Hormone Res. 24: 121–130 (1986)

0301–0163/86/0243–0121$2.75/0

Insulin-Like Growth Factors/Somatomedins: Structure, Secretion, Biological Actions and Physiological Role

J. Zapf, E.R. Froesch

Metabolic Unit, Department of Medicine, University Hospital, Zürich, Switzerland

Key Words. Insulin-like growth factors · Somatomedins · Somatomedins, structure · Somatomedins, site of production · Somatomedins, in vitro and in vivo actions

Abstract. Insulin-like growth factors (IGFs, somatomedins) are structural homologues of insulin with insulin-like biological activity. They are mainly synthesized and secreted by the liver, but may also be produced by extrahepatic tissues. In their native from in blood they are bound to specific carrier protein(s). This determines essentially their biological actions. The complexed factors stimulate growth indices in vitro and in vivo, but, in contrast to the free factors, do not exert acute effects on insulin target tissues.

Introduction

Terminology

The terms 'somatomedin' (Sm) and 'insulin-like growth factor' (IGF) are synonyms for one and the same class of polypeptides. Insulin-like growth factors can be defined as structural homologues of insulin that exert insulin-like biological effects in the presence of excess insulin antibodies. The term 'somatomedin' [1] has a somewhat more restrictive meaning in that it implies that the level of an insulin-like growth factor is under the influence of growth hormone.

IGF Species

Human serum contains two main types of IGF as defined by their immunological and structural properties and by their receptor reactivities: (1) IGF I, which is identical to somatomedin C (Sm-C) [2] and to somatomedin A (Sm-A) [3], and (2) IGF II. Besides 'classical' IGF II, two other subtypes of IGF II have been isolated: in one of them, Ser 29 of 'classical' IGF II is replaced by the amino acids Arg-Leu-Pro-Gly [R.E. Humbel, personal communication]. The other one is 'big IGF II' which differs from 'classical' IGF II by a substitution of Cys-Gly-Arg for Ser 33 and by a carboxyl terminal extension (E domain) of 21 amino acid residues [32].

In the adult rat the main somatomedin appears to be the equivalent of IGF I [4]. Bovine IGF I is identical to human IGF I [33]. The equivalent of human IGF II in the rat is multiplication stimulating activity (MSA). It appears to be the main IGF of fetal rat serum [5]. The amino acid sequence of

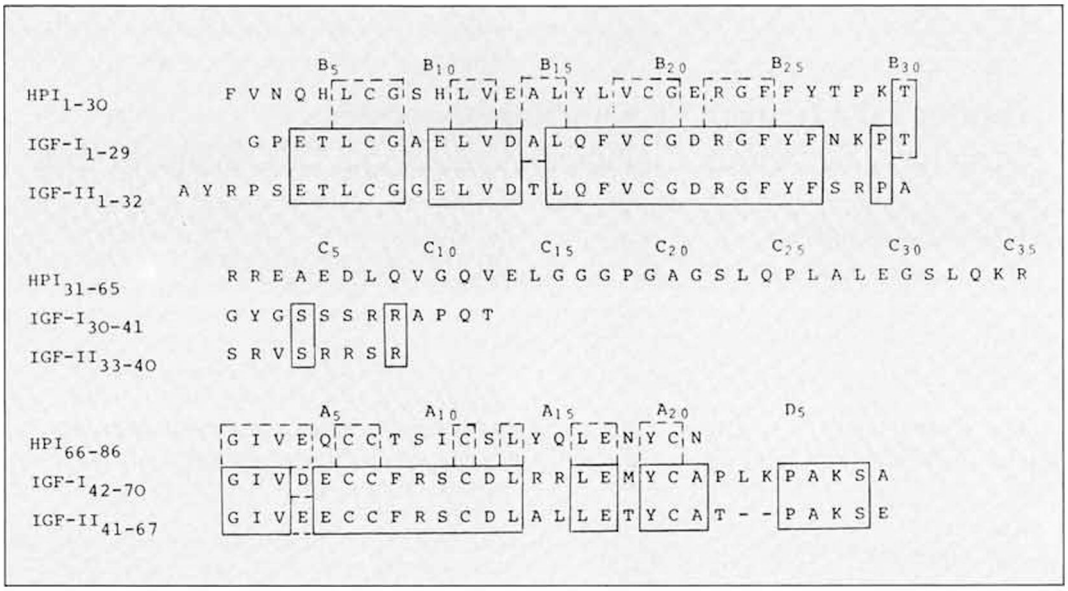

Fig. 1. Primary structures of human proinsulin (HPI), IGF I and IGF II (from ref. 31). Alignment has been chosen such as to give maximal homology. Boxes in solid lines indicate residues identical in IGF I and II; boxes in broken lines indicate residues identical in HPI and in IGF I and/or II.

MSA differs from that of human IGF II by only 5 amino acids (3 of which are located in the C domain) [6], whereas its immunological properties differ markedly from those of IGF II [7].

IGF-Carrier Protein Complexes

[for review see 8, 9]

One of the prominent features of IGFs is that they do not circulate in blood as free polypeptides, but are noncovalently bound to specific carrier proteins. The IGF-carrier complexes differ from free IGF in several respects: the carrier protein abolishes the acute insulin-like actions characteristic of free IGFs [10, 11], it restricts their permeability through capillaries and it inhibits their access to membrane receptors [10–12] and immunobinding sites [12].

Structural Features of the IGFs (fig. 1)

[for review see 8, 13]

IGF I and II are one-chain polypeptides with molecular weights of 7,646 and 7,471 daltons. They consist of an A, B, C and D domain. Large parts of the sequence within the A and B domains are homologous to the A and B chain of human proinsulin. This sequence homology is 43% for IGF I and 41% for IGF II. No sequence homology exists between the C domains of IGFs and the C peptide region of human proinsulin. In addition, the carboxyl-terminal extension (D domains) of 8 and 6 amino acid residues, respectively, is a typical structural feature of IGF I and II which is not shared with proinsulin. The sequence homology between IGF I and II is 62%.

Other prominent differences between the primary structures of IGFs and proinsulin are the relative abundance of basic amino acids and the lack of histidine in IGF I and II. Furthermore, the C domains in IGF I and II do not display the two sets of two basic amino acids (Arg-Arg and Lys-Arg) typical of the two ends of the proinsulin connecting peptide. These basic amino acids are released during the cleavage of proinsulin to insulin. There is no evidence, however, that cleavage of IGF into a two-chain molecule occurs in vivo.

Evolutionary Aspects

It appears likely that IGF I, IGF II and insulin have diverged from a common ancestor molecule. It is assumed that gene duplication has led to the subsequent divergent evolution of proinsulin and a primitive IGF molecule, and that a subsequent second duplication of the IGF gene has given rise to the divergent evolution of IGF I and II. Based on paleontological considerations it is likely that the first gene duplication of the ancestor gene has occurred before the appearance of the first vertebrates on earth, i.e. more than 600 million years ago. The second gene duplication that led to the divergence of IGF I and II has probably taken place about 300 million years ago, i.e. before the appearance of the first mammals on earth.

Biosynthesis and Secretion of the IGFs

[for review see 14]

A considerable amount of evidence exists that the liver is a major site of Sm production. This evidence stems from studies with liver cell cultures or liver explants, from in vivo studies in dogs, from investigations in patients with hepatic disease and from liver perfusion experiments.

Recently, evidence has accumulated that IGFs are produced by a variety of other tissues. For example, explants of multiple tissues from the fetal mouse or cultured human fibroblasts secrete immunoreactive IGF I/Sm-C into the culture medium. It appears likely that part of the 'IGF I-like' material found in media from cell cultures is secreted in the form of a precursor molecule. This possibility is supported by reports on the isolation of cDNA sequences that encode for IGF I [15] and IGF II precursors [16, 17].

Immunoreactive Sm-C/IGF I has been extracted from multiple tissues of rats (kidney, liver, lung, heart and testes) [18]. Tissue extracts from hypox rats contain less Sm-C/IGF I than those of normal rats. Sm-C/IGF I in the tissue extracts increases when hypox rats are injected with growth hormone prior to the extraction. Whether and to what extent these findings are relevant to the physiology of the IGFs (autocrine and/or paracrine function) has not yet been established.

As shown in figure 2, IGF I is synthesized and secreted during the perfusion of the isolated rat liver. Synthesis and secretion are drastically reduced in livers from hypox animals, but are restored to near normal after in vivo treatment of the hypox rats with growth hormone. The secretion rate of IGF I from a perfused normal rat liver is around 400 μU of insulin equivalents per hour [19]. Assuming a half-life of 3 h [20, 21] and a plasma volume of 8 ml for a rat weighing 200 g, the above production rate would result in a serum IGF level of approximately 200 μU of insulin equivalents, which is in the same order of magnitude as the normal serum IGF level in rats [130–150 μU/ml; 21].

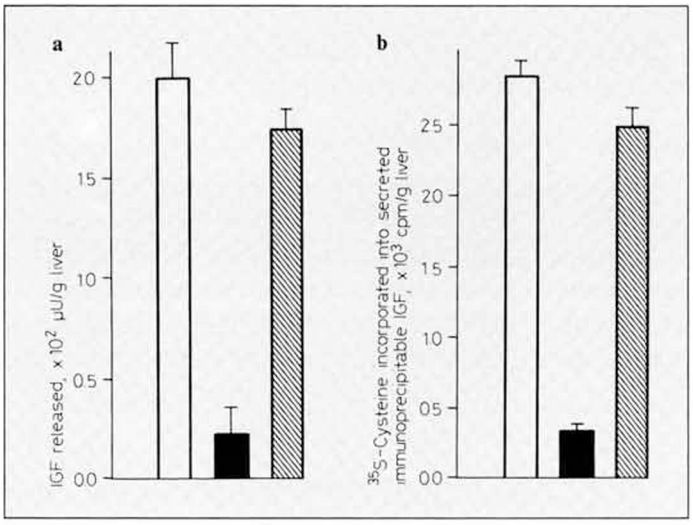

Fig. 2. Secretion (**a**) and synthesis (**b**) of IGF by isolated livers of normal (□), hypox (■) and growth-hormone treated hypox rats (▨) during 240 min of recirculating perfusion (from ref. 19).

Biological Actions of the IGFs

[for review see 22, 23]

The basis for the biological properties of IGF I and II rests in their structural homology with the insulin A and B chains. It is, therefore, not surprising that IGF I dand II elicit the same qualitative biological responses as insulin. These responses are mediated by interaction with the insulin receptor or with specific type I IGF receptors [for review see 24].

The tight association of IGFs with a specific carrier protein is an essential determinant of their biological activity. The implications of this determinant will be discussed later. The biological effects of free IGFs have been well established in a variety of tissues. Together with insulin, IGFs constitute a potent complementary anabolic principle. According to the time of onset of the biological response, two different kinds of anabolic effects can be distinguished: acute metabolic (rapid) effects and long-term (slow) effects.

In vitro Effects

The acute effects of IGF I and II are observed in the typical insulin target tissues: adipose tissue, striated and heart muscle. They are qualitatively indistinguishable from those of insulin. More recently, acute effects of the insulin-like growth factors have also been observed in osteoblast-like cells from rat calvaria. In these cells IGF I and II are potent stimulators of glycogen synthesis. The physiological significance of this effect may be related to the glycogen requirements of preosteoblasts at a stage where matrix synthesis proceeds at a high rate.

One of the early observations on somatomedins was their stimulatory effect on DNA synthesis and cell multiplication and on the incorporation of inorganic sulfate into cartilage tissue. These observations have been amply confirmed later with pure IGF I and II.

A new aspect in the long-term action of IGFs is their supportive effect on cell differentiation [25, 26]. This effect is more pronounced than the stimulatory effect on cell

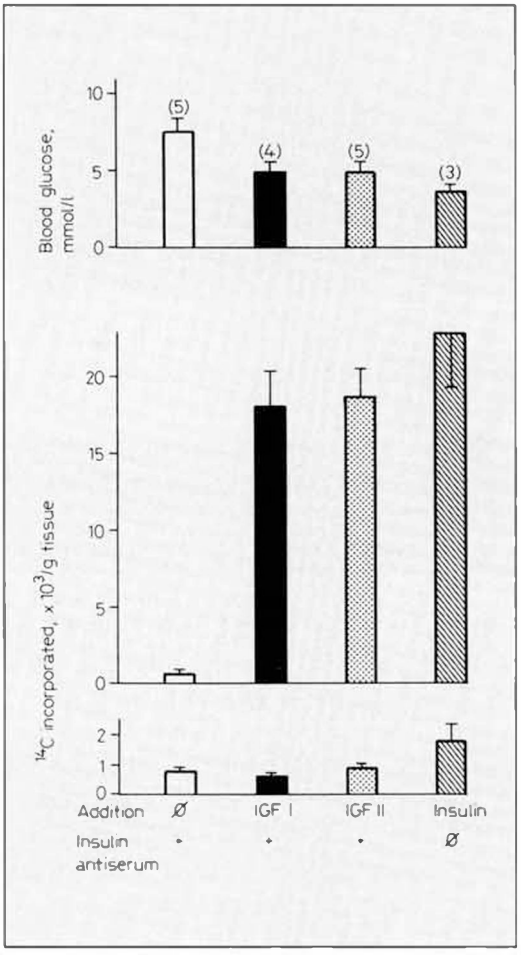

Fig. 3. Acute in vivo effects of a bolus injection of IGF I, IGF II or insulin into hypophysectomized rats. 1 ml of saline/0.2% human serum albumin, containing a trace amount (1 μCi) of U^{14}C-glucose, anti-insulin serum (neutralizing capacity 30 mU) and 20 μg (corresponding to 6 mU of insulin equivalents, standardized in the fat pad assay) of IGF I or IGF II was injected into the tail vein. The control group received anti-insulin serum without hormones. For comparison, a fourth group of rats received 6 mU of insulin without anti-insulin serum. Blood glucose (upper panel), ^{14}C-incorporation into diaphragm glycogen (middle panel) and ^{14}C-incorporation into fat pad total lipid (lower panel) were measured after 30 min. Columns give the mean ± SEM; numbers in parenthesis give the numbers of animals.

multiplication. IGFs stimulate the fusion of myoblasts to myotubes. This is accompanied by an increase of the acetylcholine esterase activity, an indicator of muscle cell differentiation. Similarly, IGF I and II stimulate alkaline phosphatase activity, a marker of osteoblast differentiation, in rat calvaria cells.

Kurtz et al. [27] have reported that IGF I enhances the proliferation of erythroid colonies from fetal mouse liver and adult bone marrow cells after 2 days in culture. Although the potency of IGF I is three orders of magnitude lower than that of erythropoietin, IGF I is the first clearly defined mitogen that stimulates the late stages of erythroid differentiation independently of erythropoietin.

Finally, IGF I and II stimulate colony formation of human chondrocytes in a semisolid methyl cellulose matrix. In this system, IGF I is more potent than IGF II on chondrocytes from adults, whereas IGF II is more potent than IGF I on fetal chondrocytes. Insulin or growth hormone are without effect [34].

Altogether, the slow effects of the IGFs are directed mainly towards an enhancement of cell differentiation accompanied by cell proliferation. All of these effects are thought to be mainly mediated by the type I IGF receptor.

In vivo Effects and the Physiological Role of the IGFs

Similar to the in vitro situation, acute effects can be differentiated from long-term effects when IGFs are administered in vivo. Whether they exert acute or long-term effects depends on the mode of administration.

Bolus Injection

When injected intravenously as a bolus into rats, IGF I and II cause pronounced insulin-like effects on glucose homeostasis and metabolism. Figure 3 shows an experi-

ment in which 20 μg (corresponding to 6 mU of insulin equivalents as standardized in the fat pad assay) of IGF I or II were injected together with a trace amount of U^{14}C-glucose and anti-insulin serum (neutralizing capacity 30 mU) into hypox rats. The control group received only anti-insulin serum and U^{14}C-glucose. For comparison, 6 mU of insulin (without anti-insulin serum) and U^{14}C-glucose were injected into a fourth group. The animals were bled by aortic puncture at different time points after the injection and the blood sugar, the ^{14}C serum radioactivity and the incorporation of U^{14}C-glucose into diaphragm glycogen and fat pad total lipids were determined. In figure 3, the results obtained 30 min after the injection are shown. IGF I and II caused the same reduction of the blood sugar level (35% of the controls) and of the half-life of the U^{14}C-serum glucose radioactivity. Insulin caused a 50% reduction of the control value. In contrast to IGF II, the hypoglycemic effect of IGF I and insulin lasted for 120 min (not shown).

A dramatic effect of IGF I and II was observed on glucose utilization in diaphragm muscle: 30 min after the intravenous injection of IGF I or II, ^{14}C-incorporation into diaphragm glycogen was stimulated 25-fold as compared to the control group. The effects of IGF I dand II were identical and comparable to the effect of insulin.

Lipid synthesis from U^{14}C-glucose was not stimulated by IGF I dand II or by insulin. This finding is consistent with in vitro observations in fat cells of hypox rats. In these cells, neither insulin nor IGF I or II are able to significantly stimulate lipid synthesis from glucose, although glucose transport proceeds at a maximal rate [28]. The reason for the lack of the stimulatory effect on lipid synthesis after hypophysectomy is the largely reduced activity of the key enzymes of lipid synthesis from glucose.

In normal rats, where insulin causes a very pronounced stimulatory effect on lipid synthesis in adipose tissue, the effects of a bolus injection of 20 μg of IGF I or II were only minor and statistically barely significant (not shown). However, both factors decreased the blood sugar levels significantly and also stimulated glycogen synthesis in the diaphragm (not shown).

Subcutaneous Infusion

When administered by subcutaneous infusion over 6 days in hypox rats, IGF I and II, like growth hormone, stimulate indices of growth [29]: as shown in figure 4, 43 and 103 μg/day of IGF I caused a dose-dependent increase in body weight, tibial epiphyseal width and in thymidine incorporating activity (in the absence of growth hormone).

The effects of the two IGF I doses are comparable to those of 12.5 and 25 mU/day of human growth hormone. In contrast to IGF I, a dose of 131 μg/day of IGF II did not cause a significant increase in body weight. However, it had a significant, although much less pronounced effect than the lower of the two IGF I doses on the two other growth indices.

The total serum IGF levels (as expressed in μU equivalents of a protein binding assay) rose from a 'background level' of 39 μU/ml to 83 and 99 μU/ml during the infusion of the two IGF I doses and to 146 μU/ml during the infusion of the IGF II dose. The infusion of 12.5 or 25 mU per day of growth hormone caused an increase in endogenous rat IGF from 39 to 67 and 75 μU/ml. With 200 mU/day of growth hormone, the endogenous rat IGF rose to 84 μU/ml, and there was a concomitant further stimulation of all three

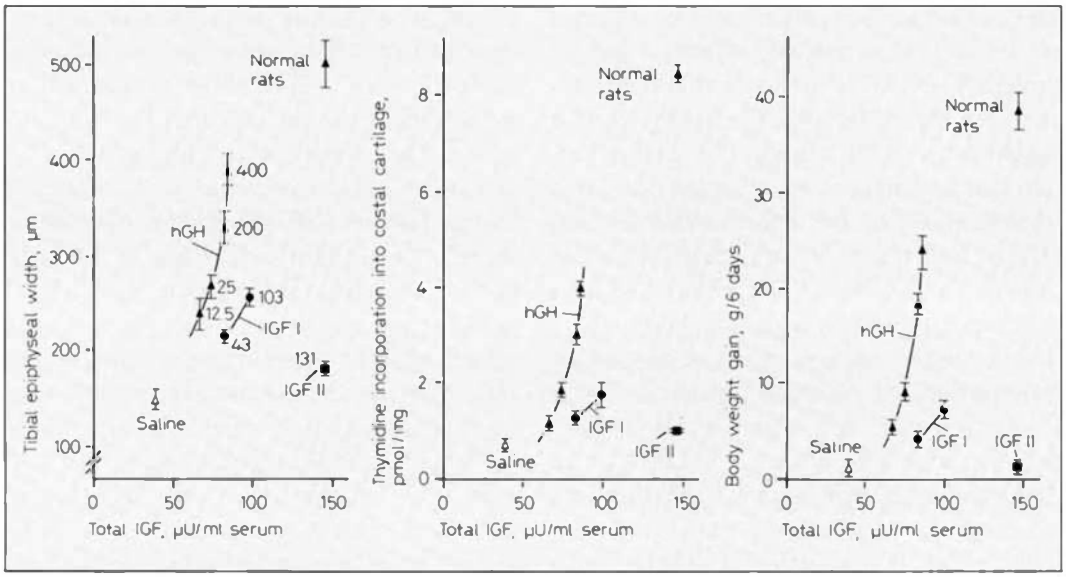

Fig. 4. Comparison of growth effects in hypophysectomized rats of infused IGF I or IGF II with effects of endogenous rat IGF induced by growth hormone administration. Hormones were infused during 6 days at the following rates: IGF I: 43 µg/day and 103 µg/day; IGF II: 131 µg/day; growth hormone (hGH): 12.5, 25, 200, 400 mU/day. Each point represents the mean ± SD of values obtained from 4 rats. Total IGF values were determined by a competitive protein binding assay in a serum pool of each group of rats and are expressed as µU equivalents of a human IGF standard (from ref. 29).

growth indices. Although the latter continued to increase under infusion of 400 mU/day of growth hormone, the endogenous IGF level did not rise further.

These findings demonstrated that IGF I, and to a smaller extent also IGF II, are capable of stimulating growth indices in the absence of growth hormone. Increases in the steady-state concentrations of IGF, whether achieved by infusion of IGF I or II, or by growth hormone-induced production of endogenous IGF, caused a dose-dependent stimulation of growth indices. These observations strongly support the somatomedin concept. It will have to be reassessed in the future whether or not higher IGF II doses are able to produce significant increases in body weight. Clearly, IGF I is the more potent of the two insulin-like growth factors in the rat. Clinical evidence suggests that this is also the case in humans: oversecretion of growth hormone in acromegalic patients leads only to an increase of IGF I, but not of IGF II. Similarly, growth hormone deficiency lowers IGF I levels more drastically than IGF II levels.

The potency difference between IGF I and II is unlikely to be due to different half-lives of the two polypeptides. A similar potency difference is observed in vitro in rat tissues.

The finding that growth hormone beyond 200 mU/day stimulates growth without further elevating endogenous rat IGF is of interest. It appears possible that growth hormone

itself has an additional direct or indirect modulatory or synergistic effect on growth processes. One way by which this might occur is via the induction of the large molecular weight form of the specific IGF carrier protein that prolongs considerably the half-life of injected IGF [30]. Neither infused IGF I nor infused IGF II are able to induce this carrier protein. In this respect, exogenous IGF does not mimic the action of growth hormone.

In contrast to the pronounced blood-sugar-lowering effect of bolus injections of IGF I and II, the blood sugar levels did not decrease with the infusion of IGF I or II. The absence of hypoglycemic effects in the long-term infusion experiments is consistent with the fact that in men and in animals the large amounts of IGF present in the circulation do not cause hypoglycemia. The explanation for this is that complexed IGF has limited access to cell membrane receptors and that, in particular, its interaction with the insulin receptor of insulin target tissues is abolished.

When IGF is injected intravenously as a bolus, the binding capacity of carrier proteins is temporarily overridden so that free IGF can reach insulin or type I IGF receptors at sufficiently high concentrations to elicit acute insulin-like effects. In long-term infusion experiments, the continuously delivered IGF equilibrates with the carrier proteins, and free IGF is not detectable in the circulation. Therefore, we do not see acute, insulin-like effects on glucose homeostasis. On the other hand, the complexed IGF resulting from the equilibration of the infused hormone with its carrier protein is apparently able to stimulate growth processes. The mechanism how complexed IGF becomes available to growth receptors is still a matter of speculation [for review see 22].

The long-term in vivo studies support the concept that IGF I is the major human somatomedin. IGF I is capable of stimulating growth indices in the absence of growth hormone. The corresponding effect of IGF II is much less pronounced, and the true physiological role of IGF II remains to be elucidated. Growth hormone is one of the main regulatory factors for the synthesis of IGFs. It is likely that growth hormone modulates the effects of IGFs on their target tissue, either directly or via the induction of a specific large molecular weight IGF carrier protein. Under normal physiological conditions, neither IGF I nor IGF II appear to be involved in the regulation of glucose homeostasis. This, however, does not exclude the possibility that IGFs might become hypoglycemic under certain physiological or pathophysiological conditions, causing an increase of free IGF in the circulation. The ancestral relationship between insulin and IGF as derived from the largely homologous structures of these molecules suggests a complementary anabolic role. Whereas IGF appears to be a major anabolic hormone responsible for mediating the growth hormone message on growth and differentiation, insulin acts in a complementary fashion by acutely providing and regulating the fuel for these processes.

References

1 Daughaday, W.H.; Hall, K.; Raben, M.S.; Salmon, W.D., Jr.; Brande, L.J. van den; Wyk, J.J. van: Somatomedin: proposed designation for sulfation factor. Nature *235:* 107 (1972).

2 Klapper, D.G.; Svoboda, M.E.; Wyk, J.J. van: Sequence analysis of somatomedin C: confirmation of identity with insulin-like growth factor I. Endocrinology *112:* 2215–2217 (1983).

3 Enberg, G.; Carlquist, M.; Jörnvall, H.; Hall, K.:

The characterization of somatomedin A, isolated by microcomputer-controlled chromatography, reveals an apparent identity to insulin-like growth factor I. Eur. J. Biochem. *143:* 117–124 (1984).

4 Rubin, J.S.; Mariz, I.; Jakobs, J.W.; Daughaday, W.H.; Bradshaw, R.A.: Isolation and partial sequence analysis of rat basic somatomedin. Endocrinology *110:* 734–740 (1982).

5 Moses, A.C.; Nissley, S.P.; Short, P.A.; Rechler, M.M.; White, R.M.; Knight, A.B.; Higa, O.Z.: Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum. Proc. natn. Acad. Sci. USA *77:* 3649–3653 (1980).

6 Marquart, H.; Todaro, G.J.; Henderson, L.E.; Oroszlan, S.: Purification and primary structure of a polypeptide with multiplication-stimulating activity from rat liver cell cultures. J. biol. Chem. *256:* 6859–6865 (1981).

7 Moses, A.C.; Nissley, S.P.; Short, P.A.; Rechler, M.M.: Immunological cross-reactivity of multiplication stimulating activity polypeptides. Eur. J. Biochem. *103:* 401–408 (1980).

8 Zapf, J.; Froesch, E.R.; Humbel, R.E.: The insulin-like growth factors of human serum: chemical and biological characterization and aspects of their possible physiological role. Curr. Top. cell. Regul. *19:* 257–309 (1981).

9 Hintz, R.L.: Plasma forms of somatomedin and the binding protein phenomenon. Clin. Endocrinol. Metab. *13:* 31–42 (1984).

10 Meuli, C.; Zapf, J.; Froesch, E.R.: NSILA-carrier protein abolishes the action of nonsuppressible insulin-like activity (NSILA-S) on perfused rat heart. Diabetologia *14:* 255–259 (1978).

11 Zapf, J.; Schoenle, E.; Jagars, G.; Sand, I.; Grunwald, J.; Froesch, E.R.: Inhibition of the action of nonsuppressible insulin-like activity (NSILA-S) on isolated rat fat cells by binding to its carrier protein. J. clin. Invest. *63:* 1077–1084 (1979).

12 Daughaday, W.H.; Mariz, I.K.; Blethen, S.L.: Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native acid-ethanol-extracted serum. J. clin. Endocr. Metab. *51:* 781–788 (1980).

13 Humbel, R.E.: Insulin-like growth factors, somatomedins and multiplication stimulating activity. I. Chemistry; in Li, Hormonal proteins and peptides, vol. 12, chap. 4 (Academic Press, New York 1984).

14 Nissley, S.B.; Rechler, M.M.: Insulin-like growth factors, somatomedins and multiplication stimulating activity. III. Biosynthesis and receptors; in Li, Hormonal proteins and peptides, vol. 12, chapt. 4 (Academic Press, New York 1984).

15 Jansen, M.; Schaik, F.M.A. van; Ricker, A.T.; Bullock, B.; Woods, D.E.; Gabbay, K.H.; Nussbaum, A.L.; Sussenbach, J.S.; Brande, J.L. van den: Sequence of c-DNA encoding human insulin-like growth factor I precursor. Nature *306:* 609–611 (1983).

16 Bell, G.I.; Merryweather, J.B.; Sanchez-Pescador, R.; Stempien, M.M.; Priestley, L.; Scott, J.; Rall, L.B.: Sequence of a c-DNA clone encoding human pre-proinsulin-like growth factor II. Nature *330:* 775–777 (1984).

17 Dull, T.J.; Gray, A.; Hayflick, J.S.; Ullrich, A.: Insulin-like growth factor II precursor gene organization in relation to insulin gene family. Nature *330:* 777–781 (1984).

18 D'Ercole, A.J.: Production and transport of somatomedin C-insulin-like growth factor I. Report of the 89th Ross Conference on Pediatric Research, Carefree, Ariz., 1984 (Ross Laboratories, Columbus, in press, 1985).

19 Schwander, J.C.; Hauri, C.; Zapf, J.; Froesch, E.R.: Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. Endocrinology *113:* 297–305 (1983).

20 Cohen, K.L.; Nissley, S.P.: The serum half-life of somatomedin activity: evidence for growth hormone dependence. Acta endocr., Copenh. *83:* 243–258 (1976).

21 Kaufmann, U.; Zapf, J.; Froesch, E.R.: Growth hormone dependence of nonsuppressible insulin-like activity (NSILA) and of NSILA-carrier protein. Acta endocr., Copenh. *87:* 716–727 (1978).

22 Zapf, J.; Schmid, C.; Froesch, E.R.: Biological and immunological properties of insulin-like growth factors (IGF) I and II. Clin. Endocrinol. Metab. *13:* 3–30 (1984).

23 Wyk, J.J. van: Insulin-like growth factors, somatomedins and multiplication stimulating activity. I. Chemistry; in Li, Hormonal proteins and peptides, vol. 12, chap. 4 (Academic Press, New York 1984).

24 Nissley, S.P.; Rechler, M.M.: Somatomedin/insulin-like growth factor tissue receptors. Clin. Endocrinol. Metab. *13:* 43–68 (1984).

25 Schmid, C.; Steiner, T.; Froesch, E.R.: Preferential enhancement of myoblast differentiation by insulin-like growth factors (IGF I and IGF II) in primary cultures of chicken embryonic cells. FEBS Lett. *161:* 117–121 (1983).
26 Schmid, C.; Steiner, T.; Froesch, E.R.: Insulin-like growth factor I supports differentiation of cultured osteoblast-like cells. FEBS Lett. *173:* 48–52 (1984).
27 Kurtz, A.; Jelkmann, W.; Bauer C.: A new candidate for the regulation of erithropoiesis. FEBS Lett. *149:* 105–108 (1982).
28 Schoenle, E.; Zapf, J.; Froesch, E.R.: Receptor binding and effect of insulin and NSILA-S on glucose transport and metabolism in adipocytes from hypophysectomized rats. Diabetologia *16:* 41–46 (1980).
29 Schoenle, E.; Zapf, J.; Hauri, C.; Steiner, T.; Froesch, E.R.: Comparison of in vivo effects of insulin-like growth factors I and II and of growth hormone in hypophysectomized rats. Acta endocr., Copenh. *108:* 167–174 (1985).
30 Zapf, J.; Schoenle, E.; Froesch, E.R.: In vivo actions of the insulin-like growth factors in the hypophysectomized rat: comparison with human growth hormone and the possible role of the specific IGF carrier proteins. In: Growth factors in biology and medicine. Ciba Symposium No. 116, pp. 169–187 (Pitman, London 1985).
31 Rinderknecht, E.; Humbel, R.E.: Primary structure of human insulin-like growth factor II. FEBS Lett. *89:* 283–286 (1978).
32 Zumstein, P.P.; Lüthy, C.; Humbel, R.E.: Amino acid sequence of a variant pro-form of insulin-like growth factor II. Proc. natn. Acad. Sci. USA *82:* 3169–3172 (1985).
33 Honegger, A.; Humbel, R.E.: Insulin-like growth factors I and II in fetal and adult bovine serum. J. biol. Chem. *261:* 569–575 (1986).
34 Vetter, U.; Zapf, J.; Heit, W.; Helbing, G.; Heinze, E.; Froesch, E.R.; Teller, W.M.: Human fetal and adult chondrocytes: Effect of insulin-like growth factors I and II, insulin and growth hormone on clonal growth. J. clin. Invest. *77:* (in press 1986).

J. Zapf, MD.
Metabolic Unit,
Department of Medicine,
University Hospital,
CH-8091 Zürich (Switzerland)