transférase des hépatocytes embryonnaires de poulet en culture.

Il est intéressant d'ailleurs de constater que les cultures de cellules fibroblastiques embryonnaires sont justement effectuées à partir d'embryons ayant 8 ou 9 jours. Cet âge correspond en effet à une concentration optimale en fibroblastes, qui ont une activité métabolique intense. De plus, des études d'incorporation de glucides effectuées, soit sur des cellules en culture, soit directement dans l'œuf, ont donné des résultats identiques[6]. On peut donc supposer que l'incorporation du précurseur glucidique dans les cellules embryonnaires de poulet est essentiellement réalisée par les fibroblastes. Cette hypothèse est corroborée par la partie descendante de la courbe de la Figure. En effet, à partir du 8e jour, la différentiation cellulaire dans l'embryon devient considérable, la proportion de fibroblastes diminue alors sensiblement, ce qui pourrait expliquer la chute de l'activité spécifique de transfert.

Les connaissances actuelles sur la biosynthèse des glycoprotéines permettent de considérer les membranes de l'ergastoplasme comme étant le site principal de transglycosylation. La localisation de la mannosyl-transférase des cellules embryonnaires de poulet dans l'ergastoplasme est en accord avec cette donnée générale. Cette activité enzymatique pourrait alors servir de marqueur pour cette fraction cytoplasmique. Elle permet également de confirmer l'évolution caractéristique des cellules embryonnaires qui voient leur réticulum endoplasmique granulaire proliférer rapidement au détriment des ribosomes libres.

Summary. The microsomal fractions from chick embryo cells were shown to carry out transfer of mannose from GDP-mannose to endogenous protein acceptors. Data also are presented which support that among the subcellular fractions only the rough microsomes are active in the mannose transfer. The maximum mannosyltransferase activity is obtained with 8-day-old embryos. This observation may be related to the proliferation of rough endoplasmic reticulum with regard to smooth microsomes.

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Solid-Phase Synthesis and Bioassay by Tibia Test of Monotetracontapeptide 81-121 and of Ditriacontapeptide 122-153 of Human Growth Hormone[1]

In his excellent paper presented at the First International Symposium on growth hormone, Li[2] stated that 'there are increasing strong evidences that the whole human growth hormone (HGH) molecule is not necessarily required for biological activities. It remains to be demonstrated that peptide fragment or fragments can be obtained in highly purified state from partial enzymic digests of HGH and that these pure fragments possess growth promoting or lactogenic activities or both'. Recently Yamasaki et al.[3] reported that one of the peptides derived from tryptic digestion of bovine growth hormone possesses remarkable biological activity on 'tibia test' and on weight gain test in rats.

To our knowledge, no data have been published concerning attempts to synthetize peptides with amino acid sequences corresponding to fragments of human growth hormone molecule, the primary structure of which was completely defined by Li[4]. In this communication we shall present essential data on the synthesis and preliminary results on the biological activity as measured by 'tibia test' of newly synthetized polypeptides corresponding to the sequence of human growth hormone from 81 to 121 (monotetracontapeptide) and from 122 to 153 (ditriacontapeptide).

Material and methods. The 2 peptides were synthetized according to the general procedure of solid-phase peptide synthesis of Merrifield[5,6]. The protection of α-amino groups of amino acids was performed using t-butyloxycarbonyl (Boc); functional groups of lateral chains were protected as follows: Asp (β-OBzl), Glu (γ-OBzl), Ser (Bzl), Thr (Bzl), Tyr (Bzl), Lys (ε-Z), Arg (NO_2), His (Dnp)[7], Met(O).

The synthesis was carried out in a stepwise manner starting in both cases from 0.55 g (0.50 mmole) of Boc-Leu-resin. Coupling was usually mediated by N,N'-dicyclohexylcarbodiimide (DCCI) (threefold excess, 3 h), only for Asn and Gln the method of *p*-nitrophenyl ester was employed. The Boc groups were removed by 1*N* HCl in HOAc for 30 min at room temperature with the exception of Boc of Gln which was removed by trifluoroacetic acid (15 min at room temperature) according to Takashima et al.[8]. The final peptides were removed from the solid support by Sakakibara method[9]: 1 g of Boc-peptidyl resin was treated with 10 ml of HF in the presence of 1 ml of anisole.

Details for peptide 81–121. From 0.55 g (0.50 mmole) of Boc-Leu-resin the yield was 2.50 g of the protected monotetracontapeptide-resin. After acidolysis by HF 1.32 g of free monotetracontapeptide were isolated. This was dissolved in Na_2CO_3 (pH 8) and the solution stirred for 1 h at room temperature. The product was purified by countercurrent distribution (130 transfers in the system 2-butanol 0.1% aqueous dichloroacetic acid 1:1, K = 15, and 130 transfers in the system chlorophorm,

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carbonium tetrachloride, methanol, NH_4OH 0.02N, 5:5:8:2, K = 7.7). Yield 250 mg.

Details for peptide 122–153. From 0.55 g (0.50 mmole) of Boc-Leu-resin the yield was 2.25 g of protected ditriacontapeptide-resin. After acidolysis by HF the 1.25 g of Dnp-ditriacontapeptide were treated with mercaptoethanol first for 2 h at pH 8 and subsequently for 24 h at pH 7. The product was purified by countercurrent distribution (300 transfers in the system 2-butanol 0.1% aqueous dichloroacetic acid 1:1, K-0.28). Yield 400 mg.

The biological assay of growth hormone-like activity on 'tibia test' of both the newly synthetized compounds and of a mixture (1:1) of them was made according to GREENSPAN et al.[10]. As reference standard bovine growth hormone BCG-1-B9 was used.

Results and discussion. The monotetracontapeptide 81–121 resulted homogeneous on paper electrophoresis at pH 2 (1.5M formic acid, 2M acetic acid, 1:1) with R_{His} = 0.11. $[\alpha]_D^{25}$ −18° (c = 1; acetic acid 80%).

The ditriacontapeptide 122–153 moved as a single spot on paper electrophoresis at pH 2 (1.5M formic acid, 2M acetic acid, 1:1) with R_{His} = 0.31. $[\alpha]_D^{25}$ −31° (c = 1, acetic acid 80%).

The analyses of amino acids of both the compounds are given on Table I. From the data reported of physical and chemical analyses applied to the synthetic polipeptides, it appears that they possess a reasonable degree of homogeneity. Nevertheless the presence of a small percentage of very closely related molecules with truncated sequences or failure sequences cannot be excluded[11]. The solid-phase method seemed also in the present work of relevance for the opportunity offered of a synthesis procedure to be easy and rapid so that it may be considered useful in the recognition of active center(s) of protein molecules through the approach of synthetizing single small fragments. The conventional method of synthesis will give subsequently the possibility of obtaining the selected compound with a higher degree of homogeneity.

From Table II, which summarizes the results of 'tibia test' bioassay, it emerges that the monotetracontapeptide 81–121 at the daily dose of 100 μg and 400 μg shows an effect which increases with the dose (width of tibial cartilage from 147.7 ± 5.4 of hypophysectomized controls to 181.1 ± 5.0 and 213.0 ± 6.4 respectively). The ditriacontapeptide 122–153 shows progressively increasing effect with the daily dose of 50, 100 and 400 μg (width of tibial cartilage from 147.7 ± 5.4 of hypophysectomized controls to 187.2 ± 4.6, 201.3 ± 5.4 and 224.0 ± 1.5 respectively).

The effect of the mixture of the 2 peptides at the daily dose for each one of 100 μg gave a value of 214.2 ± 6.4 of tibial cartilage width, which roughly corresponds to that obtained by the single peptides administered at 400 μg dose. A remarkable synergic action of the 2 polypeptides is clearly apparent.

To conclude, the present results suggest that the prepared fragments of HGH may have interesting properties and that probably the complete series of the polypeptides programmed may help to elucidate the active center(s) of HGH molecule.

Table I. Amino acid analyses[a]

	Peptide 81–121		Peptide 122–153	
	Experimental	Theoretical	Experimental	Theoretical
Asp	5.76	6	6.19	6
Thr	1.82	2	3.19	3
Ser	4.87	5	3.20	3
Glu	6.20	6	2.83	3
Pro	–	–	1.00	1
Gly	2.25	2	2.81	3
Ala	2.05	2	1.18	1
Val	2.97	3	–	–
Met	–	–	0.78	1
Ile	1.04	1	0.96	1
Leu	8.34	8	2.33	2
Tyr	1.68	2	0.86	1
Phe	0.84	1	2.21	2
Lys	1.81	2	2.23	2
His	–	–	1.16	1
Arg	0.81	1	1.88	2

[a] Samples were hydrolyzed in 6N HCl in sealed, evacuated tubes, 24 h, 110 °C, and analyzed on a Technicon amino acid analyzer.

Table II. Bioassay by 'tibia test' of the growth hormone-like activity of the monotetracontapeptide 81–121, of the ditriacontapeptide 122–153 and of a mixture of them

Groups	No. of hypophysectomized animals	Treatment		Width of tibial cartilage (μm)
		Substance	Daily dose (μg/rat/4 days)	
A	7	–	–	147.7 ± 5.4
B	7	BGH standard	12.5	219.4 ± 3.3
C	6	BGH standard	50.0	248.3 ± 1.9
D	7	peptide 81–121	100.0	181.1 ± 5.0
E	5	peptide 81–121	400.0	213.0 ± 6.4
F	4	peptide 122–153	50.0	187.2 ± 4.6
G	9	peptide 122–153	100.0	201.0 ± 5.4
H	6	peptide 122–153	400.0	224.0 ± 1.5
I	5	peptide 81–121 + peptide 122–153	100.0 100.0	214.0 ± 6.4

Zusammenfassung. Mit Hilfe der MERRIFIELD-Methode wurden zwei Polypeptide synthetisiert, die Teilsequenzen des menschlichen Wachstumshormons entsprechen. Die biologischen Eigenschaften wurden untersucht.

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