metric distribution of auxin[4]. The present results appear to suggest that, following treatment with ethrel, an asymmetric distribution of auxin might occur which results in local surplus of auxin on one side of the root. This, in turn, causes inhibition of cell elongation on that side while the other side continues to elongate thereby causing the coiling of the root. As a matter of fact ethylene is known to inhibit lateral auxin movement and also to check the capacity of the polar auxin transport system[1]. In addition, the treated seedlings usually developed much greater anthocyanin in the hypocotyls. However, at the same time a reduction in the synthesis of chlorophylls in the cotyledons was also equally evident (Table). TIBA inhibited elongation of root as well as hypocotyl, and drastically affected the production of lateral roots (Figure C).

Since root curvatures arise by the differential expansion of the two sides through a lateral migration of auxin instead of the normal symmetical and strictly polar flow[5] it seemed of interest to study interaction between ethrel and 2, 3, 5-tri-iodobenzoic acid. The latter substance is an extremely active synthetic antiauxin and is known to interfere with normal auxin transport[6]. The results presented in the Table are indicative of the fact that TIBA strongly antagonizes the effect of ethrel at root level. Therefore, the seedlings reared in TIBA-ethrel combination did not indicate any coiling (Figure D). However, the possibility of TIBA acting as competitive inhibitor of ethylene is ruled out, because the former chemical did not reverse the effect of CEPA on chlorophyll synthesis in cotyledons and seedling growth. The exact mechanism by which TIBA affects decoiling of roots in ethrel treated seedlings remains to be explored. However, since root-coiling is associated with altered auxin metabolism, more specially the altered transverse transport, it would appear that TIBA brings about decoiling by influencing auxin transport. This, however, needs further confirmation.

Résumé. L'éthrel provoque l'enroulement des racines du jeune *Ipomoea pentaphylla*. Cet effet est complètement annulé par l'adjonction de TIBA.

N. SANKHLA

Department of Botany, University of Jodhpur, New Campus, Jodhpur (India), 27 April 1971.

[4] A. V. CHADWICK and S. P. BURG, Plant Physiol. *42*, 415 (1967).
[5] L. J. AUDUS, in *Physiology of Plant Growth and Development* (Ed. M. B. WILKINS; 1969), p. 205.
[6] L. J. AUDUS, *Plant Growth Substances* (London 1959).

STUDIORUM PROGRESSUS

Sequence Comparison of Human Pituitary Growth Hormone, Human Chorionic Somatomammotropin and Ovine Pituitary Lactogenic Hormone

Human growth hormone (HGH) and ovine lactogenic hormone (LTH), secreted by the pituary gland, and human chorionic somatomammotropin (HCS), secreted by the human trophoblast during pregnancy, are all potent lactogenetic hormones[1–3]. While LTH possesses no growth promoting activity, HCS has been shown in the rat tibia test, to be 10–15% as potent as HGH in this regard[3]. HCS has also been shown to closely resemble HGH in amino acid composition[4] and immunochemical behavior[2]. Limited sequence studies, cyrried out in conjunction with these early investigations on HCS, already showed similarities to HGH in both the amino[3,5] and carboxyl[3,6] terminal regions of the molecule.

The recent publication of the complete primary structure[7] of HCS and a revised structure[8] for HGH has prompted[9] us to reevaluate our earlier[11] comparision of the HGH structure with that of LTH, and to extend this to include the newly available HCS structure. In the following discussion, we shall use the term homology to refer to instances where equivalent positions in two or more sequences are occupied either by identical amino acids, or by two different amino acids which are judged to be acceptable replacements for each other. By statistical analysisis of a large number of sequences from several types of related proteins, DAYHOFF[12] has characterized the degree of homology between all possible amino acid pairs according to the relative rates of acceptance of either member of the pair into the same residue position in a given type of polypeptide chain. As might be expected, the highest degrees of homology are found between the amino acids showing the greatest degrees of chemical similarity, corresponding to the 'conservative replacements' described by PERUTZ et al.[13]. Other pairs, in which the chemical similarity is not immediately obvious, have also been considered as homologous or acceptable replacements[12]. We shall designate as 'highly acceptable' those replacements having acceptance rates[12] equal to or greater than 40 times that predicted by chance, and as 'acceptable' those with acceptance rates from 21 to 39. In our analysis, amino acid pairs representing replacements with acceptance rates from 0 to 20 will not be considered homologous.

To examine the structues for areas of homolgy, we have aligned the three sequences according to the best possible fit of certain reference residues. Cysteine, tryptophan, tyrosine, histidine and proline were used as references because

[1] C. H. LI, Perspect. Biol. Med. *11*, 498 (1968).
[2] J. B. JOSIMOVICH and J. A. MACLAREN, Endocrinology *71*, 209 (1962).
[3] C. H. LI, Ann. Scalvo Inst., Siena *12*, 651 (1970).
[4] H. FRIESEN, Endocrinology *76*, 369 (1965).
[5] K. J. CATT, B. MOFFAT and H. D. NIALL, Science *157*, 321 (1967).
[6] L. M. SHERWOOD, Submitted to the *Atlas of Protein Sequence and Structure* (Ed. M. O. DAYHOFF, National Biomedical Research Foundation, Silver Spring, Maryland 1969,), vol. 4.
[7] C. H. LI, J. S. DIXON and D. CHUNG, Science, in press (1971).
[8] C. H. LI and J. S. DIXON, Arch. Biochem. Biophys., in press (1971).
[9] A preliminary comparison of the amino terminal regions of these three proteins showing a partial sequence for HCS and a revised partial sequence for HGH has recently been reported by NIALL[10].
[10] H. D. NIALL, Nature New Biol., Lond. *230*, 90 (1971).
[11] T. A. BEWLEY and C. H. LI, Science *168*, 1361 (1970).
[12] M. O. DAYHOFF, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Maryland 1969), vol. 4.
[13] M. F. PERUTZ, J. C. KENDREW and H. C. WATSON, J. molec. Biol. *13*, 669 (1965).

of their limited content in these proteins and their low relative mutability[12]. Occasionally, the introduction of a gap into one or the other sequence was required to obtain the best alignment. The homology between any two sequences or portions thereof, is then determined according to the criteria outlined above. Although the inclusion of acceptable replacements is certainly necessary in any evaluation of homology, it must be kept in mind that the highest emphasis should be given to the number of identical residues.

Comparison of HGH and HCS. Both HGH and HCS are single chain polypeptides[7,8] of 190 residues containing 2 disulfide bonds. The alignment of these 2 sequences presents no problem since the structure of either may be placed in a one-to-one correspondence with the other without the introduction of gaps. The several gaps appearing in the 2 sequences as depicted in the Figure are required only for concurrent alignment with the LTH structure. Out of the 190 residue positions in these 2 proteins, 160 (84%) are occupied by identical amino acids. The 30 positions showing differences, contain 19 highly acceptable replacements, and 4 acceptable replacements with only 7 positions being occupied by non-homologous amino acids. Thus, there are a total of 183 homologous positions in comparing HGH and HCS, amounting to 96% of either peptide chain. The amino terminal portion appears to contain most of the differences, with 26 changes occurring in the first 111 residue positions, and only 4 changes occurring in the remaining 79. Three short regions, each with less than 50% identity appear to be only areas in which amino acid differences are concentrated to any extent. These regions encompass residue positions 1–4, 46–56 and 103–111, containing a total of 14 changes within 24 positions. The 19 highly acceptable replacements may be further divided into 15 pairs in which one or both of the members is a polar or charged residue, and only 4 pairs in which both amino acids are hydrophobic in nature. By analogy with the results on hemoglobin[13] it might be predicted that most of the 15 polar positions occur on the surface of the molecules where they are in contact with the external aqueous solvent. In this same regard, it may be significant that all but one of the 11 replacements in the two regions bounded by positions 46–56 and 103–111, which were mentioned above as two of the areas in which there appeared to be a concentration of differences, involve changes between hydrophilic amino acids.

There appears to be an unusual conservation of the position of serine residues between these two structures. As pointed out by DAYHOFF[12], serine is the most replaceable of all the amino acids. HGH and HCS both contain 18 serine residues, 16 of which are in identical positions in the 2 molecules, amounting to 90% identity in the position of this amino acid. This value may be compared with that for methionine (70% identity) and asparagine (75% identity), which DAYHOFF lists as the next 2 most replaceable residues, and also with that of leucine (96%indentity) or glycine (93% identity) which are listed as relatively irreplaceable amino acids. All of the lysine, tyrosine, cysteine and tryptophan residues are in identical posititons, in good agree ment with the very low rates of replaceablity of these residues.

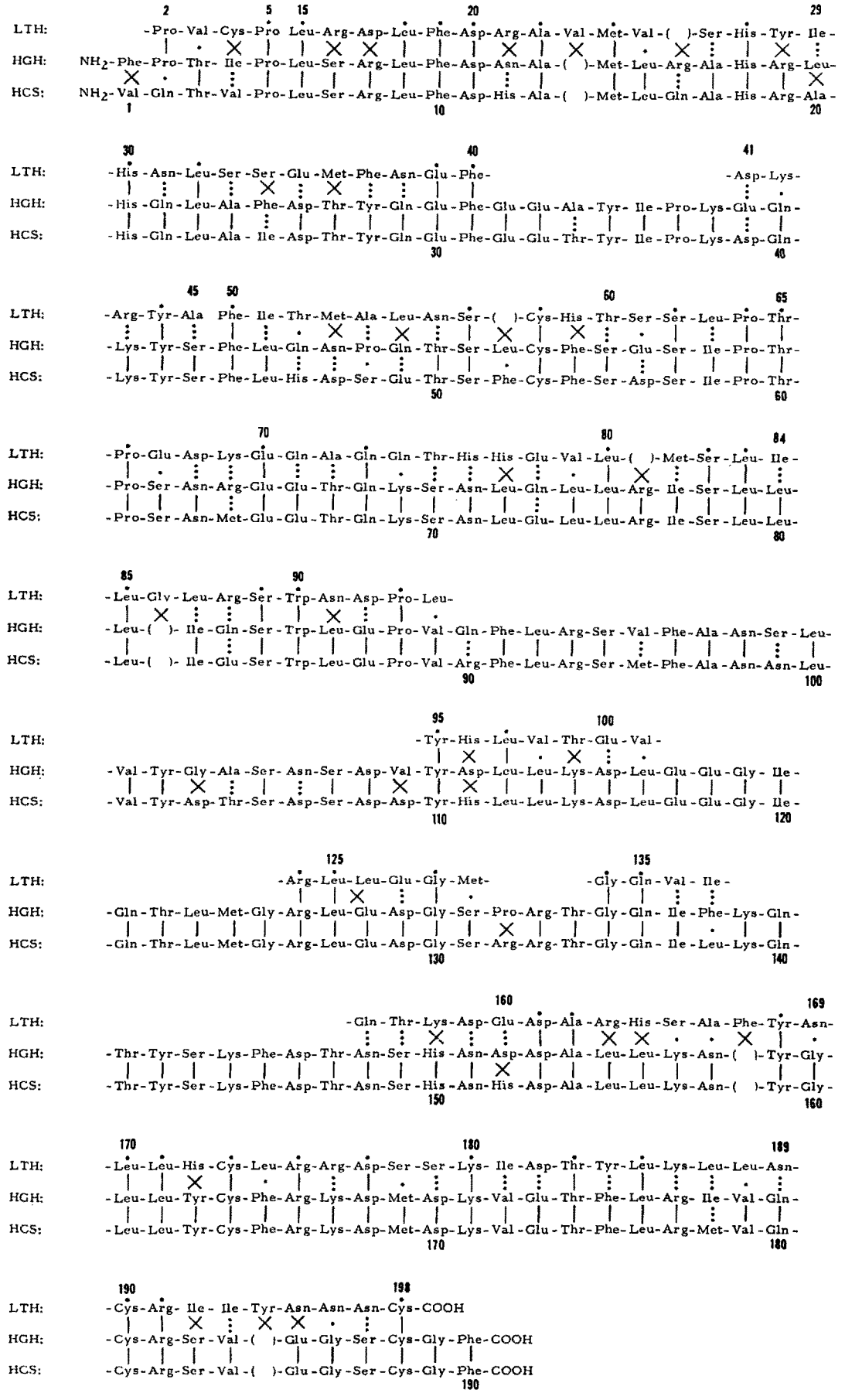
The very high degree of homology between these two protein hormones may also be seen from a consideration of the minimum number of nucleotide base differences in their genetic codes. All but one of the 30 replacements can be explained by the change of a single base in the codon triplet for that position. The replacement of Leu_{20} in HGH for Ala_{20} in HCS is the only one that requires a minimum of 2 base changes. This gives a total of only 31 base differences in the 570 nucleotides which code for these 2 proteins. We must emphasize of course, that this is the minimum number of base changes that would explain the differences in the 2 primary stuctures. Because of the degenerate nature of the genetic code, the actual number of nucleotide differences could be considerably higher than this. A summary of the homology between these 2 proteins is shown in the Table.

Comparison of HGH with LTH. The Figure also shows the regions of homology between HGH and LTH. It has been necessary to introduce several gaps into the various sequences in order to get the best alignment. 3 major areas of reasonable homology may be seen. Starting from the amino terminal end, we have aligned the tetra-peptide Pro_5–Pro_8 in LTH with the tetra-peptide Pro_2–Pro_5 in HGH. If we next align Leu_{15} of LTH with Leu_6 of HGH, the following 27 residue positions (including 2 gaps), ending with Phe_{40} of LTH and Phe_{31} of HGH contain a total of 19 homologous amino acids with only 7 unacceptable replacements. The entire region from Pro_2–Phe_{31} of HGH containing a total of 31 positions, has 13 identical, 7 highly acceptable, 2 acceptable and 8 unacceptable residue pairs, for a total homology of 71%. A second major region may

Homology between primary structures of human growth hormone (HGH), human chorionic somatomammotropin (HCS) and ovine lactogenic hormone (LTH)

Residue positions encompassed[a]	Identical pairs	Highly acceptable replacements	Acceptable replacements	Residue positions (including gaps)
HGH: 1 → 190	160	19	4	190
HCS: 1 → 190				
HGH: 2 → 31	13	7	2	31
LTH: 2 → 5 + 15 → 40				
HGH: 39 → 89	17	20	7	52
LTH: 41 → 45 + 50 → 94				
HGH: 148 → 188	14	14	7	45
LTH: 156 → 198				
HGH: 110 → 116	2	1	2	7
LTH: 95 → 101				
HGH: 126 → 131	3	1	1	6
LTH: 124 → 129				
HGH: 135 → 138	2	2	0	4
LTH: 134 → 137				

[a] Residue position numbers are taken from references; 8 (HGH), 7 (HCS) and 16 (LTH).


LTH: -Pro-Val-Cys-Pro Leu-Arg-Asp-Leu-Phe-Asp-Arg-Ala-Val-Met-Val-( )-Ser-His-Tyr- Ile-
HGH: NH2-Phe-Pro-Thr- Ile-Pro-Leu-Ser-Arg-Leu-Phe-Asp-Asn-Ala-( )-Met-Leu-Arg-Ala-His-Arg-Leu-
HCS: NH2-Val-Gln-Thr-Val-Pro-Leu-Ser-Arg-Leu-Phe-Asp-His-Ala-( )-Met-Leu-Gln-Ala-His-Arg-Ala-
LTH: -His-Asn-Leu-Ser-Ser-Glu-Met-Phe-Asn-Glu-Phe- -Asp-Lys-
HGH: -His-Gln-Leu-Ala-Phe-Asp-Thr-Tyr-Gln-Glu-Phe-Glu-Glu-Ala-Tyr- Ile-Pro-Lys-Glu-Gln-
HCS: -His-Gln-Leu-Ala- Ile-Asp-Thr-Tyr-Gln-Glu-Phe-Glu-Glu-Thr-Tyr- Ile-Pro-Lys-Asp-Gln-
LTH: -Arg-Tyr-Ala Phe- Ile-Thr-Met-Ala-Leu-Asn-Ser-( )-Cys-His-Thr-Ser-Ser-Leu-Pro-Thr-
HGH: -Lys-Tyr-Ser-Phe-Leu-Gln-Asn-Pro-Gln-Thr-Ser-Leu-Cys-Phe-Ser-Glu-Ser-Ile-Pro-Thr-
HCS: -Lys-Tyr-Ser-Phe-Leu-His-Asp-Ser-Glu-Thr-Ser-Phe-Cys-Phe-Ser-Asp-Ser- Ile-Pro-Thr-
LTH: -Pro-Glu-Asp-Lys-Glu-Gln-Ala-Gln-Gln-Thr-His-His-Glu-Val-Leu-( )-Met-Ser-Leu- Ile-
HGH: -Pro-Ser-Asn-Arg-Glu-Glu-Thr-Gln-Lys-Ser-Asn-Leu-Gln-Leu-Leu-Arg- Ile-Ser-Leu-Leu-
HCS: -Pro-Ser-Asn-Met-Glu-Glu-Thr-Gln-Lys-Ser-Asn-Leu-Glu- Leu-Leu-Arg- Ile-Ser-Leu-Leu-
LTH: -Leu-Gly-Leu-Arg-Ser-Trp-Asn-Asp-Pro-Leu-
HGH: -Leu-( )- Ile-Gln-Ser-Trp-Leu-Glu-Pro-Val-Gln-Phe-Leu-Arg-Ser-Val-Phe-Ala-Asn-Ser-Leu-
HCS: -Leu-( )- Ile-Glu-Ser-Trp-Leu-Glu-Pro-Val-Arg-Phe-Leu-Arg-Ser-Met-Phe-Ala-Asn-Asn-Leu-
LTH: -Tyr-His-Leu-Val-Thr-Glu-Val-
HGH: -Val-Tyr-Gly-Ala-Ser- Asn-Ser-Asp-Val-Tyr-Asp-Leu-Leu-Lys-Asp-Leu-Glu-Glu-Gly- Ile-
HCS: -Val-Tyr-Asp-Thr-Ser-Asp-Ser-Asp-Asp-Tyr-His-Leu-Leu-Lys-Asp-Leu-Glu-Glu-Gly- Ile-
LTH: -Arg-Leu-Leu-Glu-Gly-Met- -Gly-Gln-Val- Ile-
HGH: -Gln-Thr-Leu-Met-Gly-Arg-Leu-Glu-Asp-Gly-Ser-Pro-Arg-Thr-Gly-Gln- Ile-Phe-Lys-Gln-
HCS: -Gln-Thr-Leu-Met-Gly-Arg-Leu-Glu-Asp-Gly-Ser-Arg-Arg-Thr-Gly-Gln- Ile-Leu-Lys-Gln-
LTH: -Gln-Thr-Lys-Asp-Glu-Asp-Ala-Arg-His-Ser-Ala-Phe-Tyr-Asn-
HGH: -Thr-Tyr-Ser-Lys-Phe-Asp-Thr-Asn-Ser-His-Asn-Asp-Asp-Ala-Leu-Leu-Lys-Asn-( )-Tyr-Gly-
HCS: -Thr-Tyr-Ser-Lys-Phe-Asp-Thr-Asn-Ser-His-Asn-His-Asp-Ala-Leu-Leu-Lys-Asn-( )-Tyr-Gly-
LTH: -Leu-Leu-His-Cys-Leu-Arg-Arg-Asp-Ser-Ser-Lys- Ile-Asp-Thr-Tyr-Leu-Lys-Leu-Leu-Asn-
HGH: -Leu-Leu-Tyr-Cys-Phe-Arg-Lys-Asp-Met-Asp-Lys-Val-Glu-Thr-Phe-Leu-Arg- Ile-Val-Gln-
HCS: -Leu-Leu-Tyr-Cys-Phe-Arg-Lys-Asp-Met-Asp-Lys-Val-Glu-Thr-Phe-Leu-Arg-Met-Val-Gln-
LTH: -Cys-Arg- Ile- Ile-Tyr-Asn-Asn-Asn-Cys-COOH
HGH: -Cys-Arg-Ser-Val-( )-Glu-Gly-Ser-Cys-Gly-Phe-COOH
HCS: -Cys-Arg-Ser-Val-( )-Glu-Gly-Ser-Cys-Gly-Phe-COOH

be seen by aligning Asp_{41} of LTH with Glu_{39} of HGH and extending the LTH sequence for 5 residues to Ala_{45}. 4 residues are then dropped from the LTH sequence and the alignement is continued by pairing Phe_{50} of LTH with Phe_{44} of HGH. By including 3 gaps, this alignment may be extended to Leu_{94} of LTH and Val_{89} of HGH. This entire region (52 positions) contains 17 identical, 20 highly acceptable, 7 acceptable and 8 unacceptable replacements, the total degree of homology being 85%. The third major region is in the carboxyl terminal area of the molecules with the alignement beginning at Gln_{156} of LTH and Asn_{148} of HGH and extending 45 residue positions (including 2 gaps) to the carboxyl terminal Cys_{198} of LTH and the nonterminal Cys_{188} of HGH. These 45 positions include 14 identical, 14 highly acceptable and 8 unacceptable replacements, with a total degree of homology of 78%.

The area of the HGH sequence bounded by positions 91–147 shows only 3 short sections with homology to LTH. These 3 regions, tabulated separately in the Table, contain a total of 17 positions, with 7 identical, 4 highly acceptable, 3 acceptable and 3 unacceptable replacements.

The total of all the alignments between HGH and LTH shown in the Figure is: 51 identical residues, 46 highly acceptable, 18 acceptable and 29 unacceptable replacements. Thus, there are 115 homologous positions or about 60% of either polypeptide chain. A comparison between HCS and LTH gives very similar results, including 53 identical residues, 42 highly acceptable, 18 acceptable and 30 unacceptable replacements, with a total homology of 113 positions or again about 60% of either chain.

Residue positions common to all 3 hormones. In the Figure an asterisk has been placed above those residue positions which contain the same amino acid in all 3 structures. There are 50 such positions. These common positions would seem to be more or less randomly distributed, there being no particular area(s) in which they are clearly concentrated. They are also about equally distributed between hydrophilic and hydrophobic residues. However, in terms of the total content in all 3 proteins, some residue types appear in the common positions much more than others. For example, with the exception of the small disulfide ring near the amino terminal of LTH, each half-cysteine residue is homologous with a corresponding half-cysteine in the other 2 molecules. Thus, 12 of the 14 cysteine residues, or 86% of the total cysteine content appears in one or another homologous position in the 3 hormones. Similarly, 75% of the tryptophan residues (1 in each sequence, or 3 out of a total content of 4) appear in a single homologous position. Other residues whose positions appear to be conserved by identity are: proline, 50%; leucine, 45%; tyrosine, 39%; histidine, 33%; phenylalanine, 30%; alanine and arginine, 27%; serine, aspartic acid and glycine, each 24%. Although glutamic acid, methionine, threonine, glutamine and lysine also occur in common positions in all 3 sequences, the percentages of their total contents, conserved in this fashion are well below 25%.

Concluding remarks. While the similarities in the physiological and immunochemical properties of HGH and HCS are entirely consistent with the remarkable degree of homology in their 2 primary structures, the fact that there are so few differences between the two should help to localize the source of their disparity in growth promoting activity, to a relatively few but important residue positions. In the near future, additional comparisons with growth hormones from other species will undoubtedly further resolve this point. It might also be expected that those areas of HGH and HCS which show good homology with LTH contain one or more structural features which are intrinsically required for lactogenic activity. Of course, some or all of these same features may also be required for growth hormone activity, and we are not proposing that these 2 activities necessarily reside in mutually exclusive portions of the molecules.

Recent comparisons of these 3 proteins by means of circular dichroism studies[14,15] indicate that their secondary structures are all very similar, with an α-helix content of about 50%. These results are also in complete accord with the homologies described herein. Differences in the circular dichroism spectra of these molecules in the region of side chain absorption, however, cannot be fully explained by the one dimensional similarities seen in their sequences. More detailed knowledge of the three dimensional structure, particularly around the tryptophan and tyrosine residues will be required to answer this question.

Finally, we would like to remark on the general usefulness of this kind of investigation as an adjunct to sequence work. In our original comparison[11] of the sequences of HGH and LTH, a portion of the HGH sequence, containing 13 amino acids (plus 1 gap) had to be re-positioned in order to demonstrate the homology between the single tryptophan in HGH with Trp_{90} of LTH. A recent re-investigation of the HGH sequence showed that a sequence of 15 amino acids, including these same 13 residues plus a dipeptide (Leu-Arg), not appearing in the original HGH sequence had indeed been misplaced. In the revised HGH sequence[8], these 13 residues are found in precisely the positions indicated in our earlier study[11] of the sequence homology of these 2 hormones[17].

Zusammenfassung. Ein Vergleich der Aminosäuresequenzen von menschlichen Wachstumshormonen und menschlichem Chorion-Somatomammotropin mit denjenigen von Prolactin zeigt, dass 26% des Rückstandes in allen drei Molekülen die gleiche Position hatten mit zusätzlichen 34% als möglichen Ersatz. Alle drei Hormone scheinen einen gleichen Ursprung zu haben.

THOMAS A. BEWLEY and CHOH HAO LI

Hormone Research Laboratory, 1088 HSW, University of California, San Francisco (California 94122, USA), 10 May 1971.

[14] T. A. BEWLEY and C. H. LI, Arch. Biochem. Biophys. *144*, 589 (1971).

[15] T. A. BEWLEY and C. H. LI, Biochemistry, in press (1971).

[16] C. H. LI, J. S. DIXON, T.-B. LO, K. D. SCHMIDT and Y. A. PANKOV, Arch. Biochem. Biophys. *141*, 705 (1970).

[17] Acknowledgment. This work was supported in part by the American Cancer Society, the United States Public Health Service, the Allen Foundation and the Geffen Foundation of New York.

←

Comparison of the structures of HGH, HCS and LTH. The residue position numbers for HGH and HCS are the same, and appear below the HCS sequence. The residue position numbers for LTH appear above the LTH sequence. Homology is indicated by: identical pairs, vertical bar; highly acceptable replacements, 3 dots, and acceptable replacements, single dot. Unacceptable replacements are indicated by X. An asterisk has been placed over those residues which are commonto all 3 proteins.