Effect of estrogens and gibberellic acid (GA_3) on the growth of seedlings and cytokinin activity in extracts from 100 g of pea tissues

	Value in	Control	Estrone	Estradiol	Estriol	GA_3
Height[a] of pea seedlings	(mm)	70.8	99.6	100.4	98.1	98.7
	(%)	100.00	140.67	141.80	138.55	139.40
Yield of fresh tobacco tissue	(g/flask)	0.26	0.55	0.46	0.35	0.28
	(%)	100.00	211.53	176.92	134.61	107.69
Kinetin controls (μg/l) 0–0.12[b]						
1–0.35						
5–0.76						
25–3.16						

[a] 96 h after application. [b] Yield of fresh tobacco tissue – (g/flask).

pea seedlings deprived of cotyledons 24, 48, 72 (AbA) and 96 h (cytokinins, AbA) after application.

AbA-like substances were extracted and fractionated according to the method described by RUDNICKI[16]. TLC with benzene-acetone-acetic acid (70:30:1) as solvent was used. The abscisic acid zone (Rf 0.35–0.45) was then rechromatographed on Whatman No. 3 paper in redistilled water. The content of inhibitor was estimated by the wheat section straight growth test[17]. Growth inhibition was expressed in activity units. As activity unit 10% growth inhibition of the test plants in relation to control was taken. Cytokinins were determined according to HEIDE and SKOOG method[18]. A cation exchange column (250 ml, Dowex 50W-X4 H^+ 50–100 mesh) was used for separation. Cytokinin activity was measured by the tobacco callus bio-assay[19].

Results and discussion. The increase of 40% in the growth of the seedlings treated with estrogens and GA_3 was observed 96 h after application (Table). Thus the two kinds of different hormones had an identical physiological effect. Our results (Table, Figure) show the positive effect of estrogens on the endogenous cytokinins content and the lack of the influence on the level of AbA-like substance. On the other hand, however, gibberellic acid, while showing the same final physiological effect, lowered the AbA amounts and did not change the cytokinins content. So it seems possible that estrogens and gibberellins effect the plant metabolism in various ways. The results obtained confirm also a relationship between estrogens and other plant hormones in regulating the growth and development processes in plants. The previous[12,13] and the present papers show that estrogenic hormones influence the content of auxins, gibberellins and cytokinins in plant tissues. This may be the cause of many important metabolic reactions. The investigations of the interrelations between steroidal hormones and other groups of active substance were carried out in order to detect the strict control of growth and development processes which may exist in plants through the combined action of several regulatory substances.

Zusammenfassung. Die mit Oestrogenen (0.1 μg pro Pflanze) und Gibberellinsäure (GA_3, 0,001 μg pro Pflanze) behandelten Erbsenkeimlinge zeigten nach 96 h ein um 40% stärkeres Längenwachstum als die Kontrollpflanzen. Die Oestrogene erhöhten in den Keimlingen den Gehalt an Cytokininen, übten jedoch keinen Einfluss auf den Abscisinsäure-Gehalt aus. Die Gibberellinsäure andererseits setzte den Gehalt an Abscisinsäure herab, ohne die Konzentration der Cytokinine zu verändern.

J. KOPCEWICZ and J. H. ROGOZINSKA

Department of Plant Physiology, Institute of Biology, Copernicus University, Sienkiewicza 30/32, Torun (Poland); and
Laboratory of Plant Physiology, College of Agriculture, Bydgoszcz (Poland), 4 April 1972.

[16] R. RUDNICKI, Planta *86*, 63 (1969).
[17] J. P. NITSCH, *The Chemistry and Mode of Action of Plant Growth Substances* (Eds. R. L. WAIN and F. WIGHTMAN; Butterworths, London 1956).
[18] O. M. HEIDE and F. SKOOG, Physiologia Pl. *20*, 771 (1967).
[19] J. H. ROGOZINSKA, J. P. HELGESON, F. SKOOG, S. H. LIPTON and F. M. STRONG, Plant. Physiol. *40*, 469 (1965).

STUDIORUM PROGRESSUS

A Generalized Homology Correlation for Various Hormones and Proteins

An important archetypal connection between glucagon and secretin has been demonstrated and, in view of the disparities in both function and formation site for these two hormones, it was suggested that a search be instigated for other, less obvious genetic and biological relationships[1]. Along these lines, a computer alignment was then made between the above pair and pituitary and placental lactogen hormones[2]. The results were interesting, but inconclusive, since the complete sequences of the latter proteins were not known at the time. Recently, a different approach, based on glandular origin, was used to rank various enterosecretory proteins[3]. Here the original glucagon-secretin correlation was extended to include portions of two growth hormones. The relationships seemed probable, yet suffered from the use of erroneous primary sequences and the restriction of homology to short, selected regions. In view of continual interest in this area, a new treatment differing in various details, positioning points, and area of coverage is now presented in the Table.

Some comments are necessary on the specific primary structures selected for the various compounds. In rough

	1			5				10				15					20						25					30
EGRO		Leu	Lys	Cys	Arg	Ile	Val	Tyr	Asp	Asn	Cys	Ser																
OLAC	Thr	Pro	Val	Cys	Pro	Asn	Gly	Pro	Gly	Asp	Cys	Gln	Val	Ser	Leu	Arg	Asp	Leu	Phe	Asp	Arg	Ala	Val	Met	Val	Ser	His	Tyr	Ile	His
PPSN									Leu	Val	Lys	-	Val	Pro	Leu	Val	Arg	Lys	Lys	Ser	Leu	Arg	Gln	Asn	Leu	Ile	Lys	Asp	Gly	Lys
BPSN									Ser	Val	Lys	Leu	Ile	Pro	Val	Val	Lys	Lys	Lys	Ser	Leu	Arg	Gln	Asn	Leu	Ile	Glu	Asn	Gly	Lys
BREN							Ala	Glu	Ile	Thr	Arg	-	Ile	Pro	Leu	Tyr	Lys	Gly	Lys	Ser	Leu	Arg	Lys	Ala	Leu	Lys	Glx	His	Gly	Leu
HCAL																									Cys	Gly	Asn	Leu	Ser	Thr
PCAL																									Cys	Ser	Asn	Leu	Ser	Thr
SCAL																									Cys	Ser	Asn	Leu	Ser	Thr
PCTP																							Ser	Tyr	Ser	Met	Glu	His	Phe	Arg
MNGF																				Ser	Ser	Thr	His	Pro	-	Val	Phe	His	Met	Gly
HPIN																		Phe	Val	Asp	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val
PPIN																		Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val
BPIN																		Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val
RPIN-1																		Phe	Val	Lys	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val
RPIN-2																		Phe	Val	Lys	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val
AFIN																	Val	Ala	Pro	Ala	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val
BPTH														Ala	Val	Ser	Glu	Ile	Gln	Phe	Met	His	Asn	Leu	Gly	Lys	His	Leu	Ser
OGRO									Ala	Phe	Pro	Ala	Met	Ser	Leu	Ser	Gly	Leu	Phe	Ala	Asn	Ala	Val	Leu	Arg	Ala	Gln	His	Leu	His
BGRO									Ala	Phe	Pro	Ala	Met	Ser	Leu	Ser	Gly	Leu	Phe	Ala	Asn	Ala	Val	Leu	Arg	Ala	Gln	His	Leu	His
PGRO										Phe	Pro	Ala	Met	Pro	Leu															
HGRO										Phe	Pro	Thr	Ile	Pro	Leu	Ser	Arg	Leu	Phe	Asp	Asn	Ala	Met	Leu	Arg	Ala	His	Arg	Leu	His
HPLA										Val	Gln	Thr	Val	Pro	Leu	Ser	Arg	Leu	Phe	Asp	His	Ala	Met	Leu	Gln	Ala	His	Arg	Ala Asn	His
BBTα																											Phe	Pro	Asp	Gly
HCGT-α																														
HLUT-α																														
HCGT-β														Ser	Lys	Glx	Pro	Leu	Arg	Pro	Arg	Cys	Arg	Pro	Ile	Asn	Ala	Thr	Leu	-
BLUT-β														Ser	Arg	Gly	Pro	Leu	Arg	Pro	Leu	Cys	Glu	Pro	Ile	Asn	Ala	Thr	Leu	-
PLUT-β														Ser	Arg	Gly	Pro	Leu	Arg	Pro	Leu	Cys	Glx	Pro	Ile	Asn	Ala	Thr	Leu	Arg
BTTβ																					Phe	Cys	Ile	Pro	Thr	Glu	Tyr	Met	Met	-
PCPZ																	Lys	Ala	Pro	Ser	Gly	Arg	Val	Ser	Met	Ile	Lys	Asn	Leu
FCRN																														
CGAS																														
PGAS																														
OGAS																														
HGAS																														
PMOT																									Phe	Val	Pro	Ile	Phe	Thr
PVIP					His	Ser					Phe						Arg													
PSEC					His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Glu	Leu	Ser	Arg	Leu	Arg	Asp	Ser	Ala	Arg	Leu	Gln	Arg	Leu	Leu	Gln	Gly
PGLU					His	Ser	Gln	Gly	Thr	Phe	Thr	Ser	Asp	Tyr	Ser	Lys	Tyr	Leu	Asp	Ser	Arg	Arg	Ala	Gln	Asp	Phe	Val	Gln	Trp
PGIP					Tyr	Ala	Glu	Gly	Thr	Phe	Ile	Ser	Asp	Tyr	Ser	Ile	Ala	Met	Asp	Lys	Ile	Arg	Gln	Gln	Asp	Phe	Val	Asn	Trp

order, the data for equine growth hormone (EGRO)[4], ovine lactogen (OLAC)[5], porcine pepsinogen (PPSN)[6,7], bovine pepsinogen (BPSN)[8], bovine rennin (BREN)[9,10], human, porcine, and salmon calcitonin (HCAL, PCAL, SCAL)[11–15], porcine β-corticotropin (PCTP)[16], mouse nerve growth factor (MNGF)[17], human, porcine, bovine, and rat proinsulin (HPIN, PPIN, BPIN, RPIN-1, RPIN-2)[18–23], angler fish insulin (AFIN)[24], bovine parathyroid hormone (BPTH)[25], ovine, bovine, porcine, and human growth hormone (OGRO, BGRO, PGRO, HGRO)[26–38], human placental lactogen (HPLA)[39], bovine α- and β-thyrotropin (BTTα, BTTβ)[40], ovine α- and β-luteinizing hormone[41–43], human α- and β-chorionic gonadotropin (HCGT-α and -β)[44], human α-luteinizing hormone (HLUT-α)[45,47], bovine β-luteinizing hormone (BLUT-β)[48], porcine cholecystokinin-pancreozymin (PCPZ)[49], frog caerulein (FCRN)[50], canine, porcine, ovine, and human gastrin (CGAS, PGAS, OGAS, HGAS)[51–54], porcine motilin (PMOT)[55,56], porcine vasoactive intestinal polypeptide (PVIP)[57], porcine secretin (PSEC)[58], porcine glucagon (PGLU)[59], and porcine gastric inhibitory peptide (PGIP)[60] are taken from the literature.

Bovine prolactin differs from ovine prolactin by having alanine at position 112 and a tyrosine at 151[61,62], while bovine growth hormone is known to possess a leucine-valine interchange at 141[63,64]. Bovine α-luteinizing hormone is apparently identical to bovine α-thyrotropin[65,66], and a similarity has been discussed for the ovine compound, too[67]. The ovine hormone differs only at residue 3; however, evidence has been presented for some allele substitutions in the bovine hormone present at amino acids 106, 109, and 115[68,69]. Bovine β-luteinizing hormone seems the same as the ovine hormone, yet a few residues remain uncertain at the present time. Another interesting

1 B. WEINSTEIN, Experientia *24*, 406 (1968).
2 M. O. DAYHOFF, *Atlas of Protein Structure and Sequence* (National Biomedical Research Foundation, Silver Spring 1969), p. D-227.
3 J. W. ADELSON, Nature, Lond. *229*, 321 (1971).
4 L. OLIVER and A. S. HARTREE, *Protein and Polypeptide Hormones* (Excerpta Medica Foundation, Amsterdam 1969), p. 505.
5 C. H. LI, J. S. DIXON, T.-B. LO, K. D. SCHMIDT and Y. A. PANKOV, Archs Biochem. Biophys. *141*, 705 (1970).
6 P. V. KOEHN, and G. E. PERLMANN, J. biol. Chem. *24*, 6104 (1968).
7 B. KEIL, Phil. Trans. R. Soc. London *B 257*, 125 (1970).
8 B. KASSELL, J. KAY and J. P. MARCINISZYN, JR., Third American Peptide Meeting (1972).
9 B. FOLTMANN, Phil. Trans. R. Soc. London *B 257*, 147 (1970).
10 B. FOLTMANN, Abstracts, Seventh FEBS Meeting (1971).
11 R. NEHER, B. RINIKER, H. ZUBER, W. RITTEL and F. W. KAHNT, Helv. chim. Acta *51*, 917 (1968).
12 J. T. POTTS, H. D. NIALL, H. T. KEUTMANN, H. B. BREWER and L. J. DEFTOS, Proc. natn. Acad. Sci., USA *59*, 1321 (1968).
13 P. H. BELL, W. F. BARG, D. F. COLUCCI, M. C. DAVIES, C. DZIOBKOWSKI, M. E. ENGLERT, E. HEYDER, R. PAUL and E. H. SNEDEKER, J. Am. chem. Soc. *90*, 2704 (1968).
14 R. NEHER, B. RINIKER, W. RITTEL and H. ZUBER, Helv. chim. Acta *51*, 1900 (1968).

	31			35					40					45					50					55					60
OLAC	Asn	Leu	Ser	Ser	Glu	Met	Phe	Asn	Glu	Phe	Asp	Lys	Arg	Tyr	-	Ala	Gln	Gly	Lys	Gly	Phe	-	-	Ile	Thr	Met	Ala	Leu	Asn	Ser
PPSN	Leu	Lys	Asp	Phe	Leu	Lys	Thr	His	Lys	-	His	Asn	Pro	Ala	Ser	Lys	Tyr	Phe	-	-	Pro	Ala	Glu	Ile	Gly	Asp	Glu	Pro	Leu	Glu
BPSN	Leu	Lys	Glu	Phe	Leu	Lys	Thr	His	Lys	Val	Arg	Asn	Met	Gly	Ser	Lys	Tyr	Leu	Ile	Arg	Glu	Ala	Ala	Thr	Leu	Ser	Val			
BREN	Leu	Glu	Asp	Phe	Leu	Lys																Gly	Glu	Val	Ala	Ser	Val	Pro	Leu	Thr
HCAL	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro				
PCAL	Cys	Val	Leu	Ser	Ala	Tyr	Trp	Arg	Asn	Leu	Asn	Asn	Phe	His	Arg	Phe	Ser	Gly	Met	Gly	Phe	Gly	Pro	Glu	Thr	Pro				
SCAL	Cys	Val	Leu	Gly	Lys	Leu	Ser	Gln	Glu	Leu	His	Lys	Leu	Gln	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro				
PCTP	Trp	Gly	Lys	Pro	Val	Gly	Lys	Lys	Arg	Arg	Pro	Val	Lys	Val	Tyr	Pro	Asn	Gly	Ala	Glu	Asp	Glu	Leu	Ala	Glu	Ala	Phe	Pro	Leu	Glu
MNGF	Glu	Phe	Ser	-	-	Val	Cys	Asp	Ser	Val	Ser	Val	Trp	Val	Gly	Asp	Lys	Thr	Thr	Ala	Thr	Asn	Ile	Lys	Gly	Lys	Glu	Val	Thr	Val
HPIN	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Thr	Arg	Arg	Glu	Ala	Glu	Asp	Leu	Gln	Val	Gly	Gln	Val
PPIN	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala	Arg	Arg	Glu	Val	Glu	Gly	Pro	Gln	Val	Gly	Ala	Leu
BPIN	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala	Arg	Arg	Glu	Ala	Glu	Asn	Pro	Gln	Ala	Gly	Ala	Leu
RPIN-1	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ser	Arg	Arg	Glu	Val	Glu	Asp	Pro	Gln	Val	Pro	Gln	Leu
RPIN-2	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Met	Ser	Arg	Arg	Glu	Val	Glu	Asp	Pro	Gln	Val	Ala	Gln	Leu
AFIN	Asp	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Asp	Arg	Gly	Phe	Phe	Tyr	Asn	Pro	Lys													
BPTH	Ser	Met	Glu	Arg	Val	Glu	Trp	Leu	Arg	Lys	Lys	Leu	Gln	Asp	Val	His	Asn	Phe	Val	Ala	Leu	Gly	Ala	Ser	Ile	Ala	Tyr	Arg	Asp	Gly
OGRO	Gln	Leu	Ala	Ala	Asp	Thr	Phe	Lys	Glu	Phe	Glu	Arg	Thr	Tyr	Ile	Pro	Glu	Gly	Gln	Arg	Tyr	Ser	-	Ile	Gln	Asn	Thr	Gln	Val	Ala
BGRO	Gln	Leu	Ala	Ala	Asp	Thr	Phe	Lys	Glu	Phe	Glu	Arg	Thr	Tyr	Ile	Pro	Glu	Gly	Gln	Arg	Tyr	Ser	-	Ile	Gln	Asp	Thr	Gln	Val	Ala
HGRO	Gln	Leu	Ala	Phe	Asp	Thr	Tyr	Gln	Glu	Phe	Glu	Glu	Ala	Tyr	Ile	Pro	Lys	Glu	Gln	Lys	Tyr	Ser	Phe	Leu	Gln	Asp	Pro	Glu	Thr	Ser
HPLA	Gln	Leu	Ala	Ile	Asp	Thr	Tyr	Gln	Glu	Phe	Glu	Glu	Thr	Tyr	Ile	Pro	Lys	Asp	Gln	Lys	Tyr	Ser	Phe	Leu	His	Asp	Ser	Glx	Thr	Ser
BTTα	Glu	Phe	Thr	Met	Gln	Gly	Cys	Pro	Gln	Cys	Lys	Leu	Lys	Glu	Asn	Lys	Tyr	Phe	Ser	Lys	Pro	Asp	Ala	Pro	Ile	Tyr	Gln	Cys	Met	Gly
HCGT-α	Ala	Pro	Asx	Val	Glx	Asx	Cys	Pro	Glx	Cys	Thr	Leu	Glx	Glx	Asx	Pro	Phe	Phe	Ser	Glx	Pro	Gly	Ala	Pro	Ile	Leu	Glx	Cys	Met	Gly
HLUT-α				Val	Gln	Asp	Cys	Pro	Glu	Cys	Thr	Leu	Gln	Glu	Asn	Pro	Phe	Phe	Ser	Gln	Pro	Gly	Ala	Pro	Ile	Leu	Gln	Cys	Met	Gly
HCGT-β	Ala	Val	Glx	Lys	Glx	Gly	Cys	Pro	Val	Cys	Ile	Asn	-	-	-	-	Val	-	Thr	Thr	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Thr	Met	Thr
BLUT-β	Ala	Ala	Glu	Lys	Glu	Ala	Cys	Pro	Val	Cys	Ile	Thr	-	-	-	-	Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Ser	Met	Lys
PLUT-β	Ala	Ala	Glx	Asx	Glx	Ala	Cys	Pro	Val	Cys	Ile	Thr	-	-	-	-	Phe	Thr	Thr	Ser	Ile	Cys	Ala	Gly	Tyr	Cys	Pro	Ser	Met	Arg
B8Tβ	His	Val	Glu	Arg	Lys	Glu	Cys	Ala	Tyr	Cys	Leu	Thr	-	-	-	-	Ile	Asn	Thr	Thr	Val	Cys	Ala	Gly	Tyr	Cys	Met	Thr	Arg	Asx
PCPZ	Gln	Ser	Leu	Asp	Pro	Ser	His	Arg	Ile	Ser	Asp	Arg	Asp	Tyr	Met	Gly	Trp	Met	Asp	Phe										
FCRN										<Glu	Gln	Asp	Tyr	Thr	Gly	Trp	Met	Asp	Phe										
CGAS			Glu	Gly	Pro	Trp	Met	Glu	Glu	Ala	Glu	Glu	Ala	Tyr	-	Gly	Trp	Met	Asp	Phe										
PGAS			Glu	Gly	Pro	Trp	Met	Glu	Glu	Glu	Glu	Glu	Ala	Tyr	-	Gly	Trp	Met	Asp	Phe										
OGAS			Glu	Gly	Pro	Trp	Val	Glu	Glu	Glu	Glu	Ala	Ala	Tyr	-	Gly	Trp	Met	Asp	Phe										
HGAS			Glu	Gly	Pro	Trp	Leu	Glu	Glu	Glu	Glu	Glu	Ala	Tyr	-	Gly	Trp	Met	Asp	Phe										
PMOT	Tyr	Gly	Glu	Leu	Gln	Arg	Met	Glu	Glu	Lys	Glu	Arg	Asn	Lys	Gly	Gln														
PVIP	Leu																													
PSEC	Leu	Val																												
PGLU	Leu	Met	Asn	Thr																										
PGIP	Leu	Leu	Ala	Gln	Gln	Lys	Gly	Lys	Lys	Ser	Asp	Trp	Lys	His	Asn	Ile	Thr	Gln												

[15] H. D. Niall, H. T. Keutmann, D. H. Copp and J. T. Potts, Proc. natn. Acad. Sci. ,USA *64*, 771 (1969).
[16] B. Riniker, P. Sieber, W. Rittel and H. Zuber, Nature, New Biol. *235*, 114 (1972).
[17] W. A. Frazier, R. H. Angeletti and R. A. Bradshaw, Science *176*, 482 (1972).
[18] P. E. Oyer, S. Cho, J. D. Peterson and D. F. Steiner, J. biol. Chem. *246*, 1375 (1971).
[19] A. S. C. Ko, D. G. Smith, J. Markussen and F. Sundby, Eur. J. Biochem. *20*, 190 (1971).
[20] R. E. Chance, R. M. Ellis and W. W. Bromer, Science *161*, 165 (1968).
[21] A. Salokangas, D. G. Smyth, J. Markussen and F. Sundby, Eur. J. Biochem. *20*, 183 (1971).
[22] D. F. Steiner, S. Cho, P. E. Oyer, S. Terris, J. D. Peterson and A. H. Rubenstein, J. biol. Chem. *246*, 1365 (1971).
[23] J. Markussen and F. Sundby, Eur. J. Biochem. *25*, 153 (1972).
[24] P. Neumann, M. Koldenhof and R. E. Humbel, Z. physiol. Chem. *350*, 1286 (1969).
[25] H. B. Brewer, jr., and R. Ronan, Proc. natn. Acad. Sci., USA *67*, 1862 (1970).
[26] J. M. Dellacha, M. A. Enero, J. A. Santomé and A. C. Paladini, Eur. J. Biochem. *12*, 289 (1970).
[27] C. Peña, A. C. Paladini, J. M. Dellacha and J. A. Santomé, Eur. J. Biochem. *17*, 27 (1971).
[28] H. N. Fernandez, A. C. Paladini, J. M. Dellacha and J. A. Santomé, FEBS Lett. *17*, 17 (1971).
[29] C. H. Li, J. S. Dixon, D. Gordon and J. Knorr, Int. J. Peptide Protein Res. *4*, 151 (1972).
[30] R. E. Fellows, jr., personal communications, December 22, 1971, and June 16, 1972.
[31] J. T. Bellair, Biochem. biophys. Res. Commun. *46*, 1128 (1972).
[32] R. E. Fellows, jr. and A. D. Rogol, J. biol. Chem. *244*, 1575, (1969).
[33] J. A. Santomé, J. M. Dellacha, A. C. Paladini, C. E. M. Wolfenstein, C. Peña, E. Poskus, S. T. Daurat, M. J. Biscoglio, Z. M. M. de Sese and A. V. F. de Sanguesa, FEBS Lett. *16*, 198 (1971).
[34] M. Wallis, FEBS Lett. *21*, 118 (1972).
[35] N. Yamasaki, K. Kangawa, S. Kobayashi, M. Kikotani and M. Sonenberg, J. biol. Chem. *247*, 3874 (1972).
[36] J. B. Mills, S. C. Howard, S. Scapa and A. E. Wilhelmi, J. biol. Chem. *245*, 3407 (1970).
[37] H. Niall, Nature, New Biol. *230*, 90 (1971).
[38] C. H. Li and J. S. Dixon, Archs Biochem. Biophys. *146*, 233 (1971).
[39] L. M. Sherwood, S. Handwerger, W. D. McLaurin and M. Lanner, Nature, New Biol. *233*, 59 (1971).
[40] T.-H. Liao and J. G. Pierce, J. biol. Chem. *246*, 850 (1971).
[41] H. Papkoff, M. R. Sairam and C. H. Li, J. Am. chem. Soc. *93*, 1531 (1971).
[42] W.-K. Liu, H. S. Nahm, C. M. Sweeney, W. M. Lamkin, H. N. Baker and D. N. Ward, J. biol. Chem. *247*, 4351 (1972).
[43] W.-K. Liu, H. S. Nahm, C. M. Sweeney, G. N. Holcomb and D. M. Ward, J. biol. Chem. *247*, 4365 (1972).
[44] O. P. Bahl, R. B. Carlsen, R. Bellisario and N. Swaminathan, Biochem. biophys. Res. Commun. *48*, 416 (1972).
[45] J. Closset, G. Hennen and R. M. Lequin, FEBS Lett. *21*, 325 (1972).
[46] T. Inagami, K. Murakami, D. Puett, A. S. Hartree and A. Nureddin, Biochem. J. *126*, 441 (1972).
[47] M. R. Sairam, H. Papkoff and C. H. Li, Biochem. biophys. Res. Commun. *48*, 530 (1972).
[48] G. Maghuin-Rogister and A. Dockier, FEBS Lett. *19*, 209 (1971).
[49] V. Mutt and E. Jorpes, Biochem. J. *125*, 57 P (1972).
[50] A. Anastasi, V. Erspamer and R. Endean, Archs Biochem. Biophys. *125*, 57 (1968).
[51] K. L. Agarwal, G. W. Kenner and R. C. Sheppard, Experientia *25*, 346 (1969).

	61			65				70				75				80				85				90
OLAC	-	Cys	His	Thr	Ser	Ser	Leu	Pro	Thr	Pro	Glu	Asp	Lys	Glu	Gln	Ala	Gln	Gln	Thr	His	His	Glu	Val	Leu	Met	Ser	Leu	Ile	Leu	Gly
PPSN	Asn	Tyr	Leu	Asx	Thr	Glu	Tyr	Phe																						
BREN	Asn	Tyr	Leu	Asx	Ser	Glx	Tyr	Phe	Gly	Lys	Ile	Thr																		
PCTP	Phe																													
MNGF	Leu	Ala	Glu	Val	Asn	Ile	Asn	Asn	Ser	Val	Phe	Arg	Gln	Tyr	Phe	Phe	Glu	Thr	Lys	Cys	Arg	Ala	Ser	Asn	Pro	Val	Glu	Ser	Gly	Cys
HPIN	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Ser	Leu	Gln	Pro	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys	Arg	Gly	Ile	Val	Gly	Gln	Cys	Cys
PPIN	Glu	Leu	Gly	Gly	Gly	Leu	Gly	-	-	Gly	Leu	Gln	Ala	Leu	Ala	Leu	Glu	Gly	Pro	Pro	Gln	Lys	Arg	Gly	Ile	Val	Glu	Gln	Cys	Cys
BPIN	Glu	Leu	Ala	Gly	Gly	Pro	Gly	Ala	Gly	Gly	-	-	-	-	-	Leu	Glu	Gly	Pro	Pro	Gln	Lys	Arg	Gly	Ile	Val	Glu	Gln	Cys	Cys
RPIN-1	Glu	Leu	Gly	Gly	Gly	Pro	Glu	Ala	Asp	Gly	Leu	Gln	Thr	Leu	Ala	Leu	Glu	Val	Ala	Arg	Gln	Lys	Arg	Gly	Ile	Val	Asp	Gln	Cys	Cys
RPIN-2	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Asp	Gly	Leu	Gln	Thr	Leu	Ala	Leu	Glu	Val	Ala	Arg	Gln	Lys	Arg	Gly	Ile	Val	Asp	Gln	Cys	Cys
AFIN																								Gly	Ile	Val	Glu	Gln	Cys	Cys
BPTH	Ser	Ser	Gln	Arg	Pro	Arg	Lys	Lys	Glu	Asp	Asn	Val	Leu	Val	Glu	Ser	His	Gln	Lys	Ser	Leu	Gly	Glu	Ala	Asp	Lys	Ala	Asp	Val	Asp
OGRO	Phe	Cys	Phe	Ser	Glu	Thr	Ile	Pro	Ala	Pro	Thr	Gly	Lys	Asn	Glu	Ala	Gln	Gln	Lys	Ser	Asp	Leu	Glu	Leu	Leu	Arg	Ile	Ser	Leu	Leu
BGRO	Phe	Cys	Phe	Ser	Glu	Thr	Ile	Pro	Ala	Pro	Thr	Gly	Lys	Asn	Glu	Ala	Gln	Glu	Lys	Ser	Asp	Leu	Glu	Leu	Leu	Arg	Ile	Ser	Leu	Leu
HGRO	Leu	Cys	Phe	Ser	Glu	Ser	Ile	Pro	Thr	Pro	Ser	Asn	Arg	Glu	Glu	Thr	Gln	-	Lys	Ser	Asn	Leu	Gln	Leu	Leu	Arg	Ile	Ser	Leu	Leu
HPLA	Phe	Cys	Phe	Ser	Asx	Ser	Thr	Pro	Thr	Pro	Ser	Asx	Met	Glx	Glx	Thr	Glx	-	Lys	Ser	Asx	Leu	Glx	Leu	Leu	Arg	Ile	Ser	Leu	Leu
BBTα	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	Thr	Pro	Ala	Arg	Ser	Lys	Lys	Thr	Met	Leu	Val	Pro	Lys	Asn	Ile	Thr	Ser	Glu	Ala	Thr	Cys	Cys
HCGT-α	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	Thr	Pro	Leu	Arg	Ser	Lys	Lys	Thr	Met	Leu	Val	Glx	Lys	Asn	Val	Thr	Ser	Glx	Ser	Thr	Cys	Cys
HLUT-α	Cys	Cys	Phe	Ser	Arg	Ala	Tyr	Pro	Thr	Pro	Leu	Arg	Ser	Lys	Lys	Thr	Met	Leu	Val	Gln	Lys	Asn	Val	Thr	Ser	Glx	Ser	Thr	Cys	Cys
HCGT-β	Arg	Val	Leu	Glx	Gly	Val	Leu	Pro	Ala	Leu	Pro	Glx	Leu	-	-	Val	Cys	Asx	Tyr	Arg	Asx	Val	Arg	Phe	Glx	Ser	Ile	Arg	Leu	Pro
BLUT-β	Arg	Val	Leu	Pro	Val	Ile	Leu	Pro	Pro	Met	Pro	Glx	Arg	-	-	Val	Cys	Thr	Tyr	His	Glu	Leu	Arg	Phe	Ala	Ser	Val	Arg	Leu	Pro
PLUT-β	Arg	Val	Leu	Pro	Ala	Ala	Leu	Pro	Pro	Val	Pro	Glx	-	-	Pro	Val	Cys	Thr	Tyr	Arg	Glu	Leu	Ile	Phe	Ala	Ser	Ser	Arg	Leu	Pro
BSTβ	Val	Asx	Gly	Lys	Leu	Phe	Leu	Pro	Lys	Tyr	Ala	Leu	Ser	Gln	Asp	Val	Cys	Thr	Tyr	Arg	Asp	Phe	Met	Tyr	Lys	Thr	Ala	Glu	Ile	Pro

retention of sequence occurs with glucagon, where the bovine and human forms are the same as the porcine material[70,71]. The different heterogenetic modifications are noted in the alignment framework, but the various duplicate sequences have been omitted at this time. The use of gaps was held to a minimum between completely different proteins; however, the device was employed judiciously within similar groups.

The homology pattern for the placental and growth hormones, such as EGRO, OLAC, OGRO, EGRO, PGRO, HGRO, and HPLA, is very obvious and has been discussed in part by other workers[72–74]. Attention is drawn to the point that the present Table is the first to include data from all of these compounds. Subsequently, several improvements are apparent at this time, which includes the detection of possible sequencing errors located at positions 93–94, 139–140, and 194–195 in the various growth compounds. One must note that the relationship of OLAC to this group of hormones in the 110–160 region is poor and may reflect major placement errors or even an incorrect primary sequence. However, the sequence of HCGT-α in this area is very helpful in partially locating a relationship with OLAC. In any event, it seems OLAC and EGRO represent older, more intact, proteins based on both their longer N-terminal sections and the high degree of individual residue changes. Indeed, the present resemblance between the two proteins at the N-terminal region suggests EGRO may be the equivalent ELAC, rather than the presumed horse growth hormone. Of interest is the degree of commonality displayed in the 12–25 region between HGRO, PVIP, PSEC, PGLU, and PGIP, which implies a strong familial relationship. The completion of the sequence work on PGRO would no doubt serve to confirm these assumptions.

The gastrins are a well-known group of hormones and present no problems in terms of homology. The very recent disclosure of a structure for cholecystokinin-pancreozymin permits one to locate caerulein and the gastrins at the C-terminal sequence of PCPZ. The similarity at the 38–44 section for HGRO-HPLA and the various gastrins serves as a second reference point and ties them into the various growth hormones. Based on these arguments, a rather interesting fact emerges; namely, secretin terminates at position 32, vasoactive intestinal peptide stops at position 33, and glucagon ends at position 34, while the gastrins appear to commence in the same region. Such a break suggests the possible existence of a common zymogen, either past or present, which upon activation yields this family of biologically active hor-

[52] H. GREGORY, P. N. HARDY, D. S. JONES, G. W. KENNER and R. C. SHEPPARD, Nature, Lond. *204*, 931 (1964).
[53] K. L. AGARWAL, J. BEACHAM, P. H. BENTLEY, R. A. GREGORY, G. W. KENNER, R. C. SHEPPARD and H. J. TRACY, Nature, Lond. *219*, 614 (1968).
[54] P. H. BENTLEY, G. W. KENNER and R. C. SHEPPARD, Nature, Lond. *209*, 583 (1966).
[55] J. C. BROWN, M. A. COOK and J. R. DRYBURGH, Gastroenterology *62*, 401 (1972).
[56] M. A. COOK, Ph. D. Thesis, University of British Columbia (1972).
[57] S. I. SAID and V. MUTT, Eur. J. Biochem. *28*, 199 (1972).
[58] V. MUTT and J. E. JORPES, 4th Int. Sympl. Chem. Nat. Products, Stockholm 1966.
[59] W. W. BROMER, L. G. SINN and O. K. BEHRENS, J. Am. chem. Soc. *79*, 2807 (1957).
[60] J. C. BROWN and J. R. DRYBURGH, Can. J. Biochem. *49*, 867 (1971).
[61] B. K. SEAVEY and V. J. LEWIS, Biochem. biophys. Res. Commun. *42*, 905 (1971).
[62] M. WALLIS, personal communication, March 3, 1972.
[63] B. K. SEAVEY, R. N. P. SINGH, V. J. LEWIS and I. I. GESCHWIND, Biochem. biophys. Res. Commun. *43*, 189 (1971).
[64] H. N. FERNÁNDEZ, S. T. DAURAT, C. PEÑA, J. M. DELLACHA, J. A. SANTOMÉ and A. C. PALADINI, FEBS Lett. *18*, 53 (1971).
[65] J. G. PIERCE, T.-H. LIAO, R. B. CARLSEN and T. REIMO, J. biol. Chem. *246*, 866 (1971).
[66] D. N. WARD, L. E. PEICHERT, JR., W.-K. LIU, H. S. NAHM and W. M. LAMKIN, *Gonadotropins* (John Wiley, New York 1972), in press.
[67] C. TERTRIN-CLARY, P. DE LA LLOSA, M. HURAULT, C. COURTE and M. JUTISZ, Biochim. biophys. Acta *263*, 115 (1972).
[68] G. MAGHUIN-ROGISTER, J. CLOSSET and G. HENNEN, FEBS Lett. *13*, 301 (1971).
[69] G. MAGHUIN-ROGISTER and G. HENNEN, Eur. J. Biochem. *21*, 429 (1971).
[70] W. W. BROMER, M. E. BOUCHER and J. E. KOFFENBERGER, JR., J. biol. Chem. *246*, 2822 (1971).
[71] J. THOMSEN, K. KRISTIANSEN, K. BRUNFELDT and K. SUNDBY, FEBS Lett. *21*, 315 (1972).
[72] S. M. ALOJ and H. EDELHOCH, Proc. natn. Acad. Sci., USA *66*, 830 (1970).
[73] H. D. NIALL, M. L. HOGAN, R. SAUER, I. Y. ROSENBLUM and F. C. GREENWOOD, Proc. natn. Acad. Sci., USA *68*, 866 (1971).
[74] T. A. BEWLEY and C. H. LI, Experientia *27*, 1368 (1971).

	91				95					100					105					110					115				120
OLAC	Leu	Arg	-	Ser	Trp	Asn	Asp	Pro	Leu	Tyr	His	Leu	Val	Thr	Glu	Val	Arg	Gly	Met	Lys	Gly	Val	Pro	Asp	Ala	Ile	Leu	Ser	Arg	Ala
MNGF	Arg	Gly	Ile	Asp	Ser	Lys	His	-	Trp	Asn	Ser	Tyr	Cys	Thr	Thr	Thr	Thr	His	Thr	Phe	Val	Lys	Ala	Leu	Thr	Thr	Asp	Glu	Lys	Gln	Ala
HPIN	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn																
PPIN	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn																
BPIN	Ala	Ser	Val	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn																
RPIN-1	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn																
RPIN-2	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn																
AFIN	His	Arg	Pro	Cys	Asn	Ile	Phe	Asp	Leu	Gln	Asn	Tyr	Cys	Asn																
BPTH	Val	Leu	Ile	Lys	Ala	Lys	Pro	Gln																						
OGRO	Leu	Ile	Gln	Ser	Trp	Leu	Gly	Pro	Leu	Gln	Phe	Leu	Ser	Arg	Val	Phe	Thr	Asp	Ser	Leu	Val	Phe	Gly	Thr	Ser	Asp	Arg	-	Val	Tyr
BGRO	Leu	Ile	Ser	Glu	Trp	Leu	Glx	Pro	-	Gly	Phe	Leu	Arg	-	Val	Phe	Thr	Asn	Ser	Leu	Val	Phe	Gly	Thr	Ser	Asp	Arg	-	Val	Tyr
HGRO	Leu	Ile	Gln	Ser	Trp	Leu	Glu	Pro	Val	Gln	Phe	Leu	Arg	Ser	Val	Phe	Ala	Asn	Ser	Leu	Val	Tyr	Gly	Ala	Ser	Asn	Ser	Asp	Val	Tyr
HPLA	Leu	Ile	Glx	Ser	Trp	Leu	Glx	Pro	Val	Arg	Phe	Leu	Arg	Ser	Met	Phe	Ala	Asx	Asx	Leu	Val	Tyr	Asx	Thr	Ser	Asx	Asx	Asx	Ser	Tyr
BTTα	Val	Ala	Lys	Ala	Phe	Thr	Lys	Ala	Thr	Val	Met	Gly	Asn	Val	Arg	Val	Glx	Asn	His	Thr	Glu	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His
HCGT-α	Val	Ala	Lys	Ser	Tyr	Asx	Arg	Val	Thr	Val	Met	Gly	Gly	Phe	Lys	Val	Glx	Asn	His	Thr	Ala	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His
HLUT-α	Val	Ala	Lys	Ser	Tyr	Asn	Arg	Val	Thr	Val	Met	Gly	Gly	Phe	Lys	Val	Glx	Asn	His	Thr	Ala	Cys	His	Cys	Ser	Thr	Cys	Tyr	Tyr	His
HCGT-β	Gly	Cys	Pro	Arg	Gly	Val	Asx	Pro	Val	Val	Ser	Tyr	Ala	Val	Ala	-	-	-	-	Leu	-	-	-	-	-	-	Cys	Arg	-	Ser
BLUT-β	Gly	Cys	Pro	Pro	Gly	Val	Asx	Pro	Met	Val	Ser	Phe	Pro	Val	Ala	-	-	-	-	Leu	Ser	Cys	His	Cys	Gly	Pro	Cys	Arg	Leu	Ser
PLUT-β	Gly	Cys	Pro	Pro	Gly	Val	Asx	Pro	Thr	Val	Ser	Phe	Pro	Val	Ala	-	-	-	-	Leu	Ser	Cys	His	Cys	Gly	Pro	Cys	Arg	Leu	Ser
BBTβ	Gly	Cys	Pro	Arg	His	Val	Thr	Pro	Tyr	Phe	Ser	Tyr	Pro	Val	Ala	-	-	-	-	Ile	Ser	Cys	Lys	Cys	Gly	Lys	Cys	Asx	Thr	Asx

	121				125					130					135					140					145				150
OLAC	Ile	Glu	Ile	Glu	Glu	Glu	Asn	Lys	Arg	Leu	Leu	Glu	Gly	Met	Glu	Met	Ile	Phe	Gly	Gln	Val	Ile	Pro	Gly	Ala	Lys	Glu	Thr	Glu	Pro
MNGF	Ala	Trp	Arg	Phe	Ile	Arg	Ile	Asn	Thr	Ala	Cys	Val	Cys	Val	Leu	Ser	Arg	Lys	Ala	Thr	Arg									
OGRO	-	-	-	-	Ile	Lys	Leu	Lys	Asp	Leu	Ile	Ile	Gly	Ile	Leu	Ala	Leu	Met	Arg	Glu	Leu	Glu	Asp	Val	Thr	Pro	Arg	Ala	Gly	Gln
BGRO	-	-	-	-	Glu	Lys	Leu	Lys	Asp	Leu	Glu	Glu	Gly	Ile	-	Ala	Leu	Met	Arg	Glu	Leu/Val	Glu	Asp	Gly	Thr	Pro	Arg	Ala	Gly	Gln
PGRO																		Met	Arg	Glu	Leu	Glu	Asp	Gly	Ser	Pro	Arg	Ala	Gly	Gln
HGRO	-	-	-	-	Asp	Leu	Leu	Lys	Asp	Leu	Glu	Glu	Gly	Ile	Glu	Thr	Leu	Met	Gly	Arg	Leu	Glu	Asp	Gly	Ser	Pro	Arg	Thr	Gly	Gln
HPLA	-	-	-	-	His	Leu	Leu	Lys	Asx	Leu	Glx	Glx	Gly	Ile	Glx	Thr	Leu	Met	Gly	Arg	Leu	Glx	Asp	Gly	Ser	Arg	Arg	Thr	Gly	Glx
BTTα	Lys	Ser																												
HCGT-α	Lys	Ser																												
HLUT-α	Lys	Ser																												
HCGT-β	Thr	Thr	Asx	Cys	Gly	Gly	Pro	Lys	Asx	His	Pro	Leu	Thr	Cys	Asx	Asx	Pro	Arg	Phe	Glx	Asx	Ser	Ser	Ser	Lys	Ala	Pro	Pro	Pro	Ser
BLUT-β	Ser	Thr	Asp	Cys	Gly	Pro	Gly	Arg	Thr	Glu	Pro	Leu	Ala	Cys	Asp	His	Pro	Pro	Leu	Pro	Asp	Ile	Leu							
PLUT-β	Ser	Ser	Asx	Cys	Gly	Pro	Gly	Arg	Ala	Glx	Pro	Leu	Ala	Cys	Asx	Arg	Pro	Pro	Leu	Pro	Gly	Leu	Leu							
BBTβ	Tyr	Ser	Asx	Cys	Ile	His	Glu	Ala	Ile	Lys	Thr	Asn	Tyr	Cys	Thr	Lys	Pro	Gln	Lys	Ser	Tyr	Met								

	151				155					160					165					170					175				180
OLAC	Tyr	Pro	Val	Trp	Ser	Gly	Leu	Pro	Ser	Leu	-	Gln	Thr	Lys	Asp	Glu	-	Asp	Ala	Arg	Tyr/His	Ser	Ala	Phe	Tyr	Asn	Leu	Leu	His	Cys
OGRO	Ile	Leu	Lys	Gln	Thr	Tyr	Asp	Lys	Phe	Asp	Thr	Asn	Met	Arg	Ser	Asp	-	Asp	Ala	Leu	Leu	Lys	Asn	-	Tyr	Gly	Leu	Leu	Ser	Cys
BGRO	Ile	Leu	Lys	Gln	Thr	Tyr	Asp	Lys	Phe	Asp	Thr	Asn	Met	Arg	Ser	Asp	-	Asp	Ala	Leu	Leu	Lys	Asn	-	Tyr	Gly	Leu	Leu	Ser	Cys
PGRO	Ile	Leu	Lys	Gln	Thr	Tyr	Asp	Lys	Phe	Asp	Thr	Asn	Leu	Arg	Ser	Asp	-	Asp	Ala	Leu	Leu	Lys	Asn	-	Tyr	Gly	Leu	Leu	Ser	Cys
HGRO	Ile	Phe	Lys	Gln	Thr	Tyr	Ser	Lys	Phe	Asp	Thr	Asn	Ser	His	Asn	Asp	-	Asp	Ala	Leu	Leu	Lys	Asn	-	Tyr	Gly	Leu	Leu	Tyr	Cys
HPLA	Ile	Leu	Lys	Glx	Thr	Tyr	Ser	Lys	Phe	Asx	Thr	Asx	Ser	His	Asx	Asx	His	Asx	Ala	Leu	Leu	Lys	Asx	-	Tyr	Gly	Leu	Leu	Tyr	Cys
HCGT-β	Leu	Pro	Ser	Pro	Ser	Arg	Leu	Pro	Gly	Pro	Pro	Asx	Thr	Pro	Ile	Leu	Pro	Glx	Ser	Leu	Pro									

	181				185					190					195					200					205				
EGRO													Arg	Val	Met	Lys	Cys	Arg	Arg	Phe	Val	Glu	Ser	Ser	Cys	Ala	Phe		
OLAC	Leu	Arg	Arg	Asp	Ser	Ser	Lys	Ile	Asp	Thr	Tyr	Leu	Lys	Leu	Leu	Asn	Cys	Arg	Ile	Ile	Tyr	Asn	Asn	Asn	Cys					
OGRO	Phe	Arg	Lys	Asp	Leu	His	Lys	Thr	Glu	Thr	Tyr	Leu	Arg	Val	Met	Lys	Cys	Arg	Arg	Phe	Gly	Glu	Ala	Ser	Cys	Ala	Phe			
BGRO	Phe	Arg	Lys	Asp	Leu	His	Lys	Thr	Glu	Thr	Tyr	Leu	Arg	Val	Met	Lys	Cys	Arg	Arg	Phe	Gly	Glu	Ala	Ser	Cys	Ala	Phe			
HGRO	Phe	Arg	Lys	Asp	Met	Asp	Lys	Val	Glu	Thr	Phe	Leu	Arg	Ile	Val	Gln	Cys	Arg	-	Ser	Val	Glu	Gly	Ser	Cys	Gly	Phe			
HPLA	Phe	Arg	Lys	Asx	Met	Asx	Lys	Val	Glx	Thr	Phe	Leu	Arg	Met	Val	Gln	Cys	Arg	-	Ser	Val	Glu	Gly	Ser	Cys	Gly	Phe			

The amino acid sequences of equine growth hormone (EGRO), ovine lactogen (OLAC), porcine pepsinogen (PPSN), bovine pepsinogen (BPSN), bovine rennin (BREN), human calcitonin (HCAL), porcine calcitonin (PCAL), salmon calcitonin (SCAL), porcine β-corticotropin (PCTP), mouse nerve growth factor (MNGF), human proinsulin (HPIN), porcine proinsulin (PPIN), bovine proinsulin (BPIN), rat proinsulin-1 and -2 (RPIN-1 and -2), angler fish insulin (AFIN), bovine parathyroid hormone (BPTH), ovine growth hormone (OGRO), bovine growth hormone (BGRO), porcine growth hormone (PGRO), human growth hormone (HGRO), human placental lactogen (HPLA), bovine α-thyrotropin (BTTα), human α-luteinizing hormone (HLUT-α), human chorionic gonadotropin-α and -β (HGCT-α and -β), bovine β-luteinizing hormone (BLUT-β), porcine β-luteinizing hormone (PLUT-β), bovine β-thyrotropin (BTTβ), porcine cholecystokinin-pancreozymin (PCPZ), frog caerulein (FCRN), canine gastrin (CGAS), porcine gastrin (PGAS), ovine gastrin (OGAS), human gastrin (HGAS), porcine motilin (PMOT), porcine vasoactive intestinal polypeptide (PVIP), porcine secretin (PSEC), porcine glucagon (PGLU), and porcine gastric inhibitory polypeptide (PGIP) aligned for maximum external correspondence. Deletions are indicated with a dash.

mones. The name 'prosecgastrin' is assigned at this time to the hypothetical parent. Support for this latter proposal has already come from a different corner; namely, the detection of 'Big Gastrin', a polypeptide with a molecular weight of about 7,000 and possessing the same physiological properties as gastrin[75]. Here, on digestion with trypsin, a smaller gastrin-like peptide is released in the gut; thus, the larger molecule may be the actual precursor protein. The recent report of a 'Big, Big Gastrin' lends further weight to this proposal[76].

[75] R. S. Yalow and S. A. Berson, Gastroenterology *60*, 215 (1971).
[76] R. S. Yalow and S. A. Berson, Biochem. biophys. Res. Commun. *48*, 391 (1972).

Attention is next paid to the two bovine thyrotropins, as well as the various gonadotropins and luteinizing hormones. These proteins are related through various common sequences[77,78] and are fitted to placental lactogen at positions 61–63 and 68–70. It is recognized that these compounds have a larger number of differences with the growth hormones and are probably only distantly related at this time. The placement of BLUT-β and BTTβ with respect to BTTα is based on common residues found at positions 112–117, yet can stand improvement in the initial N-terminal section. On turning to the various proinsulins, which includes nerve growth factor and probably parathyroid hormone and β-corticotropin, alignments are easily found, especially with the cysteines located at positions 37, 62, and 89–90. Angler fish insulin is included now since it contains both an additional residue and various changes in the N-terminus of the β-chain. The homology between these groups disappears rather rapidly after the 44–47 area, possibly as a consequence of the initiation of the connecting peptide sequence found in the insulins, or of the need to introduce gap areas in order to maintain the alignments. The recent detection of a proparathyroid hormone is of much interest[79,80], since it possesses 15–20 additional residues, which presumably must lie to the left and right of the present parathyroid sequence and should support the present assignments. The inclusion of porcine pepsinogen, bovine pepsinogen, and bovine rennin at this time is based on their resemblance to the initial sections of glucagon and secretin, as well as similar regions in the various growth hormones. Finally, the three calcitonins appear to be generally related to both OLAC and the proinsulins.

The compounds discussed here must have arisen from a series of ancestral homotypes that evolved by the general mechanism of gene duplication. The original parent was probably present in the digestive tract and in time was modified to fit various roles through changes occurring in the gut and accessory regions. It is likely that the commonality in the first 30 residues for many of these compounds may be a result of the conservation of binding to a specific receptor site, followed by the activation of the adenylate cyclase system, or by the release of inorganic ions[81], transmitter substances, and by serving at the level of transcription[82]. The empirical relationships established here clearly show that the various gastric hormones constitute a homologous block of compounds, which served in time to develop the insulins, and, ultimately the growth and luteinizing hormones. On these grounds, one can find reduplicated sections beginning at positions 51, 91, and 131, suggestive of internal gene doubling patterns. Computer techniques that have been proposed for the testing of homology[83–86] will be used in the near future in an attempt to confirm these assignments. Even now indications exist that thrombin is a member of this large family. In summary, attention is called to the old folk proverb that maintains, 'the way to a man's heart is through his stomach'. This statement is undoubtedly true and probably should be modified to include other organs as well[87].

Résumé. La calcitonine, la cholécystokinine, la motilin, la pancreozymine, le peptide inhibiteur gastrique, le peptide vasoactif, la gastrine, le glucagon, et la secretine sont comparables par leur composition en amino acides a l'hormone de croissance, à l'hormone luteinigène, la gonadotropine, au facteur de croissance nerveux, à l'hormone parathyroidienne, au lactogène du placenta, à la proinsuline, à la rénine et à la thyrotropine. Ces peptides proviennent probablement d'un génotype dont l'origine se trouve dans le système digestif.

B. WEINSTEIN

Department of Chemistry, University of Washington, Seattle (Washington 98195, USA), 9 June 1972.

[77] J. E. PIERCE, T.-H. LIAO, S. M. HOWARD, B. SHOME and J. S. CORNELL, Recent Prog. Horm. Res. *27*, 165 (1971).
[78] G. HENNE, I. KLUH and G. MAGHUIN-ROGISTER, FEBS Lett. *19*, 207 (1971).
[79] B. KERNPER, J. F. HABENER, J. T. POTTS, JR., and A. RICH, Proc. natn. Acad. Sci., USA *69*, 643 (1972).
[80] D. V. COHN, R. R. MACGREGOR, L. L. H. CHU, J. R. KIMMEL and J. W. HAMILTON, Proc. natn. Acad. Sci., USA *69*, 1521 (1972).
[81] C. S. ANAST, J. M. MOHS, S. L. KAPLAN and T. W. BURNS, Science *177*, 606 (1972).
[82] J. L. R. CANDELA and A. NIETO, Nature, New Biol. *85*, 237 (1972).
[83] S. NEEDLEMAN and C. WUNSCH, J. molec. Biol. *48*, 443 (1970).
[84] J. E. HABER and D. E. KOSHLAND, JR., J. molec. Biol. *50*, 617 (1970).
[85] A. D. MCLACHLAN, J. molec. Biol. *61*, 409 (1971).
[86] H. B. WHITE III, B. E. LAUX and D. DENNIS, Science *175*, 1264 (1972).
[87] Supported by the National Institutes of Health under grant No. AM 12241.

PRO EXPERIMENTIS

The Purification of Peptic Antibody Fragments from Rabbit Immunoglobulin G

Univalent antibody fragments of the type Fab' are obtained by sequential treatment of immunoglobulins with pepsin and thiol compounds[1]. However, experience in our laboratory has shown that published methods do not produce physically homogeneous proteins. Therefore, we have devised a scheme that effectively uses gel permeation chromatography for purification[2].

To 1 volume of rabbit serum was added saturated $(NH_4)_2SO_4$ to 40% saturation[3]. The precipitated globulins were recovered by centrifugation (2,500 *g* for 10 min), dissolved in 1 volume water, and twice reprecipitated as before. The final precipitate was taken up in $^1/_6$ volume water and extensively dialyzed against 15 m*M* potassium phosphate, pH 8.0. Insoluble material was removed by centrifugation (8,000 *g* for 10 min). The supernatant was chromatographed on diethylaminoethyl (DEAE)-cellulose[4] using the same buffer. The immunoglobulin G (IgG) peak emerging in the void volume was dialyzed against 0.1 *M* sodium acetate, pH 4.5, and the contents of the dialysis bag were concentrated with Ficoll[5] to 15 mg/ml ($A_{280\ nm}^{mg/ml}$ 1.29).

To 500–750 mg IgG was added pepsin[6] at a pepsin-to-globulin ratio of 1:50. The mixture was gently agitated at 37 °C for 8 h. The reaction was stopped by raising the pH of the solution to 7.6 and by chilling. Insoluble material was removed by centrifugation (24,000 *g* for 10 min). After concentration the supernatant was applied to a column (2.8 × 45 cm) of Sephadex G-150 superfine[5].