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.

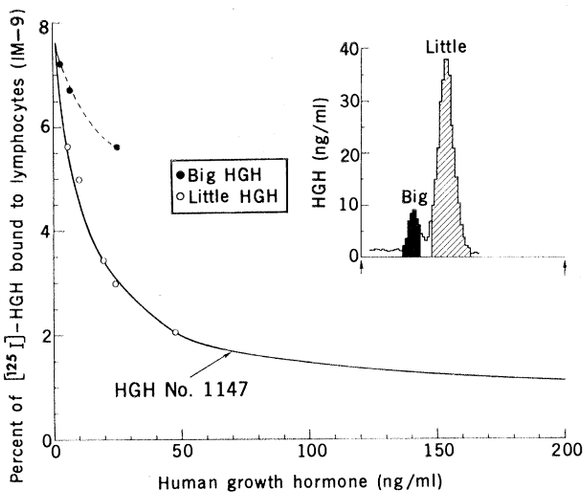

Fig. 1. Radioreceptor assay for plasma HGH components of acromegalic patient (Bog) (Table 1). Five milliliters of plasma were enriched with a tracer of [^{125}I]gamma globulin and ^{125}I and filtered over a column (1.5 × 90 cm) of Sephadex G-100 that was equilibrated and eluted with $(NH_4)_2CO_3$ (0.05M, pH 8.6). The HGH immunoreactivity was measured in 10- to 75-μl aliquots from each fraction eluted between [^{125}I]gamma globulin (left arrow on inset) and ^{125}I (right arrow on inset) [see (*1*)]. The fractions represented by the black area of the gel filtration pattern were pooled and designated as "big" HGH, and the fractions represented by the hatched area were pooled and designated as "little" HGH. The "big" and "little" components were lyophilized and reconstituted in the diluent for the radioreceptor assay; aliquots were simultaneously taken for measurement in the radioreceptor assay and radioimmunoassay. Eight million lymphocytes per milliliter from the cell line IM-9 were incubated with a tracer of [^{125}I]HGH with varying concentrations of the highly purified HGH standard No. 1147 (this same standard, which contains 5 percent big HGH, was used for the radioimmunoassay) or varying concentrations of the big and little plasma components as measured by radioimmunoassay (*4*). The solid line represents the inhibition of [^{125}I]-HGH binding by the HGH standard No. 1147; the open circles, the inhibition of binding by the little HGH plasma component; and the closed circles, joined by the dashed line, inhibition by the big HGH plasma component. The concentration of the components from the radioreceptor assay (determined from the average of all the points that produced inhibition of labeled hormone binding) and the concentration determined by radioimmunoassay were used to calculate the data shown in Table 1.

radioreceptor activity of pancreatic and plasma insulin components. The assay is sensitive to ambient basal plasma concentrations of HGH and is specific for HGH or closely related analogs such as human placental lactogen. Growth hormones from nonprimate species have no significant reactivity, nor do other pituitary polypeptide hormones. Furthermore, the ability of a given HGH preparation to compete for binding is a function of the biologic activity of the preparation, as measured in a standard rat bioassay. For many preparations the radioreceptor activity is distinctly different from the radioimmunoactivity (*4*). In this study we demonstrate that the immunoreactive HGH components present both in plasma and in the pituitary gland have very different activity as measured in the radioreceptor assay (*8*).

When plasma or pituitary extracts were filtered on Sephadex G-100 (*1*) or G-75 (*2*), two discrete immunoreactive components were identified. One component eluted from the gel as a globular protein of 22,000 molecular weight ("little" HGH) while the second component ("big" HGH) was less retarded by the gel and of presumably twice the molecular weight (Fig. 1). In normal subjects, whose growth hormone secretion was stimulated by insulin hypoglycemia or arginine infusions, big HGH comprised from 24 to 37 percent of the total circulating immunoreactive material, and in acromegalic patients big HGH comprised from 8 to 14 percent of the basal HGH (*1*).

To estimate both the radioreceptor (RR) and radioimmunoassay (RIA) potency of the Sephadex components, the column fractions comprising little HGH and big HGH were separately pooled and concentrated by lyophilization. Each component was then reconstituted in buffer, and aliquots from each were simultaneously taken for radioreceptor and radioimmunoassay. In normal subjects the (RR/RIA) × 100 of the little HGH plasma component was 50 to 78 percent; preparations from acromegalic subjects demonstrated a (RR/RIA) × 100 of 69 to 137 percent (Fig. 1 and Table 1). In marked contrast, the (RR/RIA) × 100 for the big HGH component from both normal subjects or acromegalic patients was 22 percent or less. When a pituitary extract was prepared in a fashion identical to that for the plasma components, the ratio of radioreceptor to radioimmunoactivity was, similarly, much lower for the big HGH component (Table 1). Thus, under all conditions studied, the big HGH component showed much less activity in the lymphocyte receptor assay than in the radioimmunoassay. Since after its isolation there is some conversion of big to little HGH with storage (*1*), it is possible that all of the radioreceptor activity of big HGH that we detected was due to converted material and that big HGH has essentially no receptor reactivity.

The nature of big HGH is not yet clear. The fact that it is present in both pituitary and plasma, coupled with the failure of little HGH to form big HGH in vitro, suggests that both components are secreted and that both circulate in plasma. Although it is not known whether big HGH is a precursor of little HGH, the analogy to the insulin system is striking. Both pancreatic proinsulin and the proinsulin-like components from plasma have much lower biologic activity than pancreatic insulin or the insulin component of plasma. This is reflected in an identical fashion by an in vitro bioassay with fat cells and in the radioreceptor assay in cultured human lymphocytes (*7, 9*).

The radioreceptor assay, by ascertaining the ability of a substance to compete with the labeled hormone for specific binding sites, measures the affinity of the substance for the receptor. This reactivity in the lymphocyte radioreceptor assay for growth hormone correlates closely with in vivo biologic activity (*4*). This type of correlation must be shown experimentally for each system, since it is possible for a molecule to compete strongly for receptor binding and have no biologic activity. For instance, when a molecule acts as a competitive inhibitor of binding, there may be potent radioreceptor activity in preparations that are totally inert in a standard biologic assay. In other instances the radioreceptor activity may correlate better with in vitro than in vivo biologic activity. Proinsulin has a similar ratio of activity to insulin in the lymphocyte radioreceptor and fat cell bioassay. The ratio of proinsulin to insulin activity in vivo, however, is higher, owing to the longer half-life of proinsulin. These factors must be considered in any attempt to translate radioreceptor activity to biologic activity.

When the specific characteristics of the radioreceptor technique are considered, the method becomes a powerful tool for study of polypeptide hormones because of its simplicity, sensi-

tivity, and specificity. The application of radioreceptor assay makes clear, for the first time, that immunoreactive HGH comprises components that have different capacities to displace labeled HGH from specific binding sites on viable cells. It can now be determined whether these factors are important in pathophysiologic states of growth hormone action.

P. GORDEN
M. A. LESNIAK
C. M. HENDRICKS
J. ROTH

National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

References and Notes

1. P. Gorden, C. M. Hendricks, J. Roth, *J. Clin. Endocrinol. Metab.* **36**, 178 (1973).
2. A. D. Goodman, R. Tannenbaum, D. Rabinowitz, *ibid.* **35**, 868 (1972).
3. S. A. Berson and R. S. Yalow, in *Proceedings of the 11th Reunion of French Speaking Endocrinologists* (Masson, Paris, 1971), p. 239; R. M. Bala, K. A. Ferguson, J. C. Beck, *Endocrinology* **87**, 506 (197→ J. Roth, P. Gorden, I. Pastan, *Proc. Nat. Acad. Sci. U.S.A.* **61**, 138 (1968); A. H. Rubenstein, S. Cho, D. F. Steiner, *Lancet* **1968-I**, 1353 (1968); R. S. Yalow and S. A. Berson, *Gastroenterology* **58**, 609 (1970); ———, *Biochem. Biophys. Res. Commun.* **44**, 439 (1971); S. A. Berson and R. S. Yalow, *J. Clin. Endocrinol. Metab.* **28**, 1037 (1968); L. M. Sherwood, W. B. Lundberg, Jr., J. H. Targovnik, J. S. Rodman, A. Seyber, *Am. J. Med.* **50**, 658 (1971); J. F. Habener, G. V. Segre, D. Powell, P. Dee, J. T. Potts, *J. Clin. Invest.* **51**, No. 6, abstr. 131 (1972).
4. M. A. Lesniak, J. Roth, P. Gorden, J. R. Gavin, III, *Nat. New Biol.* **241**, 20 (1973); M. A. Lesniak, P. Gorden, J. Roth, J. R. Gavin, III, *J. Biol. Chem.*, in press.
5. R. J. Lefkowitz, J. Roth, I. Pastan, *Science* **170**, 633 (197→ R. J. Lefkowitz, J. Roth, W. Pricer, I. Pastan, *Proc. Nat. Acad. Sci. U.S.A.* **65**, 745 (1970).
6. S. Y. Lin and T. L. Goodfriend, *Am. J. Physiol.* **218**, 1319 (1970).
7. J. R. Gavin, III, P. Gorden, J. Roth, J. A. Archer, D. N. Buell, *J. Biol. Chem.* **248**, 2202 (1973).
8. P. Gorden, M. A. Lesniak, C. M. Hendricks, J. Roth, *Clin. Res.* **21**, 733 (abstr.) (1973).
9. P. Gorden, J. R. Gavin, III, C. R. Kahn, J. A. Archer, M. Lesniak, C. Hendricks, D. M. Neville, Jr., J. Roth, *Pharmacol. Rev.* **25**, 179 (1973).
10. We are grateful to Dr. A. E. Wilhelmi for the growth hormone preparations and to Dr. D. M. Buell for the cultured human lymphocyte cell line.

10 August 1973

Dopamine Synthesis: Stimulation by a Hypothalamic Factor

Abstract. *The effect of treatment with the factor that inhibits the release of melanocyte stimulating hormone (MSH) identified as 1-prolyl-1-leucylglycinamide (MIF) on brain catecholamine synthesis was examined in normal and hypophysectomized rats. The tripeptide induced a dose-related increase in striatal dopamine synthesis in slices obtained from treated normal animals but not in hypophysectomized animals. Hypothalamic norepinephrine synthesis was unaltered by MIF treatment in normal as well as in hypophysectomized rats. In addition, dopamine and norepinephrine syntheses were depressed in untreated hypophysectomized animals, as compared to normal controls. These results constitute the first direct demonstration of a central neurochemical effect of a hypothalamic factor.*

The physiological role of melanocyte stimulating hormone (MSH) in mammals has not been established. Some extrapigmentary functions have been suggested (*1*, *2*); the hormone produces anxiety, motor restlessness and alterations in electroencephographs in humans (*2*), and exacerbation of symptomatology in patients suffering from Parkinsonism (*3*).

Release of MSH from the pars intermedia of the pituitary is controlled by hypothalamic releasing and release-inhibiting factors (*4*). A possible inhibiting factor of MSH release (MIF) has been isolated from mammalian hypothalamus and identified as the tripeptide, 1-prolyl-l-leucylglycinamide (*5*).

Recent pharmacological evidence has suggested a direct central role for MIF. Plotnikoff and his co-workers have shown that prior treatment with MIF potentiates the behavioral effects of L-dopa (*6*), antagonizes the central and peripheral effects induced by oxotremorine (*7*), and reverses the sedative effects of reserpine in mice and monkeys (*8*). These actions of 1-prolyl-1-leucylglycinamide suggest a possible anti-Parkinsonian or antidepressant effect through an interaction with brain dopaminergic neuronal systems. Accordingly, we studied the effect of MIF on brain dopamine metabolism.

Table 1. Concentrations of endogenous striatal dopamine and hypothalamic norepinephrine in rats treated with various single and repeated doses of MIF. Rats were killed 1.5 hours after the last injection of MIF. Each value is the mean of eight brain areas ± the standard error of the mean.

MIF (mg/kg)	Striatal dopamine (μg/g)	Hypothalamic norepinephrine (μg/g)
Saline only	4.23 ± .05	1.84 ± .02
0.5	4.12 ± .04	1.81 ± .02
1.0	4.09 ± .03	1.69 ± .03
5.0	4.21 ± .04	1.78 ± .02
4 days of 4.0	5.29 × .05*	1.73 ± .03
4 days of 1.0	4.94 × .02*	1.83 ± .03

* $P < .01$.

Normal or hypophysectomized male Sprague-Dawley rats (2 to 4 weeks after operation) housed under a regime of 12 hours of light and 12 hours of darkness (LD 12 : 12) given chow and water (normal animals) or 5.0 percent dextrose (hypophysectiomized animals) were used. Synthetic MIF (Abbott-40509) was dissolved in saline and administered intraperitoneally. Control rats were injected with an equal volume of saline. All injections were made between 2 and 4 hours after the beginning of the light cycle; the rats were killed 1.5 hours after receiving the injections. The brains were quickly removed and rinsed in ice-cold Krebs-Henseleit physiological solution; the hypothalamus and striatum were dissected out for determination of endogenous tyrosine, dopamine, and norepinephrine. The areas to be analyzed were weighed and homogenized in 0.4*N* perchloric acid, and the acid extract was poured over columns containing Dowex 50 × 4 (K^+ form). Tyrosine, dopamine, and norepinephrine were subsequently eluted with *p*H 4.5 buffer, 0.4*N* HCl, and 4*N* HCl, respectively, according to the method of Neff *et al.* (*9*). Eluates were further purified by either passing through the alumina (tyrosine) or by being adsorbed onto the alumina at *p*H 8.4, with subsequent elution (of norepinephrine and dopamine) with 0.2*N* HCl. Endogenous norepinephrine and dopamine were assayed fluorimetrically according to the methods of Anton and Sayre (*10*) and Laverty and Taylor (*11*), respectively.

Catecholamine synthesis was studied in hypothalamic and striatal slices from rats treated with MIF and saline. Brain slices were obtained with the use of a mechanical McIlwain tissue chopper (set on 0.4 mm) and placed in 25-ml flasks containing 2 ml of oxygenated, cold Krebs-Henseleit solution. Incubations were carried out in a metabolic shaker at 37°C in an atmosphere consisting of 95 percent O_2 and 5 percent CO_2. After an initial incubation period of 10 minutes 50 μl of [3,5-^{3}H]tyrosine was added to each sample to give a final concentration of $8.15 \times 10^{-6} M$ tyrosine and the incubation was continued for an additional 45 minutes. Tissue and media were rapidly separated by filtration. The tissue was washed twice with 2 ml of cold physiological solution, and the washings were added to the media. The tissue was homogenized in 0.4*N* perchloric acid. The media samples were adjusted to a final concentration of 0.4*N*