Kirk-Othmer Encyclopedia of Chemical Technology.

HORMONES, HUMAN GROWTH HORMONE

Human growth hormone [12629-01-5] (hGH), also known as somatotropin, is a protein hormone produced and secreted by the somatotropic cells of the anterior pituitary (see Hormones, anterior pituitary hormones). Secretion is regulated by a releasing factor, ie, the growth hormone-releasing hormone, and by an inhibitory factor, somatostatin. Human growth hormone plays a key role in somatic growth through its effects on the metabolism of proteins (qv), carbohydrates (qv), and lipids. Human growth hormone exerts its biological effects either through direct action of the hormone at the target tissue or indirectly through the action of a second class of peptide hormones, the somatomedins, also known as insulin-like growth factors, which are produced primarily in the liver in response to hGH binding to specific receptors there (see also Insulin and other antidiabetic drugs).

It was known as early as the 1920s that the pituitary gland contained a factor that could promote growth, but not until 1956 (1) was the factor isolated. hGH is extremely abundant in the pituitary, representing as much as 10% of this gland's dry weight, or 8 mg of hormone per gland (2).

Two well-known pathological conditions are the result of an excess or a deficiency of this hormone. The condition in which the body produces an excess of hGH is known as acromegaly or giantism. The condition in which too little is produced is dwarfism.

Human growth hormone, isolated and purified from the pituitary glands of human cadavers, was first administered to humans in 1958. In 1963, the National Institute of Arthritis, Metabolism and Digestive Diseases formed a single collection program called the National Pituitary Agency (NPA). It was the responsibility of this agency to collect human pituitary glands in the United States, organize the extraction of hGH, and distribute it within the country for hypopituitary children and for use in other studies. Pituitary-source hGH from this program was used clinically until the mid-1980s, when hGH of recombinant deoxyribonucleic acid (DNA) origin was approved for sale by the U.S. Food and Drug Administration (see Genetic engineering). One factor that led to the decline in use of pituitary-derived hGH and expedited the acceptance of the biosynthetic hGH was the diagnosis in several recipients of pituitary hGH of Creutzfeldt-Jakob disease, a severe neurological disease thought to be caused by a slow virus that was not effectively removed or deactivated in early preparations of pituitary hGH. This problem was eliminated with the use of biosynthetic hGH.

1. Chemical and Physical Properties

Human growth hormone is a single polypeptide chain of 191 amino acids (qv) having two disulfide bonds, one between Cys-53 and Cys-165, forming a large loop in the molecule, and the other between Cys-182 and Cys-189, forming a small loop near the C-terminus. The structure of hGH is shown in Figure 1; molecular mass is 22,125; the empirical formula is $C_{990}H_{1529}N_{262}O_{300}S_7$.

Purified hGH is a white amorphous powder in its lyophilized form. It is readily soluble (concentrations >10 mg/mL) in dilute aqueous buffers at pH values above 7.2. The isoelectric point is 5.2 (3) and the generally accepted value for the extinction coefficient at 280 nm is $17,700\ (M{\cdot}\mathrm{cm})^{-1}$ (4), although other values have been

Fig. 1. Primary structure of hGH showing the amino acid sequence and the disulfide bonds.

reported. The equilibrium denaturation of hGH has been studied. A two-state mechanism is indicated with a Gibbs free energy of unfolding of 60.7 ± 4 kJ/mol (14.5 ± 1 kcal/mol) (5).

In solution, hGH exists predominantly as a monomer, with a small fraction as a dimer and higher molecular weight oligomers. Under certain conditions, hGH can be induced to form larger amounts of dimers, trimers, and higher oligomers although the conditions leading to self-association are not well understood.

As of this writing the crystal structure of hGH has not been solved. A considerable amount of information is known about its tertiary structure, however. hGH is a globular protein having approximately 55% α-helical character, a value determined independently in studies using circular dichroism (6) and Raman spectroscopy

(7). A protein of a high degree of homology and structural similarity is porcine growth hormone, the crystal structure of which has been solved (8). This structure shows four antiparallel helices connected by strands of polypeptide having little structure. A crystal structure of hGH complexed with the extracellular domain of its receptor has been reported (9). In this complex, the structure of hGH is readily obvious and shows the four-helical bundle connected in an up–up–down–down fashion rather than the more common up–down–up–down. This work has convincingly shown that hGH possesses two binding sites for its receptor and that the hormone functions by dimerizing its receptor on the cell membrane. A more detailed discussion of the molecular mechanisms by which hGH functions can be found in the literature (10).

1.1. hGH Derivatives

Several derivatives of hGH are known. These derivatives include naturally occurring derivatives, variants, and metabolic products, degradation products primarily of biosynthetic hGH, and engineered derivatives of hGH produced through genetic methods.

1.1.1. Methionyl hGH

The first form of hGH to be produced through recombinant DNA technology was actually a derivative of hGH having one additional methionine residue at its N-terminus (11). Although technology has advanced to the stage where natural sequence hGH can easily be produced, as of this writing this derivative, referred to as methionyl hGH, is still produced commercially.

1.1.2. 20-K hGH

A natural, structural variant of hGH called 20-K hGH has been reported to occur in the pituitary as well as in the bloodstream (12, 13). This variant, which lacks the 15 amino acid residues from Glu-32 to Gln-46, arises from an alternative splicing of the messenger ribonucleic acid (mRNA) (14). This variant shares many but not all of the biological properties of hGH.

1.1.3. Acetylated hGH

A form of hGH that is acetylated at the N-terminus has been isolated and identified (15). It is not clear if acylation serves a regulatory role or is simply an artifact of the purification.

1.1.4. Size Isomers

In solution, hGH is a mixture of monomer, dimer, and higher molecular weight oligomers. Furthermore, there are aggregated forms of hGH found in both the pituitary and in the circulation (16, 17). The dimeric forms of hGH have been the most carefully studied and there appear to be at least three distinct types of dimer: a disulfide dimer connected through interchain disulfide bonds (8); a covalent or irreversible dimer that is detected on sodium dodecylsulfate- (SDS-)polyacrylamide gels (see Electroseparations, electrophoresis) and is not a disulfide dimer (19, 20); and a noncovalent dimer which is easily dissociated into monomeric hGH by treatment with agents that disrupt hydrophobic interactions in proteins (21). In addition, hGH forms a dimeric complex with Zn^{2+} (22). Scatchard analysis has revealed that two Zn^{2+} ions associate per hGH dimer in a cooperative fashion, and this Zn^{2+}-hGH dimeric complex was found to be more stable to denaturation than monomeric hGH (22). Because Zn^{2+} concentrations are high in hGH secretory granules of the anterior pituitary (23), it has been speculated that formation of a Zn^{2+}-hGH dimeric complex may be physiologically important in the storage and release of hGH from these granules (22).

1.1.5. Proteolytically Cleaved Two-Chain Forms

There are numerous derivatives of hGH that arise from proteolytic modifications of the molecule. The primary pathway for the metabolism of hGH involves proteolysis. The region of hGH around residues 130–150 is

extremely susceptible to proteolysis and several derivatives of hGH having nicks or deletions in this region have been described (2). This region is in the large loop of hGH (see Fig. 1), and cleavage of a peptide bond there results in the generation of two chains that are connected through the disulfide bond at Cys-53 and Cys-165. Many of these two-chain forms are reported to have increased biological activity (24).

In addition to the naturally occurring proteolytically cleaved two-chain derivatives, there are many examples of derivatives generated artificially through the use of enzymes. The enzymes trypsin and subtilisin, as well as others, have been used to modify hGH at various points throughout the molecule (25, 26). One such derivative, called two-chain anabolic protein (2-CAP), was formed through the controlled proteolysis of hGH using trypsin (27). 2-CAP was found to have biological properties very distinct from those of the intact hGH molecule. Whereas the growth-promoting activity of hGH was largely retained, most of the effects on carbohydrate metabolism were abolished.

1.1.6. Desamido hGH

Asparagine and glutamine residues in proteins are susceptible to deamidation reactions under appropriate conditions. Pituitary hGH has been shown to undergo this type of reaction, resulting in conversion of Asn-152 to aspartic acid and also, to a lesser extent, conversion of Gln-137 to glutamic acid (28). Deamidated hGH has been shown to have an altered susceptibility to proteolysis with the enzyme subtilisin suggesting that deamidation may have physiological significance in directing proteolytic cleavage of hGH. Biosynthetic hGH is known to degrade under certain storage conditions, resulting in deamidation at a different asparagine (Asn-149). This is the primary site of deamidation, but deamidation at Asn-152 is also seen (29). Deamidation at Gln-137 has not been reported in biosynthetic hGH.

1.1.7. Sulfoxide hGH

Methionine residues in proteins are susceptible to oxidation primarily to the sulfoxide. Both pituitary-derived and biosynthetic hGH undergo sulfoxidations at Met-14 and Met-125 (29). Oxidation at Met-170 has also been reported in pituitary but not biosynthetic hGH. Both desamido hGH and Met-14 sulfoxide hGH exhibit full biological activity (29).

1.1.8. Truncated Forms

Truncated forms of hGH have been produced, either through the actions of enzymes or by genetic methods. 2-CAP, generated by the controlled actions of the trypsin, has the first eight residues at the N-terminus of hGH removed. Other truncated versions of hGH have been produced by modification of the gene before expression in a suitable host. The first 13 residues have been removed to yield a derivative having distinctive biological properties (30). In this latter case the polypeptide chain is not cleaved.

1.1.9. Bound to Binding Proteins

In the circulation, it is estimated that approximately 50% of the hGH is present in a bound form in a noncovalent complex with a specific, high affinity growth hormone binding protein. Another 5% is bound to a second low affinity binding protein. The high affinity binding protein (GHBP) is a single-chain glycoprotein of ca 60,000 mol wt which binds hGH in a 1:1 stoichiometry (31). Although the human GHBP has not been sequenced, it is likely to be analogous to the situation in the rabbit where the GHBP is a fragment of the hepatic growth hormone receptor encompassing the extracellular domain (32). The GHBP is thought to play a role in influencing the *in vivo* kinetics of hGH, increasing the serum half-life and acting as a circulating reservoir of hGH that can dampen the oscillations caused by the pulsatile secretion of hGH by the pituitary.

1.1.10. Point Mutations

Since the advent of recombinant DNA technology, a number of researchers have used point mutation techniques either to delete one or more residues within the hGH molecule or systematically to change from one amino acid to another to probe hGH structure/function relationships (33).

2. Biological Properties

Human growth hormone is a very complex molecule biologically. Several diverse biological activities such as anabolic, insulin-like, diabetogenic, and lactogenic activities have been ascribed to hGH, which also appears to promote water and salt retention. An in-depth discussion of these activities may be found in several excellent reviews available in the literature (34–36).

3. Uses

Human growth hormone, used as a human pharmaceutical, is approved for only one indication in the United States, treatment of growth failure owing to hGH deficiency, a condition known as pituitary dwarfism. However, clinical trials are under way to test its efficacy in Turner's syndrome, burns, wound healing, cachexia, osteoporosis, constitutional growth delay, aging, malnutrition, and obesity.

3.1. Pituitary Dwarfism

Pituitary dwarfism is a condition characterized by an inability to produce or secrete normal levels of endogenous hGH. The condition results in reduced heights of individuals afflicted with the condition and has been treated by intramuscular or subcutaneous injection of hGH. Pituitary hGH was used prior to the approval of biosynthetic hGH. If treatment is initiated early enough, the patient can attain a final adult height well within the normal range.

3.2. Turner's Syndrome

Turner's syndrome is a genetic disorder of females characterized by short stature, nonfunctioning ovaries, and failure to develop secondary sexual characteristics. Several clinical trials in the United States, Europe, and Japan have demonstrated that hGH can accelerate short-term growth, accompanied by a relatively modest advancement of skeletal maturation. Human growth hormone has been approved for this condition in Japan and in parts of Europe. Approval is expected in the United States.

3.3. Other Uses

Other uses of hGH, eg, for burns, wound healing, cachexia, osteoporosis, aging, malnutrition, and obesity, are being investigated. These uses are in various stages of development and trials are being carried out by several different pharmaceutical companies.

4. Manufacture and Processing

Human growth hormone was originally manufactured by isolation of the natural product from human pituitaries and subsequent purification of the protein. Since 1985, manufacture of hGH has been almost exclusively by recombinant DNA technology.

4.1. Natural Product hGH

In 1944 the preparation of a highly purified growth hormone from bovine pituitary glands was reported (37). Subsequently, growth hormones derived from animal pituitaries were found to be ineffective in humans; the existence of specificity among species for growth hormone was thus established.

Numerous methods for the extraction and purification of hGH have been developed since this hormone was first isolated in 1956 (1). Initially, the only source was the pituitary gland of cadavers. The sooner after death that the glands were removed and frozen, the higher the yield and quality of the resulting hGH preparation. After thawing, the glands were used directly for extraction of the hGH or an acetone powder was prepared for later extraction. Shortages of hGH led to development of a process to extract hGH from embalmed tissues (38). Yields using embalmed glands were only 20–25% of those when fresh-frozen glands were used, but the greater availability of embalmed glands compensated for the losses. It was found that if formalin was removed from embalmed pituitary glands with large volumes of cold acetone, appreciable quantities of hGH could be isolated from these glands.

Extraction of hGH from pituitary glands has been accomplished by a variety of procedures with or without protease inhibitors. One of the first commercial procedures used glacial acetic acid at 70°C to extract the hGH from the glands (39).

Following extraction from the gland the crude hGH was purified by a number of different procedures, including precipitation steps and different types of chromatography (qv). hGH could be separated from most of the other pituitary hormones by simple size exclusion chromatography on resins like Sephadex G-100. Cation- and anion-exchange steps were introduced into the purification schemes of a number of laboratory processes. Higher quality material was produced, but in significantly lower yields. The addition of proteinase inhibitors like diisopropylfluorophosphate (DFP) before loading the material onto the anion-exchange column dramatically improved the hGH yield. Purification procedures for pituitary-derived hGH began to appear which included ion-exchange steps sometimes containing 6 *M* urea in the running buffer, ultrafiltration steps, and size exclusion steps. However, the need for increased purity was always modulated by the short supply of the starting material and the need for reasonably high yields in any purification process used for commercial purposes. Besides procedures to remove contaminating proteins from the hGH preparation, procedures were developed using 6 *M* urea and ultrafiltration to alleviate any potential contamination by slow viruses (40).

One of the goals of the early purification procedures was to produce hGH in the highest yield possible. Preparations of hGH produced in high yield were found to be satisfactory in clinical investigation, but were by no means electrophoretically homogeneous, and some were even brown in color (41). Antibodies appeared in the serum of patients treated with preparations of the order of 50% purity, and the immunogenicity was attributable to inactive components. Recognition that highly purified preparations of hGH could overcome many of the complications arising in the clinical treatment was the driving force to develop more highly purified hGH.

4.2. Recombinant hGH

Introduction of recombinant DNA technology meant an unlimited supply of hGH could be produced in a number of different systems. *Escherichia coli* was the first heterologous host used and originally produced Met-hGH, authentic hGH with the initiating methionine attached (11). This was followed by production in *E. coli* of

precursors of hGH that could be cleaved *in vitro* to yield natural sequence hGH. High level expression of hGH in *E. coli* can lead to deposition of the hormone in inclusion bodies. This mode of production offers both advantages and disadvantages, from a production point of view. It is generally quite easy to isolate the inclusion bodies by lysis of the cell and collection of the inclusion bodies by centrifugation. The isolated inclusion bodies are greatly enriched in hGH. Alternative methods of production in *E. coli* allow the hGH produced to be exported to either the periplasmic space of the bacterium (42) or to the culture medium (43). Periplasmic production of hGH generally leads to a fully soluble form of hGH that is properly folded and which can be released from the cell by osmotic lysis of the *E. coli* outer membrane (42).

If the hGH is exported to the culture medium the product can easily be collected by removal of the cells from the culture medium by centrifugation. Purification of hGH from the culture medium is facilitated by low amounts of contaminating proteins present. In fact, it has been shown that hGH can be purified on a laboratory scale by a single purification step on a reversed-phase hplc column (43). Mammalian cells growing in tissue culture have also been used as hosts to produce hGH, which is exported into the culture media (44).

The availability of large amounts of hGH produced by recombinant DNA technology made it possible to investigate other forms of chromatographic purifications such as reversed-phase hplc (rp-hplc) (45), immunoadsorbant chromatography, and chromatofocusing (46). However, the classical forms of purification such as anion-exchange and size exclusion chromatography produce hGH from a recombinant source with greater than 90% purity (42).

5. Economic Aspects

Human growth hormone is one of the largest selling therapeutic proteins produced by recombinant DNA technology. Annual worldwide sales increased from $130,000,000 in 1987 to $575,000,000 in 1992 (47). Upon approval of additional indications, the sales of hGH are expected to increase even more.

hGH preparations produced and marketed in the United States include Humatrope from Eli Lilly and Co. (Indianapolis, Ind.) and Protropin from Genentech, Inc. (South San Francisco, Calif.). Protropin differs slightly in structure from natural sequence hGH, having an additional methionine residue at the N-terminus. Both Humatrope and Protropin are marketed as vials containing 5 mg hGH plus additional excipients, ie, glycine and mannitol, in a lyophilized form. A companion diluent vial is provided, allowing the patient to reconstitute the product to the desired concentration prior to administration of the dose. Additional products are expected to become available in the United States during 1994, or thereafter. Outside the United States many commercial preparations are available in a variety of package sizes. In some cases the label quantity is expressed in international units (IUs) rather than milligrams. Package sizes of 2, 4, 12, and 16 IU/vial are available. A conversion factor of 3 IU/mg is generally accepted when calculating dosages.

6. Specifications, Standards, and Quality Control

Specifications for hGH products are defined by the governmental licensing authorities, eg, the U.S. Food and Drug Administration. Draft monographs for hGH have been prepared by both the *United States Pharmacopia* and the *European Pharmacopeia* commissions and should be formally adopted by 1995. These specifications are suitable for biosynthetic hGH. The much less purified pituitary-derived hGH has virtually disappeared from commercial production. An international reference standard for pituitary-derived hGH (lot 80/505) has been used for calibration, particularly for bioassay purposes. A highly purified biosynthetic hGH standard (lot 88/624) has been prepared and should be formally adopted by 1995, or before.

Quality control of hGH preparations includes proof of identity, potency, and purity. Purity of hGH must be carefully determined. The result can depend greatly on the specific assay used. The predominant chemical

variant in hGH at the time of manufacture is desamido hGH (29), wherein the asparagine at position 149 has been converted to aspartic acid. Desamido hGH levels are generally in the range of 2–5% at the time of manufacture, and can increase slightly over the shelf-life of the product. A second chemical derivative present in hGH preparations is hGH methionine sulfoxide (29). Methionine sulfoxide forms of hGH are generally not present at significant levels at the time of manufacture, but typically increase to a level of 3–6% over the shelf-life of the product. Both the desamido and methionine sulfoxide forms of hGH show significant biological activity, and hence the formation of these derivatives during storage has little effect on the potency of the product. The specification for total chemical variants in hGH preparations at the time of expiration is not more than 12%, in the draft USP monograph.

In addition to chemical derivatives, purity of hGH must also be established with respect to physically associated forms. The hydrophobically linked, noncovalent dimer of hGH found to exhibit relatively low biological activity (21) is present at a level of 1–2% in most hGH preparations at the time of manufacture. In addition, both noncovalent and covalent forms of hGH dimer and higher order aggregates may form during storage of the product. Typical dimer contents of hGH preparations are in the range of 2–5%, with a product specification of not more than 6%, at time of product expiration.

Although biosynthetic hGH products are of very high purity relative to pituitary-derived products, additional evidence of purity with respect to removal of DNA and proteins from the host-cell line is required. Host-cell protein levels are routinely determined and controlled to a level of not more than 10 ppm. DNA levels are demonstrated to be less than 100 pg/dose, or not more than 40 pg/mg, based on a maximal 2.5-mg dose.

7. Analytical Test Methods and Storage

Proof of identity of hGH preparations is provided by several different test methods conducted in parallel. A single test method is not considered to be sufficient evidence of identity for a molecule as complex as hGH. The most powerful assay method for identification is peptide mapping, wherein an hGH solution is treated with trypsin at neutral pH for a few hours, and the resulting peptides separated by reversed-phase high performance liquid chromatography (rp-hplc). Because trypsin cleaves on the C-terminal side of each arginine and lysine with high specificity, the resulting rp-hplc chromatogram is highly reproducible and provides a fingerprint from which identity of the test material can be deduced (48). A second method for identification of hGH also involves a highly selective rp-hplc procedure, which is capable of resolving hGH from close structural variants such as deamidated forms, methionine sulfoxides, and N-terminal derivatives (49). This method resolves hGH from *N*-methionyl hGH sold commercially as Protropin.

Potency of hGH preparations is quantitatively determined, in terms of mass per vial, by one or more chromatographic procedures (50). Biopotency is calculated from the mass-based potency using a conversion factor, typically 3 IU/mg. Traditionally a bioactivity assay using hypophysectomized rats has been used to determine potency; however, the imprecision of this assay has resulted in its use only as a semiquantitative indicator of bioactivity (1), sometimes referred to as a bioidentity test.

Chemical derivatives of hGH can be determined by a variety of chromatographic procedures. The rp-hplc procedure described for identity confirmation is also capable of determining the levels of desamido hGH and hGH methionine sulfoxide (49). Ion-exchange hplc can also be used to determine the level of desamido hGH (49). Dimeric and aggregated forms of hGH can be determined by size-exclusion hplc, using nondenaturing conditions (21). However, denaturing conditions used in rp-hplc or in sodium dodecylsulfate polyacrylamide gel electrophoresis (sds-page) cause conversion of noncovalent dimers and aggregates to monomeric hGH; thus such techniques are not suitable for this purpose.

Trace contaminants such as host cell proteins (HCPs) and DNA are determined by more specialized techniques. Host cell proteins are generally determined using an immunochemical assay, in which an antibody

preparation, raised against a mixture of the HCPs, is used to selectively detect the total level of HCPs in the product. DNA can be determined using a labeled mixture, or probe, of complimentary DNA from the host cell.

Freeze-dried hGH products are quite stable under refrigerated conditions, eg, a 24-month shelf-life is typical at this temperature. However, solutions of hGH at neutral pH readily deamidate, and storage of the reconstituted product is limited to a few weeks under refrigerated conditions, although the biological activity is relatively unaffected even after prolonged storage. Reconstituted solutions of hGH should not be frozen, because denaturation and subsequent aggregation may result.

8. Metabolism and Disposition

The pharmacokinetics of hGH have been evaluated in animals and humans. After intravenous administration, the elimination of hGH is described by first-order kinetics with a serum half-life of 12–30 min in both animals and humans (52, 53). Traditionally, intramuscular (im) injection has been the method of choice for delivery of hGH. This has given way to subcutaneous (sc) administration as the preferred route of delivery, owing to increased patient compliance as a result of lessened local discomfort (54). In humans, absorption of exogenous hGH appears to be more rapid from the im site, with a time to maximum concentration of 2–3 h compared to 4–6 h after sc administration. The disappearance phase from serum has been reported to range from 12–20 and 20–24 h after im and sc administration, respectively (55, 56). The plasma kinetics of hGH in humans has been best predicted by animal studies using the nonhuman primate and less so by rodent species. In general, no significant differences have been observed in the pharmacokinetics or biological activities of recombinant natural sequence hGH, recombinant *N*-methionyl—hGH, or pituitary-derived material in humans (52, 57).

The principal organs involved in the peripheral clearance of hGH from the plasma are the kidney and liver. hGH is cleared via glomerular filtration at the kidney and by a receptor-mediated mechanism at the liver (58, 59). In animal models, derivatives of hGH such as the 20,000 mol wt variant, oligomeric forms, and hGH complexed with GH-binding protein have been shown to be cleared from the serum at significantly lower rates than 22,000 mol wt hGH (60–62). The prolonged plasma half-life of these derivatives probably reflects a combination of decreased receptor affinity and size constraints on glomerular filtration.

Information regarding the metabolic fate of hGH in humans and animals is fragmentary. hGH has been demonstrated to be stable to proteolytic degradation in the plasma or serum from animals and humans (63). Thus it appears unlikely that intravascular degradation of hGH is relevant *in vivo.* Although a mechanism has not been established, hGH fragments formed after receptor-mediated uptake may be recycled into the circulation. *In vitro,* proteolytic processing of hGH in the large disulfide loop region along with truncation of the N-terminus resulted in hGH metabolites having masses ranging from 22,143 to 16,002 (64). These metabolites were formed by the sequential action of a chymotrypsin-like protease and a carboxypeptidase (64, 65). In addition, membrane fractions from target tissue, ie, liver, have been shown by sds-page to convert hGH to a 15,000 mol wt fragment (66). The molecular weight and the cleavage site sensitivity in these metabolites are consistent with that of endogenous pituitary-derived variants of hGH, suggesting potential physiological or pharmacological relevance. In this regard, immunoreactive hGH forms having apparent molecular weights of 12,000, 16,000, and 30,000 have been observed in human serum (67). Although the source of this material has not been elucidated, these molecules are not detectable in plasma after a secretory stimulus, suggesting their formation by peripheral tissues. At this time, the actual concentration of hGH fragments in plasma and the precise nature of this immunoreactive material has not been established. Application of sensitive and accurate methods of protein analysis such as electrospray mass spectrometry (qv) should help resolve some of the deficiencies in the information available.

BIBLIOGRAPHY

Cited Publications

1. C. H. Li and H. Papkoff, *Science* **124**, 1293 (1956).
2. U. J. Lewis and co-workers, *Recent Progr. Horm. Res.* **36**, 477 (1980).
3. B. C. Hummel and co-workers, *Endocrinology* **97**, 855 (1975).
4. Preliminary report on "Collaborative Study on the Proposed International Standard for Somatropin," National Institute for Biological Standards and Control, London, Dec. 21, 1992.
5. D. N. Brems, P. L. Brown, and G. W. Becker, *J. Biol. Chem.* **265**, 5504 (1990).
6. T. A. Bewley and C. H. Li, *Biochemistry* **11**, 884 (1972).
7. L. G. Tensmeyer, *J. Mol. Struct.* **175**, 73 (1988).
8. S. S. Abdel-Meguid and co-workers, *Proc. Nat. Acad. Sci. USA* **84**, 6434 (1987).
9. A. M. de Vos, M. Ultsch, and A. A. Kossiakoff, *Science* **255**, 306–312 (1992).
10. J. A. Wells and co-workers, *Recent Progr. Horm. Res.* **48**, 253 (1993).
11. D. V. Goeddel and co-workers, *Nature* **281**, 544 (1979).
12. U. J. Lewis and co-workers, *J. Biol. Chem.* **253**, 2679 (1978).
13. U. J. Lewis, L. F. Bonewald, and L. J. Lewis, *Biochem. Biophys. Res. Comm.* **92**, 511 (1980).
14. F. M. DeNoto, D. D. Moore, and H. M. Goodman, *Nucleic Acids Res.* **9**, 3719 (1981).
15. U. J. Lewis and co-workers, *Endocrinology* **104**, 1256 (1979).
16. M. W. Stolar, K. Amburn, and G. Baumann, *J. Clin. Endocrinol. Metab.* **59**, 212 (1984).
17. M. W. Stolar and G. Baumann, *Metabolism* **35**, 75 (1986).
18. U. J. Lewis and co-workers, *J. Biol. Chem.* **252**, 3697 (1977).
19. T. A. Bewley and C. H. Li, *Adv. Enzymol. Relat. Areas Mol. Biol.* **42**, 73 (1975).
20. N. D. Jones and co-workers, *Biotechnology* **5**, 499 (1987).
21. G. W. Becker and co-workers, *Biotechnol. Appl. Biochem.* **9**, 478 (1987).
22. B. C. Cunningham, M. G. Mulkerrin, and J. A. Wells, *Science* **253**, 545 (1991).
23. O. Thorlacius-Ussing, *Neuroendocrinology* **45**, 233 (1987).
24. R. N. P. Singh and co-workers, *Endocrinology* **94**, 883 (1974).
25. U. J. Lewis and co-workers, *Endocrinology* **101**, 1587 (1977).
26. L. Graff, C. H. Li, and M. D. Jibson, *J. Biol. Chem.* **257**, 2365 (1982).
27. G. W. Becker and C. J. Shaar, abstr. no. 342, 71st Annual Meeting, The Endocrine Society, Seattle, Wash., June 1989.
28. U. J. Lewis and co-workers, *J. Biol. Chem.* **256**, 11645 (1981).
29. G. W. Becker and co-workers, *Biotechnol. Appl. Biochem.* **10**, 326 (1988).
30. A. Gertler and co-workers, *Endocrinology* **118**, 720 (1986).
31. G. Baumann, *Acta Paediatr. Scand.* **367** (Suppl.), 142 (1990).
32. S. A. Spencer and co-workers, *J. Biol. Chem.* **263**, 7862 (1988).
33. S. Nishikawa and co-workers, *Protein Eng.* **3**, 49 (1989).
34. R. K. Chawla, J. S. Parks, and D. Rudman, *Ann. Rev. Med.* **35**, 519 (1983).
35. M. B. Davidson, *Endocr. Rev.* **8**, 115 (1987).
36. J. L. Kostyo, in L. E. Underwood, ed., *Human Growth Hormone: Progress and Challenges*, Marcel Dekker, New York 1988, p. 63.
37. C. H. Li and H. M. Evans, *Science* **99**, 183 (1944).
38. J. B. Mills and co-workers, *J. Clin. Endocrinol. Metab.* **29**, 1456 (1969).
39. M. S. Raben, *Science* **125**, 883 (1957).
40. M. Pocchiari and co-workers, *Horm. Res.* **35**, 161 (1991).
41. R. A. Reisfeld and co-workers, *Nature* **197**, 1206 (1963).
42. H. M. Hsiung, N. G. Mayne, and G. W. Becker, *Biotechnology* **4**, 991 (1986).
43. H. M. Hsiung and co-workers, *Biotechnology* **7**, 267 (1989).

44. S. Lefort and P. Ferrara, *J. Chromatogr.* **361**, 209 (1986).
45. B. S. Welinder, H. H. Sørensen, and B. Hansen, *J. Chromatogr.* **398**, 309 (1987).
46. S. Niimi and co-workers, *Chem. Pharm. Bull.* **10**, 4221 (1987).
47. A. Klausner, *Biotechnology* **11**, S35 (1993).
48. G. W. Becker and H. M. Hsiung, *FEBS Lett.* **204**, 145 (1986).
49. R. M. Riggin, G. K. Dorulla and D. J. Miner, *Anal. Biochem.* **167**, 199 (1987).
50. R. M. Riggin and co-workers, *J. Chromatogr.* **435**, 307 (1988).
51. W. Marx, M. E. Simpson, and H. M. Evans, *Endocrinology* **30**, (1942).
52. J. A. Moore and co-workers, *Endocrinology* **122**, 2920 (1988).
53. C. M. Hendricks and co-workers, *J. Clin. Endocrinol. Metal.* **60**, 864 (1985).
54. D. M. Wison and co-workers, *Pediatrics* **76**, 361 (1985).
55. K. Albertsson-Wikland, O. Westphal, and U. Westgren, *Acta Paediatr. Scand.* **75**, 89 (1986).
56. J. O. Jorgensen and co-workers, *Acta Endocrinol. (Copenhagen)* **116**, 381 (1987).
57. K. D. Jorgensson and co-workers, *Pharmacol. Toxicol.* **63**, 129 (1988).
58. F. A. Carone and co-workers, *Kidney Int.* **16**, 271 (1979).
59. M.-C. Postel-Vinay, C. Kayser, and B. Debuquois, *Endocrinol. Rev.* **111**, 244 (1982).
60. G. Baumann, K. D. Amburn, and T. A. Buchanan, *J. Clin. Endocrinol. Metab.* **64**, 657 (1987).
61. G. Baumann, M. W. Stolar, and T. A. Buchanan, *Endocrinology* **119**, 1497 (1985).
62. G. Baumann, M. W. Stolar, and T. A. Buchanan, *Endocrinology* **117**, 1309 (1985).
63. G. Baumann, *J. Clin. Endocrinol. Metab.* **43**, 222 (1976).
64. V. J. Wroblewski, R. E. Kaiser, and G. W. Becker, *Pharm. Res.* **10**, 1106 (1993).
65. V. J. Wroblewski, M. Masnyk, and G. W. Becker, *Endocrinology* **129**, 465 (1991).
66. J. M. Schepper and co-workers, *J. Biol. Chem.* **259**, 12945 (1984).
67. G. Baumann, M. W. Stolar, and K. Amburn, *J. Clin. Endocrinol. Metab.* **60**, 1216 (1985).

GERALD W. BECKER
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