of infective leafhoppers, the bacteria are attached to the cibarial pump and the lining of the esophagus in the foregut where they appear to multiply. These findings suggest that the bacterium is transmitted from the foregut by egestion during feeding by infective leafhoppers.*

The bacterium that causes Pierce's disease (PD) of grape can be transmitted to grapevines and other plants by a large number of xylem-feeding leafhopper and spittlebug species (1, 2). In addition to its wide vector range, this as yet unclassified bacterium (3) can infect a large diversity of plant species (4) and cause disease in almond, alfalfa (3, 5), and perhaps citrus (6). Another characteristic that distinguishes the transmission of the PD bacterium from other leafhopper-transmitted prokaryotic plant pathogens is that adult leafhoppers can transmit immediately after acquiring the bacterium (7), and that they continue to transmit ef-

ficiently for the remainder of their lives, which may be several months (1, 2, 7). The finding that infective nymphs do not transmit the PD bacterium after molting suggested that the PD bacteria attach to some portion of the foregut (the foregut lining is shed in molting) (8).

We have identified the location of the PD bacterium in the foreguts of infective leafhoppers by examining tissues in both infective and noninfective blue-green sharpshooters [*Graphocephala atropunctata* (Signoret)] (9) with conventional light microscopy and scanning electron microscopy. In addition, we isolated the bacterium from aseptically

dissected portions of infective leafhoppers.

Both infective and noninfective *G. atropunctata* were used in all phases of our studies. We collected blue-green sharpshooters from natural populations in Berkeley, California, and tested them for inoculativity with PD bacterium on 'Pinot Noir' grape seedlings or rooted cuttings (10). Nontransmitting leafhoppers were presumed to be noninfective. To obtain infective leafhoppers, we confined them for one or more days on grapevines with pronounced symptoms of PD. At various intervals after this acquisition feeding, the leafhoppers were caged singly on grape seedlings for one

or more days. The test plants were retained for several months to determine whether the plants were infected during leafhopper feeding.

To identify the anatomical location of the PD bacterium, we attempted to isolate the bacterium from aseptically dissected portions of surface-sterilized (10) infective leafhoppers. After removing the head, we macerated the head and the remainder of the body separately, and streaked these "inocula" over JD-3 agar medium (3). The PD bacterium as judged by microscopic examinations of Gram-stained smears and microprecipitin tests with specific antisera (3) was most consistently recovered from heads (25 of

43 insects) and less frequently from the remainder of the bodies (13 of 42). Isolations from heads usually yielded what appeared to be pure cultures of the PD bacterium, whereas the midgut and hindgut usually yielded mixtures of bacteria. In some of these isolations we cut the separated head into three regions: the stylet bundle, a short portion of the head between the stylets and the cibarial pump, and the remainder of the head, which included most of the cibarial pump and the salivary glands. The PD bacterium was isolated most frequently (20 of 32) from the portion of the head between the stylets and the cibarial pump. We also isolated the PD bacterium from the detached stylets (4 of 10) or anterior portion of the cibarial pump, but in every such case, we also isolated the bacterium from the canal between the stylets and the pump chamber. We did not recover the PD bacterium from every leafhopper that proved to be infective. However, in those leafhoppers from which we were able to recover it, we isolated the bacterium from either the entire head (in those attempts in which only entire heads were assayed) or the portion of the head between the stylets and the cibarial pump.

Microscopy of inoculative leafhoppers confirmed the location of rod-shaped bacteria within the head. Sagittal sections (11) of infective leafhoppers revealed four primary areas with high concentrations of bacteria within the foregut. Aggregates of bacteria, which stained dark blue with Giemsa stain, were observed in the "food meatus," the narrow canal opening from the stylets to the cibarium (12), within the cibarium at the base of the apodeme of the clypeal dilator muscle, in a longitudinal groove leading to the esophagus, and in the esophagus near its junction with the cibarium (Fig. 1A). The bacteria were embedded in a matrix which stained light blue. The stain reaction and appearance of this "gum" (13) in which the bacteria were embedded (2, 14) were identical to that of the matrix in which bacteria are seen in paraffin sections of infected plants (2). No such bacterial aggregates were seen in noninoculative insects, although various bacteria and trypanosomes were present in the guts of most leafhoppers.

The evidence from these observations prompted a more detailed examination with the scanning electron microscope (SEM). Twelve adult leafhoppers were placed on a PD-infected grapevine for 24 hours. Three groups of four leafhoppers were allowed various postacquisition access periods on healthy grape seedlings as follows: three, 1-day access; four, 4-

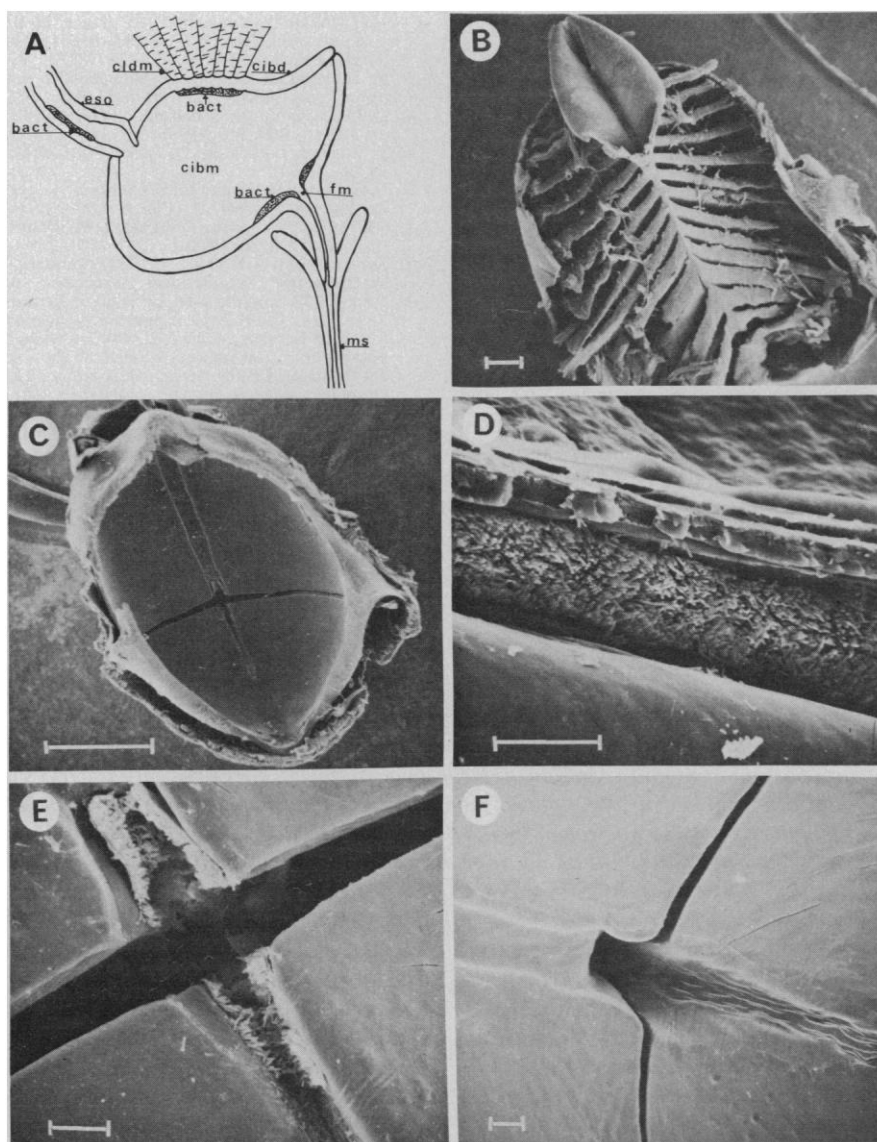


Fig. 1. Location of the Pierce's disease bacterium in the foregut of the leafhopper *Graphocephala atropunctata*. (A) Schematic sagittal section of foregut; *ms*, maxillary stylets; *fm*, food meatus; *cibm*, cibarium; *bact*, bacterial aggregation; *cldm*, clypeal dilator muscles; *cibd*, cibarial diaphragm; *eso*, esophagus. (B) Detached ventral portion of head with cibarial pump diaphragm attached to musculature (scale bar, 100 μ m). (C) Dorsal view of floor of cibarial pump with plug of bacteria at pump chamber entrance (scale bar, 100 μ m). (D) Dense growth of rod-shaped bacteria in apodemal groove in pump diaphragm. (E) Close-up of dense plug of rod-shaped bacteria lining entrance to cibarial pump chamber from food meatus. (F) Same view as (E) but from a noninfective leafhopper. Scale bar in (D) to (F), 10 μ m.

day access; and two, 12-day access. The surviving nine leafhoppers and five non-inoculative leafhoppers were examined with the SEM (15). The pump diaphragm with muscles attached (Fig. 1B) was separated from the cibarium so that the food meatus to the stylets was dorsally visible (Fig. 1C). The entire cibarium was examined, and rod-shaped bacteria were found only in those areas in which they had previously been detected with light microscopy. Seven of the nine infective leafhoppers showed rod-shaped bacteria in the medial groove at the base of the apodeme of the cibarial pump diaphragm (Fig. 1B) and five of the seven showed bacteria in the "food meatus" of the cibarium (Fig. 1, D and E). Bacteria were not found in one leafhopper from each of the 1- and 4-hour postacquisition access periods, nor in the five noninfective leafhoppers (Fig. 1F). Significantly, the bacteria were not loosely scattered, but formed distinct colonies (Fig. 1, D and E). In general, the colonies were larger and denser 4 and 12 days after acquisition feeding. The bacteria attached themselves on their narrow end on the smooth interior surface of the cibarium; this attachment appears to prevent their being dislodged despite the flow of ingested fluid estimated at an average velocity of 8 cm/sec (16). The matrix surrounding the bacterial aggregates, which was quite evident in paraffin sections, may be a gelatinous material that leaves only a thin residual film in the freeze-drying preparation for SEM (Fig. 1E). The matrix material surrounding the PD bacterium may aid their attachment and protection. Notably, we found only one other bacterium—a coccoid *Lactobacillus*-like organism, possibly *L. hordniae* (10, 17)—was found attached to the foregut. The cibarium of xylem feeders could be expected to be a highly selective microenvironment because of its low nutrient concentration and high flow velocities. Thus, bacteria which colonize such habitats should be able to securely attach to these surfaces.

The location and apparent multiplication of the PD bacteria in the foregut suggests that the bacterial pathogen is propagative, but noncirculative in its insect vector. The force of the pumping action and liquid flow probably dislodge some of the bacteria, which are then egested through the food canal to infect the plant. This mode of transmission would adequately explain the broad but well-defined spectrum of PD vectors (1, 2), the loss of inoculativity by vectors following molting (8), the lack of a required latent period in the vector (8), and the lack of bacterial transmission by *G. atropunctata*.

punctata after injections of bacterial suspensions into the body cavity (3, 18). Multiplication of the PD bacterium in the foregut would account for the indefinite persistence of inoculativity in vectors that do not molt (that is, adults) (2, 7). The wide plant host range (4) and the multiplication of the PD bacterium in the distinctly extracellular environment of the leafhopper foregut casts further doubt on the affinity of the PD bacterium to rickettsiae, as suggested on the basis of the bacterium's fastidious habitat and morphological features (14).

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15. The head, with thorax attached, was severed from leafhoppers and placed immediately in 4 percent glutaraldehyde-phosphate buffer (pH 7.4) for 24 hours, dehydrated in an ethanol series, and held in amyl acetate for 24 hours prior to drying in liquid CO₂ transition fluid. The dried specimens were dissected to remove the cibarium and attached clypeal muscle. The exposed ventral and dorsal cibarium were mounted on stubs, sputter-coated with gold, and viewed in an Etec Autoscan SEM.
16. A normal excretion volume for *G. atropunctata* is about 1 ml/day [T. E. Mittler, *Ann. Entomol. Soc. Am.* **60**, 1074 (1967)]. Given an average area of 1.5×10^{-10} m² for the food meatus (Fig. 1, E and F), a conservative estimate of the average daily flow velocity through this narrow passage is 280 m/hour or 7.8 cm/sec. Instantaneous velocities could be much higher.
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Prevention of Monocarpic Senescence in Soybeans with Auxin and Cytokinin: An Antidote for Self-Destruction

Abstract. Foliar applications of α -naphthaleneacetic acid, together with 6-benzyladenine, prevent the seed-induced degeneration (monocarpic senescence) in soybeans. In addition to halting leaf yellowing and shedding, this treatment prevents the loss of starch and nitrogen that occurs during senescence of these leaves. Although nitrogen and starch are normally redistributed to support pod growth, pod development is not impaired by this treatment, apparently because photosynthesis and nitrogen assimilation continue.

During the final phases of fruit maturation, soybean plants rapidly degenerate (monocarpic senescence) and then die. Most prominent among these changes is the yellowing and the shedding of the leaves. Since this abrupt degeneration of the whole plant is controlled by internal factors, it is a developmental self-destruct program. Because defoliation, depodding (1), and even deseeding (2) can prevent monocarpic senescence in soybeans, the seeds are the primary control centers. The primary targets appear to be the leaves, whose death causes

the demise of the rest of the plant (3).

Many factors limit vegetative development and seed production in soybeans; however, the fact that the basic life-support organs (for example, the leaves) and processes (such as photosynthesis), as well as other processes (such as nitrogen fixation), required to support seed growth degenerate at a time when they appear to be needed most to support reproductive development (4, 5) suggests that it could be of both practical and theoretical interest to prevent this decline through applications of a plant growth