In Table 1 the differences in MLTI blastogenic responses for W1 (*8*) versus W5 (*8*) lymphocytes are depicted (*11*). These findings have now been confirmed in over 30 different experiments with fresh or frozen cells. Results are similar whether cultures are performed in autologous or normal plasma. The implication that factors eluted from W1 cells are inhibitory is strengthened by the observation that W5 but not W1 cells are inhibited by the presence of the cell wash eluate in the culture scheme (Table 2). An active inhibitory component in the cell wash (CW) eluate can be fractionated and results in high and low molecular weight fractions (CW_{100} and CW_{10}). When combined, inhibition is comparable to that for the unfractionated (CW) material. Each fraction alone is inactive (Table 2). Material eluted from normal cells is inactive in a similar setting. Inhibition of W5 lymphocytes by the cell wash eluate is reversible. Since cells regain their capacity to respond following a removal of the inhibitory material, a cytotoxic effect can be ruled out.

The eluate has immunological specificity. The W5 cells from lung carcinoma patients do not show inhibition when preincubated with the eluate from the patient with adenocarcinoma of the stomach (*12*).

Furthermore, an inhibitory factor or factors present in the cell wash can be absorbed out with the patient's W5 cells but not with tumor cells. These findings differ from those of other workers where blocking factors in a cytotoxic assay could be specifically removed from serum only after absorption with target cells (*9*). In summary, the data demonstrate that peripheral leukocytes in cancer patients carry an inhibitory factor or factors. The active component resides in neither high nor low molecular weight components but in a combination of the two and suggests antigen-antibody complexes. This material is not cytotoxic but prevents the full expression of lymphocyte proliferative capacity in response to stimulation with tumor cells. Loss of inhibition might be explained on the basis of low affinity binding of an inhibitory factor or factors at the cell surface or the rapid turnover and reconstitution of lymphocyte receptors at the membrane level (*13*).

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6. Cytological examination of the cell suspensions recovered at the bottom of the gradient revealed carcinoma cells of squamous type in various stages of differentiation for the pulmonary neoplasms and for the remaining tumor, a well-differentiated adenocarcinoma of the stomach.
7. The malignant potential of the tumor cells was confirmed by their ability to grow and form histologically similar neoplasms in the thymus-deficient "nude" mice. For future testing, tumor and normal tissue were stored in a frozen state.
8. This suspension of cells underwent one 5-minute wash (W1 cells) in RPMI 1640 at 24°C. After centrifugation at 300*g* for 10 minutes the supernatant was saved and an aliquot of W1 cells was washed three more times, followed by a 12-hour incubation at 37°C in a 5 percent CO_2 and air atmosphere. Lymphocytes recovered at this point (W5 cells) were greater than 80 percent viable by trypan blue exclusion. All supernatants were combined (cell wash eluate) and concentrated tenfold by filtration. A portion of the cell wash eluate (CW) was adjusted to a *p*H of 3.1 in NaCl-glycine buffer and passed through a series of membranes of determined pore size by ultrafiltration. Material retained by the Amicon filter X M100 (CW_{100}) contains molecules above 100,000 daltons, including immunoglobulin. Subsequent passage of the filtrate from the X M100 step through an Amicon PM-10 membrane results in material of 10,000 to 100,000 daltons (CW_{10}). Collected fractions were adjusted to *p*H 7.2 prior to assaying (*8*).
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11. Responses for W5 cells were higher than those for W1 cells at all time intervals in culture.
12. Upon preincubating W5 lymphocytes or tumor cells with the CW eluate the only clear effect is upon the responding lymphocyte and not the stimulating tumor cell, that is, for patient H.S., W5 lymphocytes + CW incubated 1 hour at 24°C, rinsed once and reacted with irradiated tumor gave 1421 count/min ± 265 (S.E.); tumor + CW incubated 1 hour at 24°C, rinsed once, irradiated and reacted with W5 lymphocytes gave 7686 ± 339 (S.E.). The control consisted of W5 lymphocytes from patient H.S. incubated with an eluate from patient N.B., rinsed and reacted with irradiated tumor from H.S. gave 4234 ± 416; W5 lymphocytes + tumor (no eluate) gave 4570 ± 186.
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Pierce's Disease of Grapevines: Evidence for a Bacterial Etiology

Abstract. *A Gram-positive, rod-shaped bacterium was isolated and grown in pure culture on artificial mediums from the leafhopper* Draeculacephala minerva *Ball which had fed on plants infected with Pierce's disease. The bacterial culture was injected into uncontaminated leafhoppers which were than allowed to feed on healthy grapevines. After exposure to these leafhoppers, typical symptoms of Pierce's disease developed; however, no symptoms developed on plants exposed to leafhoppers injected with sterile culture medium. The same organism was reisolated from the experimentally inoculated plants. Electron microscopic examination of these infected plants revealed bacterial cells localized in the xylem tissues. No such cells were seen in healthy or control plants.*

Since 1884, Pierce's disease has been an important transient and insidious problem of grapevines in California and in the southeastern United States. This disease has been investigated intermittently for several decades, yet the etiologic agent remained completely obscure. At first, Pierce (*1*) believed that a microorganism was the causal agent, since bacteria-like organelles were observed in diseased plant material; but he was unable to culture any bacteria that would cause the disease. Weimer (*2*) investigated a very similar disease called alfalfa dwarf. He, too, observed bacteria or bacteria-like bodies in diseased plants but could not isolate any pathogenic bacteria. He showed, however, that the alfalfa dwarf agent was transmitted to healthy plants through grafting, and on this basis he concluded that a virus rather than a bacterium was the cause. Pierce's disease, like alfalfa dwarf, is transmissible by grafting in grapevines and, therefore, it also was concluded to be caused by a virus (*3*). In addition, Hewitt *et al.* (*3*) established that leafhoppers were natural vectors of the disease and later they concluded that Pierce's disease and alfalfa dwarf were incited by the same infectious agent. This agent was shown to be limited to xylem tissues that often became plugged with gum and tyloses

Table 1. Results of three transmission experiments demonstrating pathogenicity of the Pierce's disease agent isolated from infective leafhoppers to grapevines (*Vitis vinifera*) and electron microscopy indexing.

Experiment No.	Test plants	Bacteria-injected vector		Sterile broth–injected vector		Positive control*	
		Diseased plants (No.)	EM†	Diseased plants (No.)	EM	Diseased plants (No.)	EM
1‡	Mission	1/1§	+	0/1	−		
2‖	Carignane	4/4	+	0/4	−	2/2	+
3‖	Carignane	4/4	+	0/4	−	2/2	+

* Test plants fed with naturally infective vectors. † (+) Indicates bacteria in sections of vascular tissue as determined by electron microscopy; (—) indicates no bacteria observed. ‡ Ten insects per test plant. § Numerator is number of diseased plants; denominator is number of plants tested. ‖ Five insects per test plant.

(*4*). Recently, Hopkins and Mortensen (*5*) suggested that the agent of Pierce's disease might be a mycoplasma rather than a virus, because tetracycline treatments of diseased plants caused some remission of symptoms. Goheen *et al.* (*6*) observed rickettsia-like bodies in the xylem of leaves affected by Pierce's disease and alfalfa dwarf. In Florida, Hopkins and Mollenhauer (*7*) also reported rickettsia-like bodies in grapevines with Pierce's disease. No one has been able to culture the observed organisms on artificial medium.

We now report the isolation of a rod-shaped, Gram-positive bacterium from infective *Draeculacephala minerva* Ball leafhoppers, which can be readily cultured in an artificial medium and which can induce on healthy grapevines symptoms identical to those of Pierce's disease.

Noninfective leafhoppers, reared in captivity for more than five generations on barley plants, were fed on healthy grapevines, *Vitis vinifera* 'Mission,' and then transferred to plants with Pierce's disease. Excreta (spittle) of ten vectors were collected after they had fed first on healthy plants and then additional samples of excreta were taken from the same vectors after they had fed on diseased plants. The collected excreta from each phase of sampling was streaked on blood-dextrose agar medium (*8*) and on medium 523 agar (*9*). Also, a collodion-coated electron microscope grid was floated on some of the excreta samples. Small white bacterial colonies grew on both mediums streaked with the excreta of vectors that had fed on diseased grapevines, but similar colonies did not grow on mediums streaked with excreta from these same vectors after they had fed on a healthy grapevine (before feeding on diseased vines). Rod-shaped bacteria-like cells (0.5 to 2.0 μm) were observed with the electron microscope only in samples taken from vectors fed on diseased vines, but not in samples from vectors fed on healthy ones. In a second experiment, two groups (ten each) of noninfective leafhoppers were fed for 48 hours on healthy and diseased plants. Then the two vector groups were immersed first in 70 percent ethanol, next in 2 percent sodium hypochlorite, and then rinsed in sterile distilled water. They were finely ground and the semiliquid body material was streaked on blood-dextrose agar and medium 523 agar in petri plates. The plates were incubated for 48 to 72 hours at 32°C. Small white bactieral colonies appeared on both mediums streaked with ground vectors that had fed on diseased vines, but no such colonies appeared on mediums streaked with extracts from vectors that had fed on healthy vines.

A suspension of these bacteria (2×10^9 cells per milliliter) grown in medium 523 broth (*9*) was injected into noninfective leafhoppers with a fine glass needle, and the leafhoppers were then transferred onto healthy Mission or Carignane grapevines (five to ten vectors per plant). Another group of vectors was injected the same way with sterile medium 523 broth and placed on healthy grapevines. All the plants were kept individually isolated in insect-proof cages, and the vectors were kept on the plants for 15 days or longer, until the vectors died. After 6 weeks all the plants exposed to vectors that had been injected with the bacteria exhibited symptoms typical of Pierce's disease (Fig. 1B). No symptoms developed on plants exposed to vectors injected with sterile medium 523 broth (Fig. 1C). The results of three such transmission experiments are shown in Table 1.

Samples of the inoculated plants were prepared for electron microscopy to determine whether the organism was present in the vascular tissue. Disks of tissue, 1 to 2 mm thick, were cut from the midribs of leaves showing typical symptoms of Pierce's disease and from similar leaf tissues of the healthy plants. These tissues were fixed in 2.5 percent

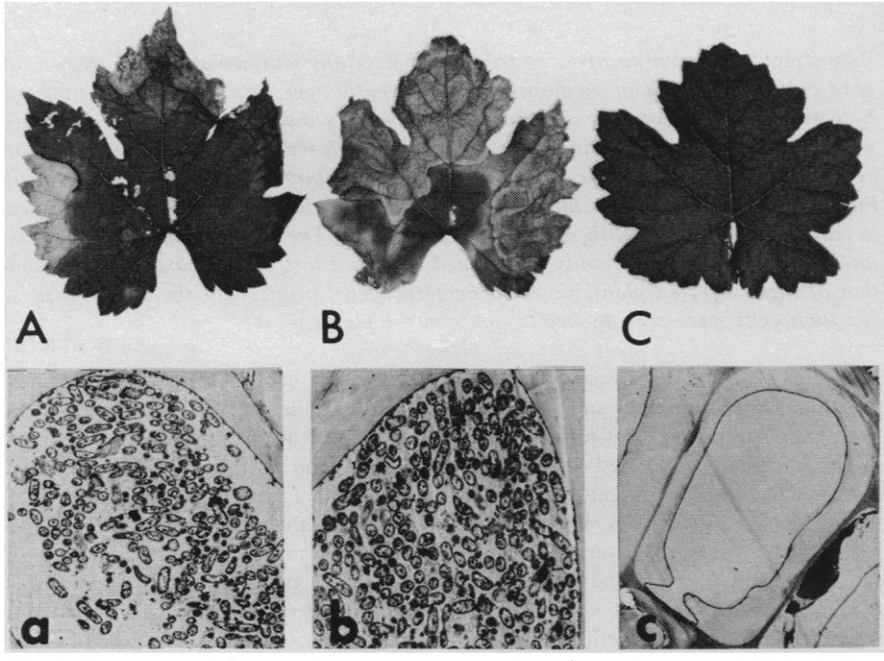

Fig. 1. Symptoms of Pierce's disease on leaves and sections of vessels of *Vitis vinifera* 'Carignane.' (A to C) Leaves from experimentally inoculated plants. (A) Leaf from a vine exposed to naturally infected vectors, (B) leaf of a vine exposed to vectors injected with bacteria, and (C) leaf from a vine exposed to vectors injected with sterile broth. (a to c) Electron micrographs of cross section of vessels in the corresponding leaves A to C. (a) Vessel member from leaf A containing bacteria-like bodies, (b) from leaf B containing identical bacteria-like bodies, and (c) from leaf C. Magnification of electron micrographs: (a to b) × 2200; and (c) × 1120.

glutaraldehyde for 2 hours, washed in 0.1*M* potassium phosphate buffer (*p*H 6.8), and postfixed in osmium tetroxide for 2 hours or longer. After dehydration in an acetone series, the tissues were embedded in Spurr's epoxy resin (*10*). Ultrathin sections were cut on a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an RCA EMU electron microscope.

Organisms similar to those seen in sections of naturally infected grapevines were observed in vessels of the plants exposed to leafhoppers injected with bacteria. The organism was not observed in the xylem vessels of the plants exposed to vectors that had been injected with sterile broth (Fig. 1c).

The same bacterium was recovered from vectors that had fed on plants experimentally infected. Identical bacteria were recovered by the same methods from naturally infected plants. As in the first experiment above, identical small white bacterial colonies grew on blood-dextrose agar and medium 523 agar. Again such colonies were absent on mediums streaked with extracts of the ground bodies of vectors that had fed on plants previously exposed to vectors injected with sterile medium 523 broth. The reisolated bacterium had the same morphology, size, and cultural and physiological characteristics as the original isolate.

The bacterium is Gram-positive, rod-shaped, 0.4 to 0.6 μm wide, 1.0 to 2.0 μm long, and nonmotile. It grows well at a temperature range of 20° to 32°C, with an optimum of 29° ± 1°C as determined by the polythermostat method (*11*), and well but slowly on blood-dextrose agar, medium 523 agar, and medium D2 agar (minus LiCl)—a selective medium for Gram-positive bacteria (*9*). The bacterium grows profusely in mediums 523 and D2 broth (minus LiCl). On medium D2 the colonies are white to white-gray in color, slightly convex, circular with entire margins, and have a smooth shiny texture. On the basal medium recommended by Hugh and Leifson (*12*) it behaved as a facultative anaerobic bacterium; it also produced acid but not gas from glucose. Tests for production of indole and methyl red were negative.

Our experiments have demonstrated that a Gram-positive bacterium is the etiological agent of Pierce's disease in grapevines. We have cultured the organism on artificial mediums. By using the leafhopper vector injected with the cultured and purified bacteria, we can consistently reproduce the disease symptoms in healthy grapevines and we can reisolate the same organism from clean leafhoppers fed on these plants and on naturally infected plants from the field. We have attempted to isolate and to culture the bacterium from diseased tissues without success. The reason for this is presently unknown. The characteristics of this bacterium, which in nature is apparently confined to its vectors and to the xylem tissues of its host plants, plus its morphological, cultural, and physiological features, suggest that the Pierce's disease agent belongs to a distinct group of plant pathogenic Gram-positive bacteria heretofore unrecognized.

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Control of Experimental Diabetes Mellitus in Rats by Transplantation of Fetal Pancreases

Abstract. *Experimental diabetes mellitus in young adult Lewis rats was successfully treated by transplantation of fetal pancreases from syngeneic fetuses. Complete or partial control lasting up to 165 days was achieved in 64 percent of recipients by using two to three pancreases of fetal age (15 to 18½ days) placed under each kidney capsule. Islets of Langerhans without exocrine elements were present in the transplants.*

Transplantation of the pancreas in patients with diabetes mellitus has been complicated in most cases by technical problems related to the exocrine secretions of the organ (pancreatitis) and to immunological rejection of the pancreatic elements plus intestinal tissues transplanted to drain the digestive enzymes. Survival has usually been measured in months or even weeks. In most cases, however, insulin treatment could be stopped, indicating at least temporary function of the endocrine elements of the pancreas (*1*).

Transplantation of isolated islets of Langerhans apparently avoids some of these problems. When 400 to 600 islets obtained from two to three rat pancreases were injected intraperitoneally into syngeneic inbred rats made diabetic with streptozotocin, the diabetic state was somewhat ameliorated (*2*). Injection of the islets into the portal vein was followed by lowering of blood glucose and urine volume to normal (*3*). When the islets from 20 to 35 rat pancreases were placed into the peritoneal cavity of syngeneic alloxan-diabetic rats, the rats showed return of blood sugar to normal (*4*).

An alternative approach to pancreas transplantation for therapy of experimental diabetes is the use of fetal or neonatal pancreases. Such tissues have survived, grown, and continued to synthesize insulin, although only barely detectable effects on diabetes in the recipient have resulted (*5*). When fetal pancreas is transplanted, atrophy of the exocrine cells usually occurs, and the epithelial cells of the ducts show mitoses and form new islets of Langerhans (*6*). This tissue may thus afford distinct advantages for the treatment of diabetes, although to date no one has reported a surgically feasible (with respect to humans) technique for transplantation of fetal pancreas which has had a physiologically significant impact on the diabetic state. The following preliminary studies were initiated in order (i) to identify a site for transplantation