dure used to prepare the antibody-ferritin conjugate. There was no significant labeling of any cellular organelle except the nucleus, which was slightly labeled.

The observations with ferritin-labeled antibody demonstrate that prolyl hydroxylase is found within the cisternae of the endoplasmic reticulum. Since separate assays of antigenicity and of enzyme activity in cell homogenates established that most of the enzyme in the matrix-free tendon cells was in an active form (*10*), the distribution of ferritin-labeled antibody reflects the location of active enzyme. The ferritin label was distributed throughout the cisternae, but the results do not exclude the possibility that the enzyme is loosely bound to the inner surface of the cisternal membrane and is displaced into the lumen by the preparative procedures. However, the results do appear to exclude the possibility that the active enzyme is on the ribosomal side of the endoplasmic reticulum, in the cytoplasm, or in the Golgi vacuoles.

The localization of prolyl hydroxylase in the cisternae has several consequences. Some hydroxylation of proline can occur in nascent, incomplete peptides (*1*), and prolyl hydroxylase cannot hydroxylate protocollagen polypeptides in a triple-helical conformation (*12*). Therefore, the newly synthesized polypeptides are fed into the cisternae during synthesis, and when the polypeptides enter the cisternae, they are in a random-coil form. Since under appropriate conditions protocollagen accumulates in connective tissue cells and can be hydroxylated 1 to 3 hours after synthesis (*13*), accumulated protocollagen must be retained in the cisternae until the hydroxylation occurs.

BJØRN R. OLSEN
RICHARD A. BERG, YASUO KISHIDA
DARWIN J. PROCKOP
Department of Biochemistry,
Rutgers Medical School,
College of Medicine and Dentistry
of New Jersey, Piscataway 08854

References and Notes

1. M. E. Grant and D. J. Prockop, *N. Engl. J. Med.* **286**, 194 (1972).
2. P. Dehm and D. J. Prockop, *Biochim. Biophys. Acta* **240**, 358 (1971); S. Jimenez, P. Dehm, B. R. Olsen, D. J. Prockop, *J. Biol. Chem.* **248**, 720 (1973).
3. R. L. Trelstad, *J. Cell Biol.* **45**, 34 (1971); M. Weinstock, *Z. Zellforsch. Mikrosk. Anat.* **129**, 455 (1972); E. D. Hay and J. W. Dodson, *J. Cell Biol.* **57**, 190 (1973).
4. R. Ross and E. P. Benditt, *J. Cell Biol.* **27**, 83 (1965); M. M. Salpeter, *J. Morphol.* **124**, 387 (1968); G. W. Cooper and D. J. Prockop, *J. Cell Biol.* **38**, 523 (1968).
5. J. D. Jamieson and G. W. Palade, *J. Cell Biol.* **34**, 577 (1967).
6. R. A. Berg and D. J. Prockop, *J. Biol. Chem.* **248**, 1175 (1973).
7. R. A. Berg, B. R. Olsen, D. J. Prockop, *Biochim. Biophys. Acta* **285**, 167 (1972).
8. P. Cuatrecasas, *J. Biol. Chem.* **245**, 3059 (1970).
9. V. A. de Saussure and W. B. Dandliker, *Immunochemistry* **6**, 77 (1969).
10. Y. Kishida, B. R. Olsen, R. A. Berg, D. J. Prockop, in preparation; B. R. Olsen, R. A. Berg, Y. Kishida, D. J. Prockop, in preparation.
11. S. Avrameas, *Histochem. J.* **4**, 321 (1972); J. P. Kraehenbuhl and J. D. Jamieson, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 1771 (1972); H. Hirano, B. Parkhouse, G. L. Nicholson, E. S. Lennox, S. J. Singer, *ibid.*, p. 2945.
12. R. A. Berg and D. J. Prockop, Abstracts of the Ninth International Congress of Biochemistry, Stockholm, July 1973, p. 423; R. A. Berg and D. J. Prockop, *Biochemistry* **12**, 3395 (1973).
13. K. Juva, D. J. Prockop, G. W. Cooper, J. Lash, *Science* **152**, 92 (1966); J. Uitto and D. J. Prockop, Abstracts of the Ninth International Congress of Biochemistry, Stockholm, July 1973, p. 425.
14. S. K. Ainsworth and M. J. Karnovsky, *J. Histochem. Cytochem.* **20**, 225 (1972).
15. T. Eskeland, E. Klein, M. Inoue, B. Johansson, *J. Exp. Med.* **134**, 265 (1971).
16. A preliminary report on this work was presented at the meeting of the New York Society of Electron Microscopists, New York, 25 May 1973. Supported in part by NIH research grant AM-16,516. We thank A. Cywinski and N. Doerr for technical assistance.

5 June 1973; revised 27 July 1973

Plant Mycoplasmas: Serological Relation between Agents Associated with Citrus Stubborn and Corn Stunt Diseases

Abstract. *Growth-inhibition and precipitin tests established that antigens of the helical mycoplasma-like organism* (Spiroplasma citri) *associated with citrus stubborn disease are serologically related to antigens in corn infected with stunt disease but not in healthy corn.*

There has previously been no reason to suspect a relationship between citrus stubborn and corn stunt diseases. Recently, however, an agent suspected to cause the citrus stubborn disease was cultured from diseased citrus (*1*), and was shown to have many of the properties of the class Mollicutes (mycoplasmas). More detailed biochemical and serological studies of the organism indicated that it was a new distinct species, but also confirmed its general similarity to other mycoplasmas (*2*). Phase-contrast microscopy of broth cultures revealed that helical filaments were the predominant form of the organism. Ultrastructural studies reaffirmed the morphological structure of these microbes and also revealed the presence of a tailed bacteriophage (*3*). It was proposed that the organism recovered from citrus stubborn be named *Spiroplasma citri* (*2*); the organism has not been assigned to higher taxons.

Table 1. Growth-inhibition tests (disc method) with *Spiroplasma citri* and antiserums to corn stunt disease and healthy corn tissues. Fractionation of corn and corn stunt antigens is described in the text. Tissue was adsorbed with freeze-dried antigen from centrifuged cultures or fractions of plant extracts.

Antiserum	Growth-inhibition zones (mm) to *Spiroplasma citri* cultures	
	Moroccan isolate	California isolate
Unadsorbed serum		
Corn stunt fraction	5–6	4–5
Corn fraction	0	0
S. citri		
Moroccan isolate	10	7–9
California isolate	7–9	6–8
A. laidlawii (PG-8)	0	0
Adsorbed serum		
Corn stunt tissue		
Unadsorbed	3–4	4–5
Absorbed (corn stunt)	3	1
Adsorbed (healthy corn)	3	4
Adsorbed (*S. citri* Morocco)	0	0
Adsorbed (*A. laidlawii* PG-8)	4	5

The helical organisms associated with citrus stubborn disease are similar to bodies found associated with, and suspected to cause, corn stunt disease (*4*), a disease that affects both plants and the insect vectors that transmit it (*5*). The helical bodies associated with corn stunt, whose appearance in ultrathin sections is mycoplasma-like, can be seen as helices in the phloem of infected corn by techniques such as freeze-etch electron microscopy (*4*). Unfortunately, although the corn stunt agent can be maintained in an infectious state for up to 48 days in primary cultures (*6*), it has not been grown in continuous cell-free culture.

Both *S. citri* and the corn stunt agent have helical structures and since *S. citri* was characterized as an unusual mycoplasma we believed that techniques employed in the serological analyses of mycoplasmas might yield useful information on the relationship between the two microorganisms.

Antiserums to the corn stunt agent were prepared from diseased plant

material. Leaves and stems (140 g) of stunted or healthy corn were cut into small pieces and ground in a blender until lightly shredded. This material was suspended in 400 ml of buffer, consisting of 0.3*M* glycine and 0.03*M* $MgCl_2$ (*p*H 8.0), and then filtered through cheesecloth to remove coarse material. Plant material was removed by differential centrifugation (10 minutes at 5000*g* and 13 minutes at 17,000*g*). The supernatant was then centrifuged at 65,000*g* for 10 minutes, and the pellet was resuspended in approximately 5 ml of the glycine buffer. Seven rabbits were immunized with the corn stunt antigen and four rabbits received the preparation from healthy corn. We prepared antiserums to the Moroccan and California strains of *S. citri* by immunizing each of two rabbits with cultured organisms (*2*). The corn preparations or *S. citri* organisms were each mixed with an equal volume of Freund's complete adjuvant, and the rabbits were inoculated at multiple intramuscular sites. After two biweekly booster immunizations, there was a large amount of antibody to *S. citri*, as estimated by growth-inhibition tests (*7*), and final bleedings were performed. With the corn stunt and healthy corn antigens, however, we gave biweekly boosters of the respective antigens for 14 to 28 weeks.

We performed growth-inhibition tests, using antiserums prepared against *S. citri*, stunted or healthy corn, or to an animal mycoplasma (*Acholeplasma laidlawii* PG-8), against 48-hour broth cultures of *S. citri*. Growth-inhibiting antibodies directed against *S. citri* were first observed 3 to 4 weeks after the first immunization of all rabbits receiving corn stunt antigen. The amount of inhibition increased as the rabbits were given biweekly booster immunizations until the inhibition zones reached a maximum diameter of 5 to 6 mm (Table 1). Growth inhibition was also observed with homologous antibody to *S. citri*, but not with antiserums obtained from rabbits receiving healthy corn or *A. laidlawii* antigen. In the growth-inhibition test with *S. citri* all serums of the rabbits were negative before immunization.

The specificity of the growth inhibition test was also examined by adsorbing antiserum to corn stunt organisms with either infected or healthy plant antigen (92 to 100 mg of dried final pellets per milliliter of antiserum), or with *S. citri* or *A. laidlawii* antigens (20 to 30 mg of dried pelleted organisms per milliliter of antiserum), and retesting the adsorbed serums against *S. citri* antigens by the growth-inhibition method. Only *S. citri* antigen was able to selectively remove the growth-inhibiting activity of the antiserum to corn stunt organisms (Table 1). The failure of dried corn stunt extract to adsorb homologous antibody from the rabbit antiserum is thought to be due to the relatively small amounts of reactive antigen present in the plant extract.

To provide further evidence that the growth-inhibiting substance in corn stunt antiserum was antibody, we performed serological tests on serum globulin fractions of the antiserum. Samples (5 ml) of corn stunt or *S. citri* antiserums were mixed with an equal volume of saturated ammonium sulfate. Precipitated globulin and supernatant were separated by centrifugation at 10,000*g*, and each fraction was dialyzed in phosphate buffered saline (*p*H 7.5) until the sulfate ions were removed. The four fractions were then frozen (−70°C) and lyophilized. Each fraction was suspended in deionized water to half the original volume of serum and tested against *S. citri* by the growth-inhibition procedure. Full serological reactivity was retained in the globulin fraction.

Table 2. Precipitin ring test of antiserums. The numbers indicate the reciprocal of the maximum twofold dilution of serum that formed a precipitin ring with the indicated antigen. The number 0 indicates that serum diluted with an equal volume of 10 percent glycerine in phosphate buffered saline failed to form a ring with the indicated antigen. Undiluted serum, including preimmune serum, occasionally and irregularly formed rings with antigens diluted in phosphate buffered saline. Nonspecific rings were not observed with diluted serums. Corn stunt and corn antigens were centrifuged and resuspended in phosphate buffered saline. They were prepared in the same way as immunizing antigen, as described in text. *Spiroplasma* antigen was sedimented from 72-hour cultures in SMC medium (*2*) and then washed in phosphate buffered saline. All antigens were subjected to ten cycles of rapid freezing and thawing at −70°C and 37°C, then centrifuged at 85,000*g* for 1 hour. The supernatants were held at 5°C before layering in the ring test.

Rabbit	Antigen			
	Plant fractions (titer)		*Spiroplasma citri* (titer)	
	Corn stunt	Corn	Moroccan	California
Serum before immunization				
445	0	0	0	0
447	0	0	0	0
486	0	0	0	0
488	0	0	0	0
502	0	0	0	0
Antiserum to corn stunt fraction				
445	32	64	8	
486	64	64	8	8
502	8	16	0	
Antiserum to corn fraction				
447	64	128	0	0
488	32	64	0	
489	32	32	0	
Antiserum to S. citri *Moroccan*				
480 to 481	32	0	256	512
Antiserum to S. citri *California*				
482 to 483	32	0	256	512

Still further evidence for the serological relation between *S. citri* and the organisms associated with corn stunt came from precipitin ring tests (*8*). Antigens for this test were prepared by ten successive cycles of freezing and thawing of resuspended pellets of cultured *S. citri* or final pellets from diseased or healthy corn. We centrifuged each of the resuspended freeze-thawed pellets and the antiserums at 85,000*g* for 60 minutes prior to testing. Antiserums were diluted in a solution of 10 percent glycerine in phosphate buffered saline and were placed in the bottom of small glass tubes (internal diameter, 4 mm). The antiserums were then overlaid with antigen that was diluted in phosphate buffered saline. Antigen that precipitated with antiserum to *S. citri* was demonstrated in extracts from diseased but not healthy corn (Table 2). Conversely, precipitating antigen was isolated from *S. citri* organisms that reacted with antiserums to the corn stunt agent. There were antigens from normal corn which reacted with serums from both diseased and healthy corn. This obscured the homologous reaction between corn stunt antigen and antibody. There was low immunological reactivity between serums and antigens in all heterologous precipitin tests, whereas in the homologous *S. citri* combination, the reactivity was high. This suggests, but does not prove, that the two organisms may have a number of different antigenic determinants.

Most antibodies against mycoplasmas are directed against components of their membranes (*9*). The determinants may be membrane proteins, or may be formed in part by lipid haptens. Although some plant glycolipids and glucose-containing polysaccharides have antigenic determinants in common with several mycoplasmas (*Mycoplasma pneumoniae* and *M. mycoides* var. *mycoides*) (*10*), it is unlikely that the cross reactivity observed in our tests is associated with such common components from plant tissue. Antigens that precipitated with antiserums to *S. citri* were not extracted from normal corn.

Since the spiroplasmas have no cell wall and yet have a helical configuration, the structure of their limiting membrane may be more complex than that of simpler mycoplasmas. Whether such complexities might be reflected in their antigenic composition is unknown. Few attempts have been made to produce specific antibody to partially purified plant mycoplasma antigens and generally with only limited success (*11*). It now seems certain that difficulties experienced by many workers (*12*) were related to inadequate periods of immunization, small amounts of immunizing antigen, and, perhaps, especially to small amounts of antigen used to detect the presence of specific antibodies.

The serological relationship presented here and the other similarities among the two plant agents suggests the existence of a unique group of prokaryotes pathogenic for plants and insects.

J. G. TULLY
National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014
R. F. WHITCOMB
Plant Protection Institute, U.S. Department of Agriculture, Beltsville, Maryland 20705
J. M. BOVE
P. SAGLIO
Station de Physiologie et de Biochimie Végétales, Centre de Recherches de Bordeaux, Institut National de la Recherche Agronomique, 33 Pont-de-la-Maye, France

References and Notes

1. P. Saglio, D. Lafleche, C. Bonissol, J. M. Bove, *Physiol. Veg.* **9**, 569 (1971); P. Saglio, D. Lafleche, M. Lhospital, G. Dupont, J. M. Bove, in *Pathogenic Mycoplasmas*, K. Elliott and J. Birch, Eds. (Elsevier, Amsterdam, 1972), p. 187; A. Fudl-Allah, E. C. Calavan, E. C. K. Igwegbe, *Phytopathology* **62**, 729 (1972).
2. P. Saglio, M. Lhospital, D. Lafleche, G. Dupont, J. M. Bove, J. G. Tully, E. A. Freundt, *Int. J. Syst. Bacteriol.* **23**, 191 (1973).
3. R. M. Cole, J. G. Tully, T. J. Popkin, J. M. Bove, *J. Bacteriol.* **115**, 367 (1973).
4. R. E. Davis, R. F. Whitcomb, T. A. Chen, R. R. Granados, in *Pathogenic Mycoplasmas*, K. Elliott and J. Birch, Eds. (Elsevier, Amsterdam, 1972), p. 205; R. E. Davis, J. F. Worley, R. F. Whitcomb, T. Ishijima, R. L. Steere, *Science* **176**, 521 (1972).
5. R. R. Granados, in preparation.
6. T. A. Chen and R. R. Granados, *Science* **167**, 1633 (1970).
7. W. A. Clyde, Jr., *J. Immunol.* **92**, 958 (1964).
8. R. F. Whitcomb and L. M. Black, *Virology* **15**, 136 (1961).
9. S. Razin, I. Kahane, J. Kovartovsky, in *Pathogenic Mycoplasmas*, K. Elliott and J. Birch, Eds. (Elsevier, Amsterdam, 1972), p. 93.
10. P. Plackett, S. H. Buttery, G. S. Cottew, in *Recent Progress in Microbiology*, N. E. Gibbons, Ed. (Univ. of Toronto Press, Toronto, 1963), p. 535; P. Plackett, B. P. Marmion, E. J. Shaw, R. M. Lemcke, *Aust. J. Exp. Biol. Med. Sci.* **47**, 171 (1969); G. E. Kenny, *Ann. N.Y. Acad. Sci.*, in press.
11. J. Pozdena, *Cesk. Biol.* **3**, 391 (1954); ——— and M. Cech, in *Stolbur a Pribuzne Virusove Bezemenosti Rastlin*, E. Spaldon, C. Blattny, V. Bojnansky, Eds. (Slovak Acad. Sciences, Bratislava, 1958), p. 198; R. Gaborjanyi and M. S. Bencsics, *Acta Phytopathol.* **3**, 31 (1968); G. Marchoux, J. Giannotti, J-B. Quiot, J. Marou, C. Vago, *C.R. Acad. Sci. Agric. France* **55**, 191 (1969).
12. A number of workers, including R. F. Whitcomb, have attempted to prepare serums in this way, but all such work has remained unpublished because the results were negative.
13. We thank N. G. Ramsburg, J. E. Nolke, C. M. Blood, M. Coan, and J. Rosen for technical assistance.

15 June 1973; revised 18 July 1973

"Big" Growth Hormone Components from Human Plasma: Decreased Reactivity Demonstrated by Radioreceptor Assay

Abstract. *Plasma as well as pituitary immunoreactive human growth hormone (HGH) comprises at least two discrete components which have been designated as "big" HGH and "little" HGH. Using a newly developed radioreceptor assay, which depends on the ability of a substance to compete with labeled HGH for binding sites on cultured human lymphocytes, we find that the big HGH component from both normal and acromegalic subjects has much less activity in the radioreceptor assay than in the radioimmunoassay, whereas the little HGH component has similar activity in both assays.*

Many polypeptide hormones have been found to exist in glands and in plasma as multiple immunoreactive components which may have different biologic activities (*1–3*). For a few hormones, either biologic or radioreceptor assays are available which are applicable to studies of plasma, while in most instances these assays lack sufficient sensitivity or specificity to measure the small quantities of hormone present in plasma. Human growth hormone (HGH) is a good example of the latter situation.

We have recently developed a sensitive and specific radioreceptor assay (*4*) which is applicable to the measurement of total plasma immunoreactive HGH or to the discrete plasma or pituitary components which have been elucidated by Sephadex gel filtration (*1*, *2*).

The radioreceptor assay for HGH is based on the original observations of Lefkowitz *et al.* for the binding of labeled adrenocorticotropin (*5*) and of Lin and Goodfriend for the binding of labeled angiotensin (*6*) to specific receptors and is similar to the method, using cultured human lymphocytes (*7*), that has been employed to measure the

Table 1. Studies of radioreceptor versus radioimmunoassay potency of plasma and pituitary growth hormone components; RR, radioreceptor; RIA, radioimmunoassay.

Subject	Growth* hormone (ng/ml)	(RR/RIA) × 100† "Little"	(RR/RIA) × 100† "Big"	Comment
		Plasma		
Acromegalic				
Bog	75	104	20	Basal
Dic	106	137	12	Basal
McD	720	130	22	Basal (jugular vein)
Obr	345	91	< 1	Basal
Bis	25	77		Basal
McC	24	69		Basal
Pool‡		135	18	
Normal				
Bog	34	61		Insulin hypoglycemia
Har	26	50		Insulin hypoglycemia
Han	30	78		Arginine stimulation
Han	24	72		Insulin hypoglycemia
Ham	23	77		Arginine stimulation
Ham	24	64		Insulin hypoglycemia
Dam	39	72		Insulin hypoglycemia
Pool No. 1§			< 10	
Pool No. 2¶		120	13	
		Pituitary		
Preparation No. 1		71	20	Partially purified pituitary extract with biological activity of 0.2 I.U./mg

* Determined by radioimmunoassay of dilute plasma. † Determined as shown in Fig. 1. ‡ Represents material combined from several of the above acromegalic patients for each of the components. § Represents material combined from several of the above normal subjects. ¶ Same as pool No. 1 except from different set of gel filtrations.