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DOI: https://doi.org/10.5169/seals-308270

Bull. Schweiz. Akad. Med. Wiss., 183 - 198 (1982)

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SOMATOMEDIN-C: MEASUREMENT BY RADIOIMMUNOASSAY, REGULATORY MECHANISMS IN HUMANS AND USES IN CLINICAL DIAGNOSIS

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Acknowledgments

This work was supported by USPHS Research grants AM01022 and HD08299; USPHS Training Grant AM07129; General Clinical Research Center Grants RR0046 and RR01066. JJVW is a recipient of a USPHS Research Career Award 4 K06A AM14115. DRC is a recipient of a National Research Service Award AM05991. AJD is a Jefferson Pilot Fellow in Academic Medicine, University of North Carolina and is supported by National Foundation March of Dimes # 5-188. PGC was a recipient of grants from the Foreign Office of the French Government, the Fondation de l'Industrie Pharmaceutique pour la Recherche, Paris and the Nordisk Insulin Laboratorium, Copenhagen. FM was supported by CNR Research Grant 68.CT.77.01367. 04/115.5482.

The somatomedins are a family of polypeptides which are believed to mediate the growth promoting actions of growth hormone. Two chemical forms of human somatomedin have been isolated and their sequences established. Somatomedin-C (Sm-C) and insulin-like growth

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factor I (IGF-I) are basic peptides which probably have identical amino acid sequences (1, 2). The basic somatomedin peptide isolated by Bhaumick and Bala is also probably identical to Sm-C/IGF-I (3). Sm-C/IGF-I is more growth hormone dependent and more active in cartilage assays than insulin-like growth factor II (IGF-II) (4), the other somatomedin which has been fully characterized. IGF-II is more insulin-like and more abundant in serum than Sm-C/IGF-I. The classification of somatomedin-A is uncertain since information on its structure is incomplete (5). Its growth hormone dependency, however, is similar to that of Sm-C/IGF-I. Also remaining to be classified is the insulin-like activity (ILAs) purified from human serum in Montreal and Paris (6). ILAs is acidic and may represent a distinct somatomedin.

Evidence is emerging that the somatomedins of the rat conform to the scheme observed in humans. Multiplication stimulating activity (MSA), a neutral rat somatomedin derived from conditioned rat liver media (7), recently has been shown to possess only 5 amino acid residues which are different from human IGF-II (8). Likewise, basic rat somatomedin which is derived from serum of rats bearing implanted growth hormone-secreting tumors, has a high degree of structural homology with human Sm-C/IGF-I (9).

Measurement of Sm-C by radioimmunoassay

The development of a sensitive radioimmunoassay (RIA) for Sm-C has made it possible to measure plasma concentrations with a high degree of precision and specificity (10). The assay utilizes Sm-C which has been purified in our laboratory from Cohn Fraction IV of human plasma by extraction in organic acid, ion exchange chromatography, gel chromatography, isoelectric focusing and reverse phase high pressure liquid chromatography (2). It is more than 90 % pure on the basis of a variety of analytical methods. Based on the concentrations of Sm-C measurable in fresh plasma by our RIA procedure, this represents a 780,000 fold purification from plasma. The RIA utilizes rabbit antisera to Sm-C which was raised by injecting somatomedin and ovalbumin which were linked by a reaction with gluteraldehyde. In this assay, Sm-C and IGF-I are equipotent in competing with ^{125}I-Sm-C for binding to antibodies (11) (Fig. 1). Somatomedin-A, IGF-II, and MSA from conditioned rat liver media are 5 %, 2 % and 1 % as potent, respectively.

Purified somatomedins have molecular weights of approximately 7500 daltons. In plasma, however, the somatomedins circulate as high molecular weight proteins, primarily in the range of 150,000 daltons. This high molecular weight somatomedin results from binding of the active, low molecular weight peptide by plasma proteins. Although these proteins have been the subject of intense study in the last several years, their precise nature remains to be

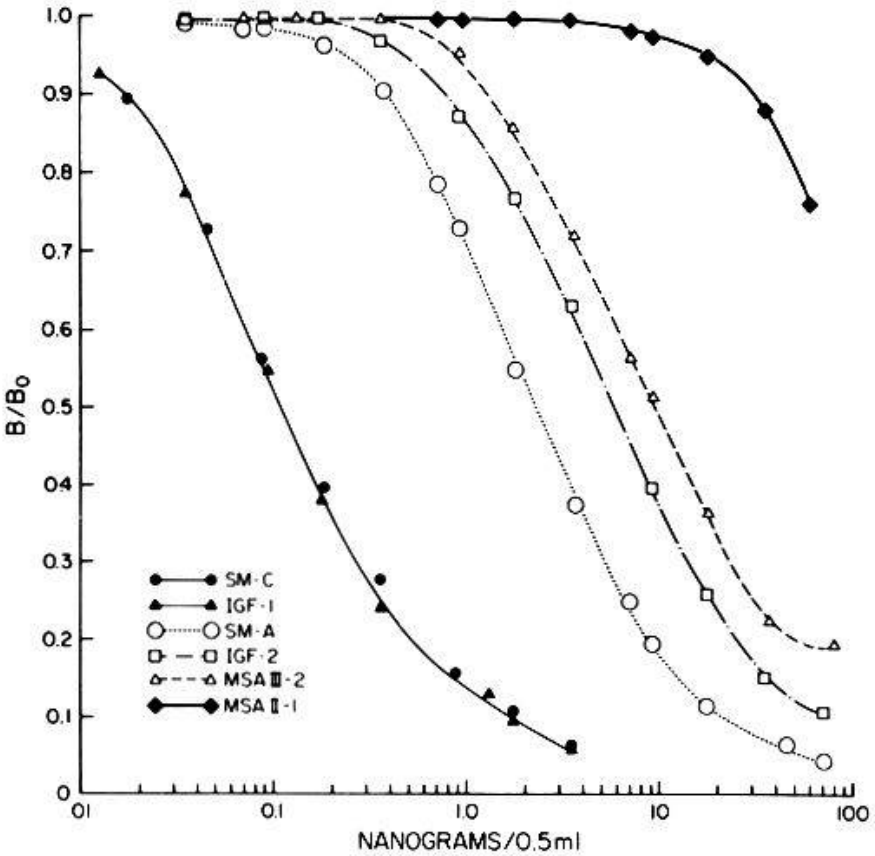

Fig. 1. Curves of competition produced by various somatomedin peptides in the Sm-C RIA. Modified from Ref. # 11.

clarified. Furlanetto (12) has proposed that they are composed of an acid stable subunit which is growth hormone (GH) dependent and able to bind Sm-C, an acid labile subunit which also is GH dependent but does not itself bind Sm-C, and a non-GH dependent binding subunit. When serum is incubated at neutral pH, we have observed that the relationship between the binding proteins and Sm-C is altered by an enzymatic process which is tempetature and pH dependent. Depending on the conditions of assay, this process can bring about a 2-3 fold increase in measurable Sm-C in serum.

One of the important effects of the binding proteins is to maintain relatively constant plasma somatomedin levels. They also undoubtedly contribute to the relatively high plasma concentrations of these factors. While these effects are advantageous for the study of regulation, the binding proteins also interfere to a variable extent with plasma radioligand assays. This interference is highly significant for some radioligand assays and makes quantitation of plasma levels impossible without processing of plasma prior to assay. In our own RIA, the binding proteins cause a reduction in the measurable Sm-C in unprocessed plasma to approximately 25 % of the total Sm-C which is present. However, dose response curves of unprocessed

Table 1. Plasma concentrations of immunoreactive Sm-C in normal adults

Age (yrs)	N	Mean Concentration (Units/ml)
18-25	77	1.22
26-35	95	0.90
36-45	31	0.74
45-65	17	0.68
All adults*	220	0.88

* Values for the all adults conform to a log-normal distribution. 95 % of subjects have values between 0.4 and 2.0 units/ml.

plasma are parallel to those of purified Sm-C, and the quantities measured in unprocessed plasma show a high correlation ($r > 0.90$) with the quantities detectable after acid chromatography of plasma. The results presented in this report, therefore, are derived from assays on unprocessed plasma or serum. Because of the lack of a recognized, purified international standard, we have used a commercially available pool of normal human serum (Ortho 1778-5, Ortho Diagnostics, Raritan, NJ) as our assay standard. Results are reported in units of somatomedin/ml.

Somatomedin-C in normal individuals

The concentrations of Sm-C in EDTA plasma from 220 normal individuals between 18 and 64 years conform to a log-normal distribution, with a mean value of 0.90 units/ml (95 % confidence limits, 0.4-2.0 units/ml). During adult life, values tend to decline with age (Table 1) and it has been reported that the relatively low Sm-C of older adults can be increased by administration of GH (13). In 122 women, the mean Sm-C was 1.06 units/ml, while in 98 men, this value was 0.87 units/ml. The mean Sm-C in cord blood is about 0.35 units/ml and values remain relatively low until 3-5 years of age. A cross-sectional study involving over 800 normal children done in conjunction with Wayne Moore (Univ. of Kansas), Michael Preece (University of London) and Alan Broughton (Nichols Institute) indicates that Sm-C concentrations have an accelerated increase around 6 years of age and reach mean levels of approximately 2.5 units/ml at a time corresponding to genital stages 3 and 4 of puberty. Even in childhood, values in girls are higher than those in boys.

We have measured Sm-C in samples drawn approximately every 30 minutes from one adult male and one female. Values changed little over a 48 hour period and do not appear to be altered by eating or other routine activities (Fig. 2). A modest decline seemed to occur during sleep. In another study (14), in which blood was collected by a portable constant flow, with-

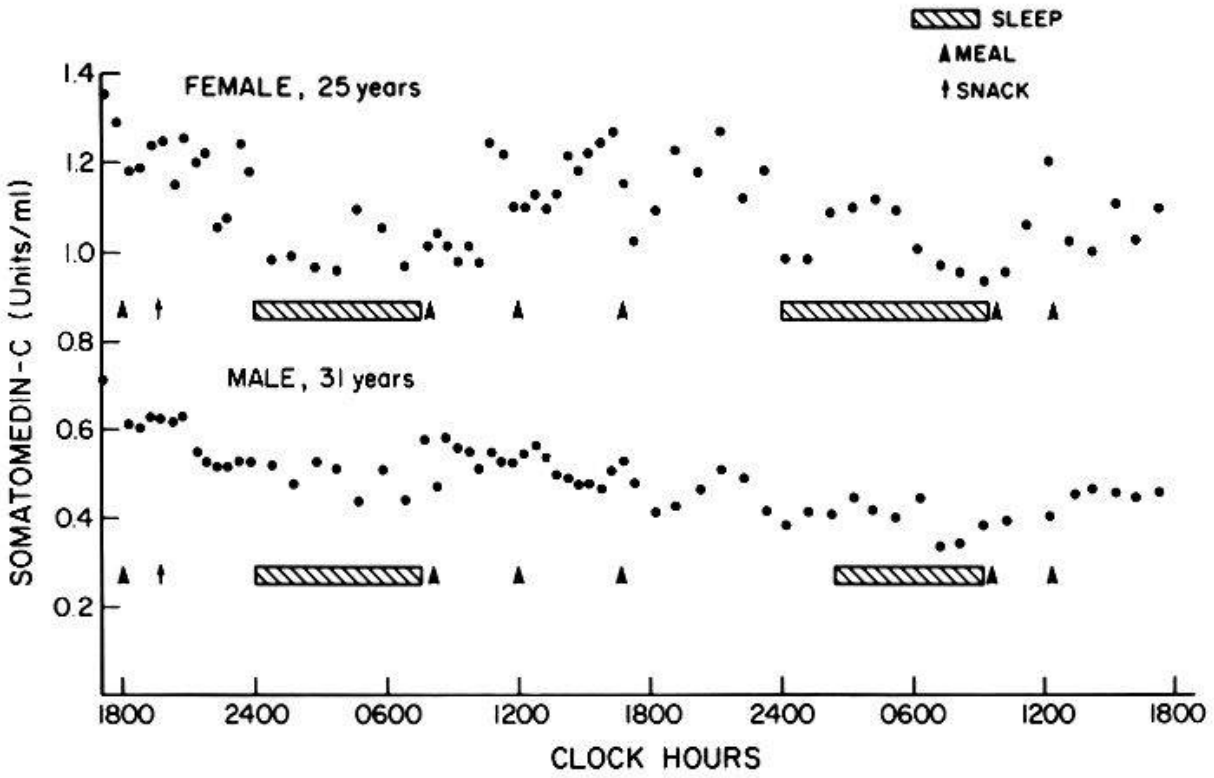

Fig. 2. Serum Sm-C concentrations by RIA in 2 normal adult volunteers over 48 hours. Individual samples were drawn at 30-60 min intervals through indwelling catheters.

drawal pump over 24 hours, 16 normal subjects showed a definite decline in Sm-C during periods of sleep (Fig. 3). The Sm-C concentrations were stable during waking hours (1.13 ± 0.09 units/ml, (mean ± SEM)) but fell after the onset of sleep to a minimum of 0.85 ± 0.08 units/ml. After awakening, the concentration rose to 1.25 ± 0.10 units/ml between 1100-1200 h. In sleep reversal experiments, 3 subjects who were deprived of sleep had a modest, slightly delayed fall in Sm-C. After the delayed onset of sleep, Sm-C declined sharply. The mechanisms for the sleep-related depression in Sm-C is not known.

Factors Regulating Sm-C

1. Stimulators

GH appears to be the most important stimulator of Sm-C in individuals who have normal nutritional status. Concentrations in plasma are reduced in GH deficiency and elevated in acromegaly. In patients with evidence of complete GH deficiency, Sm-C levels are commonly below 0.15 units/ml, less than 15 % of the mean value for adults. Following parental administration of GH in therapeutic dosages, we have observed that plasma Sm-C levels began to increase by 6-8 h, reached peak values at 16-28 h and fell to basal levels by 72 h (15). Out of 28 children receiving chronic GH therapy in our clinic, 2 failed to raise their Sm-C

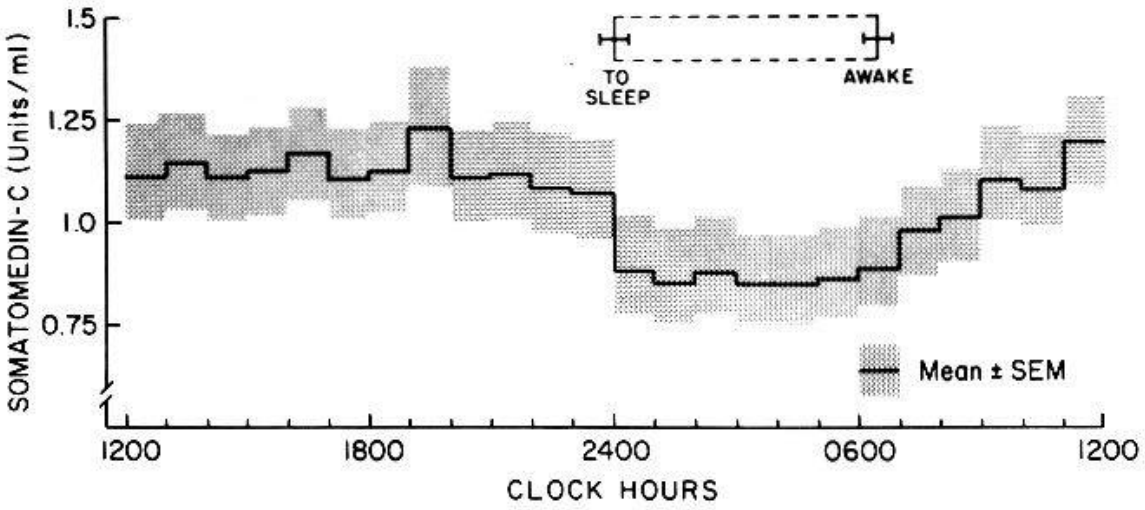

Fig. 3. Mean 24 hour profile of Sm-C in sera of 16 adult volunteers, drawn by continuous flow, constant withdrawal pump. The shaded areas represent ± 1 SEM. Redrawn from Ref. #14.

levels and in 6 others, the Sm-C responses did not climb into the normal range. These "non-inducers" were generally undernourished and had normal linear growth rates despite consistently low Sm-C levels. Studies undertaken to determine the reasons for the lack of induction revealed evidence for somatomedin binding protein deficiencies in these patients (15). When the sera of normal subjects or most GH treated hypopituitary patients were preincubated with ^{125}I-Sm-C, then chromatographed on Sephadex G-200, a substantial amount of tracer binding and almost all endogenous Sm-C activity eluted in the region of a large 150,000 (150 K) protein. In the serum of "non-inducers", however, GH treatment caused neither a significant increase in ^{125}I-Sm-C binding nor in endogenous Sm-C activity in the 150 K molecular weight region. These findings are in keeping with those reported for other somatomedins by White et al. in humans (16), and by Kaufmann et al. (17) and Moses et al. (18) in rats.
In addition to GH, placental lactogen and prolactin appear to stimulate Sm-C under certain conditions. We have observed that a single injection of ovine placental lactogen (oPL) raises the serum somatomedin of hypophysectomized rats (19) and that the Sm-C dose-response to oPL is parallel to and equipotent with ovine GH (unpublished). Furthermore, in a cross-sectional study of pregnant women, Sm-C concentrations were found to be raised after the 20th week of gestation, to be highest in the last few weeks of pregnancy, to correlate with serum levels of hPL and to fall promptly following delivery of the placenta (20). While the pregnancy-related elevation in Sm-C has not been proven to be secondary to placental lactogen, the findings in rats and the close temporal relationships between the rise and fall of Sm-C and the rise and fall of hPL suggest that this is a strong possibility.

Table 2. Somatomedin-C, growth hormone, and prolactin in 78 patients with pituitary tumors. Influence of excessive prolactin on somatomedin-C concentrations[+].

	Low GH NL Prl	Low GH High Prl	NL GH High Prl	NL GH Nl Prl
Number of subjects	23	20	17	18
Mean ages (yr)	47	36	28	29
Sex (F/M)	10/13	13/7	17/0	13/5
Peak GH after insulin (ng/ml)	<1	<1	20.4±7.2*	16.3± 7.6
Basal Prl (ng/ml)	6.7±4.5*	755±1362	51 ± 25	5.9 ± 3.0
Sm-C (units/ml)	0.23±0.10*	1.01±0.44	1.47±0.47	1.40±0.54

[+] From Clemmons et al., reference #21
* Mean ± 1 SD

We also have shown that secretion of excessive amounts of prolactin raises Sm-C values to normal in patients with GH deficiency secondary to pituitary tumors (21). Patients (n=23) with large pituitary-region tumors, GH deficiency and normal prolactin levels had Sm-C concentrations of only 0.23 units/ml (Table 2). On the other hand, 20 patients with large prolactin secreting pituitary tumors and GH deficiency, had normal Sm-C concentrations. In patients who do not have GH deficiency, increased prolactin did not raise Sm-C above the normal range (Table 2). These data suggest that in humans, prolactin is a weak stimulator of somatomedin secretion which produces a detectable effect only when GH deficiency is present. The difference in potency of GH and prolactin for Sm-C induction might be explained by the differential receptor specificity of each hormone for binding to receptors. In IM-9 lymphocytes and liver membranes, human prolactin is a weak competitor for binding to human GH receptors and these relative potencies correlate with their respective potencies for Sm-C secretion in vivo. They also explain why marked elevations in prolactin do not raise Sm-C concentrations to those observed in patients with acromegaly.

The important practical implications of the finding that excessive prolactin raises Sm-C is that hyperprolactenemia must be excluded in patients with pituitary tumors before Sm-C can be used as a screening test for GH deficiency.

2. Factors which inhibit Sm-C

With continuing investigation, it is becoming more and more apparent that nutritional status may be as important as GH in determining concentrations of Sm-C in plasma. Years ago, Van den Brande (22) and Grant (23) showed that bioassayable somatomedin was low in the blood of children with protein-calorie malnutrition. Since such plasmas contained inhibitors of the bioassays (24), it has not been proven whether the low values these investigators ob-

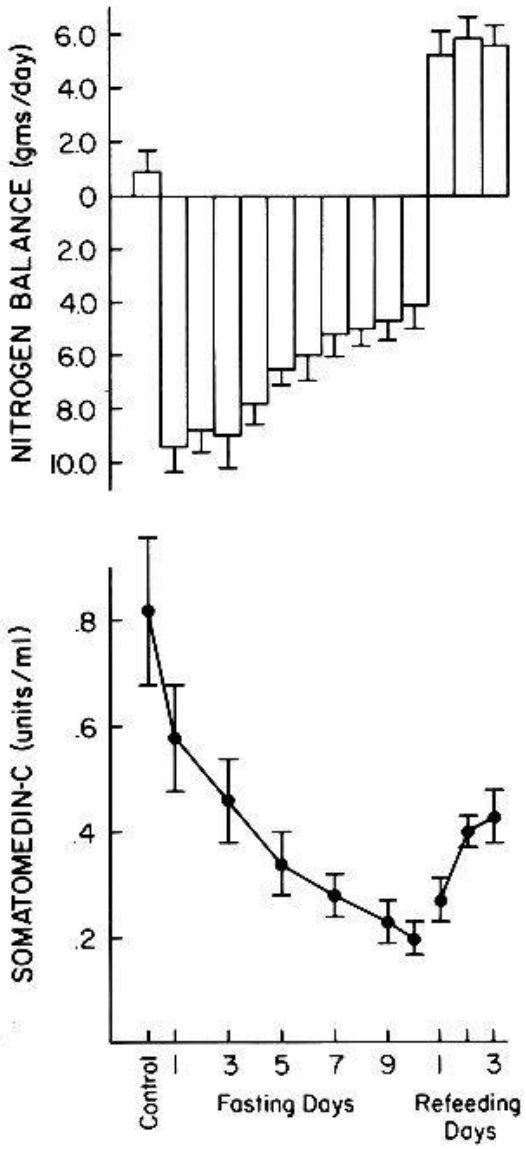

Fig. 4. Nitrogen balance and Sm-C in 7 obese adults during 10 days of fasting and 3 days of refeeding. Nitrogen balance (top) was determined by: nitrogen balance =nitrogen intake - (daily urinary urea nitrogen + 2 grams nitrogen). The latter was the estimated loss in stool, skin and urinary non-urea nitrogen. In both panels, data are expressed as mean ± SEM. (From Clemmons et al., ref. # 25, with permission.)

served were in fact due to low somatomedin or were the effects of inhibitors. We have recently completed a study in which we assessed the effect of fasting for 10 days on plasma concentrations of immunoreactive Sm-C and urinary urea nitrogen excretion in 7 obese male volunteers (25). From a mean prefast value of 0.83 units/ml, plasma Sm-C fell to 0.21 units/ml after 10 days of fasting (Fig. 4). A prompt increase was observed with refeeding. The change in Sm-C during fasting showed a highly significant correlation with the change in urinary urea nitrogen excretion ($r = 0.74$; $p < 0.0001$). It was also shown that inhibitors which interfere with quantitation in somatomedin bioassays are not factors in the fall of Sm-C observed in the RIA.

This was accomplished by showing that a pool composed of fasting-day 7 plasma from all subjects produced a dose response curve parallel to the normal serum standard and to a pool of the non-fasting plasma. Additionally, when a fasting day 7 plasma pool was incubated with multiple concentrations of highly purified Sm-C, 110 % of the original activity in the pure Sm-C could be detected in the assay. The result of this study suggests that measurement of plasma immunoreactive Sm-C provides a sensitive indicator of nitrogen loss and may be useful in monitoring the changes in protein metabolism that occur during alterations in nutritional status. We believe that with further study, it may be possible to use plasma Sm-C measurements to monitor changes in protein metabolism that occur during clinically relevant alterations in nutritional status. In particular, Sm-C might serve as an indicator of the effect of specific nutrients of nitrogen metabolism in individuals who have undergone accidental trauma, major surgery, or who have impaired intestinal function.

Administration of estrogens to acromegalic patients have been shown to reduce the serum concentrations of bioassayable somatomedin and to cause improvement in clinical status (26, 27). These effects do not appear to result from a reduction of GH secretion, since plasma GH concentrations are not consistently lowered. We have observed a prompt, significant reduction in plasma immunoreactive Sm-C in 5 acromegalic patients who were given 1 mg of ethynyl estradiol daily (28). There were also statistically significant reductions in urinary hydroxyproline and in the phosphate clearance ratio. The decline in serum Sm-C was not due to an estrogen-induced increase in somatomedin binding protein since total serum Sm-C concentrations measured after treatment of serum with acid also were reduced by estrogen therapy, and the magnitude of the reduction was equivalent to that observed in untreated serum. The result of this study indicate that the reduction in immunoreactive Sm-C correlates with estrogen-induced improvement in metabolic activity of acromegalic patients, and suggests that measurement of Sm-C may be useful in monitoring the effects of other drugs on this disease.

In pursuit of the latter point, we have studied the effect of bromocriptine on Sm-C in another group of acromegalic patients. In this study (done in collaboration with Drs. Michael Besser and J.A.H. Wass, St. Bartholomew's Hospital, London) we observed that 21 of 27 acromegalic patients treated with bromocriptine had a reduction in their serum concentrations of Sm-C. Of those who had Sm-C reduction and clinical improvement in their acromegalic status, 7 patients had no significant reduction in GH secretion. This suggests that in addition to its recognized capacity to lower GH in some patients, bromocriptine may reduce Sm-C, and thereby improve clinical status, by a mechanism other than reduction in GH levels.

In keeping with results from animal studies (29), we have found that patients with primary hypothyroidism have Sm-C values at the lower limits of normal. The mechanism for this is

not clear since values don't seem to correlate with GH status. Neither do patients with hypothyroidism appear to be resistant to GH since its injection causes Sm-C levels to rise (unpublished).

It now appears that somatomedin itself is an important factor in the inhibition of somatomedin production. One recent study (30) has shown that somatomedin increases somatostatin secretion in vitro in hypothalamic explants. In addition, in the same study it was shown that somatomedin decreases pituitary GH synthesis. Contrary to results from studies done using bioassays, we find no evidence that cortisol reduces immunoreactive Sm-C. It is possible, therefore, that cortisol directly inhibits somatomedin bioassays.

Sm-C in Clinical Diagnosis

1. Assessment of growth

While the advent of GH testing was a major diagnostic advance, we still need methods which will tell us which short children need GH therapy. Table 3 lists some of the short comings of the use of growth hormone in assessment of short stature. GH responses to provocative tests don't necessarily reflect GH secretory status. We have studied unequivocally normal children who cannot raise their GH during insulin tolerance tests (31). All investigators have had experience with short patients who are not GH deficient, but who on any given day cannot respond to a given GH provocative stimulus. For this reason, all subscribe to the convention that failure to raise GH in at least 2 provocative tests is essential for the diagnosis of GH deficiency. Interpretation of GH responses varies from one institution to another and there is no wide-spread agreement on whether peak responses of 6, 8, 10 ng/ml or some other value, segregate GH deficient from non-GH deficient children. Compounding this problem is the fact that interlaboratory standardization of RIAs is not rigorous and GH standards vary in potency. Finally, immunoreactive GH does not reflect bioactivity of serum. The work of Ellis et al. (32) and Lewis et al. (33) point to the possible importance of this. It also has been observed that immunoreactive GH and receptor reactive GH may be discrepant in short children (34).

We now have had considerable experience with the Sm-C RIA in the diagnosis of GH deficiency (35). In approximately 60 children who have hypopituitarism documented by a number of independent methods, plasma Sm-C concentrations have consistently been below 0.25 units/ml and in virtually all have been below the age-related normal values. The finding of a normal Sm-C value virtually excludes the diagnosis of hypopituitarism. On the other hand, a low Sm-C value in a growth retarded child is not diagnostic of hypopituitarism. This is because normal children under 5 years of age may have Sm-C values as low as 0.1 units/ml

Table 3. Shortcomings of the use of serum growth hormone measurements in short children

1. GH responses to provocative stimuli may not reflect GH secretory status
2. Interpretation of GH responses varies
3. Immunoreactive GH may not reflect bioactive GH

and such low concentrations may overlap with those of GH deficient children. Another complicating factor for older children is that it is not clear whether the Sm-C level should be interpreted with respect to normative data for chronological age or for "developmental age" (skeletal age and stage of puberty). A third consideration in interpreting low Sm-C concentrations in growth retarded children is the fact that values may be reduced in patients with severe primary hypothyroidism, in children with marginal nutritional status, and in children with a variety of chronic illnesses. Therefore, in growth-retarded children who have low Sm-C values, a variety of non-pituitary disorders must be excluded and provocative tests for GH secretion performed before the diagnosis of hypopituitarism can be established.
We have encountered low serum Sm-C concentrations in a number of children with growth failure who are well nourished, exhibit no evidence of systemic disease and who have apparently normal GH responses to provocative stimuli. In such case, it is important to bear in mind that the GH tests may not be providing an accurate estimate of GH secretory status and, in fact, may be misleading. Indeed, it may be necessary to assess growth responses to GH therapy in order to determine whether GH deficiency is rate limiting in growth. Should we withhold GH treatment on the basis of GH responses or should we place more faith in the Sm-C and provide a trial of therapy? We believe that this question is now ready for careful, systematic study. With the advent of recombinant DNA GH, and the availability of virtually unlimited supplies, our challenge for the next two decades will be to determine whether it is appropriate to use GH as a pharmacologic agent in short children who, by present criteria, are not candidates for such therapy.

2. Sm-C in Acromegaly

The Sm-C RIA has proved extremely useful in the evaluation of patients with acromegaly. In a study of 57 adults with active acromegaly, we found that measurement of Sm-C on a single blood sample may be a more reliable method for diagnosis than multiple GH determinations (36). In these patients, the Sm-C levels ranged from 4.3 - 36 units/ml and the mean value for the group was 10 times higher than the mean for normal adults. A single Sm-C determination correlated quite well with clinical indices of disease activity such as heel pad thickness ($r=0.73$), fasting glucose ($r=0.74$), and 1 h postprandial glucose ($r=0.77$). In contrast, the

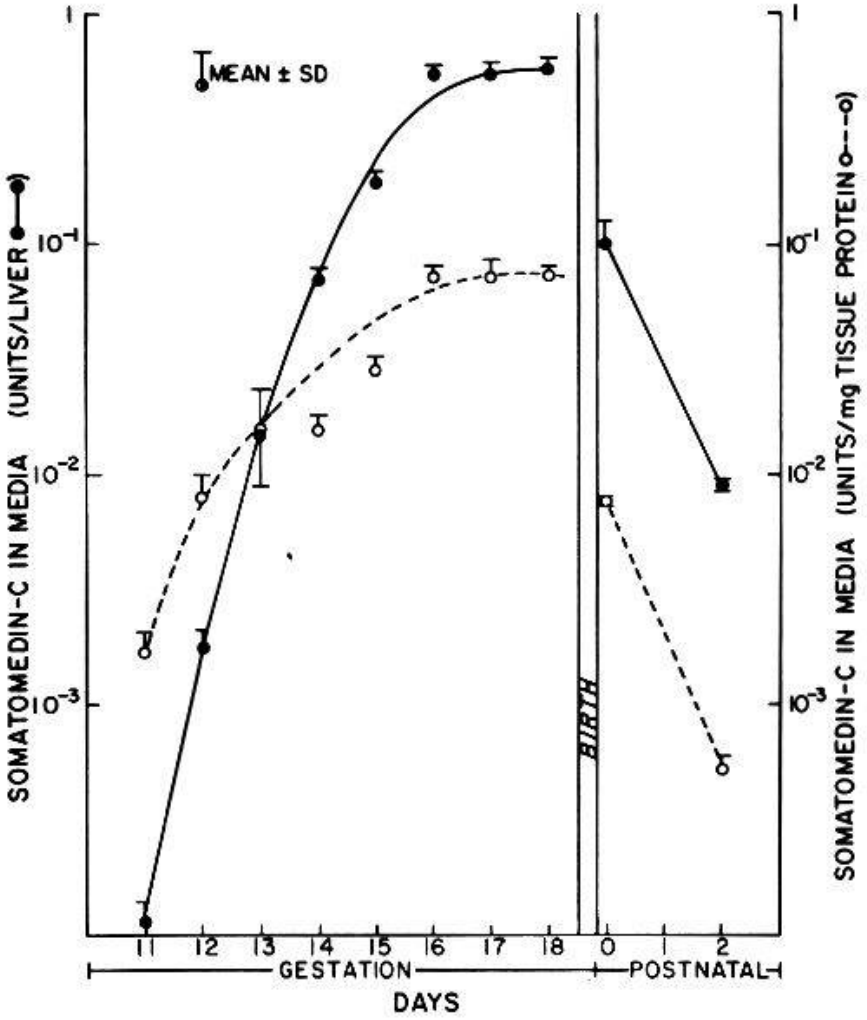

Fig. 5. Immunoreactive Sm-C in fetal and newborn mouse liver explant media. Each value represents the mean (n=4) during 72 hours of culture expressed as total content of liver or as a function of liver protein. (From D'Ercole et al., Ref. # 37, with permission.)

"glucose-suppressed" GH correlated weakly with these clinical indices of severity (r=0.34 to 0.36). In 5 patients, there was abundant clinical evidence of disease, but GH levels were suppressed normally (< 5 ng/ml) by administration of glucose. In these 5, the Sm-C values were unequivocally elevated confirming the clinical diagnosis. Preliminary data from this study suggest that changes in Sm-C concentrations parallel the degree of clinical improvement following therapy. These observations are consistent with the studies reported above in which estrogen or bromocriptine were administered.

Sm-C in blood: Origin and Function

It has been widely believed that the somatomedins are produced by the liver. Using fetal mouse liver cultured as 1 mm^3 explants in serum-free media, we have observed evidence of

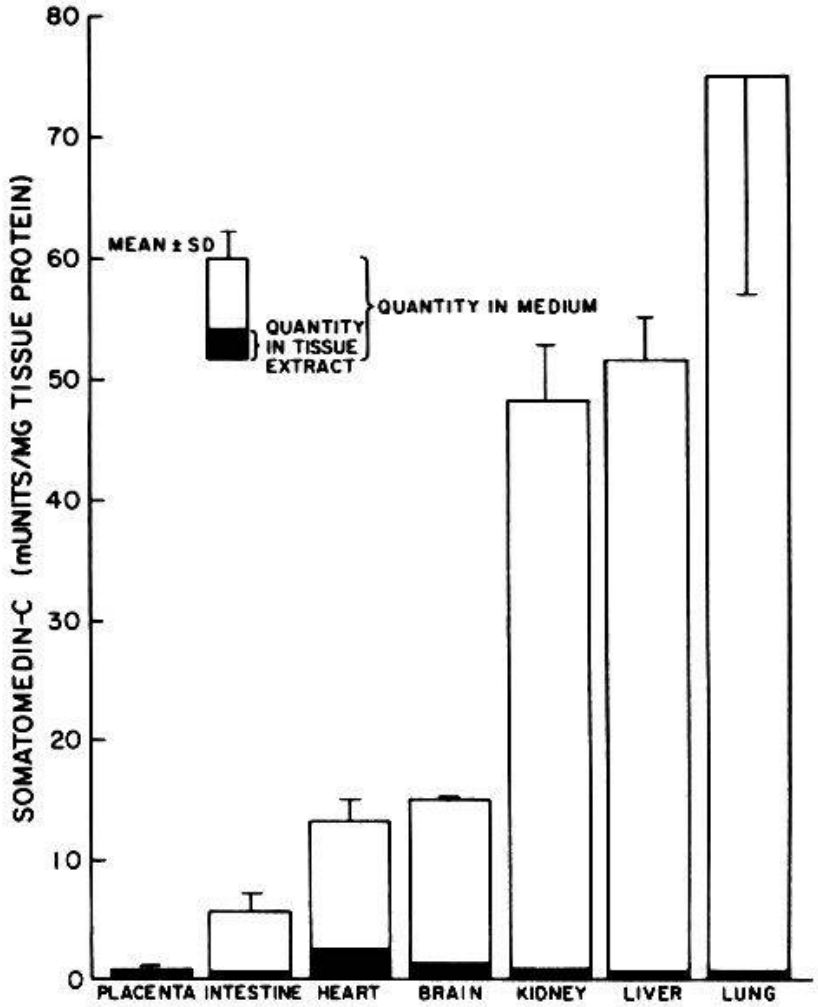

Fig. 6. Immunoreactive Sm-C in media from organ explants of 17 day gestation fetal mouse tissues. The darkened portion of each bar represents the amount of somatomedin found in tissue extracts at the beginning of incubation. The entire bar represents the amount found in the medium in which each tissue has incubated over 3 days. (From D'Ercole et al., Ref. # 37, with permission.)

somatomedin synthesis and secretion (37). We found that substances cross-reacting in the Sm-C RIA are produced by the newly formed, 11-day gestation fetal mouse liver bud and that Sm-C production by fetal liver increases exponentially in parallel with liver growth until the 16th day of gestation. Production falls postnatally (Fig. 5). We now have evidence, however, that in addition to liver, the somatomedins are produced by a variety of tissues. We have shown that mesenchymal cells grown in "micromass" (high density) cultures and explants of 17-day gestation intestine, heart, brain, kidney and lung also synthesize Sm-C in serum-free medium (Fig. 6). In all of these explants, the media somatomedin is believed to be derived by de novo synthesis, since saline extracts of tissues contained only a small portion of the somatomedin activity found in culture media, and addition of cyclohexamide totally inhibited the appearance of these substances in liver media.

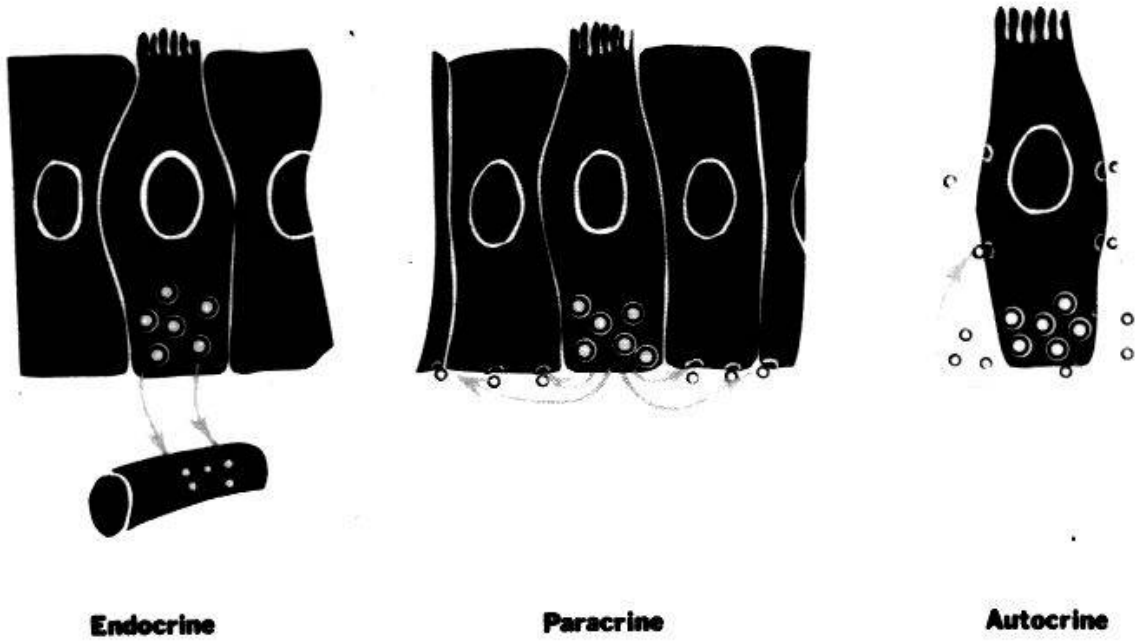

Fig. 7. Three models depicting how growth factors might reach target tissues to regulate growth. Drawing suggested by Dr. George Todaro.

These studies have been extended by showing that material active in the Sm-C RIA is produced by quiescent human fibroblast in serum-free medium (38). It has been observed that addition of GH in nanogram amounts, platelet derived growth factor, or fibroblast growth factor further stimulate Sm-C production in this in vitro system. These results are similar to those obtained by others (39).

These data have led to the hypothesis that the so-called paracrine and/or autocrine models may be operational for the somatomedins (Fig. 7). In the classic endocrine model, hormones are delivered from secretory granules into the blood stream where they are carried to distant target tissue. In the paracrine model, growth factors are secreted locally and act on neighboring cells. In the autocrine model, a cell makes its own growth factor. It is possible that, in addition to the paracrine/autocrine models, the endocrine model also may apply - that is, some tissues may not produce their own somatomedin and may be dependent on somatomedin carried in blood. No matter which model is operational, the concentrations of somatomedin in blood appear to reflect the quantities produced by the tissues and provide an accurate mirror of the growth process.

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