G.Enberg*, K.Florell* & K.Hall
Department of Endocrinology, Karolinska
Hospital, 104 01 Stockholm, Sweden.
AN IMPROVED METHOD FOR THE PURIFICATION
OF INSULIN-LIKE GROWTH FACTORS I AND II

The somatomedins insulin-like growth factors I and II (IGF-I and IGF-II) were originally isolated from human plasma by use of acid/ethanol extraction, gel filtration and isoelectric focusing (IEF). With a semi-automated chromatography system we have similarly isolated IGF-I and II from human plasma. In this system the separation between IGF-I and II was achieved by ion-exchange chromatography and not with IEF. The crossreaction of native IGF-I in the radioimmunoassay (RIA) for IGF-II has generally been found to be about 10%. Biosynthetic IGF-I has, however, a crossreaction of only 1-2% in the RIA for IGF-II. The difference is most likely due to a contamination of IGF-II in the native IGF-I preparations.

By use of a Mono-S column (FPLC system) eluted with a salt gradient at pH 5.0, we have now achieved a complete separation between IGF-I and IGF-II. This finding might be of importance when interpreting results from earlier receptor studies with IGF-I and IGF-II.

W.F.Blum*, M.B.Ranke, B.Lechner*, J.R.Bierich
University Children's Hospital, Tübingen,
Federal Republic of Germany
THE POLYMORPHIC PATTERN OF SOMATOMEDINS DURING
DEVELOPMENT

Human somatomedins (Sm) show considerable heterogeneity on separation by chromatofocusing (CF). Besides the major forms eluting at $pI=8.3$ (IGF-I/SmC) and at $pI=6.3$ (IGF-II) a number of minor peaks emerge which can be classified as IGF-I-like or as IGF-II-like. The aim of the current study was to investigate, whether there is an age-dependence of the polymorphic pattern during human development. Serum (2ml) extracts from children ($n=15$) and adults ($n=9$) were fractionated by HPLC-CF. The fractions were screened for Sm-like peptides by CPBA. The total Sm-activity of the various peaks was quantitated either by a specific IGF-I/SmC-RIA or by a IGF-II-RIA giving their relative contribution to total IGF-I/SmC and IGF-II immunoactivity resp. The results show that the various Sm-like peptides are present in all age groups studied. Although serum IGF-I/SmC rises considerably at the time of puberty, the ratio of the various IGF-I/SmC-like peptides to total IGF-I/SmC-IR did not vary. There was a small but significant increase of the two very basic SmS ($pI=9.2$ and $pI=8.7$) from 3.4% at puberty to 6.7% ($p<0.002$) in adults and from 9.3% to 14.5% ($p<0.02$) resp. The major component at $pI=8.3$ decreased accordingly from 83.1% to 72.0% ($p<0.01$). When CPBA-active peaks were tested for IGF-II-like peptides, a rather broad peak at $pI=8.1$ contained an unexpectedly high amount of IGF-II-like material ($\Sigma=21.1\%$). The relative contributions of the various other IGF-II-like components to total IGF-II-IR were, 4.5% ($pI=6.7$), 36.7% ($pI=6.3$), 14.6% ($pI=6.15$), 3.2% ($pI=6.0$), and 19.6% (salt peak) without any significant difference between age groups. These results demonstrate, that heterogeneity of somatomedins is a common phenomenon throughout human development with some age-dependence for IGF-I/SmC-like peptides and no obvious age-dependence with IGF-II-like components.

R.P. Rooman*, M.V.L. Du Caju
Department of Pediatrics, University of Antwerp,
Belgium
DECREASE OF DNA SYNTHESIS IN CULTURED HUMAN FIBROBLASTS BY A SERUM FRACTION WITH SM/IGF INHIBITING ACTIVITY.

Normal serum contains a small molecular weight fraction (Mr 1200) that inhibits the effects of somatomedins/insulin-like growth factors (SM/IGF's) on cartilage segments and epididymal fat pads (Pediatr. Res. 18:1212,1984). We examined the effects of this somatomedin inhibitory fraction (SmIF) on human skin fibroblast multiplication. The addition of SmIF (10 mEq/ml) resulted in a significant decrease of total cell numbers (130,000 cells/dish) as compared to control cultures (500,000 cells/dish) after seven days of incubation. This inhibition was partially reversible when SmIF was added to low density cultures, but returned to control values when added during the logarithmic growth phase. SmIF decreased 3H -thymidine incorporation into TCA precipitate in serum and SM/IGF stimulated synchronized human fibroblasts in a dose dependent manner to less than 10% of control values. Cell numbers, protein content and cell viability remained unchanged. Pulsing with SmIF at different time intervals during serum stimulated DNA synthesis, revealed that it only acted during the first hours after stimulation. The presence of SmIF before stimulation, during late progression or during the thymidine pulse showed no effect. We conclude that SmIF is non-toxic and reversibly inhibits cell multiplication by interfering with competence induction or early progression.

M.Claustres*, P.Chatelain* and Ch.Sultan
Department of Biochemistry B, University of Medicine
and INSERM U.58, 34100 Montpellier. INSERM U.34.
69332 Lyon, France.
COMPARATIVE EFFECTS OF HUMAN GROWTH HORMONE (GH) AND
INSULIN-LIKE GROWTH FACTOR I/SOMATOMEDIN C (IGF I/
SMC) ON ERYTHROPOIETIC CULTURES FROM NORMAL CHILDREN.

It is well established that growth-promoting effects of GH on target cells in vivo are mediated by a class of growth hormone-dependent polypeptides belonging to the group of somatomedins. We tested the effects of human GH (from France-Hypophyse) and purified IGF I/SMC in vitro on bone marrow and peripheral blood cells from children, by means of erythroid progenitor cloning technique, according to a miniaturized methylcellulose assay.

The erythropoietic proliferation was evaluated by the number of colonies derived from CFU-E (Colony Forming Units-Erythroid) and BFU-E (Burst Forming Units-Erythroid) and by the biochemical determination, in the cultured erythroblasts, of the activity of a cytosolic enzyme involved in the heme pathway (Uroporphyrinogen I synthase). In the presence of erythropoietin (0.5 IU/ml for CFU-E, 1 IU/ml for BFU-E) and fetal calf serum, human GH appeared to require very high concentrations (200-250 ng/ml) to stimulate the growth of CFU-E and BFU-E in vitro. In contrast, erythroid colony formation was significantly increased ($p<0.05$, Student's test) by as little as 1 ng/ml of IGF I/SMC, with peak activities occurring with 10-50 ng/ml.

These results suggest that IGF I/SMC exerts a direct growth promoting effect on the erythroid lineage, among other growth factors and could be involved in the regulation of erythropoiesis.

J. Bourguignon*, J. Bonaventure*, B. de La Tour*, and
MT. Corvol.
INSERM U.30 and U.12, Hôpital des Enfants-Malades, Paris,
France.
CHARACTERIZATION OF IGF1 RECEPTORS ON IN VITRO DIFFERENTIATING GROWTH PLATE (GP) CHONDROCYTES GROWN IN CULTURE.

Chondrocytes enzymatically extracted from the resting zone of GP cartilage from prepubertal rabbits, were incubated in 10% FCS-Dulbecco's medium for 48 h. They were then grown for 20 days in serum-free defined medium containing 100 ng/ml FGF as unique growth factor. Chondrocytes differentiation was observed at day 10 to 12 when cells were dividing as multilayered colonies and at day 15 to 20 when cells hypertrophied and developed important Golgi apparatus and extracellular matrix as observed by electron microscopy. At both stages of differentiation, Type II collagen was representing 95% of the newly synthesized collagenic proteins as measured after incorporation of 3H -Pro during 20 h. The specific binding of 125I-IGF1 (gift of Dr L. Van den Brande) was studied separately on dividing and hypertrophic chondrocytes. The 50% displacement was the same at both stages of differentiation at $4.5 \times 10^{-9}M$ concentration of unlabelled IGF1. The presence of several types of receptors was suggested by Scatchard analyses with similar high affinity constant ($K_a=3.3 \times 10^{-11}M^{-1}$) in dividing and hypertrophic chondrocytes. By contrast in dividing cells the maximal specific binding observed at 15°C and pH 8 was 3 times higher than in hypertrophic cells (6% and 2% respectively) and the number of specific binding sites was 1.5 times higher than in hypertrophic cells. These results suggest that GP resting chondrocytes can divide and hypertrophy in culture while they synthesize Type II collagen and possess a number of IGF1 receptors which was decreasing during the differentiation process.

MT. CORVOL, MF.DUMONTIER*, C.PREVOT*, J.WILLEPUT*.
INSERM U. 30. Hôpital des Enfants-Malades, Paris, France.
EVIDENCE THAT RABBIT CULTURED GROWTH PLATE (GP) CHONDROCYTES SYNTHESIZE 40-60K AND 7K PROTEINS WITH IMMUNOREACTIVE IGF1 ACTIVITY (IR-IGF1 A).

The local production of IGF1 like factors by cultured GP chondrocytes was studied. IR-IGF1 A in the incubation medium of prepubertal rabbit GP chondrocytes grown in serum-free defined medium during 20 days was evaluated by RIA. Chondrocyte phenotype was followed by specific proteoglycans and Type II collagen biosynthesis. The mean IR-IGF1 A measured every 48 h from day 6 to day 16 of the primary culture was constant at 40 ± 18 ImU/ 10^6 cells. This activity was further characterized after extraction of the culture medium at neutral and acid pH successively. At neutral pH IR-IGF1 A of the eluates of G100 Sephadex column appeared as two distinct peaks: a major one migrating in the 40-60K protein region plus an additive one in the 7K region with a 40-60K:7K ratio = 2:1. The 40-60K protein peak material extracted from culture medium corresponding to 10^6 cells, was able to specifically and reversibly bind 42 ± 10 fmols 125I-IGF1. At acid pH, IR-IGF1 A was similarly separated as 40-60K and 7K peaks, but their respective amount was reversed resulting in a 40-60K:7K ratio = 1:2. When chondrocytes were incubated in the presence of ^{35}S -Meth during 20 h, only the 40-60K IR-IGF1 protein peak was radiolabeled after chromatography at neutral pH. After acid extraction of the radiolabeled 40-60K peak, 60 to 80% of the radioactivity was recovered in the 7K protein peak. This radiolabeled 7K protein peak analyzed by electrophoresis on SDS 10% polyacrylamide gel pH 8.3 was comigrating with pure 125I-IGF1. These results suggest that chondrocytes synthesize and secrete IGF1-like peptide and a pool of higher MW proteins with IGF1 binding capacity.