drought in a series of stepwise regression analyses corresponding to the same models used before; the period from 1931 to 1970 was used for calibration. Again, only significant variables ($P \leq .05$) were allowed into the equations.

In general, a little explained variance is lost when factor scores instead of tree-ring indices are used as predictors. This loss is to be expected since 10 to 15 percent of the information is discarded with the fourth factor score. However, hemlock and chestnut oak factor scores still explain as much as 50.8 percent (47.9 percent adjusted) and 52.6 percent (49.8 percent adjusted), respectively, of the July PDSI variance. These values are approximately equal to the best results of the earlier regressions. Given the stability of the regression estimates made from factor scores, these newest results appear superior.

The result of one reconstruction is shown in Fig. 3. The predictors were factor scores extracted from a combined matrix of the four Shawangunk Mountain tree-ring chronologies plus the Schunemunk Mountain pitch-pine chronology. The prediction equation, developed according to stepwise regression, explained 66 percent (60 percent adjusted for degrees of freedom) of the PDSI variance for the calibration period from 1931 to 1970. In this figure, the actual drought indices are superimposed on the regression estimates for comparison.

The estimates for the calibration period are unbiased in that the number of over- and under-predictions of the actual data are virtually equal. However, the poorest estimates are almost always for the wetter years. This is not a serious drawback since in this study we are primarily concerned with occurrences of past drought, not of past wet intervals. In the latter case, different modeling techniques might be used.

Verification of the reconstructed drought indices is shown in Fig. 4. The independent data are the total precipitation from May through July for West Point, New York (1840 to 1859, 1867 to 1878, 1884 to 1895) and Mohonk Lake, New York (1896 to 1930). Both stations are near the tree-ring sites. This rainfall summation was used as a drought indicator because (i) the tree species respond directly to rainfall (Fig. 2), and (ii) the July drought indices reflect, in part, moisture conditions of the preceding months. There is generally good agreement between the two series, particularly for the drier years. Also, the low-frequency signal in the reconstructed drought indices corresponds well to that signal in the precipitation series. This comparison verifies that the reconstruction is a reasonable indicator of past July drought in the Hudson Valley.

On the basis of this initial reconstruction, the drought during the 1960's appears to be the most severe episode in terms of both intensity and duration for the past 241 years. Episodes of more persistent, but less severe, drought were apparently more common in the past. The 1766 to 1774 and 1794 to 1799 intervals stand out in this regard.

This study demonstrates the feasibility and usefulness of dendroclimatic analysis in the northeastern United States. Drought and other climatic reconstructions will be developed and improved upon as additional tree-ring chronologies are generated from the region and the biological and statistical models are refined.

EDWARD R. COOK
GORDON C. JACOBY, JR.
Lamont-Doherty Geological Observatory, Palisades, New York 10964

References and Notes

1. E. R. Cook, thesis, University of Arizona (1976).
2. C. W. Stockton and G. C. Jacoby, Jr., *Lake Powell Research Project Bull. No. 18* (Institute of Geophysics, University of California, Los Angeles, 1976).
3. A. V. Douglas and C. W. Stockton, "Long-term reconstruction of seasonal temperature and precipitation in the Yellowstone National Park region using dendroclimatic techniques" (a final report presented to the National Park Service in 1975); H. C. Fritts, *Tree Growth and Climate* (Academic Press, London, 1976).
4. C. W. Stockton, and D. M. Meko, *Weatherwise* **28**, 245 (1975).
5. W. S. Palmer, *U.S. Weather Bureau Res. Pap.* **45**, 58 (1965).
6. *Living With Climatic Change* (proceedings of the Toronto Conference Workshop, 17 to 22 November 1975) (Science Council of Canada, Ottawa, 1976).
7. C. J. Lyon, *Ecology* **17**, 457 (1936).
8. A tree-ring chronology generally consists of two or more increment cores from ten or more trees of a single species at a site. The ring widths are standardized by curve-fitting techniques and averaged for each year to produce a mean-value series for the site.
9. M. A. Stokes and T. L. Smiley, *An Introduction to Tree-Ring Dating* (Univ. of Chicago Press, Chicago, 1968).
10. Before the significance of correlation coefficients can be determined, the number of degrees of freedom must be corrected downward to account for persistence in each time series. If we assume that a first-order Markov process is operating in each series, an approximate correction can be calculated as:

$$N' = (N - 2)\frac{(1 - r_1 r_2)}{(1 + r_1 r_2)}$$

where N is the number of observations, r_1 and r_2 are the first-order serial correlation coefficients for series 1 and 2, and N' is the corrected degrees of freedom. In this case, $N = 40$ and each r is assigned a value of .5, which is actually slightly greater than that present in any of the series. After this correction degrees of freedom drops from 38 to 23 [D. R. Dawdy and N. C. Matalas, in *Handbook of Applied Hydrology*, V. T. Chow, Ed. (McGraw-Hill, New York, 1964), p. 87].
11. H. C. Fritts, T. J. Blasing, B. P. Hayden, J. E. Kutzbach, *J. Appl. Meteorol.* **10**, 845 (1971).
12. H. C. Fritts, *Quat. Res. (N.Y.)* **1**, 419 (1971).
13. The details of the modeling techniques and results will be described in greater detail (E. R. Cook, in preparation). The types of models have been described (*2*). The significance test for entering and deleting variables in stepwise regression is the partial F test. It determines whether or not the reduction in the variation of the dependent variable with the addition of an independent variable is sufficient for inclusion in the regression. The stepwise procedure terminates when the calculated partial F value falls below a predetermined significance level. [J. Neter and W. Wasserman, *Applied Linear Statistical Models* (Irwin, Homewood, Ill., 1974), pp. 382–383].
14. C. W. Stockton, thesis, University of Arizona (1971).
15. The 95 percent confidence intervals identify those months when climate is significantly correlated with tree growth. For the actual derivation of the confidence limits, see H. C. Fritts, *Tree Rings and Climate* (Academic Press, London, 1976), p. 365.
16. Supported by grant ATM 75-21226 from the Climate Dynamics Research Section of the National Science Foundation. Four of the tree-ring chronologies used were developed by E.R.C. under the supervision of M. A. Stokes at the Laboratory of Tree-Ring Research in Tucson. Lamont-Doherty Geological Observatory Contribution No. 2585.

15 March 1977; revised 31 May 1977

Peptidoglycan in the Cell Wall of the Primary Intracellular Symbiote of the Pea Aphid

Abstract. *Primary intracellular symbiotes of the pea aphid,* Acyrthosiphon pisum *(Harris), when fixed with potassium permanganate, revealed a distinctly staining area between the cytoplasmic membrane and the outer cell-wall envelope. This area is thought to be analogous to the peptidoglycan complex of the Eubacteriales. In addition, the diagnostic bacterial peptidoglycan amino compounds, muramic acid and diaminopimelic acid, were detected in a hydrochloric acid hydrolyzate of isolated symbiotes.*

Insect intracellular symbiotes are of interest both to insect physiologists and to microbiologists, to whom the evolutionary ancestry of the symbiotes presents a challenging question. Several aphid species have two distinct types of symbiotes, a rod-shaped secondary symbiote that is, morphologically, clearly bacteria-like and an oval, primary symbiote that is more difficult to categorize with existing groups of microorganisms. Whereas many early workers disputed the microbial nature of the primary symbiotes (*1*), most recent students agree that they are microorganisms although they have classified them in groups ranging from the Eubacteriales (*2–4*) to the rickettsia (*5, 6*) and mycoplasma (*7, 8*).

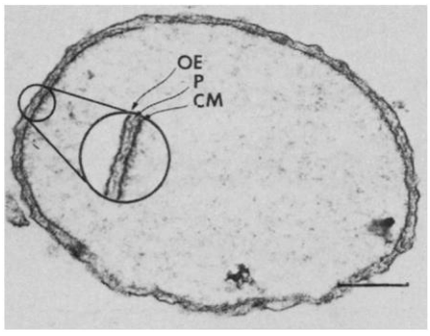

Fig. 1. Primary symbiote of pea aphid, stained with $KMnO_4$ (scale bar, 0.5 μm). The inset shows three definitive staining layers: the outer cell-wall envelope (*OE*), peptidoglycan (*P*), and the cytoplasmic membrane (*CM*).

All electron microscopic studies of aphid primary symbiotes in situ have, with a single exception (*2*), revealed the presence of three unit-membranes surrounding the microorganisms (*3–5*, *7–10*). While the inner membrane is clearly the cytoplasmic membrane of the microorganism and the outer membrane is of host origin, the middle unit-membrane is more difficult to define. By homology to the outer membrane of gram-negative bacteria, Griffiths and Beck (*11*) referred to this membrane as the outer cell-wall layer. Although the peptidoglycan complex of gram-negative bacteria (*12*) could not be resolved in the periplasmic space (that is, between the outer cell-wall layer and the cytoplasmic membrane) of the primary symbiote, it was observed in the secondary symbiote (*11*).

The first indication of a bacteria-like peptidoglycan cell-wall complex in the aphid primary symbiote came from studying the effects of penicillin, a known inhibitor of peptidoglycan biosynthesis (*10*). After treatment of the symbiotes with penicillin, accumulation of an amorphous mass of material in the periplasmic space of the primary, as well as the secondary, symbiote was described. It was suggested that this accumulation of material was analogous to the unorganized cell-wall material seen in *Bacillus megaterium* in response to penicillin (*13*).

The primary symbiote of the pea aphid, *Acyrthosiphon pisum*, was isolated previously (*14*) by means of discontinuous density gradient centrifugation. That the primary symbiote then retained its spherical morphology after exposure to a wide range of ill-defined osmotic conditions may, in retrospect, be cited as evidence in favor of an osmotically insensitive, delineating structure.

In the apparent absence of a definitive peptidoglycan complex in conventional fixation and staining procedures (*10*), the use of a more specific method seemed appropriate. Potassium permanganate has been reported to stain polysaccharide cell walls (*15*), and this stain has been used before with the primary symbiote of the pea aphid (*7*). In the experiments described here we examined at high magnifications these primary symbiotes stained with $KMnO_4$ and found a distinctly staining component between the cytoplasmic membrane and the outer cell-wall layer (Fig. 1) which suggested the presence of a typical bacterial peptidoglycan.

Ultimate proof of a bacterial-like cell wall resides in chemical identification of the cell-wall peptidoglycan components muramic acid (MurA) or diaminopimelic acid (DAPA), because one or both compounds are universal to bacterial cell walls (*16*). Daniel and Brooks (*17*) identified MurA, using thin-layer chromatography, in the intracellular symbiote of the cockroach. That this organism, now classified as *Blattobacterium* sp. (*6*), possesses a peptidoglycan complex should, perhaps, not be so surprising since, like the aphid secondary symbiote, it is morphologically very similar to rod-shaped bacteria or rickettsia.

Table 1. Amino acid analysis of isolated primary symbiotes of the pea aphid. Data are expressed as the means of three separate analyses ± standard error.

Compound	Mean percentage
Amino acid	
Aspartic acid	10.7 ± 1.4
Threonine	5.4 ± 0.4
Serine	7.3 ± 0.2
Proline	2.6 ± 0.3
Glutamic acid	6.4 ± 1.9
Glycine	9.2 ± 0.2
Alanine	5.8 ± 0.2
Valine	6.0 ± 0.5
Methionine plus methionine sulfoxides	2.2 ± 0.7
Isoleucine	10.5 ± 1.1
Leucine	8.9 ± 1.0
Tyrosine	3.0 ± 0.5
Phenylalanine	4.2 ± 0.1
Lysine	10.4 ± 0.5
Histidine	1.7 ± 0.3
Arginine	4.1 ± 0.2
Other	
Half-cystine	0.2 ± 0.2
Glucosamine	0.9 ± 0.4
Muramic acid	0.1 ± 0.0
Diaminopimelic acid	0.1 ± 0.0

During studies of lipid metabolism in isolated primary symbiotes (*18*), it seemed opportune to attempt to detect the diagnostic bacterial cell-wall amino compounds, MurA and DAPA. The primary symbiote of the pea aphid was isolated, cultured for 24 hours in Grace's insect tissue culture medium, and the lipids were extracted. The remaining pellet was flooded with 10 ml of 6*N* HCl and hydrolyzed for 12 to 16 hours at 105° C. The suspension was evaporated to dryness in a vacuum, rehydrated in the appropriate citrate buffer (*19*), and then the amino compounds were separated on an amino acid analyzer.

The separation of the component amino acids from whole, isolated, primary symbiotes is summarized in Table 1. A total of three independent determinations was made with relatively good agreement between them. The expected 16 common amino acids were detected. Tryptophan was detected in trace amounts in one analysis but was completely hydrolyzed in the other two. Odd amino compounds were also identified in all three chromatographs. Two particularly prominent peaks coincided with glucosamine and MurA, while a third peak was thought to represent DAPA (Table 1 and Fig. 2A).

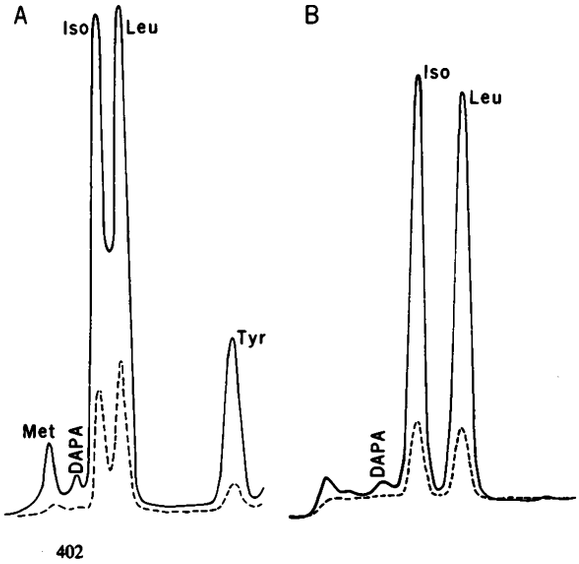

Fig. 2. Detection of diaminopimelic acid in (A) HCl hydrolyzates and (B) performic acid oxidized samples of isolated symbiotes. Tracings are at 440 nm (---) and 570 nm (—); *Met*, methionine; *DAPA*, diaminopimelic acid; *Iso*, isoleucine; *Leu*, leucine; and *Tyr*, tyrosine.

The detection of reasonable amounts of MurA and a suspected DAPA peak encouraged us to attempt to verify the identity of DAPA. This was accomplished by performic acid oxidation, which clearly separated DAPA from methionine (*20*). The performic acid–oxidized samples yielded a DAPA peak that coincided with the suspected DAPA peak from the HCl hydrolyzates (Fig. 2, A and B).

Our results show that the primary symbiote of the pea aphid does indeed contain the peptidoglycan complex of "true" bacterial cell walls. Staining with $KMnO_4$ revealed an apparent peptidoglycan complex between the cytoplasmic membrane and the outer cell-wall envelope of the primary symbiote. In addition, amino acid analysis revealed the diagnostic peptidoglycan amino compounds MurA and DAPA.

EDWARD J. HOUK
Naval Biosciences Laboratory, University of California, Oakland 94625
GARETH W. GRIFFITHS
Max-Planck-Institut für Biologische Kybernetik, 74 Tübingen 1, Germany
NICHOLAS E. HADJOKAS
STANLEY D. BECK
Department of Entomology, University of Wisconsin, Madison 53706

References and Notes

1. P. Buchner, *Endosymbiosis of Animals with Plant Microorganisms* (Interscience, New York, 1965); A. Koch, in *Symbiosis*, S. M. Henry, Ed. (Academic Press, New York, 1967), vol. 2, p. 1.
2. C. Vago and M. LaPorte, *Ann. Soc. Entomol. Fr.* **1**, 181 (1965).
3. R. Hinde, *J. Insect Physiol.* **17**, 2035 (1971).
4. F. Iaccarino and E. Tremblay, *Boll. Lab. Entomol. Agr.* **30**, 319 (1973).
5. K. Lamb and R. Hinde, *J. Invert. Pathol.* **9**, 3 (1967).
6. M. Brooks, in *Bergey's Manual of Determinative Bacteriology*, R. Buchanan and N. Gibbons, Ed. (Williams & Wilkins, Baltimore, 1974), p. 900.
7. D. McLean and E. Houk, *J. Insect Physiol.* **19**, 625 (1973).
8. L. Ehrman and R. Kernaghan, *CIBA Found. Symp.* **6**, 227 (1972).
9. G. Griffiths and S. Beck, *J. Insect Physiol.* **19**, 75 (1973).
10. ———, *Cell Tissue Res.* **148**, 287 (1974).
11. ———, *ibid.* **159**, 351 (1975).
12. J. Costerton, J. Ingram, K. Cheng, *Bacteriol. Rev.* **38**, 87 (1974).
13. P. Firz-James and D. Hancock, *J. Cell Biol.* **26**, 657 (1965).
14. E. Houk and D. McLean, *J. Invert. Pathol.* **23**, 237 (1974).
15. S. Bradbury and G. Meek, *Q. J. Microsc. Sci.* **101**, 241 (1960).
16. H. Martin, *Annu. Rev. Biochem.* **35**, 457 (1966); M. Osborn, *ibid.* **38**, 501 (1969); A. Laskin and H. Lechevalier, Eds., *Handbook of Microbiology* (Chemical Rubber Company, Cleveland, 1973), vol. 3.
17. R. Daniel and M. Brooks, *Experientia* **23**, 499 (1967).
18. E. Houk, G. Griffiths, S. Beck, *Comp. Biochem. Physiol. B* **54**, 427 (1976).
19. F. Vojtik, Department of Plant Pathology, University of Wisconsin, Madison. The short column was filled with the rehydrated sample at *p*H 4.62; elution was in two steps at *p*H 4.62 and 5.28; all buffers were 0.35*M* sodium citrate. The long column was filled with the rehydrated sample at *p*H 3.50; elution was in two steps at *p*H 3.12 and 4.25; all buffers were 0.2*M* sodium citrate.
20. J. Schroeder, Department of Biochemistry, University of Wisconsin, Madison. Performic acid was made by slowly mixing formic acid (88 percent) with H_2O_2 (30 percent) (9:1, by volume) in an acid bath. Ten milliliters of this solution was added to the samples and oxidation was allowed to proceed overnight at 5°C. The samples were neutralized with approximately 0.6 ml of HBr (40 percent), evaporated to dryness a vacuum, and analyzed by an amino acid analyzer.
21. Research was supported by the Office of Naval Research and the College of Agriculture and Life Sciences, University of Wisconsin.

25 February 1977; revised 8 June 1977

Hypertension: Increase of Collagen Biosynthesis in Arteries but Not in Veins

Abstract. *In two models of hypertension in rats, it was shown that collagen synthesis and deposition are increased in arteries where blood pressure is elevated. By contrast, there were no alterations in any of the markers of collagen synthesis in veins, where blood pressure was only slightly elevated. It would appear that the stimulus for vascular collagen synthesis is provided by a direct effect of the increased pressure on the arterial cells rather than by a humoral factor released into the general circulation.*

We have previously shown (*1*) that, in two models of hypertension in rats, there is increased synthesis and deposition of collagen in the arteries. We therefore suggested that some of the observed pathology of hypertension is related to vascular formation and deposition of collagen (*1*) and, furthermore, that it is the increased pressure itself which initiates the changes in collagen metabolism (*2*). It is known that in hypertension, the increases in pressure are predominantly in the arterial bed and that venous pressure is only slightly affected (*3*). Since the veins normally contain as much collagen as the arteries on a weight-per-weight basis, it was of interest to determine the effects of experimental hypertension on venous collagen.

Male Wistar rats were supplied by Charles River. Desoxycorticosterone acetate–salt hypertension was produced in uninephrectomized, 8-week-old rats, by subcutaneous injection of 5 mg of desoxycorticosterone acetate (DOCA) per rat twice weekly (*4*). Normotensive, unoperated male Wistar rats were used as controls. All the animals were maintained on a standard laboratory diet and were given free access to 1 percent saline in tap water for drinking. Spontaneously hypertensive rats (5 months old) were selected from the Hoffman-La Roche colony (*5*). Normotensive controls were genetically related Wistar-Kyoto male rats. Arterial blood pressures were measured by the tail-cuff and photoelectric method without anesthesia (*6*). Jugular vein blood pressures were measured by a water manometer connected with a cannula inserted directly into the jugular vein of anesthetized animals. Rats were killed by decapitation, the various blood vessels were excised, and three markers of collagen synthesis were measured.

Table 1. Arterial and venous blood pressures in hypertensive rats and controls. Rats were made hypertensive by 6 weeks of treatment with desoxycorticosterone-salt (DOCA-salt). The spontaneously hypertensive rats were 5 months old. Each value represents the mean ± the standard error of the mean obtained from at least five rats.

Model	Blood pressure	
	Arterial (mm-Hg)	Venous (mm-H_2O)
Control	120 ± 4.1	−20 ± 4.4
DOCA-salt	212 ± 4.8*	−15 ± 2.8
Control	120 ± 3.4	−23 ± 5.7
Spontaneous	208 ± 3.3*	−13 ± 3.0

*Significantly different from its appropriate control, $P < .01$.

Each tissue was homogenized in 30 volumes of 0.25*M* sucrose containing 10 m*M* tris-HCl buffer (*p*H 7.4), 100 μM dithiothreitol, and 10 μM ethylenediaminetetraacetic acid. Prolyl hydroxylase activity was measured in a 15,000*g* supernatant by the tritium release assay of Hutton *et al.* (*7*). Collagen was extracted with 0.5*M* acetic acid (*8*) and hydroxyproline was measured by the method of Kivirikko *et al.* (*9*). These values yield collagen content when multiplied by 6.98 (*10*). The incorporation of ^{14}C-labeled proline into collagenase-digestible protein was determined by incubating tissue homogenates in 1.25 ml of Earle's balanced salt solution buffered with 28m*M* Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], *p*H 7.4, containing 10 μc of ^{14}C-labeled proline (New England Nuclear) as described (*11*). Protein concentration was determined by the method of Lowry *et al.* (*12*), with bovine serum albumin as the standard.