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.

The arterial blood pressures of both types of hypertensive animals were elevated over those of the normotensive controls (Table 1). The venous blood pressures are normally very low and were, therefore, measured by a water manometer. In both types of hypertensive animals venous pressure was not significantly increased.

The prolyl hydroxylase (E.C. 1.14.11.2; proline, 2-oxoglutarate dioxygenase) activities in blood vessels are shown in Table 2. This marker of collagen biosynthesis was greatly increased in the aorta and mesenteric artery of hypertensive animals. The small increases observed in the vena cava and mesenteric vein are of questionable significance.

In another experiment, we measured in vitro collagen synthesis by blood vessels from control and hypertensive rats, as indicated by the incorporation of ^{14}C-labeled proline into collagenase-digestible protein. This marker of collagen synthesis was also increased greatly in arteries but not at all in veins (Table 3).

Hypertension resulted in an appreciable increase in collagen content in both the aorta and the mesenteric artery (Table 4). Again, no significant changes were observed in the vena cava or the mesenteric vein.

Our studies show that the alterations in collagen synthesis in the vasculature of hypertensive animals is limited to the arterial vessels as is the increased pressure. It would appear, therefore, that the increased pressure acts directly on the cells in the arterial bed and that its effects are modulated by local and, most likely, intracellular mechanisms. If this were not so and if, in hypertension, a humoral factor affecting collagen synthesis was liberated into the general circulation, the effects would most likely have been seen in veins as well as in arteries.

Additional evidence that it is the elevated blood pressure per se which is responsible for the observed changes in collagen biosynthesis is provided by the studies of Hume and Bevan (*13*). They demonstrated that experimental coarctation of the rabbit aorta results in increased collagen synthesis in that segment of the aorta which is proximal to the constriction and where blood pressure is elevated. Neither blood pressure nor collagen synthesis is increased in the segment of aorta distal to the constriction. Further supporting evidence comes from patients with coarctation of the aorta (*14*) where, again, those portions of the aorta proximal to the constriction which are subjected to increased pressure, are the ones that undergo hypertrophy and form fibrous tissue.

It remains to be seen as to just how the increased blood pressure stimulates arterial collagen synthesis and the accompanying fibrosis. Nevertheless, the present findings provide a rationale for current approaches to antihypertensive therapy (*15*) which are based on the premise that lowering blood pressure, by whatever means, is lifesaving in hypertension.

Table 2. Prolyl hydroxylase activity in arteries and veins of hypertensive rats compared to controls. The tissues were taken from the same animals that were described in Table 1, and prolyl hydroxylase was assayed in homogenates as described in the text. Enzyme activity is expressed as counts per minute per milligram of protein. Each value represents the mean ± the standard error of the mean obtained from results in five rats. The values for the mesenteric vein could not be treated statistically because the determinations were done on tissue pooled from five animals.

Model	Prolyl hydroxylase activity	
	DOCA-salt	Spontaneous
	Aorta	
Control	8,030 ± 1,020	8,730 ± 320
Hypertensive	14,690 ± 1,650*	17,850 ± 270†
	Mesenteric artery	
Control	12,710 ± 1,220	13,950 ± 580
Hypertensive	29,990 ± 2,180†	18,840 ± 330†
	Vena cava	
Control	6,590 ± 430	5,080 ± 580
Hypertensive	8,130 ± 780	6,490 ± 350
	Mesenteric vein	
Control	11,388	10,900
Hypertensive	13,078	12,270

*$P < .05$, as compared to control. †$P < .01$.

Table 3. Collagen synthesis in isolated arteries and veins from hypertensive and control rats. The same type of DOCA-salt animals were used as in Table 1. Samples of minced tissue were incubated with ^{14}C-labeled proline, homogenized, dialyzed, and treated with collagenase as described. Values for incorporation of ^{14}C-labeled proline are expressed as counts per minute per milligram of protein. The values for the aorta and mesenteric artery represent the mean ± the standard error of the mean obtained from results in five rats. The values for the vena cava and the mesenteric vein could not be treated statistically because the determinations were done on tissues pooled from five rats.

Model	^{14}C-labeled proline incorporation into collagen
Aorta	
Control	2441 ± 566
Hypertensive	5725 ± 1189*
Mesenteric artery	
Control	3482 ± 612
Hypertensive	7812 ± 1450*
Vena cava	
Control	2210
Hypertensive	2650
Mesenteric vein	
Control	2252
Hypertensive	2126

*Significantly different from control, $P < .05$.

Table 4. Collagen content of arteries and veins in hypertensive rats compared to controls. The same type of animals were used as in Table 1. The isolated vessels were homogenized and assayed for hydroxyproline; their collagen content was calculated as described. Total collagen content is expressed as milligrams of collagen per gram of tissue. Each value represents the mean ± the standard error of the mean obtained from results in five rats. The values for the mesenteric vein could not be treated statistically because the determinations were done on tissue pooled from five rats.

Model	Total collagen	
	DOCA-salt	Spontaneous
	Aorta	
Control	97.4 ± 2.5	77.9 ± 1.0
Hypertensive	116.0 ± 2.1*	101.0 ± 5.9*
	Mesenteric artery	
Control	80.8 ± 1.6	86.1 ± 3.3
Hypertensive	94.9 ± 2.2*	111.0 ± 5.8†
	Vena cava	
Control	51.1 ± 2.8	71.5 ± 2.5
Hypertensive	57.3 ± 4.3	67.0 ± 1.7
	Mesenteric vein	
Control	74.4	81.5
Hypertensive	73.1	83.8

*$P < .01$. †$P < .05$.

K. IWATSUKI, G. J. CARDINALE
S. SPECTOR, S. UDENFRIEND
Roche Institute of Molecular Biology, Nutley, New Jersey 07110

References

1. A. Ooshima, G. C. Fuller, G. J. Cardinale, S. Spector, S. Udenfriend, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3019 (197→ *Science* **190**, 898 (1975).
2. G. J. Cardinale, K. Iwatsuki, S. Spector, S. Udenfriend, *Pharmacologist* **19** (No. 2), 194 (1977).
3. B. Folkow, M. Hallbäck, Y. Lundgren, L.

Weiss, I. Albrecht, S. Julius, *Acta Physiol. Scand.* **90**, 654 (1974).
4. J. DeChamplain, L. R. Krakoff, J. Axelrod, *J. Circ. Res.* **20**, 136 (1967).
5. J. J. Tarver, B. Berkowitz, S. Spector, *Nature (London) New Biol.* **231**, 252 (1971).
6. J. R. Williams, T. R. Harrison, A. Grollman, *J. Clin. Invest.* **18**, 373 (1939).
7. J. J. Hutton, A. L. Tappel, S. Udenfriend, *Anal. Biochem.* **16**, 384 (1966).
8. M. Chvapil, D. McCarthy, D. Madden, E. E. Peackock, Jr., *Biochem. Pharmacol.* **23**, 2165 (1974); K. Iwatsuki, G. J. Cardinale, S. Spector, S. Udenfriend, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 360 (1977).
9. K. I. Kivirikko, O. Laitinen, D. J. Prockop, *Anal. Biochem.* **19**, 249 (1967).
10. D. S. Jackson and E. G. Cleary, *Methods Biochem. Anal.* **15**, 25 (1968).
11. A. Ooshima, G. C. Fuller, G. J. Cardinale, S. Spector, S. Udenfriend, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 777 (1977).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
13. W. R. Hume and J. A. Bevan, *Circ. Res.*, in press.
14. S. L. Robbins, *Pathology* (Saunders, Philadelphia, ed. 3, 1967), vol. 1, pp. 540–551.
15. E. D. Freis, D. Ragan, H. Pillsbury, III, M. Matthews, *Circ. Res.* **31**, 1 (1972); E. D. Freis, *ibid.* **29** (Suppl. 2), 70 (1971); W. Hollander, *Circulation* **48**, 1112 (1973).

15 June 1977; revised 9 August 1977

Osmotically Induced Changes in Electrical Properties of Plant Protoplast Membranes

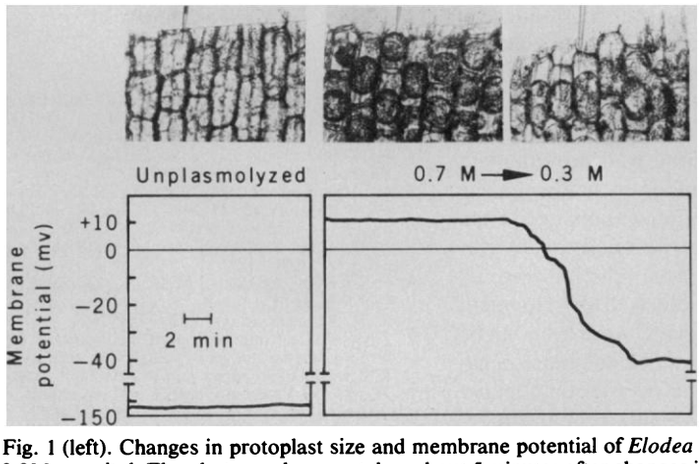
Abstract. *The internal electrical potential of protoplasts from six different plant species was positive. Plasmolyzed cells of leaves had positive voltages of similar magnitude. Both* Elodea *leaf cells and tobacco protoplasts with regenerated cell walls became more electronegative during deplasmolysis. These findings suggest that cell turgor affects membrane components that determine cellular potential.*

Since the first mass preparation of plant protoplasts by enzymatic techniques (*1*) and the regeneration of an entire plant from a protoplast (*2*), there has been much interest in the possible use of protoplasts for plant improvement (*3*). Introduction of new genetic material into the plant protoplast through transformation, transduction, or somatic hybridization becomes more feasible once the physical barrier presented by the cell wall has been removed by enzymatic digestion, but many types of protoplasts, including those from cereals, fail to develop in culture (*4*). We now report that protoplasts are electrically positive with respect to the external solution, although the leaf cells from which they were derived are electrically negative. Since isolated protoplasts must be maintained in a plasmolyzed condition during and following removal of the cell wall, we examined the effect of plasmolysis and deplasmolysis on the membrane potential of intact leaf cells, of freshly isolated protoplasts, and of older protoplasts.

For impalement of cells with microelectrodes, sections of mature leaves from oat (*Avena sativa*), corn (*Zea mays*), tobacco (*Nicotiana tabacum*), and petunia (*Petunia hybrida*) were cut and mounted in a Plexiglas chamber and bathed in 1X nutrient solution for 1 to 3 hours before impalement (*5*). Protoplasts were isolated from leaf tissue of oat, corn, tobacco, petunia, and *Datura stramonium* and stem tissue of soybean (*Glycine max*). After surface sterilization in 0.5 percent hypochlorite, the upper epidermis was stripped from the leaves and the soybean stems were split longitudinally. The areas of cells thus exposed were placed face down in an enzyme solution consisting of 1.5 percent cellulysin (Calbiochem, San Diego), 0.5 percent pectinase (Nutritional Biochemicals Corp., Cleveland), and 0.57*M* mannitol at *p*H 5.7 for 3 hours at 30°C. The released protoplasts were collected by low-speed centrifugation (75*g*) and cultured in a B-5 medium (*6*) and 0.7 percent agar. For impalement of protoplasts with microelectrodes, a 0.5 cm by 1.0 cm section was cut from the agar and placed in a Plexiglas chamber. Most measurements were made within 2 hours to 4 days of isolation; tobacco protoplasts were monitored over a 3-week period and during changes in external osmoticum.

Elodea densa was maintained in a glass beaker in 1X nutrient solution. Room air was bubbled through the medium and a combination of fluorescent and incandescent lights provided illumination at 1.2×10^4 erg cm^{-2} sec^{-1}. A leaf near the apical meristem was removed and the tip of the leaf (0.5 to 2 mm) was excised. The leaf, with the cut end up, was held in the Plexiglas chamber by a thin rubber strip on the wall of the chamber so that individual cells could be impaled with microelectrodes.

Microelectrodes with tip diameters of less than 0.5 μm were made from glass capillary tubing and filled with 3*M* KCl. The microelectrodes and a reference electrode were connected with Ag-AgCl wire to an electrometer and a chart re-



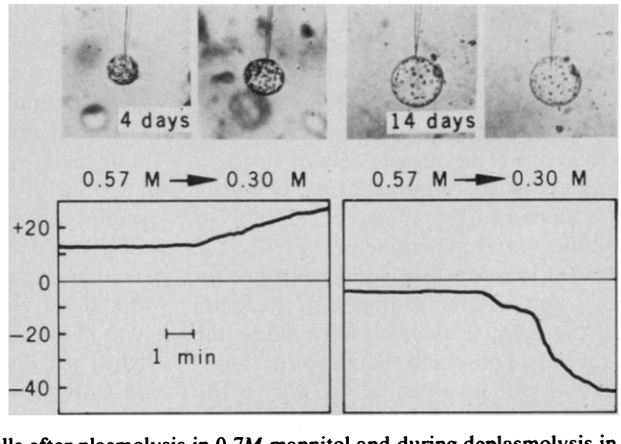


Fig. 1 (left). Changes in protoplast size and membrane potential of *Elodea* cells after plasmolysis in 0.7*M* mannitol and during deplasmolysis in 0.3*M* mannitol. The photographs were taken about 5 minutes after changes in the external osmoticum. The response of the membrane potential to external osmoticum is condensed from original chart records. This experiment was repeated three times. Fig. 2 (right). Response of young (4-day-old) and older (14-day-old) tobacco protoplasts to changes in external osmoticum. The photographs were taken about 5 minutes after perfusion of protoplasts, which were embedded in agar in 0.57*M* mannitol, with 0.30*M* mannitol. During enlargement of the 4-day-old protoplasts the potential becomes more positive. The older protoplasts have cell walls which prevent their expansion and the membrane potential becomes more negative when the osmotic concentration is lowered. Data are redrawn from chart records; these experiments were repeated six times.