Radioimmunoassay of Somatomedin B

APPLICATION TO CLINICAL AND PHYSIOLOGIC STUDIES

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ABSTRACT A radioimmunoassay has been developed for Somatomedin B, a growth hormone-dependent factor that stimulates DNA synthesis in human glia-like cells. The sensitivity permits detection of this factor in human plasma diluted 1:20,000 and in monkey plasma diluted 1:5,000. It is not measurable in nonprimate plasma diluted 1:20. The concentration in growth hormone-deficient adult patients is equivalent to 6.6±0.5 μg/ml of a highly purified somatomedin preparation. In acromegaly the concentration is 19.3±2.3 μg/ml and falls after definitive therapy that results in a decrease in plasma growth hormone. In unextracted human plasma the immunoreactive Somatomedin B is associated with a plasma protein at least as large as γ-globulin and with an electrophoretic mobility on paper resembling the α-globulins. The level of Somatomedin B in the bound form in human plasma under steady-state conditions may depend on the rate of production of the peptide and/or the concentration of the plasma-binding protein. At present there is no information concerning which of these is modulated by growth hormone. Immunoreactive Somatomedin B is found predominantly in Cohn plasma fractions III and IV, largely dissociated from the plasma-binding protein. The disappearance curves of labeled purified Somatomedin B and of immunoreactive Somatomedin B from acromegalic plasma administered intravenously to a dog were superposable; the terminal portion of the disappearance curve having a half time of almost an hour.

INTRODUCTION

The existence of a pituitary hormone required for normal growth has been recognized since the beginning of this century (1–3). However it was not until 1944 that the isolation and partial purification of a growth-stimulating factor from sheep pituitaries was effected (4). Growth hormone is unique among the peptide hormones in showing marked species specificity in its biologic action (5). The ineffectiveness in man of animal growth hormones has been shown to arise from distinct chemical differences between the hormone from primates and from lower species (6, 7). Marked species specificity is also manifest in the radioimmunoassay system, porcine and bovine growth hormones being less than 1/10,000 as immunoreactive as primate growth hormone with the anti-human growth hormone antiserum prepared in our laboratory (8) and widely distributed by the Hormone Distribution Officer of the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

Before the development of radioimmunoassay methodology, it was not possible to measure growth hormone in normal human blood. However evidence had accumulated that the action of growth hormone on skeletal tissue is mediated by a substance whose concentration in plasma is growth hormone dependent. This factor was originally called "sulfation factor" (SF)[1] because it stimulates the uptake of radioactive sulfate in the costal cartilage of hypophysectomized rats (9). It has been shown to decrease in human and rat plasma after hypophysectomy and to increase after the administration of human growth hormone to hypophysectomized patients or beef growth hormone to hypophysectomized rats (9, 10). Since the original reports (9, 10), the existence of SF has been amply confirmed, and evidence has been

[1] *Abbreviations used in this paper:* GH, growth hormone; hGH, human growth hormone; NSILA, nonsuppressible insulin-like activity; SF, sulfation factor; Som B III, preparation III.

accumulating suggesting additional properties for growth hormone (GH)-dependent serum factor(s). A more general designation, "somatomedin," has been introduced (11), which is used as a generic term for any GH-dependent substance that appears to mediate the action of GH or somatotropin. Although GH shows marked species specificity both with respect to its biologic and immunologic properties, there has until now been no evidence for similar species specificity of any of the somatomedins.

Recently at least three somatomedins with differences in chemical and biologic properties have been described (12, 13); Somatomedin A which stimulates sulfate uptake by chick cartilage (14, 15); Somatomedin B which stimulates DNA synthesis in human glia-like cells (16); and Somatomedin C, a peptide more basic than somatomedin A or B and which stimulates sulfate uptake in rat cartilage (13). In the present report we describe the development of a radioimmunoassay for purified Somatomedin B and its application to some biochemical and clinical studies. The advantages of radioimmunoassay, namely its simplicity, sensitivity, and specificity, make feasible the investigation of a host of chemical procedures and physiologic effects in numbers that would be prohibitive with the use of more tedious bioassays.

METHODS

Radioimmunoassay

Preparation of antisera. Approximately 25 mg of KABI l.uman purified Somatomedin B[2] (preparation I) was provided for immunization. Conjugation to guinea pig albumin (fraction V) was effected using the carbodiimide method of Goodfriend, Levine, and Fasman (17) with only minor variations. Six guinea pigs were immunized with the coupled preparation containing 0.2-0.5 mg of Somatomedin B homogenized in complete Freund's adjuvant according to the usual procedures of our laboratory (18). Antibody was detectable in the sera from all animals. However serum from only one animal was useful for radioimmunoassay at dilutions up to 1:4,000 (antiserum 101) and from another at 1:500 (antiserum 105). To conserve antigen, only these two animals received more than four immunizing doses. The major portion of all bleedings of guinea pig 101 with similar titer was pooled and used for the studies reported here.

Preparation of radioiodine-labeled somatomedin: Somatomedin B preparations (I, II, and III) were labeled with radioiodine using the chloramine-T technique with only minor variations (18). Specific activity of the labeled preparations ranged from 10 to 50 μCi radioiodine/μg of mate-

rial. Generally ^{125}I-labeled preparations were used for radioimmunoassay, and ^{131}I-labeled preparations were employed for studies of the distribution and metabolism of somatomedin in dogs. Purification (18) of iodination mixtures was effected either by Sephadex G-50 gel filtration or by starch gel electrophoresis using the method of Smithies (19). Generally, the eluates from starch gel were more suitable for radioimmunoassay. For most studies the less pure preparation II was used for labeling since contaminants were removed by the starch gel purification. More than 50% of the total labeled peptide could be bound in antibody excess. Using the best eluate from starch gel, generally more than 90% was bindable to antibody. Thus approximately 40% of the labeled material that did not bind to antibody was removed by starch gel purification. No differences were observed between labeled Somatomedin B II or III after purification, and these preparations were used interchangeably.

Standards. Preparation III (Som B III), which was presumed to be the most highly purified Somatomedin B preparation made available to us, was used as standard, and all concentrations are expressed in terms of immunoreactivity equivalent to a known weight of Som B III per milliliter.

Experimental conditions used for assay. The standard diluent for the assay is 4% control guinea pig serum in 0.02 M barbital buffer (pH 8.6). Antiserum 101 is used at a final dilution up to 1:4,000. 4–6 days of incubation at 4°C are required to reach equilibrium at this dilution. The volume of the incubation mixture is 0.5 ml. Separation is effected with 0.1 ml of uncoated charcoal (50 mg/ml 0.02-M barbital buffer). The double antibody method of separation of bound from free-labeled hormone can also be employed. To minimize the amount of second antibody required in these assays the standard diluent has a lower concentration of control guinea pig plasma, i.e., 1% control guinea pig plasma in the same barbital buffer.

Immunoreactive Somatomedin B in human plasma was determined in a dilution of 1:20,000 or greater and in monkey plasma in a dilution of 1:5,000 or greater. Plasma from other species including guinea pig, mouse, rat, cow, dog, sheep, pig, or rabbit was assayed at a 1:20 dilution. Somatomedin B content of several of the plasma protein fractions prepared by the Cohn method was also determined. These fractions were a gift of the Protein Foundation, Boston, Mass. They were obtained as a dry powder and were soluble at a concentration of 1 mg/ml isotonic saline. Preparations of Somatomedin A provided by KABI, of nonsuppressible insulin-like activity (NSILA) prepared by Dr. E. R. Froesch and provided to us by Dr. Jesse Roth and of Somatomedin C provided by Dr. Judson J. Van Wyck were radioimmunoassayed for their Somatomedin B content.

Fractionation procedures. All fractionation procedures were carried out at 4°C. Before application to Sephadex G-50 fine (1 × 50-cm) columns all samples including plasma, plasma protein fractions, or purified somatomedins were fortified with [^{131}I]albumin and [^{131}I]Na as marker molecules for void volume and salt peak, respectively. When fractionation was on Sephadex G-100 or G-200 columns [^{125}I]γ-globulin, [^{131}I]albumin, and [^{131}I]Na were used as markers. The zones of emergence of all components were plotted as percent of elution volume between the marker molecules. Columns were equilibrated with eluting buffer before use; this was generally 1–4% control guinea pig serum in 0.02 M barbital buffer (pH 8.6).

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[2] Three different Somatomedin B preparations were obtained from AB KABI Research Department. Preparation I was purified from human plasma by Cohn fractionation, acid ethanol extraction, and gel chromatography (12). Preparation II was similar. Preparation III had been further purified by electrophoresis and contained no Somatomedin A activity according to bioassay performed by Dr. Kerstin Hall.

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Fractionation of endogenous hormone in human plasma and of purified Som B III added to guinea pig plasma was carried out on starch gel electrophoresis (6 V/cm for 18 h). After electrophoresis, sections were cut from the gel at 1-cm intervals, frozen for 3–4 h, thawed, and eluted with 1% control guinea pig plasma in 0.02 M barbital buffer. The gel particles were removed by centrifugation, and supernatant solutions were subjected to radioimmunoassay.

Endogenous human plasma somatomedin and labeled or unlabeled purified somatomedin added to guinea pig or human plasma were fractionated on paper electrophoresis (Whatman no. 3 paper, 0.1 M barbital buffer, pH 8.6, 6 V/cm for 18 h). When labeled somatomedin was employed, the strip was heat-coagulated, autoradiographed, scanned for radioactivity with a paper strip scanner, and then stained to visualize the serum proteins. When unlabeled purified or endogenous somatomedin was fractionated, the strip was cut lengthwise. One-half of the strip was heat coagulated and stained; the other half was sectioned at 1-cm intervals and eluted with standard diluent, and the eluates were subjected to radioimmunoassay.

Rough estimates of the size of endogenous human plasma somatomedin and purified Som B III added to guinea pig plasma were also obtained by ultracentrifugal analysis. Purified Som B III was added to guinea pig plasma, equilibrated for 1 h, and then diluted 1:5. Human plasma was diluted 1:5. For comparison purposes [¹³¹I]human serum albumin and [¹²⁵I]γ-globulin in a 1:5-dilution of human plasma were also employed. 5 ml of each mixture was added in duplicate to tubes and sedimented in a Spinco model L ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) fitted with a bucket rotor (SW 50.1) for 16 h at 40,000 rpm. After the centrifuge came to rest, 0.5-ml samples were removed in succession from the top of each tube for assay of immunoreactivity or radioactivity. The concentration in each sample was expressed as percent of the concentration in the uncentrifuged specimen.

Turnover studies. Turnover studies in dogs were performed by administering [¹³¹I]Somatomedin B in a pulse injection either alone or with 2–4 ml plasma from an acromegalic subject and which contained a high concentration of endogenous Somatomedin B activity. The labeled preparation was administered with the immunoreactive somatomedin of plasma to monitor the accuracy and reproducibility of the injection procedure. Indwelling catheters were inserted into the veins of two legs of the dog, one for injection and the other for sampling. The catheter used for sampling was maintained patent with heparin. Heparinized blood samples were taken before and at regular intervals after injection. The blood was centrifuged immediately, and the supernatant plasma was stored frozen until assayed for radioactivity or somatomedin content.

Disappearance of labeled Somatomedin B and appearance of radioiodide were analyzed by fractionation of plasma radioactivity by hydrodynamic flow chromatoelectrophoresis as well as by filtration on Sephadex G-50 columns and by measuring the total radioactivity in the plasma samples. In this way the fraction of the total radioactivity that corresponded to intact labeled hormone was monitored. Disappearance of human plasma Somatomedin B after pulse injection in a dog was determined by radioimmunoassay, and its form was determined by Sephadex gel filtration.

The rates of disappearance of labeled and unlabeled species were determined from a semilogarithmic plot of the plasma concentration as a function of time. To normalize values to become independent of the weight of the animal,

the plasma concentration was expressed as percent of the injected dose per liter plasma, multiplied by the body weight of the dog.

Selection of patients. Human plasma samples assayed were from male veteran patients referred to the Reference Laboratory of the Bronx Veterans Administration for GH determinations. The diagnosis of under or over secretion of GH was based on clinical findings and GH responsiveness to standard glucose tolerance test for acromegalic subjects and insulin tolerance tests for hypopituitary subjects. Normal subjects were those with normal GH responsiveness during the appropriate test. The samples from male acromegalic subjects before and after definitive therapy were supplied by Dr. Richard C. Dimond, Department of Endocrinology and Metabolism, Walter Reed Army Institute of Research, Washington, D. C. All plasma samples were obtained in the fasting state in the morning.

RESULTS

To characterize the labeled somatomedin preparations, guinea pig plasma was added to the radioiodination mixture of each of the three somatomedin preparations of differing purity (preparations I, II, and III), and por-
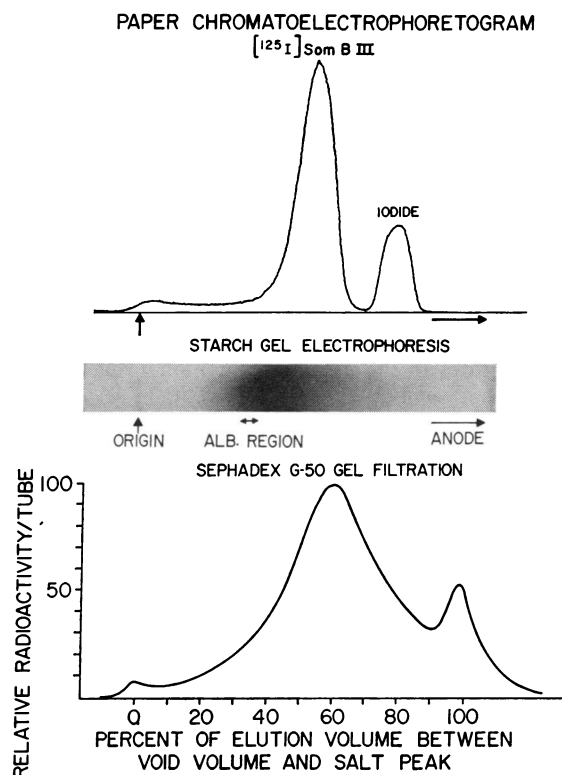

FIGURE 1 Analysis of iodination mixture of [¹²⁵I]Som B III added to guinea pig plasma on chromatoelectrophoresis (top), starch gel electrophoresis (middle) and Sephadex G-50 gel filtration (bottom). The major component of protein-bound radioactivity appears as a single band in the prealbumin region on starch gel electrophoresis and has a $K_{av} \sim 0.6$ on Sephadex.
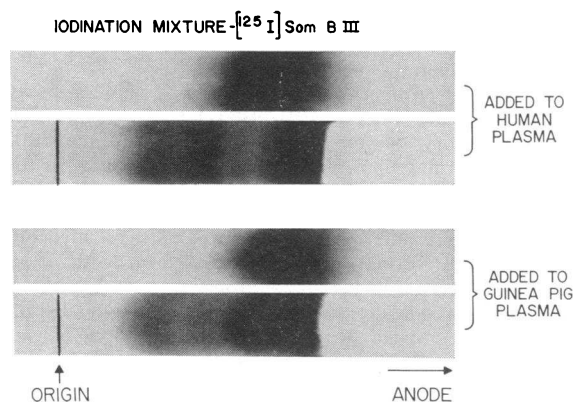
IODINATION MIXTURE-[^{125}I] Som B III

ADDED TO HUMAN PLASMA

ADDED TO GUINEA PIG PLASMA

ORIGIN → ANODE

FIGURE 2 Analysis on paper electrophoresis of iodination mixture of [^{125}I]Som B III. Autoradiographs are shown above the corresponding stained paper strips for mixtures added to human (top) and to guinea pig (bottom) plasmas.

tions of each were then fractionated by paper chromato-electrophoresis, starch gel electrophoresis, and Sephadex G-50 gel filtration (Fig. 1). The patterns obtained with all three preparations were similar on paper chromato-electrophoresis and Sephadex gel fractionation. On starch gel the major peak of labeled Som B I was just slower than serum albumin, but for the other two preparations the major peak was immediately in advance of albumin. On Sephadex G-50 gel filtration the major fraction of labeled Somatomedin B has a peak with a partition coefficient, K$_{av}$, of about 0.6.

[^{125}I]Somatomedin B added to human or guinea pig plasma has an electrophoretic mobility on paper in the region of the α-globulins (Fig. 2). This is in contrast to its mobility on starch gel where the same labeled preparation migrates in the prealbumin region (Fig. 1). The electrophoretic mobility in the paper system is dependent only on charge, and in the starch gel system, because of molecular sieving, it depends on both charge and size. The greater mobility on starch gel suggests that in this system there is no evidence for binding of labeled somatomedin to guinea pig serum proteins.

Using the radioimmunoassay system described in the section on Methods, the smallest measurable concentration of immunoreactive Somatomedin B is ~25 pg Som B III/ml (Fig. 3). This sensitivity permits detection of somatomedin in normal human plasma at dilutions of 1:20,000 or greater. No detectable immunoreactive components were found in guinea pig, mouse, rat, cow, dog, sheep, pig, or rabbit plasmas at dilutions of 1:20. Monkey plasma was assayed at a dilution of 1:5,000. The concentration in normal monkey plasma is about 2 μg Som B III/ml. In 12 normal male subjects the fasting plasma somatomedin ranged from 4 to 20 μg Som B IIII/ml with an average value of 9.8±1.5 μg Som B III/ml. The species specificity of the assay is

similar to that of the radioimmunoassay of human GH hormone, i.e., cross-reaction with primate hormone but not with that from other animal species. [^{125}I]Somatomedin B does not bind to antisera to insulin or GH when the antisera are used in marked (100- to 1,000-fold) excess. [^{125}I]Somatomedin A or [^{125}I]NSILA does not bind to the antiserum used in the immunoassay. No immunoreactive Somatomedin B was detected in the preparations of Somatomedin A, NSILA, or Somatomedin C that had been provided. Contamination of these preparations with as little as 1% Somatomedin B would have been detected.

A scattergram of the immunoreactive somatomedin content of fasting plasma from hypopituitary and acromegalic subjects is shown in Fig. 4. There is some overlap between the two groups. However the mean value for untreated acromegalic subjects (19.3±2.3 μg Som B III/ml) is about three times that of hypopituitary patients (6.6±0.5 μg Som B III/ml). In acromegalic patients, definitive therapy (surgery or radiation) that resulted in a decrease in plasma GH concentration (Table I) also effected a reduction in the somatomedin content of plasma.

Immunoreactive Somatomedin B in unextracted human plasma elutes in the void volume on Sephadex G-50 gel filtration and in the region of [^{125}I]-γ-globulin on Sephadex G-100 and G-200 gel filtration (Fig. 5). Prior incubation of plasma diluted 1:10 in 7 M urea followed by fractionation on a Sephadex G-50 column equilibrated in and eluted with 7 M urea does not effect

GP 101
1:4,000 DILUTION OF ANTISERUM

$\frac{B}{F}$ [^{125}I] Som B III
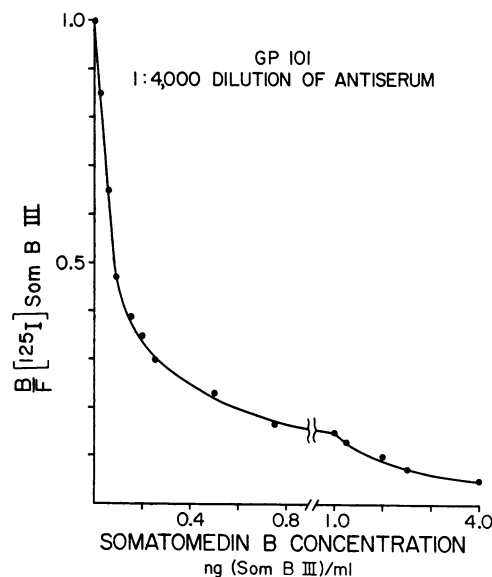
SOMATOMEDIN B CONCENTRATION
ng (Som B III)/ml

FIGURE 3 Standard curve for determination of Som B III. Standard diluent is 4% control guinea pig plasma in 0.02 M barbital buffer. Separation of bound and free [^{125}I]somatomedin was effected with uncoated charcoal.

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dissociation of somatomedin immunoreactivity from the protein-bound form.

The electrophoretic mobilities on paper of endogenous human plasma Somatomedin B and of purified Som B III added to guinea pig plasma do not differ. The peak of immunoreactivity appears to be in the intra-α-globulin region (Fig. 6a). However on starch gel electrophoresis, the major peak of endogenous human plasma somatomedin is close to the origin with some trailing up to the albumin region whereas unlabeled purified Somatomedin B added to guinea pig plasma migrates in the prealbumin region (Fig. 6b). Thus the labeled (Figs. 1 and 2) and unlabeled (Fig. 6) purified Somatomedin B in guinea pig plasma behave indistinguishably on electrophoresis.

The similar electrophoretic mobilities on paper of endogenous plasma somatomedin and purified somatomedin do not permit decision as to whether or not purified somatomedin binds to guinea pig plasma. Starch gel electrophoresis separates on the basis of size as well as charge. Endogenous somatomedin is retarded because of its binding to a human serum protein. Purified Somatomedin B does not appear to bind to guinea pig plasma proteins and therefore migrates as a prealbumin, a mobility consistent with its charge as determined from paper electrophoresis and its size as determined by Sephadex gel filtration.

The sedimentation of endogenous Somatomedin B on ultracentrifugation is consistent with its binding to a protein approximating γ-globulin in molecular weight (Fig. 7). In contrast the major fraction of purified unlabeled Somatomedin B does not bind to guinea pig plasma since its sedimentation velocity is less than that of labeled albumin (Fig. 7) and resembles that of insulin (20). It should be noted that the pellet in the bottom 0.5 ml contained the remaining Somatomedin B and labeled markers not found in the top nine portions shown in Fig. 7.

Somatomedin B in undiluted plasma is not detectably adsorbed to charcoal. However when plasma is diluted

TABLE I
Basal Concentrations of GH and Somatomedin B in Plasma of Acromegalic Subjects before and after Therapy

		hGH	Somatomedin B
		ng/ml	μg Som B III/ml
A. F.	Pre	54	14.0
	Post	8	9.5
I. B.	Pre	21	25.0
	Post	10	7.9
D. B.	Pre	72	15.7
	Post	16	9.1
J. T.	Pre	342	25.9
	Post	146	16.3
M. C.	Pre	29	17.9
	Post	<2	6.6
B. H.	Pre	138	16.0
	Post	<2	7.9
R. M.	Pre	10	11.0
	Post	<2	5.5
S. F.	Pre	36	24.1
	Post	3	15.0
J. C.	Pre	21	25.4
	Post	<2	13.2

1 : 100 in normal saline, 90% of the Somatomedin B content is adsorbed by charcoal (10 mg/ml) within a few minutes.

The Somatomedin B content of the protein fractions of human plasma prepared by the Protein Foundation, according to the Cohn ethanol scheme is given in Table II. It must be appreciated that different laboratories may use other modifications of the Cohn procedure and these modifications may result in some differences in the extraction of Somatomedin B. The Somatomedin B concentrations in fractions III and IV were about a hundredfold higher than that in fractions II and V (γ-globulin and albumin, respectively). Dilution curves of the somatomedin contained within these protein fractions are superposable on the standard curve of Som B III (Fig. 8). In contrast with the elution pattern observed in unextracted plasma (Fig. 5), the Sephadex gel filtration patterns of the Cohn fractions reveal a significant and usually predominant component with an elution volume corresponding to that of the purified Som B III (Fig. 9).

Unextracted human plasma Somatomedin B, which elutes in the void volume on Sephadex G-50 gel filtration, was administered intravenously to a dog. In post-injection samples of the dog plasma, the major component of immunoreactivity had an elution volume corresponding to that of the injected material, but there was a minor component eluting in the region of purified somatomedin ($K_{av} \sim 0.4–0.6$) (Fig. 10). Labeled puri-
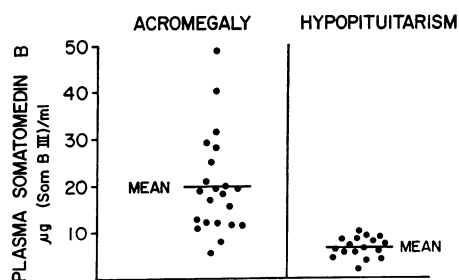

FIGURE 4 Basal Somatomedin B concentrations in 22 adult males with clinical acromegaly and proven hypersecretion of GH and in 17 adult males with diagnoses of chromophobe adenoma and GH deficiency proven by failure to respond to insulin-induced hypoglycemia.
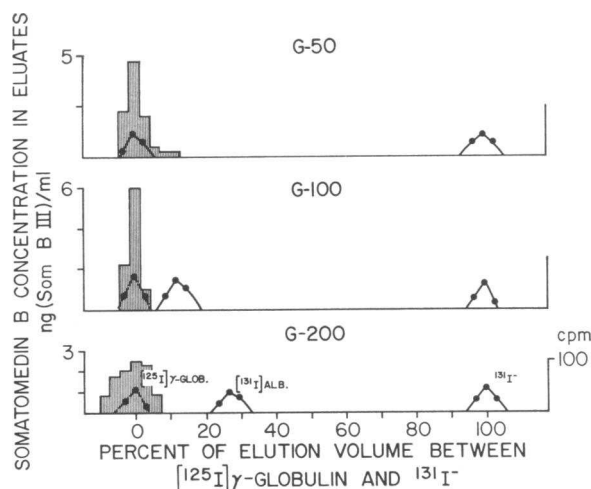
FIGURE 5 Gel filtration on Sephadex G-50, G-100, and G-200 of immunoreactive Somatomedin B in human plasma. On G-50, the $[^{131}I]$albumin and $[^{125}I]\gamma$-globulin markers superpose. Elution volume is expressed in terms of percent of volume between $[^{125}I]\gamma$-globulin and salt peak ($^{131}I^-$).

fied Somatomedin B before and immediately after intravenous administration to a dog has a peak on Sephadex G-50 gel filtration with a $K_{av} \sim 0.6$. Subsequently, when the intact hormone has disappeared, the void volume component, corresponding presumably to the damaged labeled hormone, although initially a very minor component, appears relatively more prominent and the radioiodide generated from degraded Somatomedin B shows an absolute increase (Fig. 11). In spite of the apparent difference in size between endogenous human Somatomedin B and radioiodinated Somatomedin B, as indicated by Sephadex gel filtration, their rates of disappearance after administration were superposable (Fig. 12). For each form of somatomedin, the terminal portion of the disappearance curve has a half time of 50–60 min.

DISCUSSION

The term "somatomedin" is used to designate polypeptide factors or substances in plasma that are, at least partially, GH dependent and appear to mediate the growth-promoting effect of GH in various tissues (21, 22). The absolute degree of purity of the Somatomedin B used for labeling and as a reference substance in our radioimmunoassay is not known. However, the observation that the substance that is measured with the present assay was approximately three times higher in serum in acromegaly than in hypopituitarism and consistently fell in the former group when there was a fall in plasma GH after therapy, definitely speaks in favor of the measured substance being a GH-dependent factor and therefore a somatomedin.
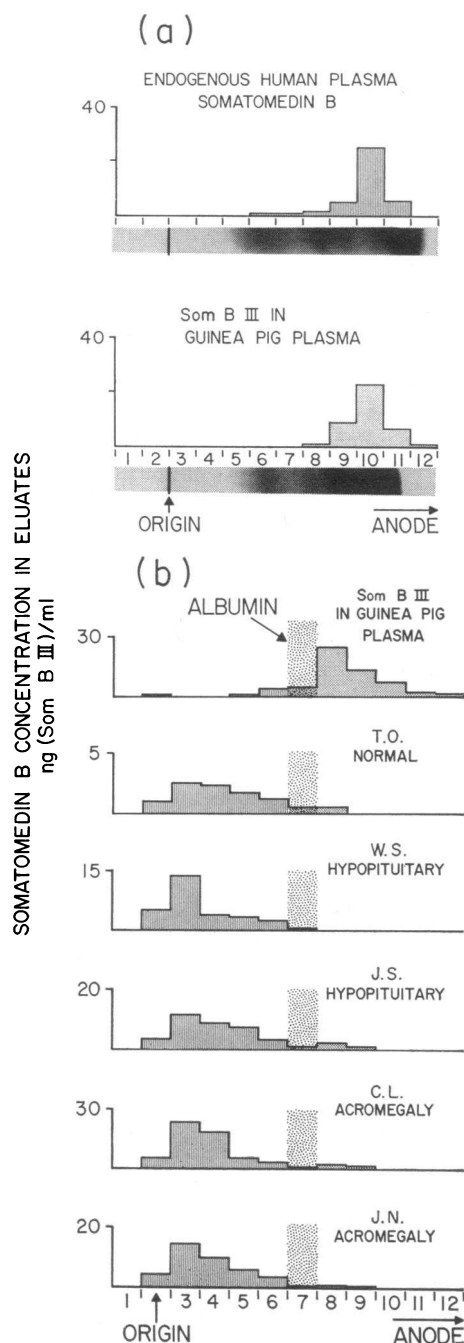
FIGURE 6 (a) Distribution on paper strip electrophoresis of endogenous Somatomedin B in human plasma (top) and Som B III added to guinea pig plasma (bottom). After completion of electrophoresis, the strip was air dried, cut down the middle, and segments from half the strip eluted; the other half-strip was heat coagulated and stained. The stained strip is shown directly below the pattern of immunoreactivity. (b) Distribution of immunoreactivity on starch gel electrophoresis of Som III added to guinea pig plasma (top) and of endogenous human plasma Somatomedin B (five bottom frames).

The guinea pig antiserum developed in response to immunization with human somatomedin B has a specificity directed to a factor in human and monkey plasma that was not found in plasma from many nonprimate mammalian species. The strong cross-reaction of monkey plasma in the radioimmunoassay system rules out the possibility that the antibody is directed against a plasma protein which is exclusively human. The observation that the antiserum produced in response to immunization with Somatomedin B derived from human plasma reacts only with primate plasma resembles the species specificity of antisera directed against human (h)GH. This species specificity is unique in antisera to peptide hormones and raises the question as to whether human Somatomedin B might be a fragment of hGH. Failure of Somatomedin B to react with a antiserum to hGH does not rule out this possibility, since the antiserum may not be directed toward that portion of the hGH molecule that might be somatomedin, or cleavage might so distort the configuration of the molecular fragment as to rule out the possibility for interaction with antibody to the hGH molecule.

Further evidence that Somatomedin B may not be directly derived from hGH is the persistance of the factor in human plasma, measurable at a dilution of 1:10,-000 in patients in whom hGH levels in the basal state and in response to various secretagogues are below levels of detectability (< 1 ng/ml).

Other evidence that Somatomedin B may not be derived as a fragment of GH is derived from an appreciation of their relative concentrations in plasma: Somatomedin B averages about 10 µg/ml and hGH levels in normal subjects are a thousandfold lower. If the somatomedin were derived from hGH, its turnover time would have to be a thousandfold slower to maintain this steady-state concentration. Our turnover studies in the dog are consistent with a turnover time for Somatomedin B perhaps twice the 20–30-min half time for hGH in man after hypophysectomy (23) but not a thousandfold longer. However, we have not had the opportunity of measuring the disappearance of endogenous Somatomedin B in man after hypophysectomy. Because of the strong binding of endogenous Somatomedin B to human plasma proteins, the disappearance of endogenous Somatomedin B from human plasma might well be considerably slower than its disappearance from the circulation of the dog.

Generation of SF after perfusion of livers from intact rats with a nutrient medium containing bovine GH has been demonstrated (24). However, we have looked for and failed to demonstrate generation of Somatomedin B immunoreactivity after perfusion of rat liver with hGH. Thus, rat liver does not cleave hGH into a Somatomedin B-like fragment measurable in our radioimmunoassay system. This does not rule out the possibility of the involvement of human liver or other organ in such cleavage.
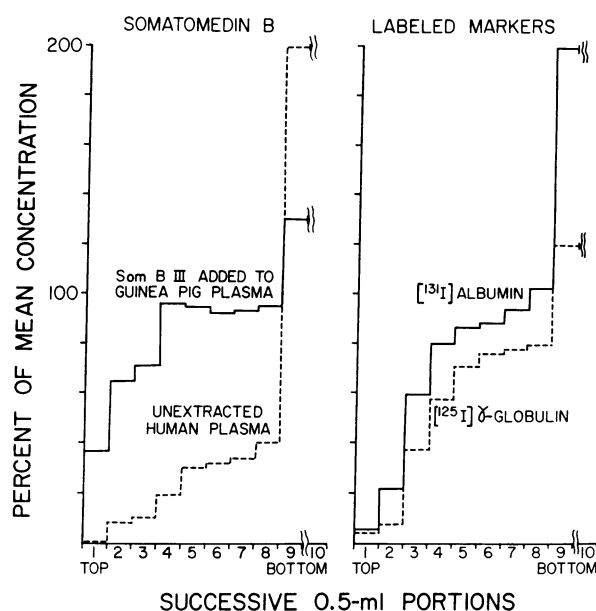

FIGURE 7 Sedimentation of endogenous Somatomedin B in human plasma, of purified Som B III added to guinea pig plasma (left) and of labeled marker molecules in human plasma (right). All plasmas were diluted 1:5. After ultracentrifugation of 5-ml samples for 16 h in a bucket rotor (SW 50.1) at 40,000 rpm, successive 0.5-ml samples were removed for radioimmunoassay of Somatomedin B content or for assay of radioactivity content and were then compared to the mean Somatomedin B or radioactivity in uncentrifuged sample. The pellet contained in the bottom 0.5 ml balanced the reduced concentrations of immunoreactivity or radioactivity of the upper portions.
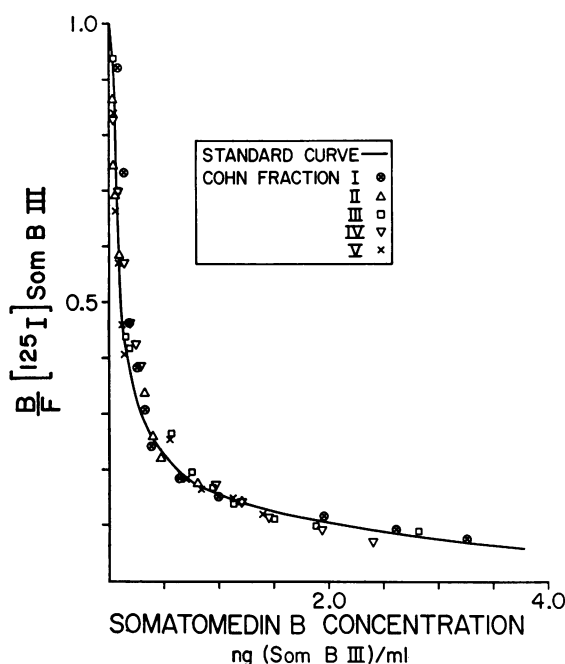
FIGURE 8 Superposition of dilution curve of Cohn fractions on standard curve of Som B III.

In studies on the incorporation of labeled thymidine into DNA of human glia-like cells, Somatomedin B purified from human plasma was used (16). Whether if a similar fraction were purified from nonprimate plasma it would have been active in this system has not been studied. Recently it has demonstrated that plasma from a variety of animal species stimulates sulfate incoropration by human and monkey as well as pig rib cartilage (25). This suggests that SF does not have the same biologic species specificity as does GH.

Somatomedin B activity in human plasma is known to be associated with plasma proteins. In this study fractionation on Sephadex gel, starch gel, and ultracentrifugation suggest that the molecular weight of the binding protein is comparable to that of γ-globulin, i.e., about 150,000 daltons or greater. The electrophoretic mobility of the binding protein corresponds to that of an α-globulin and does not differ from that of the purified unbound Somatomedin B.

We have been unable to demonstrate binding of labeled or unlabeled Somatomedin B to guinea pig plasma proteins. Because of the possibilities that the association rate might be slow or dissociation rapid, we incubated in whole guinea pig plasma for 8 h at room temperature to speed association followed by 16 h at 4°C to retard dissociation. Nonetheless on starch gel electrophoresis, Sephahex gel filtration, or ultracentrifugal analysis, Somatomedin B behaved identically whether added before

fractionation to saline or normal guinea pig plasma. A small fraction of the labeled hormone, presumed to be a damaged moiety, binds nonspecifically to serum proteins, as is commonly observed with several damaged labeled peptide hormones.

The spaces of distribution in the dog at 3 min after intravenous administration of ^{131}I-purified Somatomedin B or endogenous human somatomedin were similar and were about twice plasma volume. The human plasma somatomedin must therefore have dissociated, in part at least, quite rapidly from its human binding protein after the dilution 250–500-fold in the blood of the dog. Because the initial rates of disappearance of the somatomedins, labeled and unlabeled, were quite rapid ($t_{\frac{1}{2}} < 15$ min), their binding to dog plasma proteins could not have been significant or their egress from the capillary bed would have been more retarded.
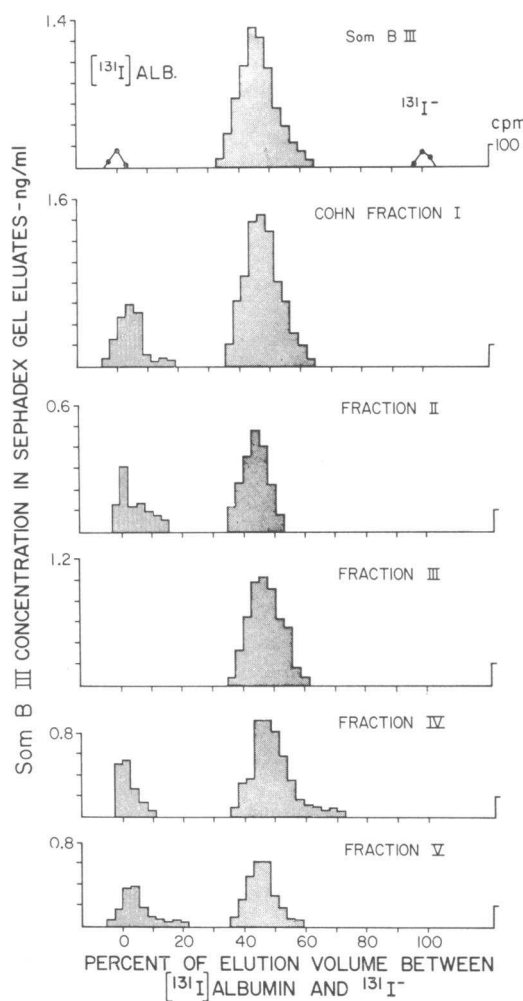

FIGURE 9 Sephadex G-50 gel filtration of purified Som B III and of immunoreactive somatomedin in Cohn fractions of human plasma.
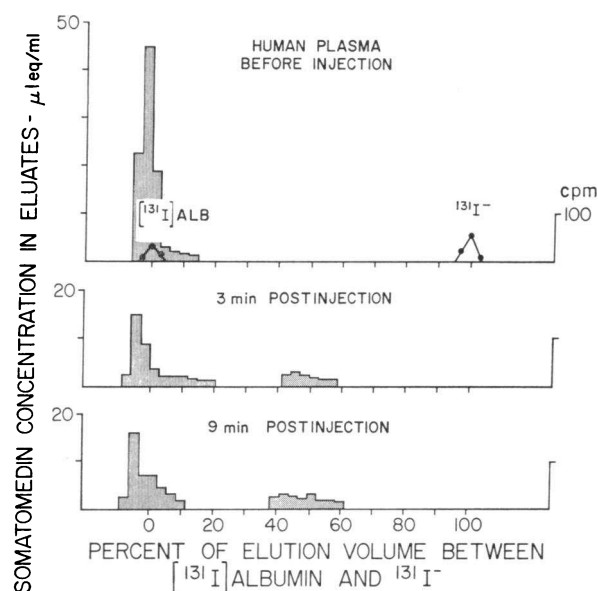
FIGURE 10 Sephadex G-50 gel filtration of immunoreactive somatomedin in human plasma (top) and in the plasma of the dog 3 min (middle) and 9 min (bottom) after intravenous administration of the human plasma. [131I]albumin and [131I]Na were added to samples before application to the columns to mark the void volume and salt peak respectively.

Consideration must be given as to whether the failure of unlabeled or labeled purified Somatomedin B to bind to serum proteins in the animal plasmas was due to alterations in structure attributable either to the initial extraction or subsequent iodination procedures. Whereas this possibility cannot be ruled out, the failure of human endogenous somatomedin, which dissociated rapidly during circulation in the dog, to rebind to dog plasma proteins cannot be accounted for by chemical alteration. The most likely explanation appears to be that the dog and guinea pig, at least, lack a specific binding protein for human Somatomedin B. However, we have also been unable to demonstrate rebinding of labeled Somatomedin B to human plasma. Whether this is due to saturation of the human binding protein and a slow rate of exchange or alteration of the labeled Somatomedin B is a problem as yet unresolved. Hintz, Orsini, and VanCamp (26) have demonstrated dissociation of somatomedin activity from a binding protein in human plasma during Sephadex gel filtration at an acid pH. The dissociated material reassociated at neutral pH with plasma proteins that had been freed of somatomedin.

The observation that the major fraction of immunoreactive Somatomedin B in Cohn fractions III and IV is in the free form could be explained in three different ways: (a) Somatomedin B was altered by the fractionations so that it could no longer bind to the binding pro-
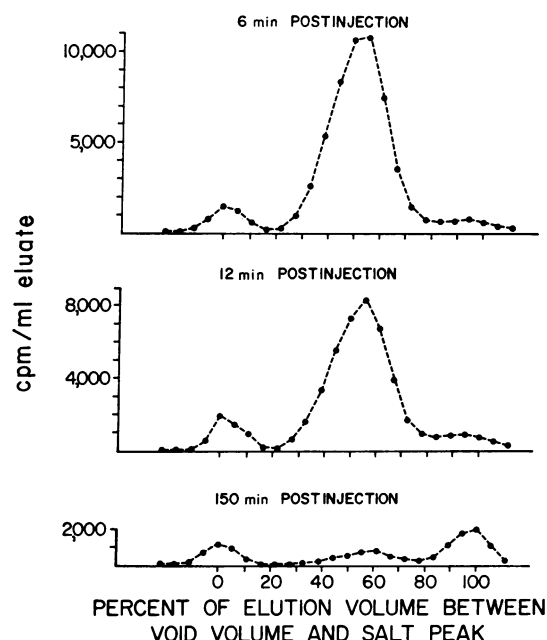

FIGURE 11 Sephadex G-50 filtration patterns of radioactivity in dog plasma after intravenous administration of [131I]Somatomedin B. The small peak in the void volume presumably is altered labeled material bound nonspecifically to serum protein; the central peak corresponds to intact labeled somatomedin; the peak on the right is radioiodide returned to the circulation after degradation of the [131I]-Somatomedin B.
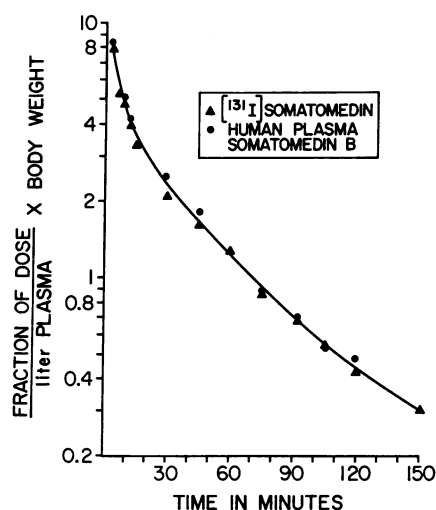

FIGURE 12 Superposable disappearance curves of intact purified [131I]Somatomedin B and immunoreactive Somatomedin B obtained from acromegalic plasma after intravenous administration to a dog.

tein; (b) the binding protein was altered; (c) the binding protein is in another Cohn fraction. The last is the least likely since the binding protein appears to have the characteristics of an α-globulin and hence should appear in fraction III and/or fraction IV. The failure to demonstrate rebinding of the labeled Somatomedin B to unfractionated human plasma cannot be accounted for by alteration of the binding protein and is more consistent with explanation (a). If Somatomedin B is modified during the extraction process so that it cannot rebind to the serum protein, what would be the effect on reactivity in the immune system employed in this assay? The evidence is that bound and unbound Somatomedin B have almost equivalent immunoreactivity. Consider that total plasma protein is about 70 mg/ml, that fractions III and IV account for about 25% of the total protein and that (from Table II) the mean concentration of Somatomedin B (primarily in the unbound form) is about 0.3 μg/mg protein. The Somatomedin B concentration in unfractionated plasma should therefore be about $N(70 \times 0.25 \times 0.3)$ or ~ 5 μg/ml plasma. This agrees well with the mean concentration in normal plasma of 10 μg/ml, allowing for losses in the Cohn fractionation.

It should be emphasized that the concentrations reported in the assay for Somatomedin B are in terms of the weight of preparation III standard. This standard is not necessarily completely pure. Nonetheless the observation that the major fraction of the iodinated peptide, even before purification, binds in excess antiserum suggests that the assay is not based on a minor peptide component in the preparation.

The level of Somatomedin B in the bound form in human plasma under steady-state conditions may depend on the rate of production of the peptide and/or the concentration of the plasma binding protein. At present there is no information concerning which of these is modulated by GH.

ACKNOWLEDGMENTS

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