activity. In all experiments a saturating concentration of dipalmitin was employed; however, in early experiments on rabbits 3 days after surgery, nonsaturating amounts of CDP-choline were used. Determination of the apparent Michaelis constant ($3 \times 10^{-5} M$) permitted use of a saturating concentration of CDP-choline (1 mM) in later measurements. In all cases velocity was proportional to protein concentration in the ranges employed for these assays.

The results of lung lecithin analyses are presented in Table 1. Values for saline-injected rabbit fetuses agreed with those for fetuses of comparable gestation who did not undergo surgery, and also agreed with the data of Gluck *et al.* (*8*). Steroid-treated fetuses showed a statistically significant ($P < .005$) elevation in lung lecithin from 70 mg per gram of dry lung (control value) to 96 mg per gram of dry lung. The latter figure is even higher than the lecithin content in lungs of full-term rabbit fetuses (*8*). Fractionation of isolated lecithin by precipitation with cold acetone revealed that the lecithin synthesized is precipitable by acetone and therefore is presumably surface-active (*9*).

Although the analyses of lung lecithin were consistent with a net increase in biosynthesis after corticosteroid injection, it was important to directly demonstrate an elevated rate of synthesis and to determine which pathway was involved. Incorporations of [^{14}C]choline and [^{14}C]methionine into lecithin by lung slices are shown on Table 1. Whereas there were no differences in methylation, choline incorporation occurred at a significantly greater rate in lung exposed to corticosteroid as compared to control lung. The 150 percent increase in choline incorporation was noted in lung slices both 6 and 72 hours after steroid administration.

Choline phosphotransferase activity was significantly increased in the steroid-treated rabbit fetuses both 6 and 72 hours after administration of the compound. Of 32 rabbit fetuses examined, the mean increase (counts per minute in lecithin per milligram of protein per minute) was from 211 ± 23 for controls to 299 ± 27 for the Predef-treated animals ($P < .05$). Similar changes in choline incorporation by lung slices and in choline phosphotransferase activity were also observed after injection with hydrocortisone; however, for an equivalent response approximately 50 times as much steroid (by weight) was required.

Prior treatment of the rabbit fetuses with actinomycin D (*10*) (1 μg per gram of body weight, given intraperitoneally 30 minutes before either steroid or saline) did not block the steroid-mediated rise in choline incorporation and enzyme activity (Fig. 1). In contrast, a paradoxical effect of actinomycin D was observed; choline incorporation was higher in animals first treated with the antibiotic as compared with those first treated with intraperitoneal saline. Such a phenomenon was noted for cortisol induction of tyrosine aminotransferase (E.C. 2.6.1.5) (*11*). In contrast, prior treatment with cycloheximide (*12*) (7 μg/g, given as described for actinomycin D) abolished the corticosteroid effects.

Numerous biochemical effects of hydrocortisone in the whole animal have been noted, including induction of phenylethanolamine methyltransferase in the adrenal medulla (*13*) and of several hepatic enzymes involved in gluconeogenesis (*5*). It is conceivable that the observed elevation in lecithin biosynthesis might be secondary to increased production of another metabolite such as epinephrine or glucose. However, the concurrent and proportional rise in choline phosphotransferase activity, along with inhibition of both effects by cycloheximide, suggests that the increased lecithin production requires de novo synthesis of this enzyme. Moreover, since actinomycin D did not prevent the proposed induction in doses that are effective in other systems (*13*), it would appear that the corticosteroid effect is mediated on the level of messenger RNA translation. Further studies will be required to substantiate this impression.

Extrapolations to the human fetus and newborn may be hazardous. However, these observations suggest that hydrocortisone may be beneficial as a specific therapy in the respiratory distress syndrome.

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Rickettsia-like Bacterium Associated with Pierce's Disease of Grapes

Abstract. *A pleomorphic bacterium was observed by electron microscopy in grape plants infected with Pierce's disease. The organism was located in xylem tissue, and its occurrence was closely associated with symptoms of Pierce's disease. The bacterium resembled a rickettsia in morphology, in its failure to grow on cell-free media, and in its sensitivity to tetracycline antibiotics.*

Pierce's disease, a killer of grapevines (*Vitis* species), is one of the principal factors limiting grape production in Florida (*1*). The pathogen has long been considered a virus, but the successful suppression of the development of symptoms with tetracycline antibiotics in 1970 indicated that this may not be the case (*2*). We now report that an obligately parasitic bacterium resembling a rickettsia is constantly associated with the disease.

'Thompson Seedless' grape plants in their second year of growth were used

in this study. Healthy grapevines were maintained in this planting by building screen cages over the plants to exclude the abundant leafhopper (family Cicadellidae) vectors of the Pierce's disease pathogen. Small samples of tissue, about 0.5 to 1.0 mm long, were taken from the petiole, midvein, and smaller veins of grape leaves. The tissue samples were fixed in 2 percent glutaraldehyde–2 percent paraformaldehyde in 0.05*M* collidine buffer, *p*H 7.3, for 2 hours at room temperature; rinsed with a buffer (five changes of 10 minutes each); and then fixed in 1 percent osmium tetroxide overnight in the refrigerator. The tissues were washed in distilled water, dehydrated in an alcohol and acetone series, and embedded in a Spurr epoxy resin mixture (*3*). Thin sections were cut on a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi electron microscope (model HU-11E).

A pleomorphic bacterium was observed in the tracheary elements of the petioles, midveins, and small veins of leaves from vines infected with Pierce's disease (Fig. 1A). It was found in more than 90 percent of the thin sections taken from the leaves of ten infected plants. The bacterium was usually found in only four or five elements per vascular bundle. Often, one element of a vascular bundle contained dense masses of the bacteria (Fig. 1A), while two or three nearby elements contained lower concentrations of the organism. The organism was usually observed in more than one vascular bundle per thin section. It was never found in thin sections from ten healthy vines. This bacterium was thus constantly associated with the symptoms of Pierce's disease.

Oxytetracycline (Pfizer, Agricultural Terramycin) drench treatments were applied regularly to several plots in the 'Thompson Seedless' planting from its establishment (*4*). All antibiotic drench treatments suppressed the development of Pierce's disease during the first year, but untreated vines were severely diseased. In the second growing season, due either to a lack of oxytetracycline uptake or to a dilution factor in the larger vines, some of the vines treated with antibiotic developed symptoms of Pierce's disease. Thin sections from eight of these vines contained the bacterium, but it was not found in any of eight vines in which oxytetracycline drenches still suppressed the development of symptoms during the second year of growth.

The morphology of the organism was quite similar to that of the rickettsiae (*5*). The bacterium was usually a rod-shaped body 0.4 to 0.5 μm wide by 1.0 to 3.0 μm long (Fig. 1A) with a well-defined cell wall (Fig. 1B). The cell wall was rippled and appeared in some instances to be invaginated (Fig. 1B). The walls of some of the bacteria were relatively thin and appeared to have three layers, while others, possibly representing different developmental stages, had thicker walls in which the triple layering was not so obvious (Fig. 1B). A plasma membrane could be found in some sections. The internal ultrastructure consisted of electron-dense areas and one or more areas of low electron density that contained strands of material similar in appearance to DNA strands in other bacteria (Fig. 1B). A substance that was mucilaginous or fibrous (or both) was usually associated with the surface of the bacteria or was present in the space between the bacteria (Fig. 1B).

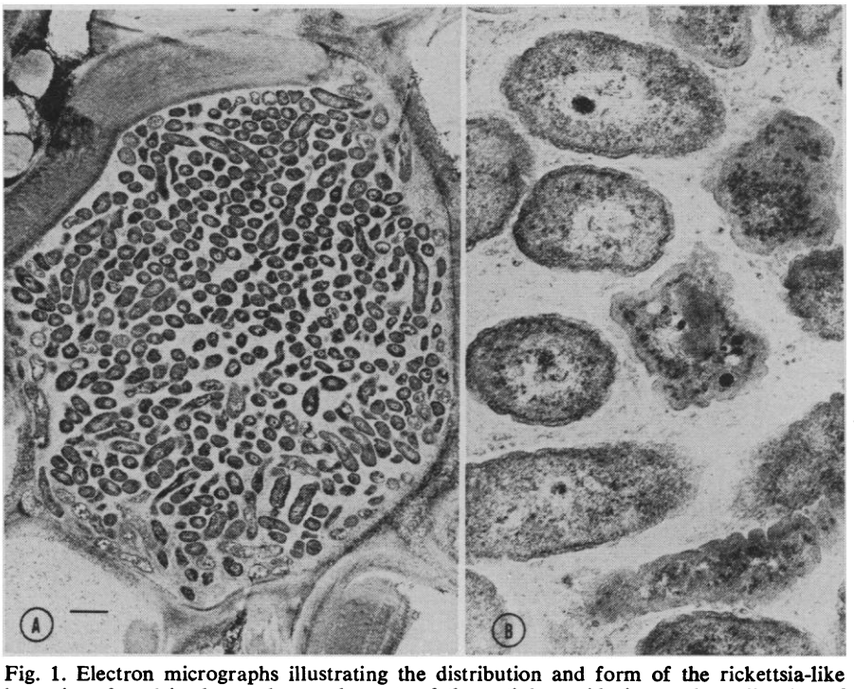

Fig. 1. Electron micrographs illustrating the distribution and form of the rickettsia-like bacterium found in the tracheary elements of the petioles, midveins, and small veins of leaves from vines infected with Pierce's disease. The marker line in (A) is 1 μm (A, × 4,600; B, × 36,000).

Standard plant-pathogenic techniques for isolating bacteria from diseased plant material were used with tissue from plants with Pierce's disease. After leaves from infected vines were rinsed two or three times in sterile water, 12-mm sections of the petiole were excised and triturated in 2 ml of water or buffered saline. The resultant suspensions were streaked onto nutrient agar; nutrient agar plus glucose; King's medium B; agar medium containing yeast extract, glucose, and calcium carbonate; and skim-milk agar. Petiole sections were also squeezed with forceps so that sap was expressed; this was then streaked over the surfaces of the media. The bacterium did not grow on any of the nonselective, general-purpose media used, and is presumed, therefore, to be obligately parasitic.

The proper taxonomic status of this bacterium is unknown. It does, however, closely resemble members of the Rickettsiaceae. It is rickettsia-like in morphology and ultrastructure, in its failure to grow readily in cell-free media, and in its sensitivity to the tetracycline antibiotics.

Members of the Rickettsiaceae cause a number of serious diseases in animals and man, but they are primarily parasites of arthropods. The presence of rickettsia-like organisms in homopterous insects, especially their reported occurrence in the saliva of a leafhopper (*6*), indicates an access to plant tissues. To our knowledge, rickettsia-like organisms have not previously been reported in any plant containing chlorophyll. It seems feasible that the grape bacterium could be a member of the Rickettsiaceae that has become adapted to plant tissue.

The rickettsia-like organism was closely associated with Pierce's disease, but proof that it is the causal agent awaits the fulfillment of Koch's

postulates. Several facts implicate this bacterium as the causal agent: (i) the bacterium was found in the xylem tissue where the causal agent of Pierce's disease is known to be (*7*); (ii) the correlation between symptoms and occurrence of the bacterium was high; (iii) the bacterium was never found in plants in which the disease had been controlled with tetracycline antibiotics and (iv) the exclusion of leafhopper vectors prevented the occurrence of both the symptoms of Pierce's disease and the bacterium.

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Urinary Catecholamine Metabolites during Behavioral Changes in a Patient with Manic-Depressive Cycles

Abstract. *3-Methoxy-4-hydroxyphenylglycol and normetanephrine were analyzed in daily urine specimens of a patient with manic-depressive cycles who was studied longitudinally. The quantities of these catecholamine metabolites excreted into urine were decreased during periods of depression as compared with periods of mania. Urinary excretion of 3-methoxy-4-hydroxyphenylglycol varied cyclically with a period length of approximately 20 days. Changes in this metabolite, and perhaps in normetanephrine, preceded the affective and behavioral shifts.*

The concentration of 3-methoxy-4-hydroxyphenylglycol (MHPG) in urine was found to be significantly decreased in a group of depressed patients as compared with normal controls (*1*). The excretion of this metabolite was decreased in depressed patients and increased in manic patients (*2*). Bond *et al.* (*3*), in a longitudinal study of two patients with manic-depressive illness, also reported that urinary MHPG excretion was significantly higher during the manic phases and lower in the depressed phases. They suggested that the increase in the urinary excretion of MHPG precedes the switch into mania and that the amount of this metabolite reflects a cyclic variation in brain norepinephrine turnover, with decreased turnover triggering the episode of depression. Because of the suggested relation between brain norepinephrine and the affective disorders (*4*), that is, mania and depression, urinary MHPG determinations are of particular interest because this metabolite is the principal product of brain norepinephrine degradation in several species of mammals (*5*, *6*). Early reports indicated that approximately 25 to 30 percent of urinary MHPG in dogs is derived from the metabolism of brain norepinephrine (*5*). However, this figure may be too conservative, because it has been found that approximately 30 to 60 percent of urinary MHPG in nonhuman primates may be derived from the metabolism of brain norepinephrine (*7*).

Urinary norepinephrine and normetanephrine, which are derived from pools of norepinephrine outside the central nervous system (*8*), were also reported to be altered in patients with manic-depressive illness. Strom-Olsen and Weil-Malherbe (*9*) found that urinary excretion of norepinephrine and epinephrine was greater during the manic phase in patients with manic-depressive disorders. Bergman (*10*) reported elevated excretion of norepinephrine and epinephrine in a series of manic patients, whereas no significant changes in the excretion of either amine were observed in patients with endogenous depressions (retarded depressions were not separately characterized). Shinfuku *et al.* (*11*) reported increases in urinary excretion of norepinephrine during mania in a single patient with regular manic-depressive mood changes. Greenspan *et al.* (*12*) found that excretion of norepinephrine and normetanephrine was greater during hypomania than during normothymic periods or during periods of retarded depression. In a longitudinal study of depressed patients, Schildkraut *et al.* (*13*) observed a gradual rise in normetanephrine excretion during the period of definitive clinical improvement in depressed patients treated with imipramine. Bunney *et al.* (*14*) measured urinary catecholamines daily in a group of patients. Norepinephrine and dopamine were elevated before and during the manic episode. In particular, norepinephrine was significantly increased 1 day before the shift from depression to mania. In contrast, MHPG was not elevated just before or during the manic episode. In considering changes in catecholamine excretion, Bunny *et al.* commented that the days just before the manic episode may represent an initial phase of the mania.

Although the cited studies are in essential agreement as to the changes in urinary norepinephrine and its metabolites during shifts between depression and mania, they do not indicate whether these changes are secondary to the behavioral change or whether they reflect a change in catecholamine disposition and metabolism which is related to affective illness. We report longitudinal data on the urinary excretion of normetanephrine, which is derived from peripheral adrenergic pools, and of MHPG, a significant fraction of which may derive from norepinephrine metabolism in brain, by a patient who switched twice from depression to mania and once from mania to depression.

The patient was a middle-aged, married woman who at the time of admission had been suffering from a manic-depressive illness for 5 years. The illness first manifested itself as a severe depression of 3 months' duration, with spontaneous recovery occurring early in a pregnancy. The patient has since had several episodes of depression or mania (or both) of sufficient severity to require hospitalization. In a depressed phase the patient generally looks sad, expresses feelings of guilt, has psychomotor retardation, and may be actively suicidal, and while in a manic phase she is elated or boisterous, angry, and at times combative. During periods spent outside the hospital there was