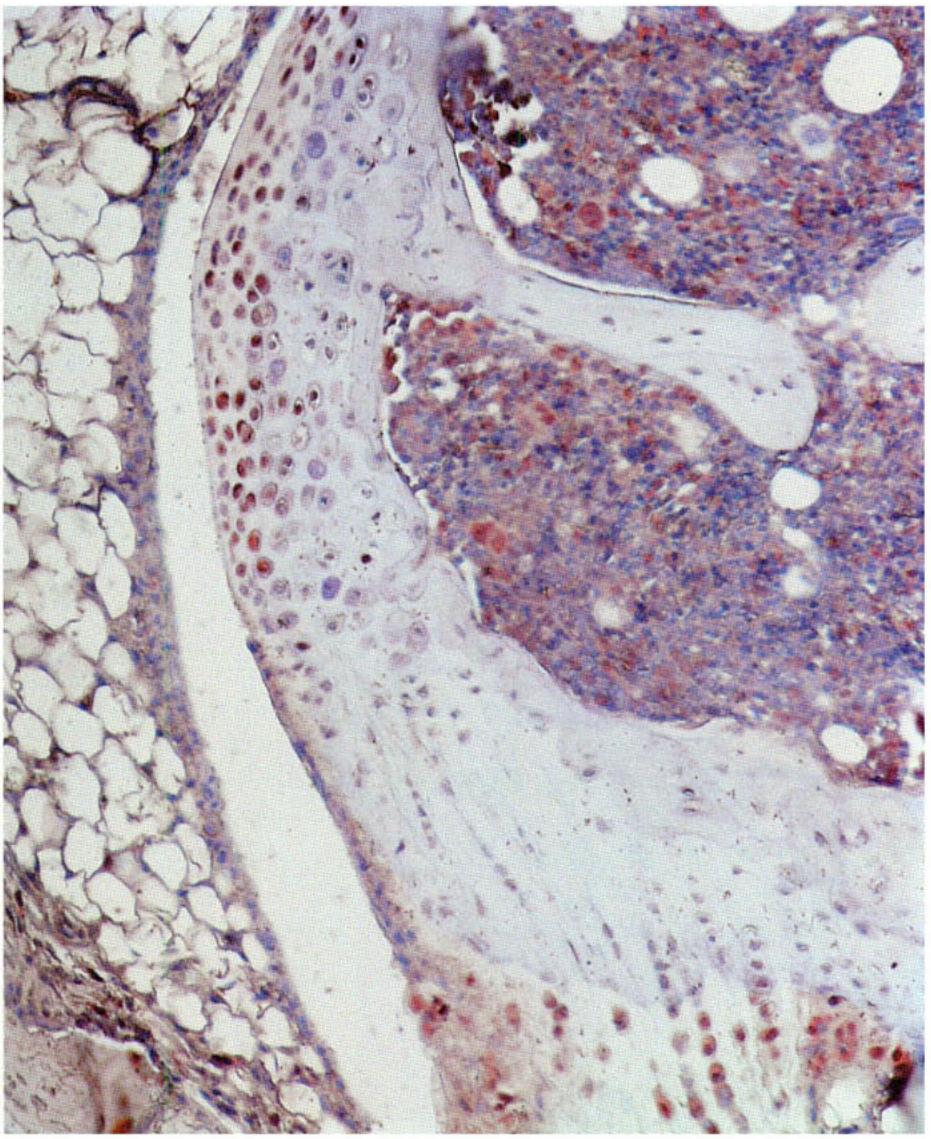
SYMPOSIUM ON INSULIN-LIKE GROWTH FACTORS/

SOMATOMEDINS

NAIROBI, KENYA - NOVEMBER 13-15, 1982



Immunoperoxidase staining of insulin-like growth factor-I (Somatomedin-C) in the articular cartilage of a growth hormone deficient Little (lit/lit) mouse treated with growth hormone. The reddish color indicates positive staining, except in the bone marrow which contains a high amount of endogenous peroxidase.

Insulin-Like Growth Factors Somatomedins

Basic Chemistry · Biology Clinical Importance

Proceedings of a Symposium on
Insulin-Like Growth Factors/Somatomedins
Nairobi, Kenya, November 13–15, 1982

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Preface

The Symposium on Insulin-Like Growth Factors/ Somatomedins was held in Nairobi, Kenya on November 13-15, 1982, as a satellite symposium to the 11th Congress of the International Diabetes Federation. The last international symposium on insulin-like growth factors had been held 4 1/2 years previously. During this time many major discoveries were made culminating in the direct demonstration that pure insulin-like growth factors promote growth in vivo. The increasing importance of this area of research is attested to by the large number of new workers who have entered the field and the exponential increase in papers. Because of the truly multinational character of research in this area, an international forum was desired for this meeting. The genetic relationship of the insulin-like growth factors to insulin made it appropriate to hold this meeting in conjunction with the International Diabetes Federation meeting in Nairobi, Kenya. The Symposium was held during the weekend recess of the parent conference. Representatives were present from many countries, including Switzerland, Great Britain, Australia, United States, Japan, Canada, France, West Germany, Kenya, Hong Kong, Denmark, South Africa, and Nigeria. The format consisted of invited lecturers, oral communications, and two poster sessions. The surroundings afforded a conducive environment for interaction between investigators, both during the conference and afterwards on safari.

The organizers are particularly indebted to the following sponsors, without whose generous contributions the Symposium could not have been held: Hoffman-La Roche, Inc., 11th Congress of the International Diabetes Federation, Shionoge Corporation, International Mineral & Chemical Corporation, Pfizer Central Research, Monsanto Company, Smith, Kline Clinical Laboratories,

KabiVitrum, Nichols Institute, Sumitomo Corporation, Mead Johnson & Company, Miles/Bayer Laboratories, Sandoz Pharmaceuticals, Children's Hospital of San Francisco, Hoechst A.G., Genentech, Inc., Connaught, Labs, Serono Laboratories, Inc., Pharmacia Laboratories, Inc., Dako Corporation, Beckman Instruments, Swissair, Speywood Labs. Contributions were also obtained from: AMGen, LKB Produkter AB, Endocrine Sciences, Cetus, Upjohn Company, Adria Laboratories, Lilly Research Laboratories, Becton Dickinson, Ross Laboratories, Merck Sharp & Dohme Research Laboratories, Schering Corporation, New England Nuclear.

San Francisco, May 1983

E. Martin Spencer

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The Three Historical Origins of Insulin-Like Growth Factor Research

THE SOMATOMEDIN HYPOTHESIS: ORIGINS AND RECENT DEVELOPMENTS

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The roots of the somatomedin hypothesis can be traced to the recognition by workers in Herbert Evans' laboratory in the 1940's that cartilage is the main target organ for growth hormone stimulated skeletal growth. Most of the early studies were carried out on epiphysial plate cartilage using tedious histologic morphometric techniques. After World War II, there was a boom in interest in radioactive isotopes as markers of metabolic processes and a great improvement in availability. Among the isotopes in the hands of investigators was ^{35}S sulfur. H. Böstrom in Stockholm (1) and D.D. Dzlewietkowski (2) of the Rockefeller Institute were pioneers in the use of ^{35}S sulfate uptake in cartilage as a marker of chondroitin-sulfate synthesis.

In this pre-RIA era, I was attracted to the possibility that ^{35}S-sulfate uptake in cartilage could be used as an end point for an in vitro bioassay of GH by the observation of Murphy in my laboratory that there was a defect in sulfate uptake in cartilage of hypophysectomized rats which could be corrected with 24 hours by GH administration (3).

At this time I was fortunate to have a very bright, hard-working but extremely modest and retiring young research fellow, Dr. William D. Salmon, Jr., join my laboratory. He rapidly established the conditions for in vitro incubaton of cartilage and a simplified method of measurement of ^{35}S sulfate uptake. He found that GH given in vivo to hypophysectomized rats restored the in vitro uptake of ^{35}S-sulfate by nasal, xiphoid and costal cartilage to the level of normal cartilage but when he added GH to the incubation medium either alone or with hypophysectomized rat serumto hypox rat cartilage, he observed little or no stimulation of ^{35}S-sulfate uptake (4).

This observation greatly disappointed us and aborted our plans to develop an in vitro

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bioassay. We could not attribute our failure to metabolic deterioration of cartilage during incubation because cartilage has no intrinsic blood supply and is normally adapted to anoerobic metabolism. Functional viability of cartilage in tissue culture media is maintained for long periods.

We next considered the possibility that GH could be acting indirectly through some serum component. Such a mechanism had been proposed earlier by Bornstein and Park (5) to explain the inhibitor of glucose uptake which appeared in hypophysectomized alloxan diabetic rat serum after GH treatment. As I had previously worked with Rollo Park, I was familiar with this hypothesis. Our initial experiments showed that normal serum stimulated two-fold increase in ^{35}S-sulfate uptake but serum from hypox rats was virtually inactive. The effect was not immediate but increased progressively over 24 hours of study. Treatment of hypox rats with GH restored the sulfate uptake stimulatory activity of serum to nearly normal levels. In these GH treated hypox rats there was a good temporal correlation between increase in ^{35}S-sulfate uptake of their cartilage with the appearance of the ^{35}S-sulfate uptake stimulatory activity of their serum. We showed that the stimulatory effect of GH was not attributable to insulin or glutamine. We went on to show that a difference between hypox and hormonal rat serum persisted after dialysis. The term "sulfation factor" was proposed for the activity. A quantitative bioassay for sulfation factor was proposed.

The assay for sulfation factor developed by Salmon for rat serum was applicable to human serum (6). Extremely low concentrations of sulfation factor were observed in patients after total hypophysectomy and in pituitary dwarfs. Most patients with acromegaly had elevations of serum sulfation factor.

Our observations of sulfation factor attracted little attention except in Stockholm where Professor Rolf Luft recognized their potential importance and interested one of his doctoral candidates, Sven Almqvist, in sulfation factor. Almqvist made improvements inthe statistical design of the hypox rat costal cartilage assays and by personally hypophysectomizing his rats and dissecting their costal cartilage he was able to minimize assay variance which has been the bane of this assay. In a series of 7 papers, Almqvist confirmed the basic findings of my laboratory and went on to

describe the changes of serum SF with age (7). He was the first to recognize that sulfation factor was low during the first 4 years of life. He also described a fall in sulfation factor concentration of two acromegalic patients treated with estrogens and the kinetics of decline of sulfation factor after hypophysectomy of acromegalic patients.

After this brilliant start in somatomedin research, it was decided by Professor Luft that the Department of Endocrinology and Metabolism at the Karolinska Hospital was more in need of a thyroidologist than a sulfation factorologist and Almqvist was sent to the NIH in Bethesda to become a thyroidologist.

The next years brought conclusive evidence that sulfation factor effects were not limited to hormonal sulfation of proteoglycans but included stimulation of collagen synthesis (8), synthesis of non-collagen protein (9), DNA synthesis (10) and RNA synthesis (9).

A clinical study of my laboratory in collaboration with Zvi Laron and associates at Petah Tikva Israel in patients with dwarfism and high serum growth hormone, commonly referred to as Laron dwarfism, attracted general interest in sulfation factor (11). We found that sulfation factor levels were as low in these patients as were found in patients with hypopituitarism but treatment with human growth hormone failed to restore normal serum factor activity . This dissociation of growth hormone levels and sulfation factor levels supported the essentiality of sulfation factor in human growth but other interpretations are possible.

The hiatus of interest in sulfation factor in Stockholm ended when Kerstin Hall (12) began her doctoral studies in the late 1960's in Professor Luft's Department. She developed an embryonic chick cartilage bioassay which had virtues of economy and convenience at the price of some loss of specificity. With Judson Van Wyk, who spent a sabbatical year in Stockholm, and others, she undertook a full scale attempt at isolation of the sulfation factor. An initial acid ethanol extraction was utilized to free the active peptide from binding proteins. Subsequent purification steps were monitored by measuring sulfate uptake, thymidine uptake in cartilage and insulin-like effects on epididymal fat. It was observed that throughout the various purification

steps sulfation factor, thymidine factor and insulin-like activity all co-purified and it was suggested that they were properties of the same molecules. It is notable that isoelectric separation provided clear evidence of separate basic and neutral peptides with growth factor activity. Subsequent purification of the neutral peptide was pursued in Stockholm by Kerstin Hall and the Kabi group and the basic peptide in Chapel Hill by Van Wyk and associates. The Zurich group with Rene Humbel was following a parallel duality in their isolation and characterization of IGF-I and IGF-II.

At this stage it was recognized that the operational name of sulfation factor was too restrictive for the multiple actions of the GH dependent tissue growth factors. The term somatomedin was arrived at by consensus of most of the investigators of the time (13). This Greco-Roman hybrid has been useful as a generic term for hormonal peptides mediating GH action. The neutral peptide under study in Stockholm was designated somatomedin A, an acidic peptide subsequently shown to contain EGF as a contaminant was called somatomed B and the basic peptide studied in Chapel Hill was named somatomedin C.

The changing of names from sulfation factor to somatomedin can also be looked upon as the coming of age of the somatomedin hypothesis. It marked the time when many new investigators entered the field and progress became more rapid with further characterization of the chemical nature of these substances culminating in the accomplishments of Rinderknecht and Humbel in establishing the sequences of IGF-I and II (14, 15). Our knowledge of the serum binding proteins for somatomedin increased. New radioreceptor and radioimmunoassays for somatomedins largely replaced bioassays. Separate receptors for IGF-I/Sm C and IGF-II were recognized and characterized. Seious study was undertaken of inhibitors of somatomedin actions.

The hypothesis that the somatomedins are important regulators of skeletal growth has not gone unchallenged. The in vitro biological activity of somatomedin complexed to its binding protein has been questioned. It has been impossible to demonstrate unequivocally the presence of unbound somatomedins in serum. All detectable somatomedin exists in specific binding protein complexes. In a number of test systems such as the isolated rat heart (16), rat adipocytes (17) and chick embryo

fibroblast (18) bound somatomedin is virtually inactive. We have observed that less than one-third of the somatomedin in whole serum has access to the somatomedin receptor on human placental membranes (19). In addition, the large complexes of protein bound somatomedins must be greatly hindered in crossing the capillary epithelium. Despite these limitations of action of protein bound somatomedins on certain tissues, somatomedin, in whole serum can effectively stimulate cartilage metabolism in vitro even at high dilutions. With our current in vitro conditions, 100-200% stimulation of sulfate incorporation by hypophysectomized rat cartilage is achieved with only 2% rat serum in the incubation medium. It is unknown whether the extremely small concentration of unbound somatomedin which escapes detection could be responsible for receptor activation or whether one or another species of bound somatomedin can activate the receptor directly. We find that the binding properties of the serum are altered at high dilution. This could act to increase dissociation of bound somatomedin in interstitial tissues. Whatever the explanation, somatomedin can reach cartilage in sufficient concentration and availability to exert its stimulatory effects.

While I do not wish to reject the insulin-like effects of somatomedins on non skeletal tissues, cartilage is the only mammalian tissue which has been studied in vitro which so specifically responds to somatomedin containing serum as compared to somatomedin poor serum.

A second major objection to the somatomedin hypothesis has been the lack of confirmation of the growth promoting actions of somatomedins in vivo. Until recently, investigators have been handicapped by limitations in availability of highly purified somatomedin peptides. Relatively large amounts of the purified somatomedins must be given to restore and maintain the serum concentration of somatomedin of hypophysectomized animals to normal. This is a consequence of the high serum concentration of the somatomedin peptides as compared to other hormones and the rapid clearance of administered somatomedin when not bound in the normal binding protein complex. In contrast to the need for relatively large amounts of somatomedin for replacement treatment of hypophysectomized animals, smaller amounts of GH are required. GH acts on liver and perhaps other tissues to produce somatomedins. It is likely that a single molecule of GH can promote the secretion of multiple somatomedin molecules.

The first positive demonstration that somatomedins can stimulate growth in vivo was provided by Van Buul-Offers et al. (20) who injected partially purified human somatomedin into immature, Snell dwarf mice. Growth in length and weight occurred and sulfate and thymidine uptake in isolated cartilage was stimulated. This study was not conclusive because the preparation administered, although devoid of significant GH or insulin contamination, was admittedly crude.

This criticism cannot be applied to the important observations of Schoenle et al. (21) who obtained unequivocal stimulation of growth of hypophysectomized rats with IGF-I infused continuously by implanted osmotic minipumps. IGF-II administered in a similar fashion was much less effective (22). Dr. Zapf will describe these studies in greater detail in his text.

The demonstration that IGF-I is more potent than IGF-II in stimulating growth in vivo and cartilage metabolism in vitro and the recognition that growth hormone dependence of IGF-I is much greater than IGF-II all lead to the conclusion that it is the major somatomedin of serum. IGF-II, possessing a separate dedicated receptor, probably will be shown to have different physiologic roles.

Isaksson et al. (23) have challenged the somatomedin hypothesis by demonstrating that GH can stimulate longitudinal bone growth directly. These investigators injected the epiphysial growth plates of hypophysectomized rats with 10 μg of GH on three occasions over a five day period. Appositional bone growth measured by a tetracycline labeling technique, demonstrated a 44% increase on the injected side as compared to the uninjected side. At face value this contradicts the somatomedin hypothesis. There are certain aspects of the experiment that need to be considered. The injection of 10 μg of hormone into an avascular tissue undoubtedly created unphysiologically high concentrations of hormone. The response was relatively small compared to a 227% stimulation of growth induced by 5 μg/day of GH subcutaneously in a similar experimental system by Thorngren and Hansson (24). Isaksson and associates certainly have not shown that exposure of the epiphyseal plate to physiologic concentrations of GH can restore normal appositional bone growth in the absence of somatomedins.

Another challenge to the hormonal role of somatomedin exists. It has been observed by Atkison et al. (25) and Clemmons et al. (26) that certain fibroblasts release RIA detectable Sm C/IGF-I-like peptides and that this release is stimulated by GH. These same cells are capable of being stimulated by Sm C/IGF-I. If this type of local production of somatomedins and their paracrine action is important in vivo, the somatomedins might not be true hormonal agents. These experiments would not explain the lack of effect of GH on isolated cartilage and the exquisite sensitivity of this tissue to somatomedin.

In conclusion I have reviewed the genesis of the somatomedin hypothesis and some of the early evidence on which it was founded. The years have brought additional clinical and experimental evidence in its support. The recent demonstration of the in vivo growth promoting potency of IGF-I has provided a long awaited and welcome addition to the evidence supporting the hypothesis. As of 1982, the role of somatomedins in mediating some or all of GH action on skeletal tissue remains an attractive and viable hypothesis.

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FROM NSILA TO IGF: A LOOK BACK ON THE MAJOR ADVANCES AND BREAKTHROUGHS

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The discovery of insulin-like activity of serum and of nonsuppressible insulin-like activity

The discovery of insulin-like activity (ILA) of serum dates back before the time when radioimmunoassays for the measurement of insulin in serum became available. The main observations were the following: When the diaphragm or adipose tissue of the rat are incubated in serum, glucose uptake of these tissues is stimulated as if they were in the presence of approximately 200 μU of insulin per ml (1,2). These findings were followed up by incubation experiments with adipose tissue and serum in the presence of anti-insulin serum from guinea pigs which inhibits the action of insulin. It was found that 90 % of the insulin-like effect of serum on adipose tissue was not suppressed by anti-insulin serum and it was reasoned that the insulin-like substance in serum could not be immunologically identical with insulin (3). Rat adipose tissue was mostly used for the detection and measurement of nonsuppressible insulin-like activity (NSILA) of serum and this bioassay proved to be very reproducible. It served as the major bioassay for NSILA until these substances were purified and chemically characterized. The activity of NSILA of serum before and after extraction was always expressed in terms of microunits of insulin which served as a well defined standard hormone.

Insulin-Like Growth Factors / Somatomedins

Extraction procedures for NSILA-s

In analogy to the extrcation of insulin from pancreas, acid/ethanol was originally used to extract NSILA from serum. A small portion of total NSILA of serum was found to be soluble in acid/ethanol. It was called NSILA-s, the s standing for soluble. The molecular weight of NSILA-s was estimated to be around 7'500 which was in sharp contrast to the molecular weight of NSILA in native serum which lies in the order of magnitude of 200'000 daltons (4). During acid/ethanol extraction of serum most of the NSILA remains in the precipitate. This fraction of serum NSILA was called NSILA-P, P standing for precipitated (5). These findings were erroneously interpreted to mean that the acid/ethanol extraction led to a denaturation and precipitation of the major part of serum NSILA. NSILA-P was biologically characterized and it was found to be active on both adipose tissue and diaphragm in vitro and after intraperitoneal injection into the rat (6). In vivo experiments with intravenous injections of impure preparations of NSILA-P ended with the rapid death of the rats (unpublished obersvation). NSILA of a high mol. wt. was also prepared by Dowex-chromatography and several subsequent purification steps by Poffenbarger who called the substance NSILP, P standing for protein (7). Antibodies against NSILP were produced in rabbits and a radioimmunoassay for NSILP was developed (8). According to some reports in the literature NSILP may be elevated in patients with tumor hypoglycemia. However, NSILP levels were also found to be elevated in patients with tumors which did not go along with hypoglycemia. Thus, the physiological significance of NSILP still is in the dark. It is also possible to separate large mol. wt. NSILA from NSILA-s by Sephadex chromatography (9). These large molecular forms of NSILA, i.e. NSILA-P, NSILP and large mol. wt. NSILA cannot be converted to NSILA-s and probably are not related to NSILA-s. They have

not been further characterized, neither chemically, nor biologically, nor physiologically. These forms of NSILA are not to be confounded with NSILA-s or IGF I and IGF II bound to their carrier proteins because acid treatment of any kind does not dissociate IGF I or IGF II from these forms of large mol. wt. NSILA.

For the analytical measurement of NSILA-s in individual sera the dissociation of NSILA-s from their binding proteins is mandatory. This was realized by acid chromatography over Sephadex by Schlumpf et al. in our laboratory (10). By this one-step procedure NSILA-s is reproducibly dissociated from the carrier protein and can then be determined in any of the assay systems which are now available (bioassays using fat cells or fat pads, chick embryo or other fibroblasts, sulfation of cartilage of various animals, protein binding assay using the carrier protein of human or other sera, radioimmunoassay for IGF I and IGF II), (for details see Zapf, this issue).

Large scale production of NSILA-s

Many tissues were extracted with acid/ethanol in the hope that 1) large amounts of NSILA-s might be obtained and 2) that the problem of the origin of this hormone might be resolved. However, we and others found that more NSILA-s is present in serum per mg of protein than in any other tissue. Therefore, the large scale production of NSILA-s had to start from serum as raw material. Precipitate B which is similar to Cohn fraction IV and which is a by-product of the preparation of human albumin and human gamma globulins and which cannot be used for any better purpose was found to contain large amounts of NSILA-s bound to its binding proteins (5). A method was devised in our laboratory to extract and purify NSILA-s in

small amounts from serum and from this Cohn fraction IV . This procedure was then addopted by Dr. Richard at Hoffmann-La Roche who extracted 6 tons of Cohn fraction IV with acetone and acid ethanol and sent the acetone powder to Humbel in the Department of Biochemistry of the University of Zürich (11). Rinderknecht and Humbel purified this crude preparation of NSILA-s using a total of about 6 steps for their purification procedure and finally managed to identify two pure polypeptides, IGF I and IGF II.

PURIFICATION SCHEME LEADING TO THE ISOLATION OF IGF I AND II

Purification step	Specific biological activity: mU/mg protein (fat pad assay)	Purification (compared to IGF in whole serum)
Native serum	0.0015–0.003[a]	—
Precipitate B	0.005–0.01	2–6×
Acid ethanol extract (acetone powder)	0.05–0.08	20–50×
Acetic acid (0.5 *M*) extract	0.1–1.0	50–500×
Sephadex G-75 (0.5 *M* acetic acid)	5–15	2000–10,000×
Sephadex G-50 (1.0 *M* acetic acid)	20–40	7000–25,000×
PAGE (20%) in 0.1% SDS, pH 8.8	200–400	70,000–250,000×
SE-Sephadex C-25 in 0.05 *M* pyridine-acetate, pH 2.5, 55°		
pool I = IGF I	336 ± 20	300,000–500,000×[b]
pool II → PAGE (20%) pH 4.3 → IGF II	308 ± 26	70,000–100,000×[b]

[a] Determined after acidic gel filtration of native serum on Sephadex G-50 (medium) in small-molecular-weight pool.

[b] Purification as compared to the level of IGF I and IGF II in serum measured by RIA. Purification factors for all other purification steps refer to the mixture of the two polypeptides.

Table 1 (from 15)

The chemical nature or IGF I and IGF II

Rinderknecht and Humbel published the amino acid sequence of IGF I and IGF II in 1978 (12,13). Both molecules resemble insulin relatively closely. Approximately 45 % of the amino acids in IGF I and in IGF II are located in the same positions

as in the human insulin molecule and these regions of homology are the A- and the B-chain. The three disulfide

Primary structure of IGF II (from 13)

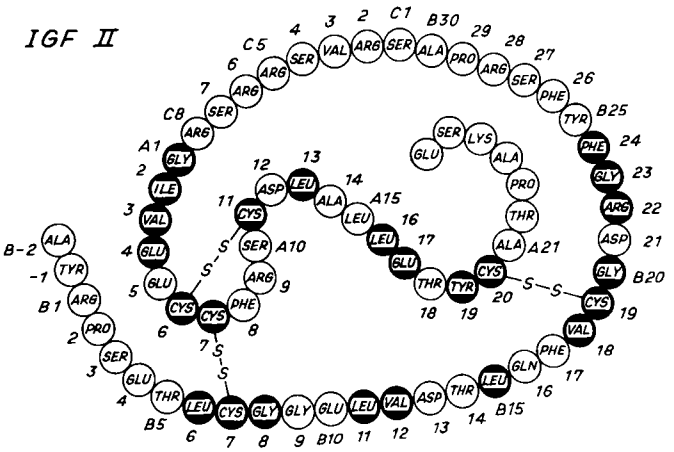

Figure 1

bonds present in the insulin molecule are also placed in the same locations in IGF I and IGF II. In contrast to insulin, the C-region is maintained in the IGF I (12 amino acids) and IGF II (8 amino acids) molecule and there is an extension of the A-chain which was called D-region. The latter consists of 8 amino acids in the case of IGF I and 6 amino acids in the case of IGF II. Blundell et al. estimate that the gene duplication which led to the diverson of the common ancestor molecule to insulin on one hand and IGF on the other hand occurred about 600 million years ago, i.e. when the vertebrates appeared on earth (14). The gene duplication which led to the existence of IGF I and IGF II is dated to about 300 million years ago, i.e. to about the time when mammals appeared on earth. These figures are based on the assumption that the substitution of amino acids in IGF I and IGF II took place at a rate similar to that of insulin.

This discovery of Rinderknecht and Humbel is of paramount importance in the NSILA-s/IGF story for several reasons:

1) there appears to be not just one insulin gene, but rather a family of genes which code for insulin, insulin-like growth factors and maybe still some other not yet defined factors.

2) the structural similarity between IGF I and II on one side and insulin on the other explains easily why these hormones crossreact at the level of their membrane receptors. In all likelihood the insulin receptor and the IGF receptors have regions which are similar and which recognize both species of hormones.

Proposed receptor binding region of insulin projected on the 3-dimensional model of IGF I

Antibody regions of insulin projected on the 3-dimensional model of IGF I

Figure 2 (from 15)

3) the species differences among insulin molecules and there relative biological protency (receptor binding and bioactivity) has yielded some information about the biologically essential regions in the insulin molecule. The discovery of the primary structure of the IGFs adds a great deal of new

knowledge to what had been speculated about these regions that are important for biological activity. One may in the future be able to design new molecules which may have even greater affinity for the insulin receptor than insulin itself or which bind better to the IGF receptor than IGF I and II, so that a large spectrum of insulin-like molecules can be envisaged.

Insulin-like effects of IGF I and IGF II

As already mentioned above most cells studied so far have membrane receptors for IGF I and IGF II. Lately, two different IGF receptors have been characterized and a topography of IGF receptors is presented in this volume by Rechler. NSILA was originally detected and could later be measured because it acts like insulin on adipose tissue and muscle. The effects of IGF I and IGF II on the fat pad and adipose tissue cells are identical to those of insulin (for review see Zapf et al.; 15). IGF stimulates glucose transport in and out of the adipose tissue cell, glycogen synthesis and lipogenesis and inhibits glycogen breakdown and lipolysis in the same way as insulin. IGF is between 50 and 100 times less potent than insulin. IGF acts on the rat heart muscle also in the same way as insulin and it is about 3 to 5 times less potent. The soleus muscle of the mouse and rat is stimulated by IGF as by insulin and IGF is approximately 10 % as potent as insulin (16). Qualitatively, the effects of insulin and of IGF cannot be distinguished. IGF stimulates glucose transport, glycogen synthesis, glycolysis, amino acid transport and protein synthesis. Liver cells have a membrane receptor which binds IGF II particularly well. A function has not yet been ascribed to the hepatic IGF II receptor.

IGF I and IGF II are potent hypoglycemic agents when injected as a bolus i.v.

Growth promoting effects of IGF

Morell et al. (17) showed for the first time that impure preparations of NSILA-s stimulate DNA-synthesis of cultured chick embryo fibroblasts and their replication. In the meantime similar experiments have been carried out with both IGF I and IGF II and it has been shown that these two growth factors are equipotent on chick embryo fribroblasts (18). On a molar basis of comparison they are approximately 100 times as potent as insulin and it is believed that insulin acts via the IGF receptor. Many cells in culture have one or two IGF receptors and react to IGF by increasing protein synthesis, RNA-synthesis and DNA-synthesis. This is particularly true for all cells deriving from mesenchymal tissues. IGF I and IGF II are potent sulfation factors (chick, rat and pig cartilage; 15).

Replication of chick embryo fibroblasts with different growth factors.

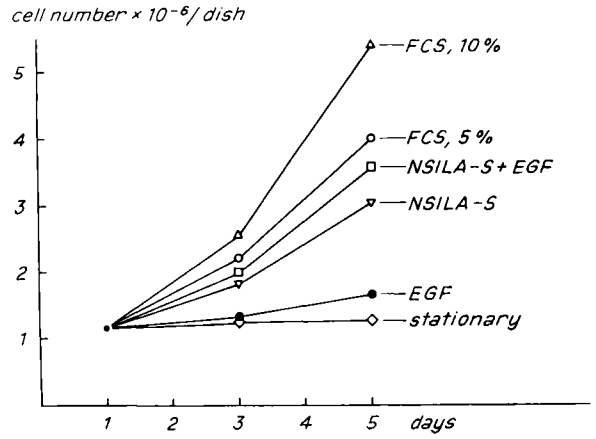

Figure 3

The hypothesis has been put forward that serum is a good growth medium because it contains the two most important growth factors in excess, namely the IGFs on one hand and platelet derived growth factor on the other hand (29). Together these two groups of factors render the cell competent for dividing and they stimulate cell division (progression). In many cell systems IGF does not only induce replication of cells but also their differentiation. This has been shown for premyoblasts (Schmid et al., this issue), from chicken embryos and also for erythroid cells from mice (19). In any case, it is of great interest that serum can be substituted by a mixture of IGF and platelet derived growth factor. Human fibroblasts grow just as well in the presence of IGF and PDGFs as with 5 % fetal calf serum. It should be possible in the future to culture most cells in the presence of defined growth factors and hormones.

Myotube formation in cultures of primary chick embryo "fibroblasts" seeded without serum but in the presence of 30 ng of IGF I. (from Schmid et al., in prep.)

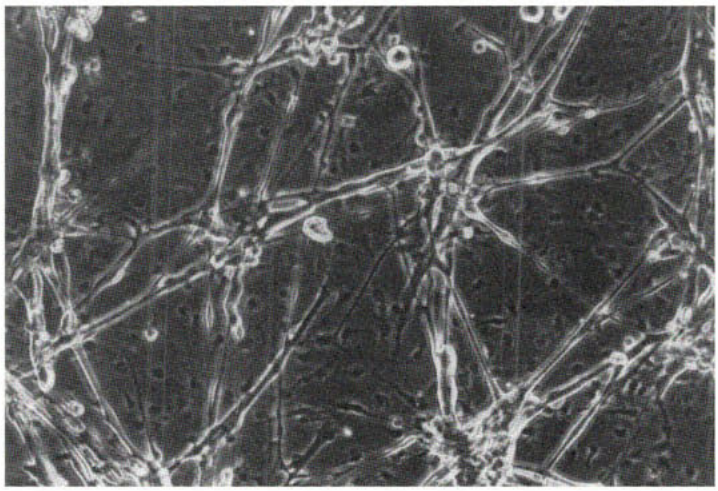

Figure 4

The IGF binding proteins and their physiological significance

As already discussed under the first 3 headings, NSILA in its native form in serum has a molecular weight of around 200'000 but IGF only one of 7'500. Acid ethanol extraction or acid chromatography of serum on Sephadex leads to a decrease of the mol. wt. of NSILA to that of the IGFs. Zapf et al. have shown that this phenomenon is due to the stripping of IGF from specific binding proteins of serum (20). There are two major IGF binding proteins in serum one with a mol. wt. of 200'000, the other one with a mol. wt. of 50'000. In all likelihood more than 99 % of IGF circulates in the blood bound to these binding proteins. Most of the total IGF I and IGF II is present in the peak with a mol. wt. of 200'000 (21). If labeled IGF I or II is injected intravenously to normal rats the labeled IGF binds rapidly to the small mol. wt. binding peak and then shifts over to the large mol. wt. binding peak (22). When bound to the latter peak ^{125}I-IGF has a half life in the rat of about 90 minutes. In the hypophysectomized rat and also in humans with pituitary insufficiency the large mol. wt. binding peak is diminished or altogether absent and most of the intravenously injected ^{125}I-IGF binds to the small mol. wt. binding peak (23). In this bound form ^{125}I-IGF has a half-life in the rat of 20 to 30 minutes. As demonstrated by Meuli et al. (23) and Zapf et al. (24) binding of IGF to the IGF binding proteins precludes their interaction with the insulin receptor so that insulin-like effects of IGF are no longer observed in the presence of a saturating amount of binding protein. The effect of IGF on adipocytes in vitro is inhibited by adding IGF binding protein (24). The perfused rat heart does no longer respond to IGF if a large enough amount of binding protein has been added to the perfusion medium (24).

Distribution of ^{125}I-IGF among serum proteins after i.v. injection to normal rats from 1 minute to 4 hours (from 24)

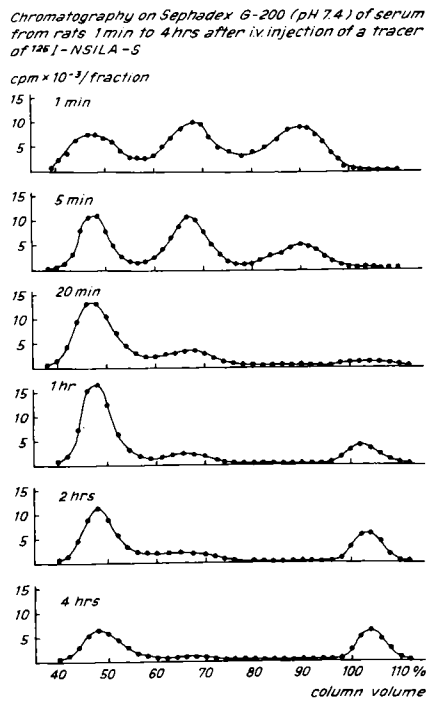

Figure 5

This phenomenon can be interpreted to mean that the organism protects itself against the hypoglycemic effects of IGF. There is enough IGF in serum that glucose uptake of adipose tissue and muscle would always be maximally stimulated and could not be regulated by insulin if the IGF did circulate as the free hormone. The binding proteins are responsible for the fact that IGF can play its role as growth promoting and regulating agent and that it does not influence glucose homeostasis to any major extent. With the sulfation assay one measures the

total amount of somatomedins/IGFs present in serum, i.e. somatomedins attached to their binding proteins. It appears that cartilage and maybe some other tissues and cells have a mechanism available which allows them to detect IGF in the bound form or to dissociate it from the binding protein so that the IGF receptor can recognize the free molecule and can then respond to it. How these cells and tissues do this, remains heretofore a mystery.

IGF I, the major somatomedin in human serum

When radioimmunoassays for the determination of IGF I and IGF II became available it was possible to determine these two hormones for the first time separately from each other. Zapf et al. have shown that the levels of IGF I are elevated in acromegalic patients and low in pituitary dwarfs (25). Some dwarfs with normal or high growth hormone concentrations in the serum also have low IGF I levels, in particular the so-called Laron dwarfs and an entire ethnic group of small people, the pygmies (25, 26). The pygmies seem to have a short stature not because they lack growth hormone but because they are deficient in IGF I. Infusion of growth hormone to a pituitary dwarf restores IGF I levels back to normal and, concomittantly, incites a growth spurt but it has no such effects in the pygmy (27). IGF I levels rise during puberty in normal children when the growth spurt occurs (25). All these clinical findings point to the major physiological effect of IGF I as a growth hormone.

The final proof that IGF I is a growth promoting hormone has been obtained in our laboratory when we showed that slowly infused IGF I induces growth in hypophysectomized rats in the absence of any other pituitary or pituitary-dependent peripheral hormones (28). These animals were not substituted

with thyroid hormone, sex or adrenal steroids. IGF I was clearly more potent than IGF II. These results support the somatomedin hypothesis of Salmon and Daughaday (29): Growth hormone induces the synthesis of IGF I in men and IGF I then acts on fibroblasts, chondrocytes, osteoblasts etc. and leads to replication of these cells and to the production of collagen and proteoglycans. The results of these experiments do not preclude direct effects of growth hormone on other cell systems or effects of growth hormone on these same cells.

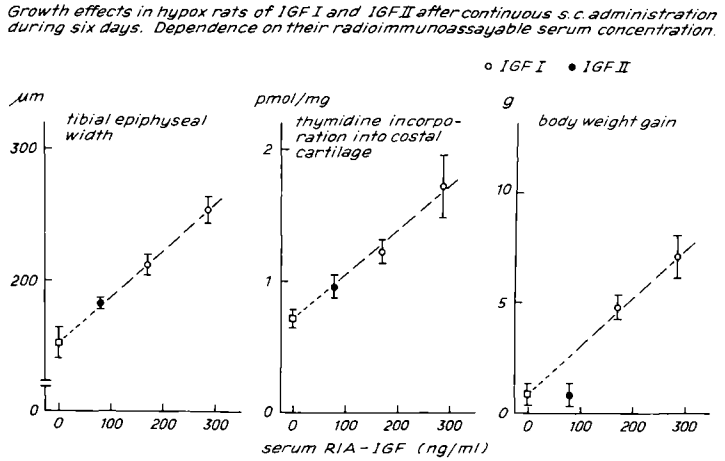

Figure 6 (adapted from 28)

Outlook on the possible therapeutic usefulness of the IGFs

It seems as if the digression of the ancestor molecule of the insulin family into insulin and IGFs had brought about a diversification of one major anabolic principle in lower animals into two major anabolic principles in higher animals, insulin becoming responsible for the homeostasis of fuel in the body and the IGFs taking on the responsibility together with other growth factors, for the initiation and maintenance

of growth processes in mesenchymal tissues. It is quite clear that the net loss of fuel in the absence of insulin does not permit growth in the face of an extremely catabolic situation. The absence of IGF I in man, on the other hand, does not lead to an acute metabolic decompensation but rather to failure to grow. Therefore, these two groups of insulin-like hormones, insulin and the IGFs have complementary effects on fuel homeostasis and on growth and replication of cells. Their respective receptors mediate similar processes in the cells, but IGF is kept from acting on the insulin receptor by its binding to the IGF binding proteins.

Insulin has become a standard drug for more than 60 years and it saves the lives of millions of insulin-dependent diabetics. It also makes it possible for diabetic children to grow normally. Diabetic children who are well regulated with insulin do have normal amounts of IGF I in their plasma. Insulin alone, however, does not guarantee a normal level of IGF I as demonstrated by pituitary dwarfs, pygmies and Laron dwarfs. Besides insulin, growth hormone is needed to maintain a normal level of IGF I. Furthermore, in situations of extreme stress, growth hormone may be very high and IGF I levels low so that the organism remains in a catabolic state. It is to be expected that the replacement therapy with IGF would be useful in the following clinical situations: polytraumatized and burned patients just to name two important clinical situations in which IGF I might switch the organism from a catabolic into an anabolic state. IGF I might help in conditions of acute osteoporosis like that occurring during massive steroid therapy or immobilization. Finally, the IGFs could be helpful in local healing processes: wound healing in general, fracture healing, and maybe in some situations where degenerative processes are destroying joints. All this may still sound very speculative and indeed, it is. However, the astounding biological effectiveness and the broad spectrum of the biological

effects of IGF make it likely that these substances will one day become useful therapeutic tools in a number of clinical situations (30).

View of the complementary anabolic effects on insulin and IGF mediated by the respective hormone receptors in different cells and tissues (from 30)

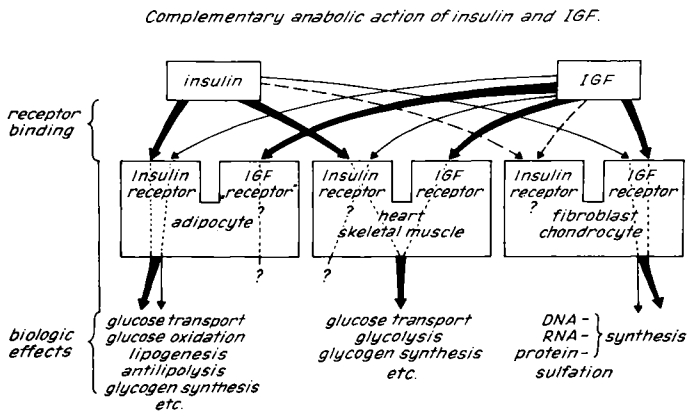

Figure 7

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MULTIPLICATION STIMULATING ACTIVITY FOR CELLS IN CULTURE

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Introduction

While endocrinologists were isolating the growth hormone dependent factor(s) which stimulate [^{35}S]sulfate incorporation into cartilage proteoglycan, and investigators in diabetes research were characterizing nonsuppressible insulin-like activity in human plasma, cell biologists were attempting to purify and characterize serum macromolecules which are required for the multiplication of cells in culture. Temin called this factor(s) multiplication stimulating activity (MSA) (1). Although the term, MSA, was first applied to the activity in serum, in the literature MSA has usually referred to a family of polypeptides identified in serum-free medium conditioned by the rat liver cell line BRL-3A. In this paper we will recount the research on MSA including chemistry, biological effects, physiology in the rat, cell surface receptors, and production by cells in culture.

Multiplication Stimulating Activity in Serum

The observation by Temin (2) that high concentrations of insulin partially replaced the serum requirement for the multiplication of chick embryo fibroblasts led Pierson and Temin (3) to apply the purification schemes that had previously been

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employed in the isolation of plasma insulin-like activity, to the purification of MSA from calf serum. Using an assay that measured the incorporation of [^{3}H]thymidine into DNA in chick embryo fibroblasts, Pierson and Temin achieved a 6,000-8,000 fold purification of multiplication stimulating activity from bovine serum. In retrospect, this MSA preparation from bovine serum probably represented an impure mixture of all of the members of the insulin-like growth factor (IGF)/somatomedin family. That growth hormone dependent factors are important serum components for the multiplication of chick embryo fibroblasts in culture is supported by the finding that these cells

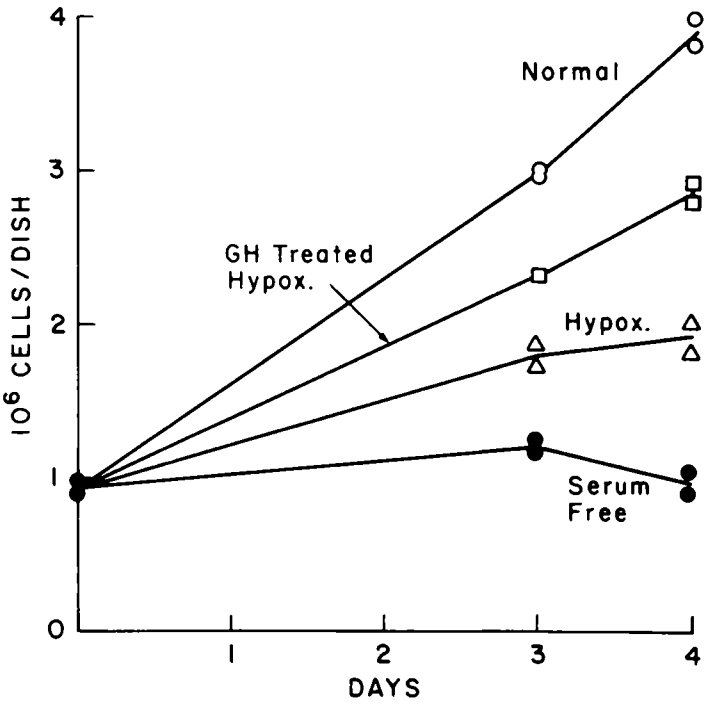


Fig. 1. Growth of tertiary cultures of chick embryo fibroblasts in extracts of sera from normal, hypophysectomized, and growth hormone-treated hypophysectomized rats. The extract was prepared by addition of physiologic saline to serum (3:1, vol/vol), adjusting the pH to 5.5 and boiling for 15 min (from Cohen et al. (4)).

grow better in an extract of normal rat serum than in a hypophysectomized rat serum extract and this deficiency can be corrected by growth hormone treatment of the hypophysectomized rat (Fig. 1) (4).

MSA from Medium Conditioned by a Rat Liver Cell Line (BRL-3A)

Reasoning that cells that are able to multiply in culture without serum do so because they produce MSA, Dulak and Temin (5) applied the purification scheme that had been used for the partial purification of MSA from calf serum, to purify MSA from serum-free medium conditioned by the rat liver cell line BRL-3A. In addition to multiplication stimulating activity for chick embryo fibroblasts, this MSA preparation had insulin-like metabolic activity and sulfation activity in the hypophysectomized rat cartilage assay.

MSA chemistry

Dulak and Temin (6) used isoelectric focusing and acrylamide gel electrophoresis to show that MSA from the BRL-3A rat liver cells actually consisted of a family of polypeptides. Moses et al. (7) demonstrated that there were at least 7 MSA species ranging in molecular weight from 7,100 to 16,250. There was a single species Mr = 16,250, 4 with Mr = 8,700 and 2 with Mr = 7,100. It was proposed that the sequences of the smaller MSA species were contained within the larger forms. This was supported by similar behavior of the different MSA species in bioassays and radioreceptor assays (7). In addition, the different size MSA species all recognized a rabbit antiserum raised against the Mr = 8,700 species (Fig. 2) (8). Recently a Mr = 21,600 species (presumably preproMSA) has been specifically immunoprecipitated with antisera raised against MSA (Mr = 8,700) from a mixture of [^{35}S]proteins synthesized in a

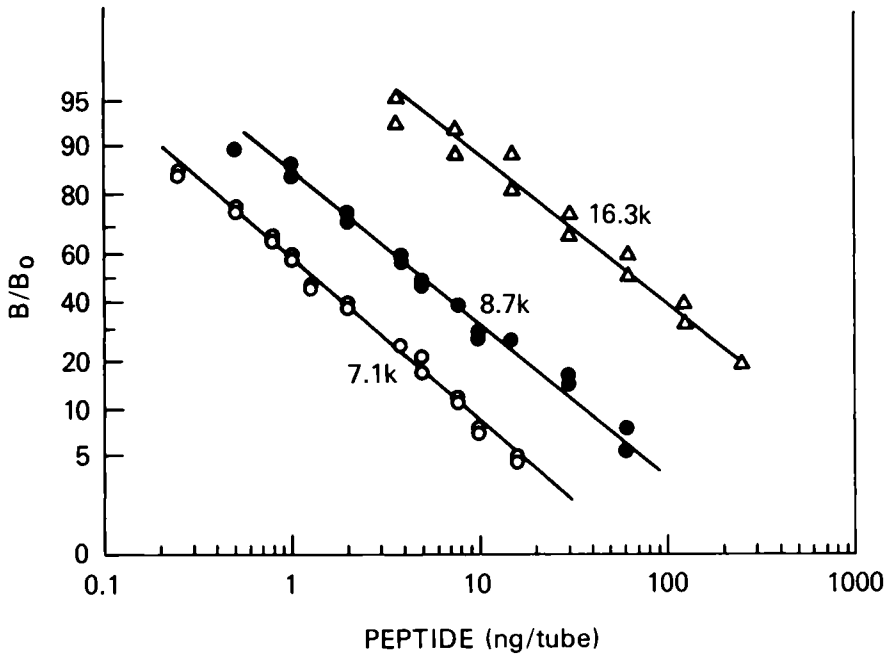


Fig. 2. Crossreactivity of MSA polypeptides (Mr = 7,100, 8,700, 16,250) in an MSA radioimmunoassay which utilizes a rabbit antiserum raised against MSA II-I (Mr = 8,700) (from Moses et al. (8)).

reticulocyte lysate translation system directed by BRL-3A mRNA (9). A slightly smaller MSA species (Mr ≅ 20,000), presumably proMSA, has also been identified at early labeling times in whole cell (BRL-3A) experiments which examined the incorporation of [^{35}S]cysteine into polypeptides specifically immunoprecipitated by 4 different antisera raised against MSA (Mr = 8,700) (10) (see the paper by Yang et al. in this volume). Taken together, these experiments strongly support the conclusion that the MSA polypeptides first described in BRL-3A conditioned medium by Dulak and Temin (5, 6) are all derived from a single precursor molecule Mr = 21,600.

In an important paper, Marquardt and his colleagues (11) reported the complete amino acid sequence of a Mr = 7,484 MSA

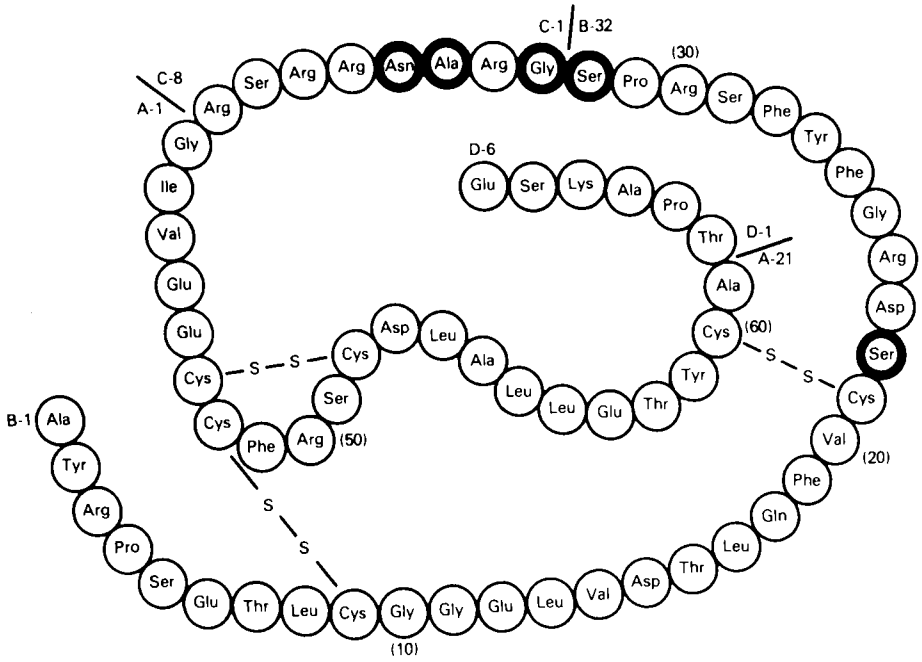


Fig. 3. Amino acid sequence of MSA (Mr = 7,484) purified from serum-free medium conditioned by the BRL-3A rat liver cell line. Residues which are homologous with residues in human IGF-II are shown by the solid circles. Domains corresponding to proinsulin and human IGF sequences are indicated by the letters A-D (drawn from the data of Marquardt et al. (11)).

species purified from the BRL-3A rat liver cell line. Larger MSA species were also found by Marquardt et al., but they chose to purify the Mr = 7,484 species. They reported that there are only 5 amino acid differences between MSA and human IGF-II; 3 of the differences are in the C-peptide region (Fig. 3). They concluded that MSA is the rat homologue of human IGF-II. On the basis of behavior on Biogel P10 gel filtration and high pressure liquid chromatography, as well as amino acid composition (Yvonne W-H. Yang, unpublished data), it seems likely that the MSA species which Marquardt and his colleagues sequenced is one of our MSA III species (Mr = 7,100) (7). (Since the error of molecular weight measurement by gel filtration in

Table I

Biologic Effects of MSA

A. Insulin-like metabolic activity
    1. Glucose transport (12, 13)
    2. Glucose oxidation, lipogenesis (14, 15)
B. Growth stimulation
    1. DNA synthesis and cell multiplication (12, 16-20)
    2. Incorporation of radioactive uridine into RNA (20, 21)
    3. Incorporation of radioactive amino acids into protein, protein accumulation (20, 22)
    4. Incorporation of [^{35}S]sulfate into proteoglycan, proteoglycan accumulation (5, 20, 22)
    5. Transport of amino acids (12, 13, 23-26)
    6. Rubidium uptake (27)
    7. Increase in ornithine decarboxylase activity (28-30)
    8. Meiotic maturation of Xenopus laevis oocytes (31)
C. Differentiation of myoblasts and chondrocytes (32, 33)
D. Other
    1. Tyrosine amino transferase induction (24)
    2. Estrogen-mediated induction of the ovalbumin gene (34)
    3. Inhibition of adenylate cyclase (35)

guanidine hydrochloride is at least 10%, the Mr = 7,484 calculated from amino acid sequence is not significantly different from Mr = 7,100.)

Biological effects of MSA

Table I lists the biological activities of MSA. Other than some systems in which other IGFs have not been tested, we can conclude that these biological activities are shared by the

other IGFs. The experiments referenced in Table I were performed with MSA that had been separated from the binding protein. Knauer and Smith (36) showed that addition of purified binding protein resulted in inhibition of MSA stimulated [^{3}H]-thymidine incorporation into chick embryo fibroblast DNA. The

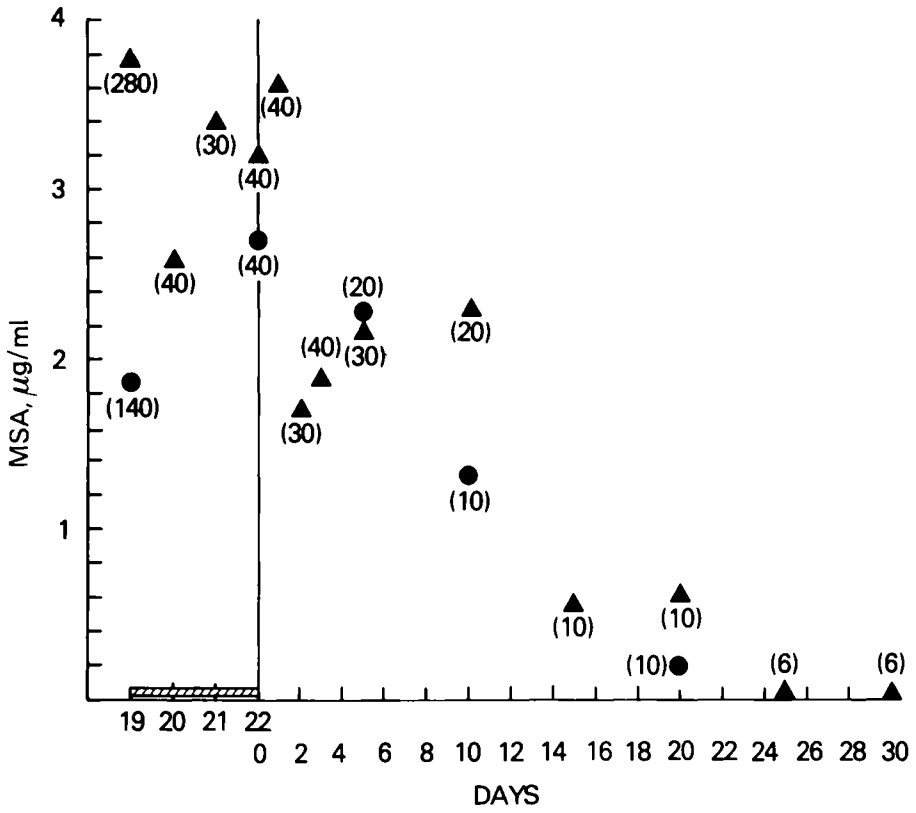


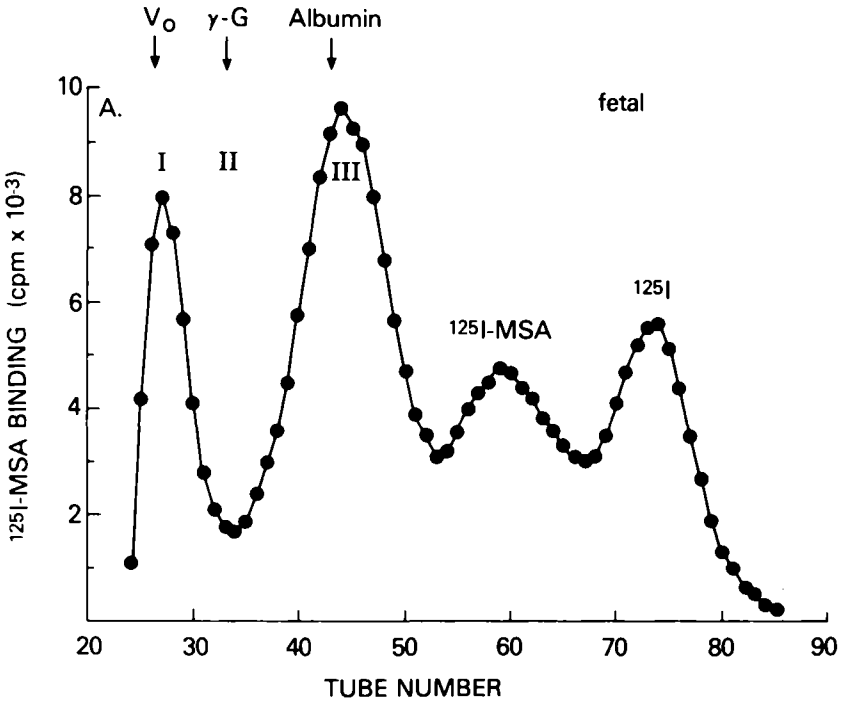
Fig. 4. Age dependence of MSA levels by radioimmunoassay in sera of maternal (hatched bar), fetal, neonatal, and young rats. The number of fetuses for each pool is indicated in parentheses; when two different pools from rats of the same age were assayed, the results are indicated by different symbols. Pools were chromatographed on Sephadex G-75 in 1 M acetic acid and the radioimmunoassay was performed on a pool of fractions corresponding to the elution volume of MSA standards (Mr = 7,100 and 8,700) (from Moses et al. (39)).

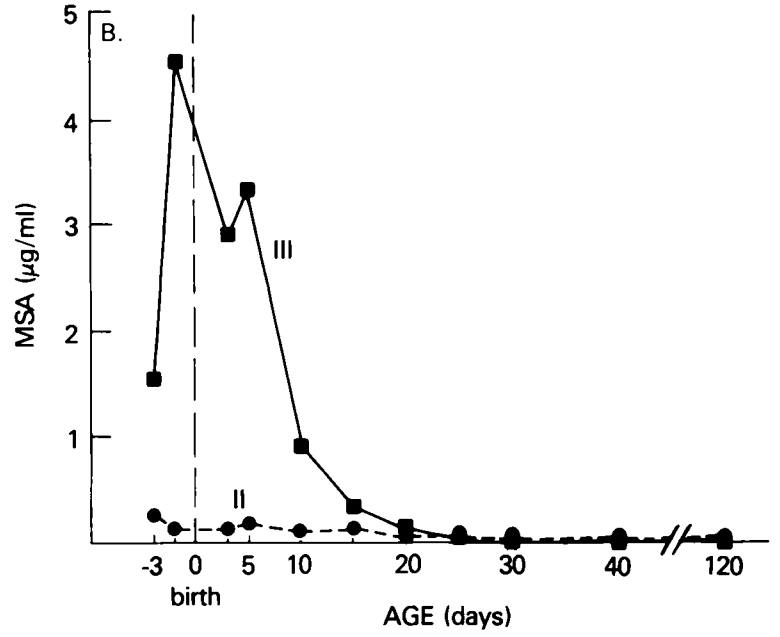
conclusion drawn from this type of experiment is that the native MSA-binding protein complex is biologically inactive. However, since the purified binding protein may have been partially denatured during purification, it is possible that the native complex is biologically active.

MSA physiology in the rat

The development of a specific radioimmunoassay for MSA (8) made it possible to measure MSA in biologic fluids. Although it could be determined that MSA levels by radioimmunoassay were growth hormone dependent in young adult rats (37), the levels were very low and account for a fraction of the total somatomedin activity. The finding that polypeptides indistinguishable from MSA were synthesized by fetal rat liver explants in organ culture (38) led us to measure the MSA concentration in fetal rat serum. Moses et al. (39) found that MSA levels were 20-100 fold higher in fetal rat serum than in maternal serum and gradually declined after birth to reach maternal levels by day 25 of extrauterine life (Fig. 4). Using a rat placental membrane radioreceptor assay which is specific for IGF-II, Daughaday et al. (40) have also found that rat IGF-II levels are higher in fetal than in maternal serum. In these experiments the developmental pattern is somewhat different from that found with the MSA radioimmunoassay; there was a peak of activity at day 5 and

Fig. 5. (opposite) Panel A. Chromatography of fetal (19-day gestation) rat serum on Sephadex G-200 after incubation with ^{125}I-MSA. The elution volumes of gammaglobulin and albumin are shown. Panel B. Radioimmunoassay of MSA associated with the gammaglobulin-size (II) and albumin-size (I) binding proteins in sera of rats of different ages. Prior to assay sera were gel filtered as in Panel A and fractions corresponding to peak III and gammaglobulin region (II) of the column were pooled and gel filtered on Sephadex G-50 in 1 M acetic acid to dissociate and separate MSA from the binding proteins. The MSA radioimmunoassay was performed on the Sephadex G-50 post-void pool (redrawn from White et al. (42)).


V_o
γ-G
Albumin
A.
fetal
I
II
III
^{125}I-MSA
^{125}I
^{125}I-MSA BINDING (cpm x 10^{-3})
TUBE NUMBER


B.
III
II
MSA (μg/ml)
birth
AGE (days)

levels at day 25 were not different from that late in gestation. It is not known why the MSA radioimmunoassay and the rat placental radioreceptor assay provide somewhat different developmental patterns in the rat since both assays measure rat IGF-II (MSA). In any case, these developmental patterns for rat IGF-II are different from that described for rat IGF-I; rat IGF-I levels are higher in adult rats than in fetal rats (40, 41).

White et al. (42) found that MSA in fetal and neonatal rat serum was exclusively associated with a binding protein slightly smaller than albumin (Fig. 5); the growth hormone dependent, gammaglobulin-size binding protein was absent in fetal rat serum. Accompanying the decline in serum MSA levels and the increase in rat IGF-I postnatally, there is a change in the binding protein pattern from the fetal, albumin-size binding protein to predominance of the gammaglobulin-size binding protein. Interestingly, the fetal binding protein is the same size as the binding protein produced by fetal rat liver explants in organ culture (43) and synthesized by the BRL-3A2 rat liver cell line (44). Knauer et al. (45) have purified the binding protein synthesized by the BRL-3A cell line; it appears to be made up of 2 subunits of Mr = 30,000 and 31,500.

MSA receptors

An explanation for the mitogenic effect of pharmacologic concentrations of insulin on chick embryo fibroblasts (2) was provided by early experiments which examined the binding of radiolabeled MSA to these cells (15, 46, 47). Insulin was found to compete with ^{125}I-MSA for binding. The relative potency of MSA, insulin, and proinsulin in competing for binding of ^{125}I-MSA to the chick embryo fibroblasts was the same as their relative potency in stimulating [^{3}H]thymidine incorporation into DNA (47), suggesting that insulin was mitogenic for these cells by

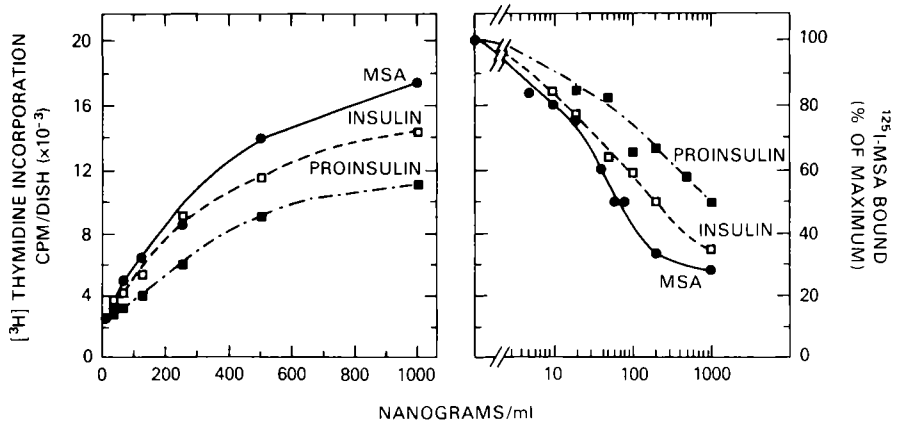


Fig. 6. Left panel. Stimulation of [^{3}H]thymidine incorporation into DNA in chick embryo fibroblasts by different concentrations of MSA, insulin, and proinsulin. Right panel. Competition for ^{125}I-MSA binding to chick embryo fibroblasts by different concentrations of MSA, insulin, and proinsulin (redrawn from (47)).

interacting with the MSA receptor (Fig. 6). However, the mitogenic effect of insulin is not always explained by insulin acting through an IGF receptor. In rat hepatoma (H35) cells (48) and F9 teratocarcinoma cells (49) insulin appears to exert its mitogenic effect by acting through the insulin receptor.

As competitive binding studies were performed on a variety of cells and membrane preparations with both radiolabeled IGF-I and IGF-II or MSA it became apparent that there was more than one type of IGF receptor (50). (See papers by Rechler et al. and Rosenfeld et al. in this volume for a detailed discussion of IGF receptors.) More recently, crosslinking studies have confirmed the presence of two types of IGF receptors (51-53). The receptor type which prefers IGF-II or MSA is insulin insensitive and has a Mr = 260,000 by SDS-gel electrophoresis under reducing conditions (51, 52). This IGF-II type receptor has recently been purified from rat chondrosarcoma cells by MSA

affinity chromatography (54). Interestingly, the purified receptor has the same relative affinity for IGF-II, MSA, and IGF-I as shown previously for the receptor on intact cells (55), and the molecular weight (250,000) by SDS-gel electrophoresis is the same as the value determined by crosslinking studies on intact cells. In some tissues (rat placenta (56)) and cells (chondrosarcoma chondrocytes (55), rat embryo fibroblasts (57)), this receptor is highly specific for IGF-II; IGF-I interacts only weakly. Of interest is the observation that this receptor type predominates in some cells of fetal origin or having fetal characteristics such as rat embryo fibroblasts (57) and Swarm rat chondrosarcoma chondrocytes (55). Using membranes prepared from fetal and neonatal rat livers, Gavin et al. (58) have described a prepartum peak of IGF-II binding. The predominance of this receptor in fetal tissue and the specificity for IGF-II raise the possibility that this receptor type may be important in mediating the biologic response to MSA in fetal rat tissue.

Synthesis of MSA by rat embryo fibroblasts

The reports by Atkison et al. (59) and Clemmons et al. (60) showing that human skin and lung fibroblasts produce IGF-I/SM-C led us to ask whether rat embryo fibroblasts might synthesize MSA. Actually Temin had demonstrated in 1970 that medium conditioned by rat embryo fibroblasts would support the multiplication of chick embryo fibroblasts (61). Adams et al. (57) found that rat embryo fibroblasts synthesized MSA polypeptides indistinguishable from MSA species produced by the BRL-3A rat liver cell line (Fig. 7). IGF-I levels in the conditioned media were very low and could be accounted for by MSA crossreacting in the IGF-I/SM-C radioimmunoassay. Postnatally there was a switch to IGF-I production (62). Thus measurement of MSA and IGF-I levels in medium conditioned by fibroblasts derived from skin and lung of rat pups and young rats showed that the fibroblasts from the older animals produced IGF-I but very little

MSA. Therefore cultured fibroblasts mimicked the developmental pattern of MSA (rat IGF-II) and IGF-I noted in rat serum.

Accompanying the developmental switch from MSA production to IGF-I production in the fibroblast cultures there was a change in the regulation of these IGFs by placental lactogen and growth hormone. In rat embryo fibroblasts, ovine placental lactogen, but not growth hormone, stimulated synthesis of MSA (62). In the fibroblasts from the older animals both growth hormone and

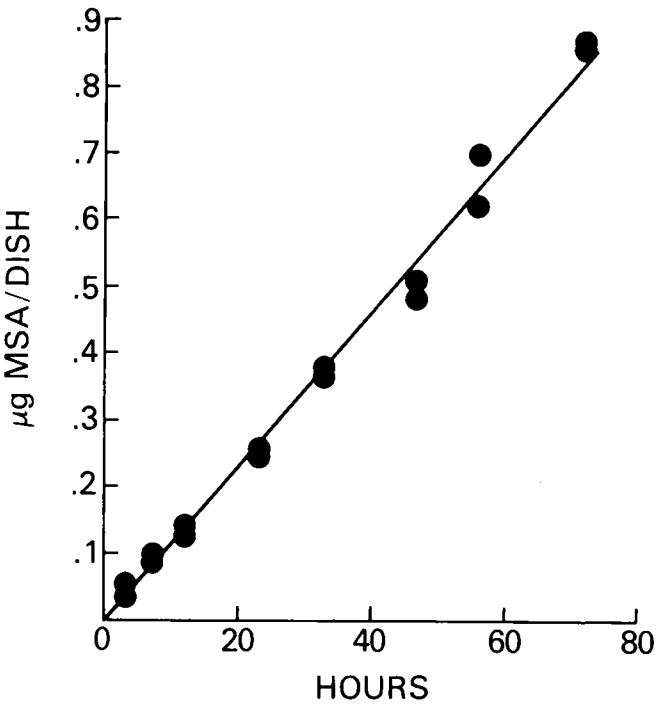


Fig. 7. Time course of appearance of MSA in serum-free medium conditioned by tertiary cultures of rat embryo fibroblasts (57). MSA was measured by radioimmunoassay (8). Cell number remained constant over the 72-hr period.

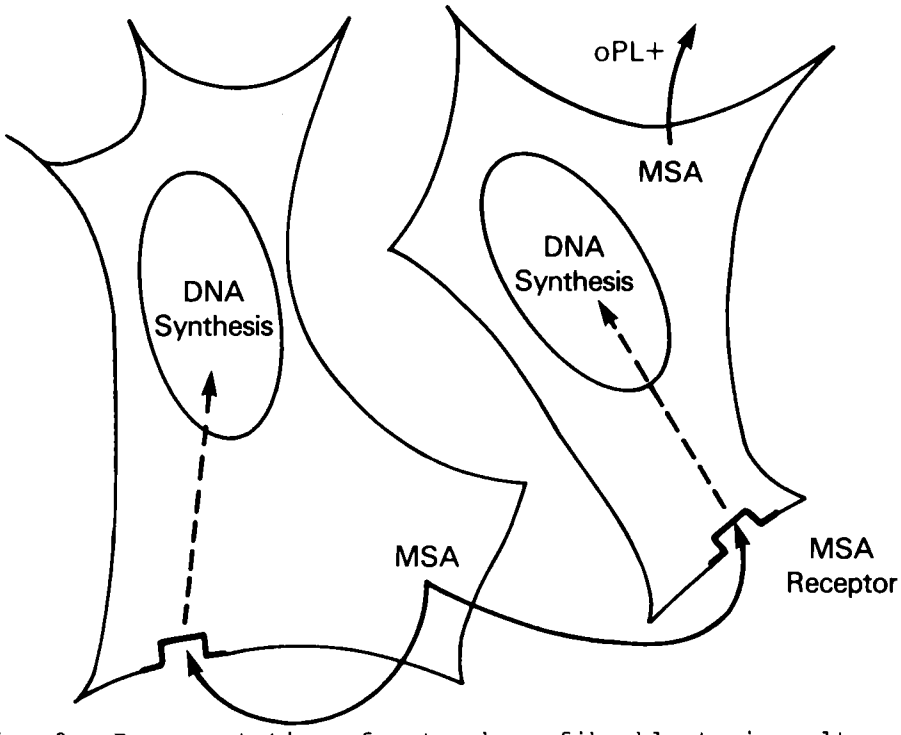


Fig. 8. Representation of rat embryo fibroblasts in culture showing synthesis and secretion of MSA into the medium (57), and interaction of MSA with cell surface receptors leading to a biologic response (DNA synthesis) (57). Ovine placental lactogen (oPL) stimulates the synthesis of MSA (62). We can not distinguish between a model in which MSA is produced by and acts on the same cell versus production by one cell and interaction with another; both possibilities are shown here.

ovine placental lactogen stimulated IGF-I but not MSA production. These studies provided a mechanism whereby placental lactogen could regulate fetal growth (Fig. 8).

Conclusion

Led by the observation that pharmacologic concentrations of insulin are mitogenic for cells in culture, cell biologists isolated a family of polypeptides called multiplication

stimulating activity (MSA), later shown to be the rat counterpart of human IGF-II. Studies of MSA in the rat showing high blood levels, exclusive association with an albumin-size binding protein in fetal blood, predominance of an IGF-II type receptor on some fetal cells, production by fetal liver explants and fetal fibroblasts, and stimulation of MSA synthesis in fetal fibroblasts by placental lactogen, suggest that MSA may be a fetal growth factor in the rat.

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In Vivo Action of Insulin-Like Growth Factors

LONG-TERM IN VIVO EFFECTS OF INSULIN-LIKE GROWTH FACTORS (IGF) I AND II ON GROWTH INDICES: DIRECT EVIDENCE IN FAVOR OF THE SOMATOMEDIN HYPOTHESIS

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Introduction

According to the somatomedin (SM) hypothesis (1,2) growth hormone (GH) stimulates growth indirectly through SM or IGF (fig. 1).

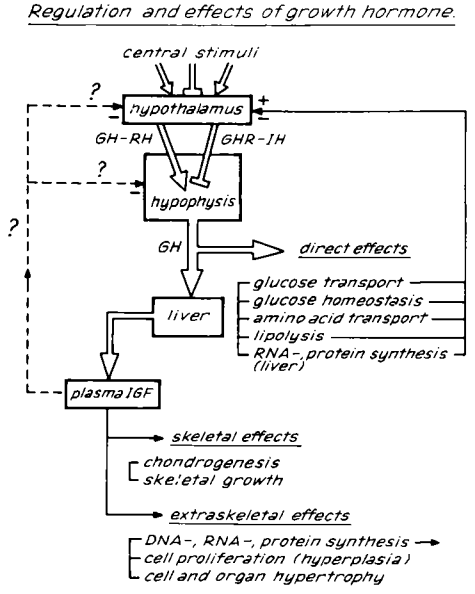

Figure 1

Insulin-Like Growth Factors / Somatomedins

IGF I and II, which are closely related to insulin in structure (3,4) and function (5), have SM activity in vitro: they are potent stimulators of cell replication, DNA-, RNA- and protein synthesis in fibroblasts (5,6), chondrocytes (7) and calvarian cells (9,10) and they stimulate sulfate incorporation into rat and chick cartilage (5,7).

Evidence in favor of the SM concept has so far mostly been indirect. It has been based on a) experimental and b) clinical findings.

a) experimental: GH or serum from hypox rats do not stimulate the incorporation of sulfate into cartilage of hypox rats in vitro. However, GH treatment of hypox rats restores sulfation activity of their serum (1,11).
b) clinical: IR-IGF I/SM-C is decreased in hypopituitarism and in patients with isolated GH deficiency (12,13) and it is increased in acromegalics (12,13). GH treatment of GH-deficient children or hypox rats induces growth and is accompanied by an increase of IGF (IR-IGF I and II in man: ref. 14,15; total IGF in the rat: ref. 16).

Although the above in vitro results and clinical findings support the SM hypothesis, unequivocal direct evidence has been lacking. Some authors have shown that sc administration (3 times a day for several weeks) of plasma peptide fractions containing SM activity cause an increase in body wt. and sulfate incorporating activity of cartilage (17,18) and of nose-tail length (18) in Snell dwarf mice. In one of these studies, 10 % glucose had to be added to the drinking water to prevent hypoglycemia (18) due to the acute insulin-like action of the injected material. In a more recent study, Ellis et al. circumvented the latter obstacle by using a

continuous sc infusion system (Alzet minipumps) to test the in vivo effects of a partially purified IGF preparation in hypox rats (19). Indeed, they could demonstrate a dose-dependent increase in body wt., organ weights and in the tibial epiphyseal width of the treated animals. These experiments have now been repeated with pure IGF I and II (20).

43 and 103 ug/day of IGF I, 131 ug/day of IGF II or 12.5 and 25 mU/day of GH were administered to groups of 4 rats by continuous 6 d infusions via sc implanted minipumps (flow rate 1 ul/h) and the effects on body wt., tibial epiphyseal cartilage width and on thymidine incorporating activity of the costal cartialge of the experimental animals were measured.

Results

As shown in fig. 2 for IGF I, body wt. had increased by 4.8 ± 0.5 g and 7.1 ± 0.6 g after 6 d of infusion of the two IGF I doses.

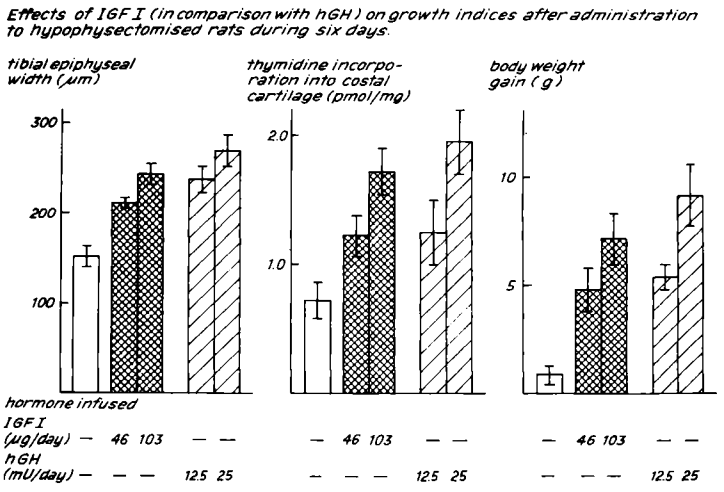

Figure 2

The two doses of GH caused an increase of 4.5 ± 0.4 and 9.6 ± 0.8 g, whereas 131 ug of IGF II had no significant effect as compared to saline-infused controls (not shown).

A dose-dependent increase of the tibial epiphyseal width similar to that observed with the two GH doses was observed with the two IGF I doses, and a less pronounced, but statistically significant increase was demonstrable with IGF II (not shown).

Thymidine incorporating activity of the costal cartilage of both IGF I-infused rats was enhanced to the same extent as in the two GH-infused groups. Again, IGF II had a smaller, but significant effect (not shown).

Conclusions

1) These data demonstrate that IGF I and, although less potently, IGF II stimulate growth indices in the absence of GH and thus provide direct evidence in favor of the SM concept.

2) The potency difference between IGF I and IGF II is similar to that observed in vitro in the rat cartilage sulfation assay (5) and is not due to their different half-lives: the steady state serum levels of IGF I and II measured at the end of the 6 d infusion of 103 ug/day of IGF I and 131 ug/day of IGF II were 286 and 372 ng/ml, respectively. Thus, the potency differences in the growth promoting actions of IGF I and II seem to reflect intrinsic properties of the two IGF molecules. So far, we have not been able to detect IGF II-like material in rat serum crossreacting at the antibody towards human IGF II.