Table 1. *Amino acid compositions of human growth hormone and human placental lactogen*

	Composition (g./100g. of total amino acid residues)				
	HGH		HPL		
Amino acid	Li & Liu (1964)	C5–G-100*	Prep. 1†	Prep. 2‡	Prep. 3†
Lys	4·97	6·39	5·30	5·67	5·38
His	1·67	1·98	4·31	4·35	4·50
Arg	7·12	7·52	7·58	7·32	7·78
Asp	10·88	10·89	12·21	11·70	11·78
Thr	4·84	4·21	5·54	4·67§	4·62
Ser	7·23	6·60	6·81	5·29§	6·20
Glu	16·23	14·75	15·21	15·68	14·66
Pro	3·93	3·91	2·44	2·56	2·39
Gly	2·46	2·47	2·05	2·11	1·94
Ala	2·50	2·70	2·36	2·30	2·12
CyS	1·80	0·60	0·93	1·23	0·92
Val	3·09	3·60	3·30	3·60	3·25
Met	1·83	1·78	2·71	3·29	3·29
Ile	3·34	3·81	3·26	3·66	3·48
Leu	12·15	13·23	12·31	13·37	13·05
Tyr	5·47	6·86	5·87	5·82§	6·01
Phe	8·15	8·38	7·81	7·38	7·60
Trp‖					1·03

* Prepared by a modification of the method of Raben (1957), followed by gel filtration.

† Hydrolysed in 6N-HCl for 48hr.; the results given for preparation 3 are mean values of two determinations.

‡ Mean values of three hydrolysis times (24, 48 and 64hr.) in 6N-HCl at 110°.

§ Values of threonine, serine and tyrosine extrapolated by the method of Hirs, Stein & Moore (1954).

‖ Tryptophan estimated only for HPL preparation 3.

42300. These values were based on an apparent specific volume of 0·74 calculated from the amino acid composition of the protein together with the specific volumes of the known residues.

The yield obtained by quantitative *N*-terminal analysis by the Edman procedure was 1mole of valine/21500g. of protein, after correction for moisture content. This value, taken with the molecular-weight estimations based on the physical properties of the molecule in solution, indicates that HPL may consist of a dimer. The possibility that this dimer is composed of two HGH-like molecules is suggested by the similarity between the amino acid compositions of HPL and HGH, reported by Friesen (1965*a*) and confirmed in this study (Table 1). The development of a method for obtaining large amounts of an HPL preparation that is homogeneous by several physical and chemical criteria, as outlined in the present communication, should make possible a closer structural comparison with HGH.

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The Use of Photolysis of Dinitrophenyl-peptides in Structural Studies on the Cell-Wall Mucopeptide of *Corynebacterium poinsettiae*

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The photochemical behaviour of DNP-amino acids and peptides was studied by Russell (1963*a*). He found that when the DNP-derivatives of most α-amino acids were dissolved in 1% (w/v) $NaHCO_3$ and exposed to light, the link between the amino group and the aliphatic chain was cleaved to yield NNA†, carbon dioxide and the aldehyde produced

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† Abbreviation: NNA, 4-nitro-2-nitrosoaniline.

from the amino acid by conversion of C-2 into -CHO (Russell, 1963*b*). If the DNP derivative of an amino acid was stable to light, then the DNP group was almost certainly not located on an amino group in the α-position relative to a free carboxyl group. On the other hand, photolability indicated the presence of the structure DNP-NH·CRR′·CO_2H, and NNA was often a product.

During work on the structure of cell-wall mucopeptides, peptides were isolated from partial acid hydrolysates that contained ornithine and glutamic acid. The photolability of the DNP-peptides has proved useful in establishing their structure. The feasibility of this reaction for investigation of peptide structure was examined with model compounds. Under the conditions described below the DNP derivative of α-glutamyl-L-alanine was completely stable to light, whereas DNP-alanine or DNP-ophthalmic acid (DNP-L-γ-glutamyl-L-α-amino-*n*-butyrylglycine) was rapidly decomposed to yield NNA. The photolysed DNP-ophthalmic acid contained α-aminobutyric acid and glycine, but no glutamic acid. Hence the photochemical degradation successfully distinguished between α-linked and γ-linked *N*-terminal glutamic acid residues. By a similar argument DNP-peptides with a *C*-terminal ornithine residue should be photostable when the α-amino group of the ornithine bears the aminoacyl substituent, and photolabile when substitution is on the δ-amino group (Russell, 1963*a*).

The cell-wall mucopeptide of *Corynebacterium poinsettiae* (N.C.P.P. 177) contains approximately equimolecular proportions of glutamic acid, glycine, alanine, homoserine and ornithine (Perkins, 1965), whereas the UDP-*N*-acetylmuramyl-peptide, accumulated in the presence of vancomycin or penicillin, contains one more mole of alanine and no ornithine (Chatterjee & Perkins, 1966). The amino sugar nucleotide was heated at 100°, first in 0·01N-HCl for 5min. to remove UDP, and then in Na_2CO_3 solution at pH 10·8 for 4min. to eliminate the lactyl-peptide side chain from C-3. The product was dried and incubated at 37° for 4 days in acetic acid–conc. HCl (1:1, v/v). The acid was removed *in vacuo*, peptides with free amino groups were adsorbed on a column of cation-exchange resin Zeo-Karb 225 (H^+ form) and eluted with 3N-ammonia. The peptides were then separated by chromatography on washed paper in butan-1-ol–acetic acid–water (63:10:23, by vol.), eluted and subjected to electrophoresis in 0·25N-formic acid for 3hr. at 9v/cm. Isolated peptides were hydrolysed to their constituent amino acids with 6N-HCl at 105° for 18hr. or were converted into their DNP derivatives by reaction with fluorodinitrobenzene in alkaline solution.

Peptides were also isolated from the cell walls of *C. poinsettiae*. Samples were incubated with lysozyme (100μg./ml.) in 0·1M-ammonium acetate buffer, pH 6·5, at 37° for 24hr. The soluble product was heated at 100° in Na_2CO_3 solution, pH 10·8, for 15min. and peptides were then isolated as described above.

The ether-soluble DNP-amino acids derived from the DNP-peptides by acid hydrolysis were characterized by thin-layer chromatography on Kieselgel in chloroform–methanol–acetic acid (95:5:1, by vol.), and the water-soluble DNP-amino acids by paper chromatography in 1·5M-phosphate buffer (Levy, 1954). The two mono-DNP derivatives of ornithine, prepared by the method of Sanger (1946), had R_F values as follows: α-DNP-ornithine, 0·52; δ-DNP-ornithine, 0·44. NNA was characterized by its absorption spectrum and by chromatography in 2-methylpropan-2-ol–4·25N-ammonia (4:1, v/v) (Russell, 1963*a*).

To study the action of light, DNP-peptides (0·1–0·3μmole) were dissolved in 1% (w/v) $NaHCO_3$ (1·8ml.) and placed in a water-jacketed tube (7mm. diam.) 20cm. from a 400w mercury discharge lamp (General Electric Co.). The extinctions at 348 and 284mμ were read at 5min. intervals for 15min., after which time no further changes occurred. Photolability was indicated by a rapid rise in E_{284} and a corresponding fall in E_{348}, representing the conversion of the chromophore of a DNP-amino acid into NNA. Any NNA produced was extracted from the alkaline solution with ethyl acetate. Unchanged DNP-peptide or degraded DNP-peptide was then extracted with ethyl acetate from the acidified solution and hydrolysed in acid. The amino acid or DNP-amino acid components were identified by chromatography.

The peptides derived from the cell walls and amino sugar nucleotides of *C. poinsettiae* and the effects of light on their DNP-derivatives are shown in Table 1. To show whether the peptide chain in the amino sugar nucleotide was straight or branched, it was hydrazinolysed by the method of Schocher, Jusic & Watson (1961). Alanine was the only *C*-terminal amino acid, so that the chain must have been straight. The lactic acid residue in peptide A presumably arose from muramic acid by elimination at C-3. The results are therefore consistent with a peptide chain of muramylglycyl-γ-glutamylhomoserylalanylalanine.

The products from the walls were more complicated. On hydrolysis, peptide E gave equimolar amounts of glutamic acid and ornithine, and the DNP-peptide gave only glutamic acid and α-DNP-ornithine. No simple peptide of glutamic acid and ornithine could give this result. Since the δ-amino group of ornithine yielded no DNP derivative, it must have been substituted by glutamic acid. The

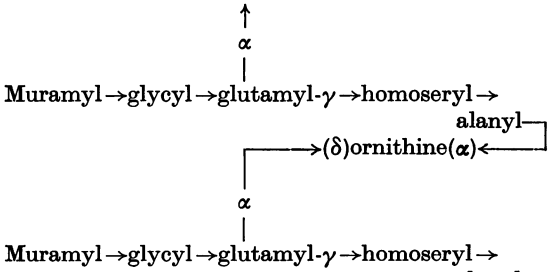
Table 1. *Peptides isolated from the cell walls and amino sugar nucleotides derived from* C. poinsettiae

Source	Peptide	Components of fluorodinitrobenzene-treated peptide	Components in photolysed DNP-peptide	Proposed structure
Amino sugar nucleotide	A*	Lactic acid, Gly, Glu	—	Lactyl-Gly-Glu
	B	Ala, DNP-Ala†	Not done	Ala-Ala
	C	Glu, DNP-Gly	Not done	Gly-Glu
	D	Homoserine, DNP-Glu	NNA, homoserine	γ-Glu-homoserine
Cell wall	E	Glu, α-DNP-Orn	NNA, Glu	N^5-(Pyrrolid-2-one-5-carboxyl)-Orn
	F	Orn, DNP-Glu, DNP-Ala	Unchanged	N^2-(Ala)-N^5(α-Glu)-Orn
	G‡ (derived from F)	DNP-Ala, δ-DNP-Orn	Unchanged	N^2-(DNP-Ala)-Orn
	H‡ (derived from F)	DNP-Glu, α-DNP-Orn	NNA, DNP-Glu	N^5-(DNP-α-Glu)-Orn

* Not adsorbed by cation-exchange resin. Lactic acid was shown by a colour reaction with H_2SO_4 and *p*-hydroxybiphenyl.

† One molar proportion of each was found.

‡ The DNP derivative of peptide F was hydrolysed in 4 N-HCl at 100° for 30 min. After extraction with ethyl acetate the aqueous layer was stored, and the organic phase was dried and rehydrolysed. The whole procedure was repeated. The combined aqueous layers were subjected to electrophoresis on paper at pH 7 in collidine–acetate buffer. The yellow material separated into two bands, one neutral (G) and one anionic (H).



Scheme 1.

carboxyl group of the ornithine must have been free since the DNP derivative was photolabile. It seems likely that peptide E contained a cyclized *N*-terminal glutamic acid residue, no doubt produced during acid hydrolysis of a γ-carboxyl group in amide linkage (Sanger, Thompson & Kitai, 1955). Under the hydrolytic conditions used in the present work, glutamine gave a considerable yield of pyrrolid-2-one-5-carboxylic acid. Presumably peptide E arose by the same mechanism, the glutamic acid γ-carboxyl group originally having been bound in peptide linkage to homoserine (see peptide D). The peptides E–H suggest that ornithine forms the cross-link from one chain to another, being joined by its δ-amino group to the glutamic acid α-carboxyl group of one chain and by its α-amino group to the alanine residue of another, as shown in Scheme 1. Since the nucleotide precursor contains two alanine residues and the wall unit only one, presumably the terminal alanine is lost during the second stage of cross-linking, as proposed for other bacteria (Wise & Park, 1965; Tipper & Strominger, 1965). Four types of cross-linkage fron the ε-amino group of a diamino acid in one chain to the terminal D-alanine of another have been described: bridging by tri-L-alanine, tri-L-alanyl-L-threonine or pentaglycine and a direct link with no additional amino acid (Petit, Muñoz & Ghuysen, 1966; Weidel & Pelzer, 1964; Araki, Shirai, Shimada, Ishimoto & Ito, 1966). The link now described, involving the glutamic acid α-carboxyl group and a cross-linking ornithine residue, is very different, the bond to a *C*-terminal alanine residue of the second chain being the only common feature.

The walls of *C. poinsettiae* contain a few free amino groups. These yield α-DNP-ornithine. The DNP derivative of the cell walls was photolabile, so that these ornithine residues must have been linked only by their δ-amino groups, both the α-amino group and the carboxyl group being free.

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Activation of Liver Pyruvate Kinase by Fructose 1,6-Diphosphate

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It has been reported by Hess, Haeckel & Brand (1966) that yeast pyruvate kinase (ATP–pyruvate phosphotransferase, EC 2.7.1.40) is stimulated by FDP.* This is apparently not a general property of pyruvate kinases, since in tests on extracts from *Lactobacillus fermentii* and several animal tissues stimulation by FDP was detected only with the enzyme from bovine heart muscle. Stimulation of rat-liver pyruvate kinase by very low concentrations of FDP is now reported, the pattern of the kinetics of stimulation supporting the view that this is an allosteric effect.

Preparation of enzyme extract. Rat liver was homogenized with 5vol. of cold distilled water in a Waring Blendor and the homogenate centrifuged at 15000*g* for 1hr. (MSE 18 centrifuge, 6 × 250 head, 12000 rev./min.). The protein of the supernatant fluid was fractionated by differential ammonium sulphate precipitation in the cold. The fraction precipitated by between 30% and 40% saturation with ammonium sulphate, which contained 50% of the total liver pyruvate-kinase activity, was redissolved in a small volume of water and stored at 4°. Crude extracts prepared in this way had a specific activity of about 1unit/mg. of protein and were used without further purification (1unit is defined as 1μmole of substrate utilized/min. at 25°).

Assay. Pyruvate kinase was assayed by the method of Bücher & Pfleiderer (1955). The reaction is coupled with lactate dehydrogenase to give oxidation of $NADH_2$, which is followed by measuring the change in E_{340}. Except where modified for a particular purpose the final reaction mixture contained: triethanolamine buffer, pH7·5, 40mM; KCl, 70mM; $NADH_2$, 0·15mM; ADP, 1·0mM; $MgSO_4$, 8·0mM; lactate dehydrogenase [rabbit-muscle enzyme; Boehringer Corporation (London) Ltd., London, W.5], 10units. The reaction, carried out at 25°, was started with PEP.

Results. The factors governing stimulation of pyruvate kinase by FDP are complex, the stimulated/unstimulated activity ratio, which may be as high as 40, depending not only on the FDP concentration but also on PEP concentration and pH. The interrelationship between FDP and PEP is best illustrated by the data showing the influence of each of these on the velocity–concentration curve for the other. In the absence of FDP the curve for initial velocity against PEP concentration is sigmoidal (Fig. 1), the plot of $1/v$ against $1/s$ curves upwards, the Hill plot gives a maximum slope of approximately 2 and half-maximal activity is attained at 0·6mM-PEP. In the presence of high concentrations of FDP (0·5mM), the response to PEP concentration is transformed to give a Michaelis–Menten curve. The reciprocal plot is now linear, the Hill plot has a slope of 1 and the half-maximal concentration of PEP is 60μM, i.e. tenfold lower than in the absence of FDP.

The data for the converse relationship (varying FDP concentration at fixed concentrations of PEP) are less reliable, since by reason of the saturation characteristics of the system the most informative portions of the curves occur at low reaction rates and very low concentrations of FDP. Under these conditions measurements of reaction rate are imprecise and the presence of small quantities of

* Abbreviations: FDP, fructose 1,6-diphosphate; PEP, phosphoenolpyruvate.