after labelling has also been observed by Lissitzky, Roques, Torresani & Simon (1965). The maturation of newly synthesized thyroglobulin into a more-stable compact molecular configuration may be a function of iodine content. Nunez *et al.* (1965) have shown that chemical iodination of amino acid-labelled thyroglobulin derived from sheep-thyroid slices results in an increase in sedimentation coefficient from 17s to 19s. Seed & Goldberg (1965*b*) incubated rat-thyroid slices for periods as long as 5hr. During this time-interval labelled amino acids accumulated in 16s, 12s and smaller proteins, but labelled 19s thyroglobulin was not formed. These authors postulated a block in maturation peculiar to the system *in vitro*. An alternative explanation for the findings of Seed & Goldberg (1965*b*) may be that the periods of incubation were too short to obtain labelling in 19s thyroglobulin.

The question whether 3–8s proteins contain one or more precursors of 12s sub-units remains unanswered. The relative specific activity of proteins in the 3–8s fractions did not change within the time-interval of the present study. It is likely that the 3–8s proteins are heterogeneous with regard to turnover. A rapidly labelled precursor might be only one component of a group of proteins and thus escape detection by the present methods.

The possibility of contamination of thyroid extracts by plasma proteins labelled *in vivo* was examined. Sedimentation analyses of plasma

collected at intervals between 2 and 20hr. after injection of [^{14}C]leucine showed more than 96% of radioactive protein in the 3–8s fractions. From the content of ^{14}C in thyroids from rats given intravenous injections of ^{14}C -labelled plasma proteins it was estimated that contamination could account for 20–30% of the labelled 3–8s proteins and only 1% of the 12–19s proteins in the thyroid extracts.

In summary, the results of the present study are consistent with the following scheme of thyroglobulin biosynthesis in the rat: (a) formation of a 12s sub-unit, possibly from smaller polypeptide sub-units; (b) aggregation of two 12s sub-units into a 16s molecule; (c) maturation of 16s protein into 19s thyroglobulin.

This work was supported in part by Grant AM08410 from the U.S. Public Health Service. The expert technical assistance of Miss Shirley Craig is gratefully acknowledged.

Edelhoc, H. (1960). *J. biol. Chem.* **235**, 1326.

Lissitzky, S., Roques, M., Torresani, J. & Simon C. (1965). *Bull. Soc. Chim. biol., Paris*, **47**, 1999.

Nunez, J., Mauchamp, J., Macchia, V. & Roche, J. (1965). *Biochim. biophys. Acta*, **107**, 247.

Seed, R. W. & Goldberg, I. H. (1963). *Proc. nat. Acad. Sci., Wash.*, **50**, 275.

Seed, R. W. & Goldberg, I. H. (1965*a*). *J. biol. Chem.* **240**, 764.

Seed, R. W. & Goldberg, I. H. (1965*b*). *Science*, **149**, 1380.

Biochem. J. (1967) **102**, 270

Purification and Physicochemical Properties of Human Placental Lactogen

By K. J. CATT, BARBARA MOFFAT, H. D. NIALl and B. N. PRESTON

Departments of Medicine and Biochemistry, Monash University, Melbourne, Victoria, Australia

(Received 21 November 1966)

Several procedures have been reported for the preparation of HPL,* a placental hormone that possesses certain biological and immunological features in common with HGH. Typical of these preparative methods are those of Josimovich & MacLaren (1962), Cohen, Grumbach & Kaplan (1964) and Friesen (1965*a,b*). Recently Turtle, Beck & Daughaday (1966) have described a modification of the procedure of Cohen *et al.* (1964) to give a more purified preparation.

* Abbreviations: HPL, human placental lactogen; HGH, human growth hormone.

To enable structural studies of HPL to be performed, the purification of this protein from human placental tissue was carried out by a modification of the method of Friesen (1965*b*). The preparation of this material in considerable quantity was facilitated by the use of a side fraction obtained during the preparation of human serum albumin from term placentas by Commonwealth Serum Laboratories, Melbourne. Immunodiffusion studies of all fractions of this process against antiserum to HGH revealed that a considerable quantity of immunoreactive material was present in fraction

II + III, a globulin-rich precipitate obtained early in the procedure. The precipitin line obtained with this fraction showed a reaction of identity with the line formed between antiserum to HGH and pregnancy serum, and showed partial immunological identity with purified HGH.

To prepare fraction II + III, minced placenta was extracted for 2 hr. at 0° with 1 l. of 0.9% NaCl/kg. of placenta. The saline extract was adjusted to 25% (v/v) ethanol at pH 6.6–6.9, stirred for 30 min. and then centrifuged to give fraction II + III as a precipitated paste.

Then 50 kg. of this paste, equivalent to 875 kg. of fresh placenta, was extracted overnight with 200 l. of 0.1 M-NH₄HCO₃. This and all succeeding steps were performed at 0–5°. The supernatant solution was stirred overnight with DEAE-cellulose (5 kg. dry wt.) that had been equilibrated with the same buffer. DEAE-cellulose was washed twice with 200 l. of 0.1 M-NH₄HCO₃, then eluted once overnight, and again for 8 hr., with 75 l. of 0.4 M-NH₄HCO₃. The yield of protein obtained by freeze-drying the combined eluates was 453 g., the expected yield based on laboratory-scale extractions being about 500 g. Disk electrophoresis of extracts of this red powder showed a prominent band, more anodal than albumin, and small quantities of serum proteins and haemoglobin. Combined polyacrylamide-disk electrophoresis and agar-gel diffusion against antiserum to HGH showed this band to be the immunoreactive component of fraction II + III.

Further purification of the HPL was performed by gel filtration and ion-exchange chromatography as follows. Samples (10 g.) of powder were extracted for 2 hr. with 90 ml. of 0.1 M-NH₄HCO₃ buffer, pH 9.0. The small residue was discarded, and the supernatant applied to a column (100 cm. × 8 cm.) of Sephadex G-75 equilibrated with 0.1 M-NH₄HCO₃ buffer, pH 8.5. The u.v. absorption of the eluate showed a single large peak with a trailing shoulder. Disk electrophoresis and immunodiffusion against antiserum to HGH showed that the HPL was eluted on the shoulder of this peak, immediately after albumin. The relevant fractions were pooled, dialysed and freeze-dried, to yield 1400 mg. of protein. This material was then redissolved in 0.1 M-NH₄HCO₃ and dialysed for 24 hr. against several changes of 0.01 M-sodium-potassium phosphate buffer, pH 5.5. The precipitate that formed was separated by centrifugation and saved, and the supernatant was applied to a CM-cellulose column (50 cm. × 2.5 cm.) that had been equilibrated with the same phosphate buffer. The column was developed with a linear gradient from 0.01 M-phosphate buffer, pH 5.5, to 0.2 M-phosphate buffer pH 6.0. A front peak of non-immunoreactive material was followed by a sharp peak rich in HPL,

which was pooled, dialysed and freeze-dried. This material was redissolved and applied to a column (85 cm. × 2.5 cm.) of Sephadex G-100 in 0.1 M-NH₄HCO₃ buffer, pH 8.5, whence it was eluted as a single, almost symmetrical, peak. The front part of this peak, which contained traces of a haem-containing component, was discarded, and the rest pooled and freeze-dried to yield 200 mg. of purified placental lactogen. The precipitate obtained at pH 5.5 was also redissolved, dialysed and freeze-dried and applied in 200 mg. portions to the same Sephadex G-100 column. The elution pattern revealed a small front peak and two adjacent retarded peaks, the first of which contained the red haem-containing component, and the second the HPL component. The HPL-containing fractions when pooled and freeze-dried yielded a total of 800 mg.

The HPL fractions obtained from the pH 5.5 residue and the supernatant were identical on disk electrophoresis, and gave a single precipitin line on immunodiffusion against antiserum to HGH. They were also free of albumin, as shown by immunodiffusion against antiserum to human albumin, and substantially free of 410 mμ-absorbing material.

The N-terminal amino acid was found to be valine by the phenylisothiocyanate (Edman, 1950) and fluorodinitrobenzene (Levy, 1954) methods. A further cycle of the Edman procedure showed that the adjacent residue was glutamine. The C-terminal amino acid revealed by carboxypeptidase digestion of HPL was phenylalanine.

The HPL prepared by this extraction procedure is substantially homogeneous, as indicated by the symmetrical elution peak obtained by a further gel filtration on Sephadex G-100, the absence of other components on disk electrophoresis and the presence of a single species of amino acid on each end-group analysis. Further evidence of homogeneity was provided by ultracentrifugation. Sedimentation-velocity experiments carried out at pH 6.80 in 0.1 M-NaCl gave evidence of a single symmetrical peak over the concentration range 1–4 mg./ml. The concentration-dependence of the sedimentation coefficient was very slight and extrapolated to give $S_{20,w}$ 3.34 s.

The molecular weight of the HPL was estimated to be approx. 37 500 by measurement of its elution volume from a Sephadex G-100 column (Andrews, 1965) that had been calibrated with human serum albumin and HGH. The molecular weight of the protein was determined by equilibrium ultracentrifugation by following the technique of Yphantis (1964). At pH 6.80 in 0.1 M-NaCl, some heterogeneity of molecular weight was apparent. A graphical estimate of the monomer molecular weight yielded a value of 39 000 whereas the weight-average molecular weight of the entire sample was

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

	Composition (g./100 g. 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 48 hr.; the results given for preparation 3 are mean values of two determinations.

‡ Mean values of three hydrolysis times (24, 48 and 64 hr.) 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 1 mole of valine/21 500 g. 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 (1965a) 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.

This work was supported by a grant from the Anti-Cancer Council of Victoria. We are most grateful to Mr C. Guthrie of Commonwealth Serum Laboratories, Melbourne, for his assistance with this work. The amino acid analyses were performed by Mr G. Nicol, Department of Biochemistry, Monash University, Melbourne.

Andrews, P. (1965). *Biochem. J.* **96**, 595.

Cohen, H., Grumbach, M. M. & Kaplan, S. L. (1964). *Proc. Soc. exp. Biol., N.Y.*, **117**, 438.

Edman, P. (1950). *Acta chem. scand.* **4**, 283.

Friesen, H. (1965a). *Endocrinology*, **76**, 369.

Friesen, H. (1965b). *Nature, Lond.*, **208**, 1214.

Hirs, C. H. W., Stein, W. H. & Moore, S. (1954). *J. biol. Chem.* **211**, 941.

Josimovich, J. B. & MacLaren, J. A. (1962). *Endocrinology*, **71**, 209.

Levy, A. L. (1954). *Nature, Lond.*, **174**, 216.

Li, C. H. & Liu, W. K. (1964). *Experientia*, **20**, 169.

Raben, M. S. (1957). *Science*, **125**, 883.

Turtle, J. R., Beck, P. & Daughaday, W. H. (1966). *Endocrinology*, **79**, 187.

Yphantis, D. A. (1964). *Biochemistry*, **3**, 297.

Biochem. J. (1967) **102**, 29c

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

By H. R. PERKINS*

Twyford Laboratories, Twyford Abbey Road, London, N.W. 10

(Received 22 November 1966)

The photochemical behaviour of DNP-amino acids and peptides was studied by Russell (1963a). He found that when the DNP-derivatives of most

* Present address: National Institute for Medical Research, Mill Hill, London, N.W. 7.

α -amino acids were dissolved in 1% (w/v) NaHCO₃ 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

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