A. Introduction and Nomenclature of the Somatomedins

The somatomedins, also referred to as insulin-like growth factors, are a family of peptides whose serum concentrations are regulated principally by growth hormone and nutrient status. Evidence is now emerging that most or all of the biologic effects of the somatomedins can be attributed to two peptides, somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) and IGF-II (VAN WYK 1984). Sm-C/IGF-I has a molecular weight of 7649 and a pI of 8.0–8.7. It contains 70 amino acid residues in a single chain with 3 disulfide bridges. It is highly growth hormone dependent, and has potent growth-promoting activity in many in vitro systems (ZAPF et al. 1984). IGF-II is also a single-chain peptide, which has a more nearly neutral pI and a molecular weight of 7471. IGF-II is less growth hormone dependent and appears to have less growth-promoting activity than Sm-C/IGF-I. Both Sm-C/IGF-I and IGF-II possess nearly 50% homology with proinsulin in regions of the molecule that correspond to the A and B chains of insulin. As in humans, two forms of somatomedin have been isolated from rat plasma. One of these, multiplication-stimulating activity (MSA) is the rat homolog of human IGF-II, differing from human IGF-II by only five amino acid residues (MARQUARDT et al. 1981). Although not certain, it is expected that other species will be found to have two somatomedins, similar to those in humans and rats. The term somatomedin-A has been applied to a substance that now appears to be a deamidated form of Sm-C/IGF-I, and the peptide referred to as somatomedin-B proved to be a fragment of a plasma-spreading factor contaminated with epidermal growth factor (EGF) (HELDIN et al. 1981; BARNES et al. 1984).

In plasma, the somatomedin peptides of molecular weight 7500 are bound to larger carrier proteins, and to date, no free somatomedins have been identified (SMITH 1984). The major class of plasma-binding protein is growth hormone dependent, has a molecular weight of approximately 150,000, and can be dissociated with acid into at least two subunits. The other distinct somatomedin-binding protein is not growth hormone dependent, and has a molecular weight of approximately 40,000 (DROP et al. 1984). These binding proteins account for the fact that the concentrations of somatomedins in the circulation are constant throughout the day, and are relatively high, being 100–180 ng/ml for Sm-C/IGF-I and even higher for IGF-II. The effects of these binding proteins on measurement of somatomedins in RIAs and membrane-binding assays will be discussed in Sect. B.II.

While it is clear that the somatomedins (particularly Sm-C/IGF-I) in plasma are responsive to growth hormone, nutritional status, and a variety of other...
modulators, it is less clear what biologic function the somatomedins in plasma might serve. The reason for this is that there is considerable circumstantial evidence that the somatomedins might act by paracrine and/or autocrine mechanisms, as well as (or instead of) the traditional endocrine mechanisms (Underwood et al. 1986). The evidence for paracrine/autocrine mechanisms of action include the observations that: (a) no somatomedin-rich organ reservoir has been found; (b) somatomedins appear to be produced by many cells in many tissues; (c) the somatomedins exert biologic effects on diverse types of cells; and (d) after growth hormone injection, Sm-C/IGF-I concentrations in tissues of hypophysectomized rats rise prior to the rise in blood concentration.

I. Methods for Measuring Somatomedins Before the Development of Radioimmunoassays

1. Bioassays

The biologic assays that have been used for measurement of somatomedins in serum reflect the diversity of actions of these peptides and the focus of early investigations into these biologic effects. Daughaday and colleagues (see Van Wyk 1984), exploring the effects of somatomedins on cartilage growth in vitro, showed that serum from hypopituitary animals had little stimulatory effect on uptake of sulfate by cartilage. Likewise, growth hormone added to cartilage had no significant stimulatory effect. However, after hypopituitary animals were treated with growth hormone, their serum stimulated sulfate uptake in vitro. The “sulfation factor,” and subsequently the “thymidine factor” assays were developed from these somatomedin effects and became the principal means for study of somatomedin. While such assays are quite sensitive, they are not specific for somatomedins and they measure the net effect of somatomedins, somatomedin-like stimulators, and inhibitors of somatomedin action.

Because of their interest in the serum insulin-like activity that cannot be removed by addition of antibodies to insulin, Froesch and colleagues (see Zapf et al. 1984) developed a bioassay dependent on the oxidation of radiolabeled glucose to CO₂ by fat cells. This assay was used to purify the nonsuppressible insulin-like activity (NSILA) of serum, and led to the characterization of IGF-I and IGF-II.

Researchers interested in cell culture have monitored purification of serum factors that stimulate cell growth by measuring the incorporation of thymidine into cultured chick embryo fibroblasts (Pierson and Temin 1972). This line of investigation led eventually to the purification of MSA and the characterization of IGF-II in the rat. In addition to their lack of specificity, these bioassays are time-consuming, expensive to perform, and somewhat unpredictable when used to assay serum samples.

2. Radioreceptor Assays and Plasma Protein Binding Assays

The first radioreceptor assay for Sm-C/IGF-I was based on the observation that partially purified Sm-C/IGF-I competed with radiolabeled insulin for the insulin receptor (Hintz et al. 1972). This assay proved quite useful for monitoring the