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PTO/SB/21 (6-99)

Approved for use through 09/30/2000. OMB 0651-0031
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Patent Number	5,681,814 (08/071,819)
Issued Date	OCTOBER 28, 1997 (JUNE 4, 1993)
First Named Inventor	ROSS G. CLARK
Group/Art Unit	1642
Examiner Name	HUFF, SHEELA JENDRA 2005
Attorney Docket Number	39766-0190

RECEIVED

OFFICE OF PETITIONS

Total Number of Pages in This Submission

ENCLOSURES (check all that apply)

<input checked="" type="checkbox"/> FEE TRANSMITTAL FORM <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment / Response <input type="checkbox"/> After Final <input type="checkbox"/> Version With Markings Showing Changes <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Response to Missing Parts/ Incomplete Application <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53 <input type="checkbox"/> Copy of Notice	<input type="checkbox"/> Copy of an Assignment <input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition Routing Slip (PTO/SB/69) and Accompanying Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, by Assignee to Exclusion of Inventor Under 37 C.F.R. §3.71 With Revocation of Prior Powers <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Small Entity Statement <input type="checkbox"/> Request for Refund	<input type="checkbox"/> After Allowance Communication to Group <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to Group (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> ADDITIONAL ENCLOSURE(S) (PLEASE IDENTIFY BELOW): <input checked="" type="checkbox"/> APPLICATION FOR EXTENSION OF PATENT TERM BASED ON REGULATORY REVIEW OF A NEW DRUG APPLICATION PURSUANT TO 35 U.S.C. §156 WITH EXHIBITS A-F; TWO COPIES OF ENTIRE DOCUMENTS WITH EXHIBITS; and STAMPED RETURN POSTCARD
Remarks AUTHORIZATION TO CHARGE DEPOSIT ACCOUNT 08-1641 FOR ANY FEES DUE IN CONNECTION WITH THIS PAPER, REFERENCING ATTORNEY'S DOCKET NO. 39766-0190.		

SIGNATURE OF APPLICANT, ATTORNEY OR AGENT

Firm or Individual name	HELLER EHRMAN LLP 275 Middlefield Road, Menlo Park, California 94025	GINGER R. DREGER (Reg. No. 33,055) Telephone: (650) 324-7000	Facsimile: (650) 324-0638
Signature			
Date	OCTOBER 27, 2005	Customer Number:	25213

CERTIFICATE OF EXPRESS MAILING

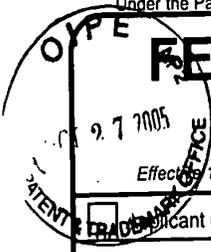
I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated below and addressed to: MAIL STOP PATENT EXT., Commissioner for Patents, PO Box 1450, Alexandria, Virginia 22313-1450, on this date: **OCTOBER 27, 2005**

Express Mail Label **EV 582 623 652 US**

Typed or printed name	C. FONG		
Signature		Date	OCTOBER 27, 2005

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FEE TRANSMITTAL for FY 2005

Effective 10/01/2003. Patent fees are subject to annual revision.

Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ 1,740.00)

Complete if Known

Application Number	08/071,819 (5,681,814)
Filing Date	JUNE 4, 1993 (OCTOBER 28, 1997)
First Named Inventor	ROSS G. CLARK
Examiner Name	HUFF, SHEELA JITENDRA
Art Unit	1642
Attorney Docket No.	39766-0190

METHOD OF PAYMENT (check all that apply)

Check Credit card Money Order Other None

Deposit Account:

Deposit Account Number: **08-1641 (39766-0190)**
 Deposit Account Name: **HELLER EHRMAN LLP**

The Director is authorized to: (check all that apply)

Charge fee(s) indicated below Credit any overpayments

Charge any additional fee(s) or any underpayment of fee(s)

Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity		Small Entity		Fee Description	Fee Paid
Fee Code	Fee (\$)	Fee Code	Fee (\$)		
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for <i>ex parte</i> reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	120	2251	60	Extension for reply within first month	
1252	450	2252	225	Extension for reply within second month	
1253	1,020	2253	510	Extension for reply within third month	
1254	1,590	2254	795	Extension for reply within fourth month	
1255	2,160	2255	1,080	Extension for reply within fifth month	
1401	500	2401	250	Notice of Appeal	
1402	500	2402	250	Filing a brief in support of an appeal	
1403	1,000	2403	500	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	500	2452	250	Petition to revive - unavoidable	
1453	1,500	2453	750	Petition to revive - unintentional	
1501	1,400	2501	700	Utility issue fee (or reissue)	
1502	800	2502	400	Design issue fee	
1503	1,100	2503	550	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	790	2809	395	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	790	2810	395	For each additional invention to be examined (37 CFR 1.129(b))	
1801	790	2801	395	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify)				APPL FOR EXT OF PATEN TERM	1,740.00
*Reduced by Basic Filing Fee Paid				SUBTOTAL (3)	(\$ 1,740.00)

FEE CALCULATION

1. BASIC FILING FEE

Large Entity		Small Entity		Fee Description	Fee Paid
Fee Code	Fee (\$)	Fee Code	Fee (\$)		
1001	300	2001	150	Utility filing fee	
1002	200	2002	100	Design filing fee	
1003	200	2003	100	Plant filing fee	
1004	300	2004	150	Reissue filing fee	
1005	200	2005	100	Provisional filing fee	
SUBTOTAL (1)					(\$)

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims	Fee from below	Fee Paid
Total Claims	<input type="text"/> -20** = <input type="text"/>	X <input type="text"/>	= <input type="text"/>
Independent Claims	<input type="text"/> -3** = <input type="text"/>	X <input type="text"/>	= <input type="text"/>
Multiple Dependent	<input type="text"/>	= <input type="text"/>	= <input type="text"/>

Large Entity		Small Entity		Fee Description	Fee Paid
Fee Code	Fee (\$)	Fee Code	Fee (\$)		
1202	50	2202	25	Claims in excess of 20	
1201	200	2201	100	Independent claims in excess of 3	
1203	360	2203	180	Multiple dependent claim, if not paid	
1204	200	2204	100	** Reissue independent claims over original patent	
1205	50	2205	25	** Reissue claims in excess of 20 and over original patent	
SUBTOTAL (2)					(\$)

**or number previously paid, if greater; For Reissues, see above

SUBMITTED BY				(Complete if applicable)	
Name (Print/Type)	GINGER R. DREGER	Registration No. (Attorney/Agent)	33,055	Telephone	(650) 324-7000
Signature		Date	OCTOBER 27, 2005		

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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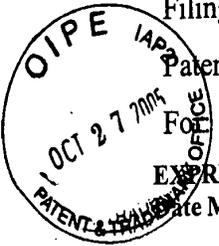
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: CLARK, Ross G., et al. Docket No.: 39766-0190
Serial No.: 08/071,819 Group Art Unit: 1642
Filing Date: June 4, 1993 Examiner: Huff, Sheela Jitendra
Patent No.: U. S. Patent No. 5,681,814 Issued: October 28, 1997

FORMULATED IGF-I COMPOSITION

EXPRESS MAIL LABEL NO. EV 582 623 652 US

Date Mailed: OCTOBER 27, 2005



**APPLICATION FOR EXTENSION OF PATENT TERM BASED ON REGULATORY
REVIEW OF A NEW DRUG APPLICATION PURSUANT TO 35 U.S.C. §156**

MAIL STOP PATENT EXT.

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

The Applicant, Genentech, Inc., of 1 DNA Way, South San Francisco, California 94080, represents that it is the owner of record of the entire right, title and interest in and to U. S. Patent No. 5,681,814 as evidenced by the Assignment recorded on June 13, 1994 under Reel/Frame: 7020/0348. A copy of the recorded Assignment, along with a copy of the Notice of Recordation of Assignment Document is submitted herewith as **Exhibit A**.

Genentech, Inc. hereby applies, pursuant to 35 U.S.C. §156(d)(1) and 37 C.F.R. §1.740, for extension of the term of the above-identified U. S. Patent No. 5,681,814 issued on October 28, 1997, and based on U. S. Application Serial No. 08/071,819 filed on June 4, 1993. U. S. Application Serial No. 08/071,819 is a continuation-in-part application of U.S. Application Serial No. 07/806,748 filed December 13, 1991, now abandoned, which is a divisional application of U.S. Application Serial No. 07/535,005 filed June 7, 1990, now issued as U.S. Patent No. 5,126,324.

U. S. Patent No. 5,681,814 results from an application filed before the date that is 6 months after the date of the enactment of the Uruguay Round Agreements Act (December 8, 1994); accordingly, its date of expiration under 35 U.S.C. §154(c)(1) is October 28, 2014, the greater of the 20-year term from the effective date (June 7, 2010 and June 4, 2013), or 17 years from grant (October 28, 2014).

The patent term extension is requested until September 20, 2017, *i.e.*, for a period of 1,058 days, or such greater or lesser period as the Director may deem Genentech, Inc. to be entitled.

This application is based on the regulatory approval of INCRELEX™ (mecasermin [rDNA origin] injection) to Tercica, Inc. Tercica, Inc. acquired exclusive rights to develop, commercialize and manufacture INCRELEX™ (mecasermin [rDNA origin] injection) from Genentech, Inc. INCRELEX™ (mecasermin [rDNA origin] injection) is an aqueous solution for injection, containing, as the sole active ingredient, human insulin-like growth factor-1 (rhIGF-1) produced by recombinant DNA technology in *E. coli* bacteria, which have been modified by the addition of the gene for human IGF-1. rhIGF-1 consists of 70 amino acids in a single chain with three intramolecular disulfide bridges and a molecular weight of 7649 daltons. The amino acid sequence of rhIGF-1 is identical with that of endogenous human IGF-1, and is shown in the Product Description attached as **Exhibit B**. INCRELEX™ is a sterile aqueous solution intended for subcutaneous injection. Each multi-dose vial of INCRELEX™ contains 10 mg/mL mecasermin, 9 mg/mL benzyl alcohol, 5.84 mg/mL sodium chloride, 2 mg/mL polysorbate 20, and 0.05 M acetate at a pH of approximately 5.4.

The INCRELEX™ (mecasermin [rDNA origin] injection) formulation is covered by U. S. Patent No. 5,681,814.

The date of the New Drug Application (NDA) Approval for INCRELEX™ is August 30, 2005. This is the first permitted commercial marketing or use of this product. This application is accordingly being made within the 60-day statutory period provided in 35 U.S.C. §156(d).

In accordance with 37 C.F.R. §1.740, Genentech, Inc. provides the following information.

(1) *A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics.*

Genentech, Inc. submits herewith as **Exhibit B** to this application the prescribing information for INCRELEX™ (mecasermin [rDNA origin] injection) as approved by the U.S. Food and Drug Administration (FDA). INCRELEX™ is a sterile, aqueous solution of recombinant human insulin-like growth factor (rhIGF-1), produced in *E. coli*. Each multi-dose

vial of INCRELEX™ contains 10 mg/mL mecasecmin, 9 mg/mL benzyl alcohol, 5.84 mg/mL sodium chloride, 2 mg/mL polysorbate 20, and 0.05 M acetate at a pH of approximately 5.4. INCRELEX™, as approved, is indicated for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency (Primary IGFD) or with growth hormone (GH) gene deletion who have developed neutralizing antibodies to GH.

- (2) ***A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred.***

The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act, 21 U.S.C. §355.

- (3) ***An identification of the date on which the product received permission for commercial marketing or use under the provisions of law under which the applicable regulatory review period occurred.***

The approved product received permission for commercial marketing or use under §505(b) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355) on August 30, 2005. A copy of the approval letter received from the FDA is attached as Exhibit C.

- (4) ***An identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.***

The active ingredient in INCRELEX™ is the above-described rhuIGF-1, which has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

- (5) ***A statement that the application is being submitted within the sixty-day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted.***

This application is being submitted on or before October 28, 2005, the last day of the sixty-day period permitted for submission pursuant to 37 C.F.R. §1.720(f), *i.e.*, the last day of the sixty-day period following the August 30, 2005 approval for commercial marketing of

INCRELEX™, that is not a Saturday, Sunday, or Federal holiday, as provided in 35 U.S.C. §156(d)(1); 37 C.F.R. §1.720(f) and 37 C.F.R. §1.7.

- (6) *A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration.*

This application seeks extension for U. S. Patent No. 5,681,814, issued to Ross G. CLARK, Douglas A. YEUNG, and James Q. OESWEIN, on October 28, 1997. The patent will expire on October 28, 2014.

- (7) *A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings.*

A copy of U. S. Patent No. 5,681,814, including claims and drawings, is enclosed as **Exhibit D.**

- (8) *A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent.*

Copies of Maintenance Fee Statements attesting to the payment of the 4th and 8th year annuities are enclosed as **Exhibit E.**

U. S. Patent No. 5,681,814 has not been subject to any disclaimer, certificate of correction, or reexamination.

- (9) *A statement that the patent claims the approved product or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which one such patent claim reads on: (i) The approved product, if the listed claims include any claim to the approved product; (ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product; and (iii) The method of manufacturing the approved product, if the listed claims include any claim to the method of manufacturing the approved product.*

U.S. Patent No. 5,681,814 claims the approved product, *i.e.*, Claims 1, 2, 3, and 4 encompass the approved product.

The showing listing each applicable claim and demonstrating the relationship between one such claim of U. S. Patent No. 5,681,814 and the approved product (INCRELEX™ (mecasermin [rDNA origin] injection)) is as follows:

Claims of U. S. Patent No. 5,681,814	INCRELEX™ (mecasermin [rDNA origin] injection)
<p>Claim 1. An IGF-1-containing composition for subcutaneous administration comprising about 8-12 mg/ml of IGF-1, about 5-6 mg/ml of sodium chloride, a stabilizer consisting of about 8-10 mg/ml of benzyl alcohol or about 2-3 mg/ml of phenol, or both[,] about 8-10 mg/ml of benzyl alcohol and about 2-3 mg/ml of phenol, and an about 50-mM sodium acetate buffered solution at a pH of about 5.4.</p>	<p>INCRELEX™ (mecasermin [rDNA origin] injection) is an IGF-1-containing composition for subcutaneous injection, comprising 10 mg/ml IGF-1 (mecasermin), 5.84 mg/ml of sodium chloride, 9 mg/ml benzyl alcohol, 2 mg/ml polysorbate 20, and 50 mM acetate buffer at a pH of approximately 5.4.</p>
<p>Claim 2. The composition of claim 1 additionally comprising about 1-5 mg/ml of a surfactant.</p>	<p>INCRELEX™ comprises 2 mg/ml polysorbate 20.</p>
<p>Claim 3. The composition of claim 2 wherein the surfactant is polysorbate or poloxamer in an amount of about 1-3 mg/ml.</p>	<p>INCRELEX™ comprises 2 mg/ml polysorbate 20.</p>
<p>Claim 4. The composition of claim 3 wherein the surfactant is polysorbate</p>	<p>INCRELEX™ comprises 2 mg/ml polysorbate 20.</p>

- (10) *A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. 156 (g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period, particularly as follows: (i) For a patent claiming a human drug, antibiotic, or human biological product: (A) The effective date of the investigational new drug (IND) application and the IND number; (B) The date on which a new drug application (NDA) or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and (C) The date on which the NDA was approved or the Product License issued.*

For the New Drug Application (NDA) Approval of INCRELEX™ (mecasermin [rDNA origin] injection) the following dates and related information are applicable:

Effective Date for IND: June 16, 1992

IND Number: 39,679

Initial Submission Date of NDA: February 24, 2005, received by FDA February 28, 2004.

FDA Approval Date for NDA: August 30, 2005

NDA Number: 21-839

- (11) A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities.***

The regulatory review period began on May 13, 1992 with the submission of the IND by Genentech, Inc. Genentech inactivated the IND on March 13, 2000, and transferred it to Tercica, Inc. on April 19, 2002. Tercica, Inc. accepted the transfer and sponsorship of the IND on October 4, 2002, and reactivated the IND on December 5, 2003. During the period beginning May 13, 1992 and ending March 13, 2000, efforts were underway by Genentech, Inc., the original marketing applicant, to proceed to filing a New Drug Application (NDA). During the period beginning October 4, 2002 and continuing through August 30, 2005, efforts were underway by Tercica, Inc., the current marketing applicant, to complete the New Drug Application (NDA), which was filed on February 24, 2005.

During this period, the following significant activities and dates are applicable:

Document/Event	Date
Submit IND Application	May 13, 1992
Receipt of IND Application confirmed, IND #39,679 assigned	May 18, 1992
Submission of Safety Report #068 (see IND #33,487)	May 22, 1992
Telephone conference re clinical hold for IV use of IGF-1	May 28, 1992
Letter forwarding text portion of Phase III Protocol #F0375g on a diskette	June 9, 1992
FDA letter re completion of review of IND #39,679; study may proceed; request for revision of Protocol F0375g	June 16, 1992
Start Efficacy Study # F0375g	August 3, 1992
Telephone conferences with FDA	August 28, 1992 and August 31, 1992
Letter to FDA with proposal to amend Protocol #F0375g	September 25, 2005
Submit revised Protocol #F0375g	October 8, 1992
Letter to FDA re 1992 Annual Report	December 21, 1992
Submission of Protocol Amendment - new investigator	August 13, 1993
Submission of Protocol Amendment - change of protocol	August 26, 1993
Submission of Protocol Amendment - change of protocol	December 1, 1993
Meeting with FDA re status of Study #F0206s	December 22, 1993
Submission of Initial 10-day Alert Report No. 14999	January 28, 1994
Submission of Initial 10-day Alert Report No. 15044	February 3, 1994

Telephone conference re plans to amend Phase III protocols	March 22, 1994
Submission of Annual Report for IND # 39,679	March 29, 1994
Telephone conference with FDA re questions concerning Annual Report submission	April 17, 1994
Telephone conference with FDA to answer questions concerning Annual Report submission	June 3, 1994
preIND meeting	July 12, 1994
Start of Efficacy Study #F0632g	July, 1994
Telephone conference with FDA re long term toxicology study protocol	August 30, 1994
Submission of Change in Protocol #F0375g and New Protocol #F0632g	December 1, 1994
Submission of Annual Report for IND #39,679	January 9, 1995
Submission of New Protocol #F0671g	July 18, 1995
Submission of Annual Report for IND # 39,679	August 16, 1995
Submission of Request for Orphan Drug Designation	October 31, 1995
Letter acknowledging receipt of Request for Orphan Drug Designation	November 3, 1995
Start of Efficacy Study # F0671g	November 6, 1995
Submission of Protocol Amendment - new investigator	November 8, 1995
Grant of Orphan Drug Designation	December 12, 1995
Submission of Protocol Amendment - new investigators	December 18, 1995
Complete Efficacy Study #F0632g	February, 1996
Complete Efficacy Study #F0375g	March 12, 1996
Submission of Changes in Chemistry, Manufacturing, and Controls (IND #33,487)	September 11, 1996
Submission of Annual Report (IND #39,679)	September 30, 1996
Submission of Revised Investigator Brochure (IND #33,487)	February 19, 1997
Submission of Pharmacology/Toxicology Amendment (IND #33,487)	July 23, 1997
Submission of Annual Report (IND #39,679)	August 11, 1997
FDA Request for Annual Report re Application # 95-936	November 3, 1997
Submission of Annual Report re IND # 39,679	February 20, 1998
Change of Designated Officials re IND #33,487	April 2, 1998
Submission of Amendment to IND #46,212	April 23, 1998
Submission of IND Safety Report (IND #39,679)	May 22, 1998
Follow up on IND Safety Report (IND #39,679)	June 29, 1998
Completion of Efficacy Study	June 29, 1998
Submission of Annual Report (IND # 39,679)	September 11, 1998
Submission of Annual Report (IND # 39,679)	May 25, 1999

Submission of Final Reports for Clinical Studies F0375g, F0632g, and F0671g	February 22, 2000
Request to inactivate IND #39,679	March 13, 2000
FDA Confirmation that IND #39,679 is considered inactive	March 17, 2000
Transfer of IND #39,679 from Genentech, Inc. to Tercica, Inc.	April 19, 2002
Acceptance of Transfer and Sponsorship of IND #39,679 by Tercica, Inc.	October 4, 2002
Tercica's Request for Type C meeting re IND #39,679	October 18, 2002
Submission of request for reinstatement of IND #39,679	October 28, 2002
Acknowledgement of change of sponsorship of IND #39,679	November 20, 2002
Tercica's Request for Type C Meeting re IND #39,679	December 18, 2002
Submission of Information Package for Type C Meeting	January 23, 2003
Tercica-FDA Meeting	March 5, 2003
Tercica's submission of Characterization Plan	November 7, 2003
Tercica's submission of two new protocols and a general investigation plan to reinstate IND #39,679; notice of intent to reactivate IND #39,679	December 5, 2003
Notice of intent to reactivate IND #39,679 received by FDA	December 8, 2003
Tercica's letter to FDA concerning the extension of expiration dating for rhIGF-1	December 23, 2003
FDA letter confirming that IND is now considered to be on active status	January 7, 2004
Extension of expiration dating orally granted	January 21, 2004
FDA response to Tercica's questions for Protocol MS301.	February 3, 2004
Start of PK Study MS302	February 11, 2004
Completion of PK Study MS302	February 29, 2004
Tercica's request for Type B pre-NDA Meeting	March 29, 2004
Submission of Amendment to Protocol MS301	May 6, 2004
Pre-NDA Meeting	May 27, 2004
Submission of Proposed Proprietary Name for Review	June 8, 2004
Submission of Protocol Amendment - New Protocol MS302a	June 11, 2004
Submission of newly revised Investigator Brochure for rhIGF-1	June 16, 2004
Response to FDA Request for Information re Proposed Proprietary Name for Review	July 7, 2004
Submission of Amendment #2 to Protocol MS301	July 16, 2004
Submission of Amendment #1 to Protocol MS302a	August 6, 2004

Submission of Dataset Proposal for NDA #39,679	August 9, 2004
Submission of Chemistry, Manufacturing, and Controls Information	August 19, 2004
Submission of New Protocol MS308	September 1, 2004
Submission of Amendment #2 to Proccotol MS302a	September 10, 2004
Submission of further details re NDA Dataset Proposal	September 16, 2004
FDA request to present safety and efficacy data in DNA Dataset Proposal in separate folders	September 27, 2004
Amendment #1 to Protocol MS308	November 29, 2004
Submission of Request for Type C Meeting	December 2, 2004
Submission of Amendment # 3 to Protocol MS301	December 7, 2004
Response to Agency request for information re Proposed Proprietary Name Review	January 11, 2005
Type C Meeting	January 24, 2005
Submission of Annual Report	January 27, 2005
Submission of Amendment #2 to Protocol MS301	May 24, 2005
Submission of NDA	February 24, 2005
Submission of NDA received	February 28, 2005
Agency Approval of Proprietary Name INCRELEX	April 13, 2005
Submission of information in follow up to Type C Meeting	May 9, 2005
Tercica-FDA Meeting at OOPD	June 16, 2005
Submission of slides presented at June 16, 2005 meeting to participants	June 24, 2005
NDA Approved	August 30, 2005

- (12) *A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of extension claimed, including how the length of extension was determined.*

Genentech, Inc. believes that it is entitled to an extension of term for U. S. Patent No. 5,681,814 (the Patent) in accordance with the provisions of 35 U.S.C. §156. Genentech, Inc. believes that the period of extension applicable to the patent is **1,058 days**, based on the following chronology. In accordance with 37 C.F.R. §1.775 the extension was calculated as follows:

1.	Number of days for the testing phase as defined in 37 CFR 1.775(c)(1)	4,636	
2.	Number of days for the approval phase as defined in 37 CFR 1.775(c)(2)	187	
3.	Sum of lines 1 and 2		4,823
4.	Number of days of the period in line 2 which occurred prior to the issue date of the patent	0	
5.	Number of days of the period in line 2 during which the applicant failed to act with due diligence as defined in 37 CFR 1.775(d)(1)(ii)	0	
6.	Sum of line 4 and line 5		0
7.	Subtract line 6 from line 3		4,823
8.	Number of days of the period of line 1 which occurred prior to the issue date of the patent	1,960	
9.	Number of days of the period of line 1 during which the applicant failed to act with due diligence as defined in 37 CFR 1.775(d)(1)(ii)	935	
10.	Sum of lines 8 and 9		2,895
11.	Subtract line 10 from line 7		1,928
12.	Number of days from line 1	4,636	
13.	Number of days from line 10	2,895	
14.	Subtract line 13 from line 12	1,741	
15.	One-half of line 14		870
16.	Subtract line 15 from line 11		1,058
17.	Original expiration date of patent	10/28/2014	
18.	Expiration date of patent if extended by the number of days on line 16	9/20/2017	
19.	Date of FDA final approval	8/30/2005	
20.	Limitation set forth in 37 CFR 1.775(d)(3)	14 years	
21.	Add number of years on line 20 to the date on line 19	8/30/2019	
22.	Earlier date appearing on line 18 or line 21		9/20/2017
23.	Original expiration date of patent	10/28/2014	
24.	The patent issued after 9/24/1984	5 years	
25.	Add number of years on line 24 to the date on line 23	10/28/2019	
26.	The earlier date appearing on line 22 or line 25		9/20/2017
27.	Original expiration date of patent		10/28/2014
28.	Length of patent term extension		1,058 days

- (13) *A statement that applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought (37 C.F.R. §1.765).*

Genentech, Inc. acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office (and to the United States Patent and Trademark Office), and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

- (14) *The prescribed fee for receiving and acting upon the application for extension (37 C.F.R. §1.20 (j)).*

Genentech, Inc. hereby requests that the Commissioner charge the amount of \$1,740.00 as the prescribed fee under 37 C.F.R. §1.20(j) to Deposit Account No. 08-1641 (referencing Attorney's Docket No. 39766-0190). If for any reason this payment is insufficient, Applicant hereby authorizes that any deficiency may be charged, or any overpayment credited, to Deposit Account No. 08-1641 (referencing Attorney's Docket No. 39766-0190).

- (15) *The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed*

Please direct all correspondence relating to this application to:

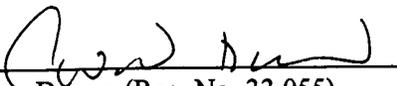
Ginger R. Dreger
Registration No. 33,055
Attorney of Record
Heller Ehrman, LLP
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7115
Facsimile: (650) 324-0638
E-mail: gdreger@hellerehrman.com

This application is accompanied by two additional copies thereof (for a total of three copies), under section 37 C.F.R. §1.740(b).

Genentech is providing herewith, in **Exhibit F**, a Power of Attorney and General Authority for the undersigned to execute this application.

Respectfully submitted,

Date: October 27, 2005

By: 
Ginger Dreger (Reg. No. 33,055)

HELLER EHRMAN, LLP
Customer No. 25213
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7000
Facsimile: (650) 324-0638

SV 2163809 v1

10/27/05 11:12 AM (39766.0190)

Exhibit A



UNITED STATES DEPARTMENT OF COMMERCE
 Patent and Trademark Office
 ASSISTANT SECRETARY AND COMMISSIONER
 OF PATENTS AND TRADEMARKS
 Washington, D.C. 20231

DATE: 07/11/94
 TO:

N08B

JANET E. HASAK
 MAIL STOP 49
 460 POINT SAN BRUNO BOULEVARD
 SO. SAN FRANCISCO, CALIFORNIA 94080

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GENENTECH, INC. LEGAL DEPT.

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NOV 03 2005

OFFICE OF PETITIONS

UNITED STATES PATENT AND TRADEMARK OFFICE
 NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT BRANCH OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE U.S. PATENT AND TRADEMARK OFFICE ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT ASSIGNMENT PROCESSING SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT BRANCH, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231

ASSIGNOR: CLARK, ROSS G. DOC DATE: 06/06/94

ASSIGNOR: CRONIN, MICHAEL J. DOC DATE: 06/06/94

ASSIGNOR: YEUNG, DOUGLAS A. DOC DATE: 06/07/94

ASSIGNOR: OESWEIN, JAMES Q. DOC DATE: 06/07/94

RECORDATION DATE: 06/13/94 NUMBER OF PAGES 003 REEL/FRAME 7020/0348

DIGEST : ASSIGNMENT OF ASSIGNORS INTEREST

ASSIGNEE:
 GENENTECH, INC.
 MAIL STOP #49
 460 POINT SAN BRUNO BOULEVARD
 SO. SAN FRANCISCO, CALIFORNIA 94080

SERIAL NUMBER 8-071819 FILING DATE 06/04/93
 PATENT NUMBER ISSUE DATE 00/00/00

[Handwritten Signature]
 EXAMINER/PARALEGAL

ASSIGNMENT BRANCH
 ASSIGNMENT/CERTIFICATION SERVICES DIVISION

ASSIGNMENT

WHEREAS, Ross G. Clark, a citizen of New Zealand, residing at 711 Ursula Avenue, Pacifica, California 94044, Michael J. Cronin, a citizen of United States of America, residing at 338 Portola Drive, San Mateo, California 94403, Douglas A. Yeung, a citizen of United States of America, residing at 2628 Benchmark Avenue, Fremont, California 94536, and James Q. Oeswein, a citizen of the United States of America, residing at 123 Madrona Avenue, El Granada, California 94018, have invented a new and useful invention in

FORMULATED IGF-I COMPOSITION

for which an application Serial No. 08/071,819 (Docket No. 646D1P2) for Letters Patent has been filed by them on 4 June 1993; and

WHEREAS, GENENTECH, INC., a corporation organized and existing under and by virtue of the laws of the State of Delaware, having a place of business at 460 Point San Bruno Boulevard, South San Francisco, California 94080, is desirous of acquiring an interest in and to said invention, and in and to the Letters Patents to be obtained therefor;

NOW, THEREFORE, be it known by all whom it may concern;

That for good and valuable consideration the receipt of which is hereby acknowledged, the said Ross G. Clark, Michael J. Cronin, Douglas A. Yeung and James Q. Oeswein have and do hereby sell, assign, transfer and set over unto the said GENENTECH, INC., its successors and assigns, the full and exclusive right, title and interest including all rights under the Paris Convention for the Protection of Industrial Property, in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor, not only for, to, and in the United States of America, its territories and possessions, but for, to and in all other countries; and it has been and is hereby authorized and requested that the appropriate government agencies issue said Letters Patents to said GENENTECH, INC., in accordance with this Assignment.

Said Ross G. Clark, Michael J. Cronin, Douglas A. Yeung and James Q. Oeswein covenant and agree to cooperate with GENENTECH, INC., to enable said GENENTECH, INC. to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said Ross G. Clark, Michael J. Cronin, Douglas A. Yeung and James Q. Oeswein includes prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said GENENTECH, INC., (a) for perfecting the right, title and interest herein conveyed (b) for prosecuting any of said applications; (c) for filing and prosecuting applications for reissuance of any of said patents; (e) for interference or other priority proceedings involving said invention; and (f) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said Ross G. Clark, Michael J. Cronin, Douglas A. Yeung and James Q. Oeswein in providing such cooperation shall be paid for by said GENENTECH, INC.

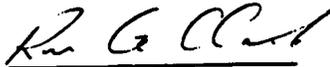
The terms and covenants of this assignment shall inure to the benefit of said GENENTECH, INC., its successors, assigns and other legal representatives, and shall be binding upon said Ross G.

Clark, Michael J. Cronin, Douglas A. Yeung, and James Q. Oeswein, their respective heirs, legal representatives and assigns.

Said Ross G. Clark, Michael J. Cronin, Douglas A. Yeung and James Q. Oeswein hereby warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF we undersign as follows;

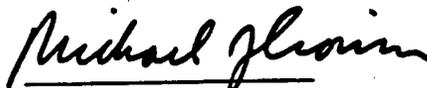
South San Francisco



Ross G. Clark

Dated: 6.6.94

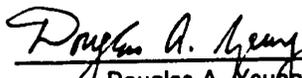
South San Francisco



Michael J. Cronin

Dated: 6-6-94

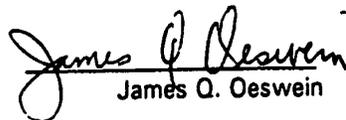
South San Francisco



Douglas A. Yeung

Dated: 6-7-94

South San Francisco



James Q. Oeswein

Dated: 6/7/94

REF 1020 FRAMES 50

JUN 13 94

Page 2

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Exhibit B

benzyl alcohol, 5.84 mg/mL sodium chloride, 2 mg/mL polysorbate 20, and 0.05M acetate at a pH of approximately 5.4.

CLINICAL PHARMACOLOGY

General

Insulin-like growth factor-1 (IGF-1) is the principal hormonal mediator of statural growth. Under normal circumstances, growth hormone (GH) binds to its receptor in the liver, and other tissues, and stimulates the synthesis/secretion of IGF-1. In target tissues, the Type 1 IGF-1 receptor, which is homologous to the insulin receptor, is activated by IGF-1, leading to intracellular signaling which stimulates multiple processes leading to statural growth. The metabolic actions of IGF-1 are in part directed at stimulating the uptake of glucose, fatty acids, and amino acids so that metabolism supports growing tissues.

The following actions have been demonstrated for endogenous human IGF-1:

Tissue Growth – 1) Skeletal growth occurs at the cartilage growth plates of the epiphyses of bones where stem cells divide to produce new cartilage cells or chondrocytes. The growth of chondrocytes is under the control of IGF-1 and GH. The chondrocytes become calcified so that new bone is formed allowing the length of the bones to increase. This results in skeletal growth until the cartilage growth plates fuse at the end of puberty. 2) Cell growth: IGF-1 receptors are present on most types of cells and tissues. IGF-1 has mitogenic activities that lead to an increased number of cells in the body. 3) Organ growth: Treatment of IGF-1 deficient rats with rhIGF-1 results in whole body and organ growth.

Carbohydrate Metabolism – IGF-1 suppresses hepatic glucose production and stimulates peripheral glucose utilization and therefore has a hypoglycemic potential. IGF-1 has inhibitory effects on insulin secretion.

Pharmacokinetics

Absorption – While the bioavailability of rhIGF-1 after subcutaneous administration in healthy subjects has been reported to be close to 100%, the absolute bioavailability of INCRELEX™

given subcutaneously to subjects with primary insulin-like growth factor-1 deficiency (Primary IGFD) has not been determined.

Distribution – In blood, IGF-1 is bound to six IGF binding proteins, with > 80% bound as a complex with IGFBP-3 and an acid-labile subunit. IGFBP-3 is greatly reduced in subjects with severe Primary IGFD, resulting in increased clearance of IGF-1 in these subjects relative to healthy subjects. The total IGF-1 volume of distribution after subcutaneous administration in subjects with severe Primary IGFD is estimated to be 0.257 (\pm 0.073) L/kg at an INCRELEX™ dose of 0.045 mg/kg, and is estimated to increase as the dose of INCRELEX™ increases.

Metabolism – Both the liver and the kidney have been shown to metabolize IGF-1.

Excretion – The mean terminal $t_{1/2}$ after single subcutaneous administration of 0.12 mg/kg INCRELEX™ in pediatric subjects with severe Primary IGFD is estimated to be 5.8 hours. Clearance of INCRELEX™ is inversely proportional to IGF binding protein-3 (IGFBP-3) levels and CL/F is estimated to be 0.04 L/hr/kg at 3 mcg/mL IGFBP-3.

**Summary of INCRELEX™ Single-Dose Pharmacokinetic
Parameters in Children with Severe Primary IGFD
(0.12 mg/kg, SC)**

	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-8} (hr*ng/mL)	$t_{1/2}$ (hr)	Vd/F (L/kg)	CL/F (L/hr/kg)
n	3	3	3	3	12 ^a	12 ^a
Mean	234	2	2932	5.8	0.257	0.0424
CV%	23	0	50	64	28	38

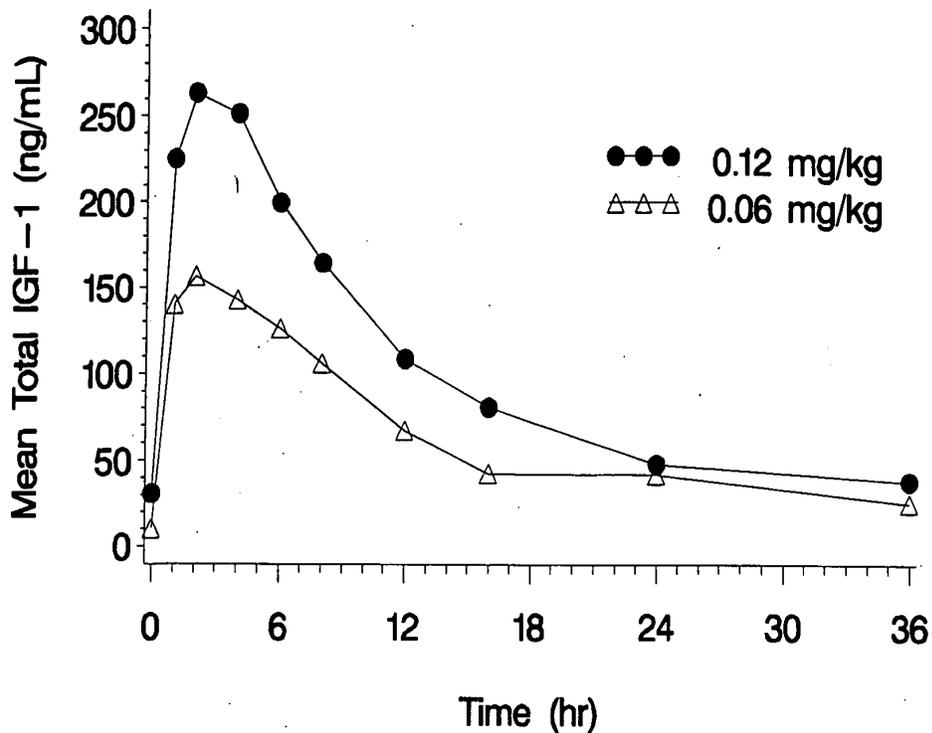
C_{max} = maximum concentration; T_{max} = time of maximum concentration;
 AUC_{0-8} = area under the curve; $t_{1/2}$ = half-life; Vd/F = volume of
distribution; CL/F = systemic clearance; SC = subcutaneous injection;
CV% = coefficient of variation in %.

Male/female data combined, ages 12 to 22 years.

^a Data represents 3 subjects each at doses 0.015, 0.03, 0.06, and
0.12 mg/kg SC.

PK parameters based on baseline adjusted plasma concentrations.

Mean Total IGF-1 Concentration after a Single Subcutaneous Dose of INCRELEX™ in Children with Severe Primary IGFD (0.06 mg/kg and 0.12 mg/kg, n = 3 per group)



Special Populations

Geriatric – The pharmacokinetics of INCRELEX™ have not been studied in subjects greater than 65 years of age.

Gender – In children with Primary IGFD and in healthy adults there were no apparent differences between males and females in the pharmacokinetics of INCRELEX™.

Race – No information is available.

Renal insufficiency – No studies have been conducted in Primary IGFD children with renal impairment.

Hepatic insufficiency – No studies have been conducted to determine the effect of hepatic impairment on the pharmacokinetics of rhIGF-1.

CLINICAL TRIALS

Effects of INCRELEX™ Treatment in Children with Severe Primary Insulin-like Growth Factor-1 Deficiency (Primary IGFD)

Five clinical studies (four open-label and one double-blind, placebo-controlled), with subcutaneous (SC) doses of INCRELEX™ generally ranging from 0.06 to 0.12 mg/kg (60 to 120 µg/kg) administered twice daily (BID), were conducted in 71 pediatric subjects with severe Primary IGFD. Patients were enrolled in the trials on the basis of extreme short stature, slow growth rates, low IGF-1 serum concentrations, and normal growth hormone secretion. Data from these 5 clinical studies were pooled for a global efficacy and safety analysis. Baseline characteristics for the patients evaluated in the primary and secondary efficacy analyses were (mean, SD): chronological age (years): 6.7 ± 3.8 ; height (cm): 84.8 ± 15.3 cm; height standard deviation score (SDS): -6.7 ± 1.8 ; height velocity (cm/yr): 2.8 ± 1.8 ; height velocity SDS: -3.3 ± 1.7 ; IGF-1 (ng/mL): 21.6 ± 20.6 ; IGF-1 SDS: -4.3 ± 1.6 ; and bone age (years): 4.2 ± 2.8 . Sixty-one subjects had at least one year of treatment. Fifty-three (87%) had Laron Syndrome; 7 (11%) had GH gene deletion, and 1 (2%) had neutralizing antibodies to GH. Thirty-seven (61%) of the subjects were male; forty-eight (79%) were Caucasian. Fifty-six (92%) of the subjects were pre-pubertal at baseline.

Annual results for height velocity, height velocity SDS, and height SDS are shown in Table 1. Pre-treatment height velocity data were available for 58 subjects. The height velocities at a given year of treatment were compared by paired t-tests to the pre-treatment height velocities of the same subjects completing that treatment year.

Table 1: Annual Height Results by Number of Years Treated with INCRELEX™

	Pre-Tx	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
Height Velocity (cm/yr)									
N	58	58	48	38	23	21	20	16	13
Mean (SD)	2.8 (1.8)	8.0 (2.2)	5.8 (1.5)	5.5 (1.8)	4.7 (1.6)	4.7 (1.6)	4.8 (1.5)	4.6 (1.5)	4.3 (1.1)
Mean (SD) for change from pre-Tx		+5.2 (2.6)	+2.9 (2.4)	+2.3 (2.4)	+1.5 (2.2)	+1.5 (1.8)	+1.5 (1.7)	+1.0 (2.1)	+0.7 (2.5)
P-value for change from pre-Tx [1]		<0.0001	<0.0001	<0.0001	0.0045	0.0015	0.0009	0.0897	0.3059
Height Velocity/SDS									
N	58	58	47	37	22	19	18	15	11
Mean (SD)	-3.3 (1.7)	1.9 (3.0)	-0.2 (1.6)	-0.2 (2.0)	-0.7 (2.1)	-0.6 (2.1)	-0.4 (1.4)	-0.4 (1.9)	-0.4 (1.9)
Mean (SD) for change from pre-Tx		+5.2 (3.1)	+3.1 (2.3)	+2.9 (2.3)	+2.2 (2.2)	+2.5 (2.2)	+2.7 (1.7)	+2.5 (2.1)	+2.7 (2.8)
Height SDS									
N	61	61	51	40	24	21	20	16	13
Mean (SD)	-6.7 (1.8)	-5.9 (1.8)	-5.6 (1.8)	-5.4 (1.8)	-5.5 (1.9)	-5.6 (1.8)	-5.4 (1.8)	-5.2 (2.0)	-5.2 (2.0)
Mean (SD) for change from pre-Tx		+0.8 (0.5)	+1.2 (0.8)	+1.4 (1.1)	+1.3 (1.2)	+1.4 (1.3)	+1.4 (1.2)	+1.4 (1.1)	+1.5 (1.1)

Pre-Tx = Pre-treatment; SD = Standard Deviation; SDS = Standard Deviation Score

[1] P-values for comparison versus pre-Tx values are computed using paired t-tests.

Forty-nine subjects were included in an analysis of the effects of INCRELEX™ on bone age advancement. The mean ± SD change in chronological age was 4.9 ± 3.4 years and the mean ± SD change in bone age was 5.3 ± 3.4 years.

INDICATIONS AND USAGE

INCRELEX™ (mecasermin [rDNA origin] injection) is indicated for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency (Primary IGFD) or with growth hormone (GH) gene deletion who have developed neutralizing antibodies to GH. Severe Primary IGFD is defined by:

- height standard deviation score ≤ -3.0 and
- basal IGF-1 standard deviation score ≤ -3.0 and

- normal or elevated growth hormone (GH).

Severe Primary IGFD includes patients with mutations in the GH receptor (GHR), post-GHR signaling pathway, and IGF-1 gene defects; they are not GH deficient, and therefore, they cannot be expected to respond adequately to exogenous GH treatment.

INCRELEX™ is not intended for use in subjects with secondary forms of IGF-1 deficiency, such as GH deficiency, malnutrition, hypothyroidism, or chronic treatment with pharmacologic doses of anti-inflammatory steroids. Thyroid and nutritional deficiencies should be corrected before initiating INCRELEX™ treatment.

INCRELEX™ is not a substitute for GH treatment.

CONTRAINDICATIONS

INCRELEX™ should not be used for growth promotion in patients with closed epiphyses.

INCRELEX™ is contraindicated in the presence of active or suspected neoplasia, and therapy should be discontinued if evidence of neoplasia develops.

Intravenous administration of INCRELEX™ is contraindicated.

INCRELEX™ should not be used by patients who are allergic to mecasermin (IGF-1) or any of the inactive ingredients in INCRELEX™.

WARNINGS

INCRELEX contains benzyl alcohol as a preservative. Benzyl alcohol as a preservative has been associated with neurologic toxicity in neonates.

If sensitivity to INCRELEX™ occurs, treatment should be discontinued.

PRECAUTIONS

General. Treatment with INCRELEX™ should be directed by physicians who are experienced in the diagnosis and management of patients with growth disorders.

INCRELEX™ has not been studied in children less than 2 years of age or in adults.

INCRELEX™ should be administered shortly before or after a meal or snack, because it has insulin-like hypoglycemic effects. Special attention should be paid to small children because their oral intake may not be consistent. Patients should avoid engaging in any high-risk activities (e.g., driving, etc.) within 2-3 hours after dosing, particularly at the initiation of INCRELEX™ treatment, until a well-tolerated dose of INCRELEX™ has been established.

Lymphoid tissue (e.g., tonsillar) hypertrophy associated with complications, such as snoring, sleep apnea, and chronic middle-ear effusions have been reported with the use of INCRELEX™. Patients should have periodic examinations to rule out such potential complications and receive appropriate treatment if necessary.

Intracranial hypertension (IH) with papilledema, visual changes, headache, nausea and/or vomiting have been reported in patients treated with INCRELEX™, as they have been reported with therapeutic growth hormone administration. IH-associated signs and symptoms resolved after interruption of dosing. Funduscopic examination is recommended at the initiation and periodically during the course of INCRELEX™ therapy.

Slipped capital femoral epiphysis and progression of scoliosis can occur in patients who experience rapid growth. These conditions and other symptoms and signs known to be associated with GH treatment in general should be monitored during INCRELEX™ treatment.

As with any exogenous protein administration, local or systemic allergic reactions may occur. Parents and patients should be informed that such reactions are possible and that if an allergic reaction occurs, treatment should be interrupted and prompt medical attention should be sought.

Geriatric Use. The safety and effectiveness of INCRELEX™ in patients aged 65 and over has not been evaluated in clinical studies.

Carcinogenesis, mutagenesis, impairment of fertility. INCRELEX™ was administered subcutaneously to Sprague Dawley rats at doses of 0, 0.25, 1, 4, and 10 mg/kg/day for up to

2 years. An increased incidence of adrenal medullary hyperplasia and pheochromocytoma was observed in male rats at doses of 1 mg/kg/day and above (≥ 1 times the clinical exposure with the maximum recommended human dose [MRHD] based on AUC) and female rats at all dose levels (≥ 0.3 times the clinical exposure with the MRHD based on AUC). An increased incidence of keratoacanthoma in the skin was observed in male rats at doses of 4 and 10 mg/kg/day (≥ 4 times the MRHD) and in female rats treated with 10 mg/kg/day (7 times the MRHD based on AUC). An increased incidence of mammary gland carcinoma in both male and female rats was observed in animals treated with 10 mg/kg/day (7 times the MRHD based on AUC). Based on excess mortality secondary to IGF-1 induced hypoglycemia, these skin and mammary tumor findings were only observed at doses that exceeded the maximum tolerated dose (MTD).

Mutagenesis: INCRELEX™ was not clastogenic in the in vitro chromosome aberration assay and the in vivo mouse micronucleus assay.

Impairment of fertility: INCRELEX™ was administered intravenously to rats at doses of 0.25, 1, and 4 mg/day to conduct the fertility study. No effects on fertility were observed in male or female rats treated with doses up to 4 mg/kg/day (4 times the clinical exposure with the MRHD based on AUC.)

Pregnancy Category C. Embryo-fetal toxicity studies were conducted in Sprague Dawley rats with doses of 1, 4, and 16 mg/kg/day, and in New Zealand White rabbits with doses of 0.125, 0.5, and 2 mg/kg/day administered intravenously. No embryo-fetal developmental abnormalities were observed in rats with doses up to 16 mg/kg/day (20 times the MRHD based on body surface area [BSA] comparison). In the rabbit study, the NOAEL for maternal toxicity was 2 mg/kg (8 times the MRHD based on BSA) and the NOAEL for fetal toxicity was 0.5 mg/kg (2 times the MRHD based on BSA). INCRELEX™ displayed no teratogenicity at doses up to 2 mg/kg (8 times the MRHD based on BSA).

The effects of INCRELEX™ on an unborn child have not been studied. Therefore, there is insufficient medical information to determine whether there are significant risks to a fetus.

Nursing Mothers. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when INCRELEX™ is administered to a nursing woman.

Information for Patients. Patients and/or their parents should be instructed in the safe administration of INCRELEX™. INCRELEX™ should be given shortly before or after (20 minutes on either side of) a meal or snack. **INCRELEX™ should not be administered when the meal or snack is omitted.** The dose of INCRELEX™ should never be increased to make up for one or more omitted doses. INCRELEX™ therapy should be initiated at a low dose and the dose should be increased only if no hypoglycemia episodes have occurred after at least 7 days of dosing. If severe hypoglycemia or persistent hypoglycemia occurs on treatment despite adequate food intake, INCRELEX™ dose reduction should be considered. Providers should educate patients and caregivers on how to recognize the signs and symptoms of hypoglycemia.

Patients and/or parents should be thoroughly instructed in the importance of proper needle disposal. A puncture-resistant container should be used for the disposal of used needles and/or syringes (consistent with applicable state requirements). Needles and syringes must not be reused.

ADVERSE REACTIONS

As with all protein pharmaceuticals, some patients may develop antibodies to INCRELEX™. Anti-IGF-1 antibodies were present at one or more of the periodic assessments in 14 of 23 children with Primary IGFD treated for 2 years. However, no clinical consequences of these antibodies were observed (e.g., allergic reactions or attenuation of growth).

In clinical studies of 71 subjects with Primary IGFD treated for a mean duration of 3.9 years and representing 274 subject-years, no subjects withdrew from any clinical study because of adverse events. Adverse events considered related to INCRELEX™ treatment that occurred in 5% or more of these study participants are listed below by organ class.

Metabolism and Nutrition Disorders: hypoglycemia

General Disorders and Administrative Site Conditions: lipohypertrophy, bruising

Infections and Infestations: otitis media, serous otitis media

Respiratory, Thoracic and Mediastinal Disorders: snoring, tonsillar hypertrophy

Nervous System Disorders: headache, dizziness, convulsions

Gastrointestinal Disorders: vomiting

Ear and Labyrinth Disorders: hypoacusis, fluid in middle ear, ear pain, abnormal tympanometry

Cardiac Disorders: cardiac murmur

Musculoskeletal and Connective Tissue Disorders: arthralgia, pain in extremity

Blood and Lymphatic System Disorders: thymus hypertrophy

Surgical and Medical Procedures: ear tube insertion

Hypoglycemia was reported by 30 subjects (42%) at least once during their course of therapy. Most cases of hypoglycemia were mild or moderate in severity. Five subjects had severe hypoglycemia (requiring assistance and treatment) on one or more occasion and 4 subjects experienced hypoglycemic seizures/loss of consciousness on one or more occasion. Of the 30 subjects reporting hypoglycemia, 14 (47%) had a history of hypoglycemia prior to treatment. The frequency of hypoglycemia was highest in the first month of treatment, and episodes were more frequent in younger children. Symptomatic hypoglycemia was generally avoided when a meal or snack was consumed either shortly (i.e., 20 minutes) before or after the administration of INCRELEX™.

Tonsillar hypertrophy was noted in 11 (15%) subjects in the first 1 to 2 years of therapy with lesser tonsillar growth in subsequent years. Tonsillectomy or tonsillectomy/adenoidectomy was performed in 7 subjects; 3 of these had obstructive sleep apnea, which resolved after the procedure in all three cases.

Intracranial hypertension occurred in three subjects. In two subjects the events resolved without interruption of INCRELEX™ treatment. INCRELEX™ treatment was discontinued in the third subject and resumed later at a lower dose without recurrence.

Mild elevations in the serum AST and LDH were found in a significant proportion of patients before and during treatment and no rise in levels of these serum enzymes led to treatment discontinuation. ALT elevations were occasionally noted during treatment. Renal and splenic lengths (measured by ultrasound) increased rapidly on INCRELEX™ treatment during the first years of therapy. This lengthening slowed down subsequently; though in some patients, renal and/or splenic length reached or surpassed the 95th percentile. Renal function (as defined by serum creatinine and calculated creatinine clearance) was normal in all patients, irrespective of renal growth. Elevations in cholesterol and triglycerides to above the upper limit of normal were observed before and during treatment. Echocardiographic evidence of cardiomegaly/valvulopathy was observed in a few individuals without associated clinical symptoms. Because of underlying disease and the lack of control group, the relation of the cardiac changes to drug treatment cannot be assessed.

Thickening of the soft tissues of the face was observed in several patients and should be monitored during INCRELEX™ treatment.

OVERDOSAGE

There is no clinical experience with overdosage of INCRELEX™. Based on known pharmacological effects, acute overdosage would be predicted to lead to hypoglycemia. Long-term overdosage may result in signs and symptoms of acromegaly. Treatment of acute overdose of INCRELEX™ should be directed at reversing hypoglycemia. Oral glucose or food should be consumed. If the overdose results in loss of consciousness, intravenous glucose or parenteral glucagon may be required to reverse the hypoglycemic effects.

DOSAGE AND ADMINISTRATION

Preprandial glucose monitoring should be considered at treatment initiation and until a well tolerated dose is established. If frequent symptoms of hypoglycemia or severe hypoglycemia occur, preprandial glucose monitoring should continue. The dosage of INCRELEX™ should be individualized for each patient. The recommended starting dose of INCRELEX™ is 0.04 to 0.08 mg/kg (40 to 80 µg/kg) twice daily by subcutaneous injection. If well-tolerated for at least

one week, the dose may be increased by 0.04 mg/kg per dose, to the maximum dose of 0.12 mg/kg given twice daily. Doses greater than 0.12 mg/kg given twice daily have not been evaluated in children with Primary IGFD and, due to potential hypoglycemic effects, should not be used. If hypoglycemia occurs with recommended doses, despite adequate food intake, the dose should be reduced. INCRELEX™ should be administered shortly before or after (\pm 20 minutes) a meal or snack. If the patient is unable to eat shortly before or after a dose for any reason, that dose of INCRELEX™ should be withheld. Subsequent doses of INCRELEX™ should never be increased to make up for one or more omitted dose.

INCRELEX™ injection sites should be rotated to a different site with each injection.

INCRELEX™ should be administered using sterile disposable syringes and needles. The syringes should be of small enough volume that the prescribed dose can be withdrawn from the vial with reasonable accuracy.

STABILITY AND STORAGE

Before Opening - Vials of INCRELEX™ are stable when refrigerated [2° to 8°C (35° to 46°F)]. Avoid freezing the vials of INCRELEX™. Protect from direct light. Expiration dates are stated on the labels.

After Opening – Vials of INCRELEX™ are stable for 30 days after initial vial entry when stored at 2° to 8°C (35° to 46°F). Avoid freezing the vials of INCRELEX™. Protect from direct light.

Vial contents should be clear without particulate matter. If the solution is cloudy or contains particulate matter, the contents must not be injected. INCRELEX™ should not be used after its expiration date. Keep refrigerated and use within 30 days of initial vial entry. Remaining unused material should be discarded.

HOW SUPPLIED

INCRELEX™ is supplied as a 10 mg/mL sterile solution in multiple dose glass vials (40 mg/vial).

NDC-15054-1040-5

Rx only

Manufactured for: Tercica, Inc.
Brisbane, CA 94005 USA

by: Baxter Pharmaceutical Solutions LLC
Bloomington, IN 47402 USA 7/05

Issued: August 2005

3-1015-267

Patient Information**INCRELEX™ (EENK-ruh-lex)
(mecasermin [rDNA origin] injection)**

Read the Patient Information that comes with INCRELEX™ before your child starts taking INCRELEX™ and each time you get a refill. There may be new information. This leaflet does not take the place of talking with your child's doctor about your child's condition or treatment.

What is INCRELEX™?

INCRELEX™ is a liquid that contains man-made insulin-like growth factor-1 (IGF-1), which is the same as the IGF-1 made by your body. INCRELEX™ is used to treat children who are very short for their age because their bodies do not make enough IGF-1. This condition is called primary IGF-1 deficiency. IGF-1 should not be used instead of growth hormone.

INCRELEX™ has not been studied in children under 2 years of age.

Who Should Not Use INCRELEX™?

Your child should not take INCRELEX™ if your child:

- Has finished growing (the bone growth plates are closed)
- Has cancer
- Has other causes of growth failure
- Is allergic to mecasermin or any of the inactive ingredients in INCRELEX™. Check with your child's doctor if you are not sure.

Your child should never receive INCRELEX™ through a vein.

What should I tell my child's doctor before my child starts INCRELEX™?

Tell your child's doctor about all of your child's health conditions, including if your child:

- Has diabetes
- Has kidney problems
- Has liver problems
- Has a curved spine (scoliosis)
- Is pregnant or breast-feeding.

Tell your child's doctor about all the medicines your child takes, including prescription and nonprescription medicines, vitamins, and herbal supplements. Especially tell your child's doctor if your child takes insulin or other anti-diabetes medicines. A dose adjustment may be needed for these medicines.

How Should My Child Use INCRELEX™?

- Use INCRELEX™ exactly as prescribed for your child. Your doctor or nurse should teach you how to inject INCRELEX™. Do not give your child INCRELEX™ unless you understand all of the instructions. See the **"Instructions for Use"** at the end of this leaflet.
- Inject INCRELEX™ under your child's skin shortly (20 minutes) before or after a meal or snack. **Skip your child's dose of INCRELEX™ if your child cannot eat for any reason.** Do not make up the missed dose by giving two doses the next time.
- Inject INCRELEX™ just below the skin in your child's upper arm, upper leg (thigh), stomach area (abdomen), or buttocks. **Never inject it into a vein or muscle.** Change the injection site for each injection ("rotate the injection site").

- Only use INCRELEX™ that is clear and colorless. If your child's INCRELEX™ is cloudy or slightly colored, return it for a replacement.

What are the Possible Side Effects of INCRELEX™?

INCRELEX™ may cause the following side effects, which can be serious:

- **Low blood sugar (hypoglycemia).** INCRELEX™ may lower blood sugar levels like insulin. It is important to only give your child INCRELEX™ right before or right after (20 minutes on either side of) a snack or meal to reduce the chances of low blood sugar. Do not give your child INCRELEX™ if your child is sick or cannot eat. Signs of low blood sugar are:
 - Dizziness
 - Tiredness
 - Restlessness
 - Hunger
 - Irritability
 - Trouble concentrating
 - Sweating
 - Nausea
 - Fast or irregular heartbeat

Severe hypoglycemia may cause unconsciousness, seizures, or death. If you take INCRELEX™, you should avoid participating in high risk activities (such as driving) within 2 to 3 hours after INCRELEX™ injection, especially at the beginning of INCRELEX™ treatment.

Before beginning treatment with INCRELEX™ your doctor or nurse will explain to you how to treat hypoglycemia. You/your child should always have a source of sugar such as orange juice, glucose gel, candy, or milk available in case symptoms of hypoglycemia occur. For severe hypoglycemia, if your child is not responsive and cannot drink sugar-containing fluids, you should give an injection of glucagon. Your doctor or nurse will instruct you how to give the injection.

Glucagon raises the blood sugar when it is injected. It is important that your child have a well-balanced diet including protein and fat such as meat and cheese in addition to sugar-containing foods.

- **Enlarged tonsils.** INCRELEX™ may enlarge your child's tonsils. Some signs of enlarged tonsils include: snoring, difficulty breathing or swallowing, sleep apnea (a condition where breathing stops briefly during sleep), or fluid in the middle-ear. Sleep apnea can cause excessive daytime sleepiness. Call your doctor should these symptoms bother your child. Your doctor should do regular exams to check your child's tonsils.
- **Increased pressure in the brain (intracranial hypertension).** INCRELEX™, like growth hormone, can sometimes cause a temporary increase in pressure within the brain. The symptoms of intracranial hypertension can include headache and nausea with vomiting. Tell your doctor if your child has headache with vomiting. Your doctor can then check to see if intracranial hypertension is present. If it is present, your doctor may decide to temporarily reduce or discontinue INCRELEX™ therapy. INCRELEX™ therapy may be started again after the episode is over.
- **A bone problem called slipped capital femoral epiphysis.** This happens when the top of the upper leg (femur) slips apart. Get medical attention for your child right away if your child develops a limp or has hip or knee pain.
- **Worsened scoliosis** (caused by rapid growth). If your child has scoliosis, your child will need to be checked often for an increase in the curve of the spine.
- **Allergic reactions.** Your child may have a mild or serious allergic reaction with INCRELEX™. Call your child's doctor right away if your child gets a rash

or hives. Get medical help immediately if your child has trouble breathing or goes into shock.

INCRELEX™ can cause reactions at the injection site including:

- Loss of fat (lipoatrophy)
- Increase of fat (lipohypertrophy)
- Pain, redness, or bruising

Injection site reactions can be avoided by changing the injection site at each injection ("injection site rotation").

Call your child's doctor if your child has side effects that are bothersome or that do not go away.

These are not all the side effects of INCRELEX™. Ask your child's doctor or pharmacist for more information.

How Should I Store INCRELEX™?

- **Before Opening** – Store new unopened vials of INCRELEX™ in the refrigerator (not the freezer) between 35° to 46°F (2° to 8°C). Do not freeze INCRELEX™. Keep INCRELEX™ out of direct heat and bright light. If a vial freezes, throw it away.
- **After Opening** – Once a vial of INCRELEX™ is opened, you can keep it in the refrigerator between 35° to 46°F (2° to 8°C) for 30 days after you start using the vial. Do not freeze INCRELEX™. Keep INCRELEX™ out of direct heat and bright light. If a vial freezes, throw it away.

Keep INCRELEX™ and all medicines out of reach of children.

General Information About INCRELEX™

Medicines are sometimes prescribed for conditions other than those described in patient information leaflets. Do not give INCRELEX™ to your child for a condition for which it was not prescribed. Do not give INCRELEX™ to a person other than your child. It may be harmful.

This leaflet summarizes the most important information about INCRELEX™. If you would like more information, talk to your child's doctor. You can also ask your child's doctor or pharmacist for information that is written for health professionals.

More information is available at 1-800-TERCICA (1-800-837-2422).

What are the Ingredients in INCRELEX™?

Active ingredient: mecasepmin

Inactive ingredients: sodium chloride, polysorbate 20, benzyl alcohol, and acetate.

INSTRUCTIONS FOR USE

INCRELEX should be administered using sterile disposable syringes and needles. The syringes should be of small enough volume that the prescribed dose can be withdrawn from the vial with reasonable accuracy.

Preparing the Dose:

1. Wash your hands before getting INCRELEX™ ready for your child's injection.
2. Use a new disposable needle and syringe every time you give a dose. Use syringes and needles only once. Throw them away properly. **Never** share needles and syringes.
3. Check the liquid to make sure it is clear and colorless. Do not use after the expiration date or if it is cloudy or if you see particles.
4. If you are using a new vial, remove the protective cap. Do not remove the rubber stopper.
5. Wipe the rubber stopper of the vial with an alcohol swab to prevent contamination of the vial by germs that may be introduced by repeated needle insertions (see Figure 1).

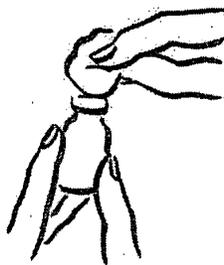


Figure 1: Wipe top with alcohol

6. Before putting the needle into the vial, pull back on plunger to draw air into the syringe equal to the INCRELEX™ dose. Put the needle through the rubber top of the vial and push the plunger to inject air into the vial (see Figure 2).

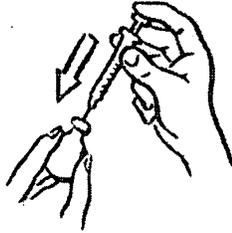


Figure 2: Inject
air into vial

7. Leave the syringe in the vial and turn both upside down. Hold the syringe and vial firmly (see Figure 3).

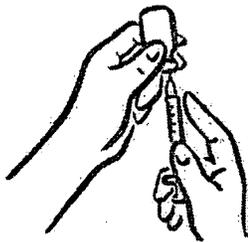


Figure 3: Prepare
for extraction

8. Make sure the tip of the needle is in the liquid (see Figure 4). Pull the plunger to withdraw the correct dose into the syringe (see Figure 5).

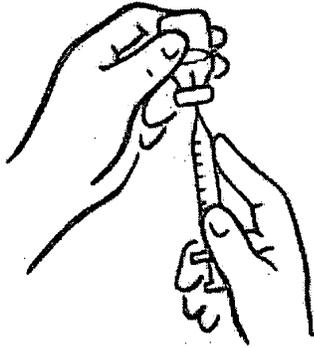


Figure 4: Tip in liquid

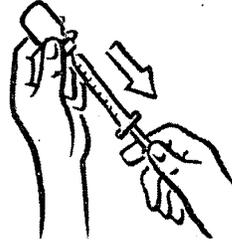


Figure 5: Extract Correct Dose

9. Before you take the needle out of the vial, check the syringe for air bubbles. If bubbles are in the syringe, hold the vial and syringe with needle straight up and tap the side of the syringe until the bubbles float to the top. Push the bubbles out with the plunger and draw liquid back in until you have the correct dose (see Figure 6).

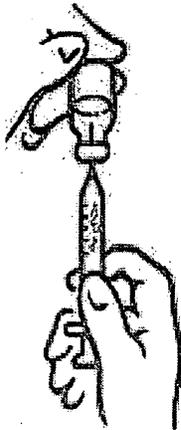


Figure 6: Remove air bubbles and refill syringe

10. Remove the needle from the vial. Do not let the needle touch anything. You are now ready to inject (see figure 7).



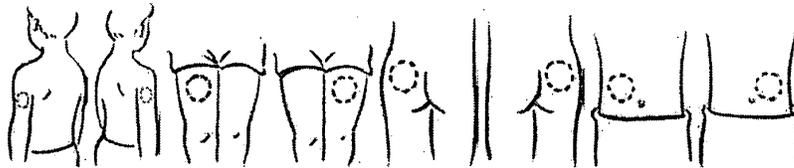
Figure 7:
Ready to inject

Injecting the Dose:

Inject INCRELEX™ as instructed by your child's doctor.

Do not give the INCRELEX™ injection if your child is unable to eat within 20 minutes before or after the injection.

1. Decide on an injection area – upper arm, thigh, buttock, or abdomen (see below). The injection site should be changed for each injection (“rotate the injection site”).



Upper arm

Thigh

Buttock

Abdomen

2. Use alcohol or soap and water to clean the skin where you are going to inject your child. The injection site should be dry before you inject.

3. Lightly pinch the skin. Stick the needle in the way your child's doctor showed you. Release the skin (see figure A).



Figure A: Lightly pinch the skin and inject as instructed

4. Slowly push in the plunger of the syringe all the way, making sure you have injected all the liquid. Pull the needle straight out and gently press on the spot where you injected your child with gauze or a cotton ball for a few seconds. **Do not rub the area** (see figure B).

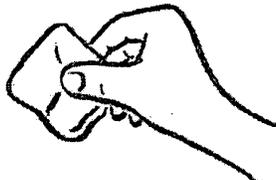


Figure B: Press (don't rub) with gauze or cotton

5. Follow your child's doctor's instructions for throwing away the needle and syringe. Do not recap the syringe. Used needle and syringe should be placed in a sharps container (such as a red biohazard container), hard plastic container (such as a detergent bottle), or metal container (such as an empty coffee can). Such containers should be sealed and disposed of properly.

For additional information, call 1-800-TERCICA (1-800-837-2422)

Manufactured for: Tercica, Incorporated
Brisbane, CA 94005 USA

www.tercica.com

Issued: August 2005

1/8"

8 mm

1/8"

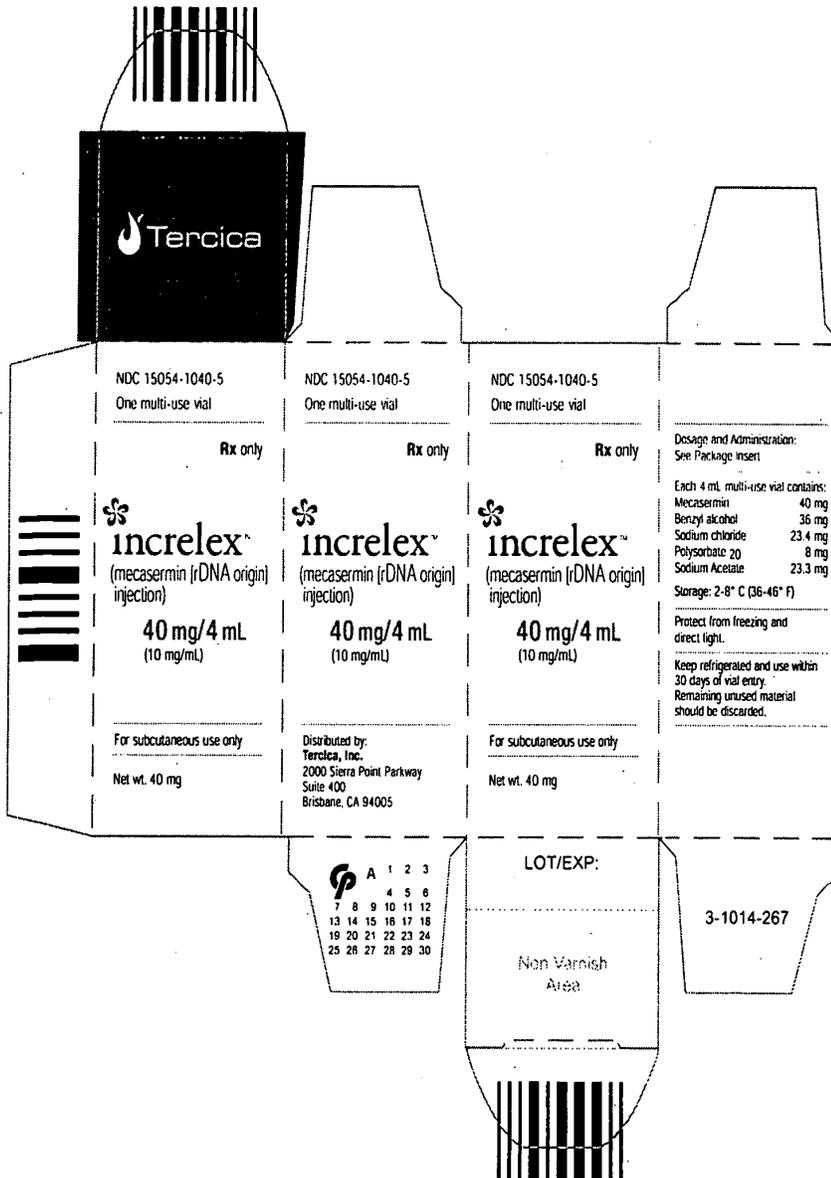
13/16"

1/16"

<p>Storage 2.8" (72.14 mm) Keep refrigerated and use within 30 days of first entry Refrigerated material return to cold as described</p> <p>Protect from light and moisture.</p>	<p>NDC 15254-1049-5 Rx only</p> <p>increlex (mexasermin (pDNA origin) injection) 40 mg/4 mL</p> <p>Net wt. 40 mg (10 mg/mL) For subcutaneous use only</p>	<p>Each 4 mL multi-use vial contains:</p> <p>Mexasermin 40 mg Sodium acetate 20 mg Sodium chloride 22.4 mg Polysorbate 20 8 mg Sodium formate 22.3 mg</p> <p>Compare and Administration: See Package Insert</p> <p>Distributed by: Teraco, Inc. 2000 Eureka Road Parkway Suite 400 Brea, CA 92605</p> <p>LOT/EXP EXP. DATE PREF. PERIOD</p>
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Creative Press Job No: 19226 Die Cut Label Size: 13/16" x 2 1/4"
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 Colors: Black PMS Green
 Roll Stock: Pharma High Gloss Plus Pharmacode: 1114
 Adhesive: E828
 Liner: 40#
 Item No. 3-1013-257
 Corner Radius: 1/8"

NDA 21839



Creative Press, Inc. Job Number: 42987
 Colors: Black, PMS Green
 Increlex Unit Carton 3-1014-267
 Stock: .018 C1S SBS
 Pharma Code: 1115 UPC Code:

NDA 21839

Exhibit e



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 21-839

Tercica, Inc.
Attention: Ira Wallis
Vice President, Regulatory Affairs
2000 Sierra Point Parkway, Suite 400
Brisbane, CA 94005

RECEIVED
AUG 30 2005

BY:.....

Dear Mr. Wallis:

Please refer to your new drug application (NDA) dated February 24, 2005, received February 28, 2005, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for INCRELEX™ (mecasermin [rDNA origin] injection), 10 mg/mL.

We acknowledge receipt of your submissions dated May 9, June 29, July 13 and 28, and August 23 and 26, 2005.

This new drug application provides for the use of INCRELEX™ (mecasermin [rDNA origin] injection) for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency (Primary IGF1D) or with growth hormone (GH) gene deletion who have developed neutralizing antibodies to growth hormone.

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the agreed-upon labeling text.

The final printed labeling (FPL) must be identical to the enclosed labeling (package insert submitted by secure email August 26, 2005, patient package insert submitted by secure email August 25, 2005, immediate container and carton labels submitted August 23, 2005). Marketing the products with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

Please submit an electronic version of the FPL according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format - NDAs*. Alternatively, you may submit 20 paper copies of the FPL as soon as it is available but no more than 30 days after it is printed. Individually mount 15 of the copies on heavy-weight paper or similar material. For administrative purposes, designate this submission "**FPL for approved NDA 21-839.**" Approval of this submission by FDA is not required before the labeling is used.

In addition, submit three copies of the introductory promotional materials that you propose to use for this product. Submit all proposed materials in draft or mock-up form, not final print. Send one copy to the Division of Metabolism and Endocrinology Products (DMEP) and two copies of both the promotional materials and the package inserts directly to:

Division of Drug Marketing, Advertising, and Communications
Food and Drug Administration
Center for Drug Evaluation and Research
5901-B Ammendale Road
Beltsville, MD 20705-1266

We note your agreements dated July 28, 2005, to submit the following information: (1) data from 12-month stability studies on three lots of formulated drug substance stored in bags to establish shelf-life dating for bulk drug substance; (2) results of studies to identify and characterize the additional IEF bands in certain rhIGF-1 drug product samples stored at 25°C/60% RH; and (3) results of the ongoing preservative effectiveness study.

Mecasermin has been designated as an orphan drug for the indication being approved. Therefore, the pediatric study requirements of the Pediatric Research Equity Act of 2003 do not apply to this application. However, we note that clinical studies were conducted in pediatric patients two years of age and older.

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

Effective immediately, **ALL** regulatory submissions, whether sent by U.S. Postal Service, overnight mail service, or courier, should be sent to the following address. Processing of submissions sent to other addresses may be delayed.

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Metabolism and Endocrinology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Enid Galliers, Chief, Project Management Staff, DMEP, at 301-827-6429.

Sincerely,

{See appended electronic signature page}

Robert J. Meyer, M.D.
Director
Office of Drug Evaluation II
Center for Drug Evaluation and Research

Enclosures:

Package Insert (PI)

Patient Package Insert (PPI)

Vial Label

Carton Label

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Robert Meyer

8/30/2005 04:40:32 PM

Exhibit D



US005681814A

United States Patent [19]

[11] Patent Number: **5,681,814**

Clark et al.

[45] Date of Patent: **Oct. 28, 1997**

[54] **FORMULATED IGF-I COMPOSITION**

- [75] Inventors: Ross G. Clark, Pacifica; Douglas A. Yeung, Fremont; James Q. Oeswein, Moss Beach, all of Calif.
- [73] Assignee: Genentech, Inc., South San Francisco, Calif.
- [21] Appl. No.: 71,819
- [22] Filed: Jun. 4, 1993

Related U.S. Application Data

- [60] Continuation-in-part of Ser. No. 806,748, Dec. 13, 1991, abandoned, which is a division of Ser. No. 535,005, Jun. 7, 1990, Pat. No. 5,126,324.
- [51] Int. Cl.⁶ A61K 38/00; A01N 37/18
- [52] U.S. Cl. 514/12; 514/2; 514/21
- [58] Field of Search 514/2, 12, 21

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(List continued on next page.)

Primary Examiner—Toni R. Scheiner
 Assistant Examiner—Sheela J. Huff
 Attorney, Agent, or Firm—Janet E. Hasak

[57] **ABSTRACT**

A formulation for IGF-I is disclosed that is useful in treating hyperglycemic disorders and, in combination with growth hormone, in enhancing growth of a mammal. Also disclosed is a process for preparing a formulation of growth hormone and IGF-I from the IGF-I formulation. The IGF-I formulation comprises about 2-20 mg/ml of IGF-I, about 2-50 mg/ml of an osmolyte, about 1-15 mg/ml of a stabilizer, and a buffered solution at about pH 5-5.5, optionally with a surfactant.

8 Claims, 25 Drawing Sheets

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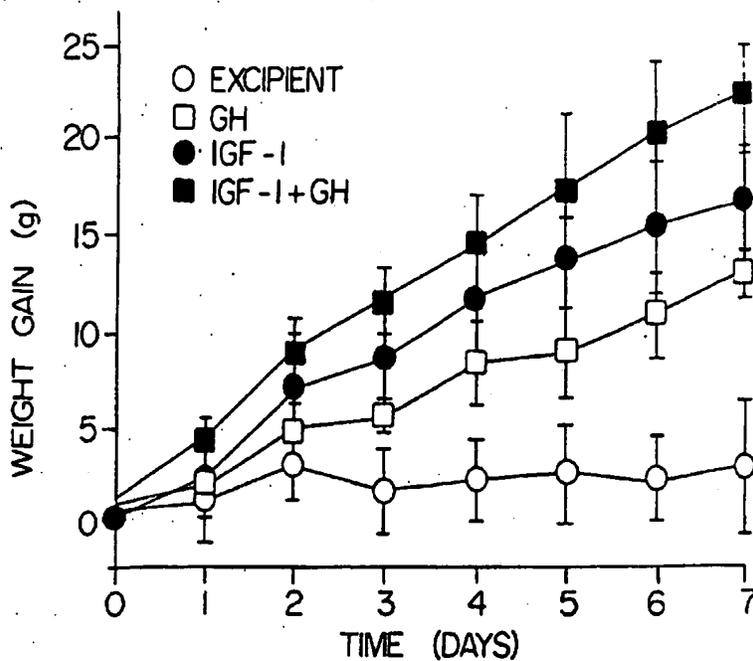


FIG. IA

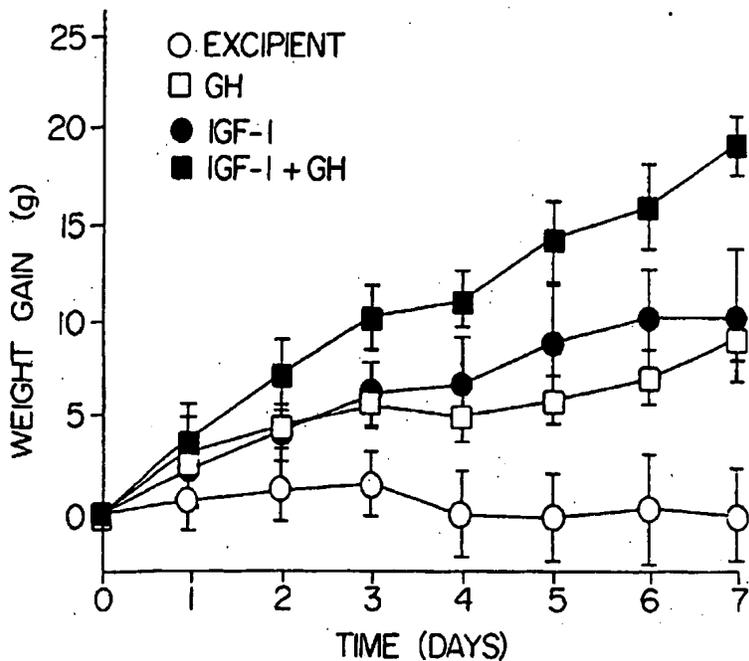


FIG. IB

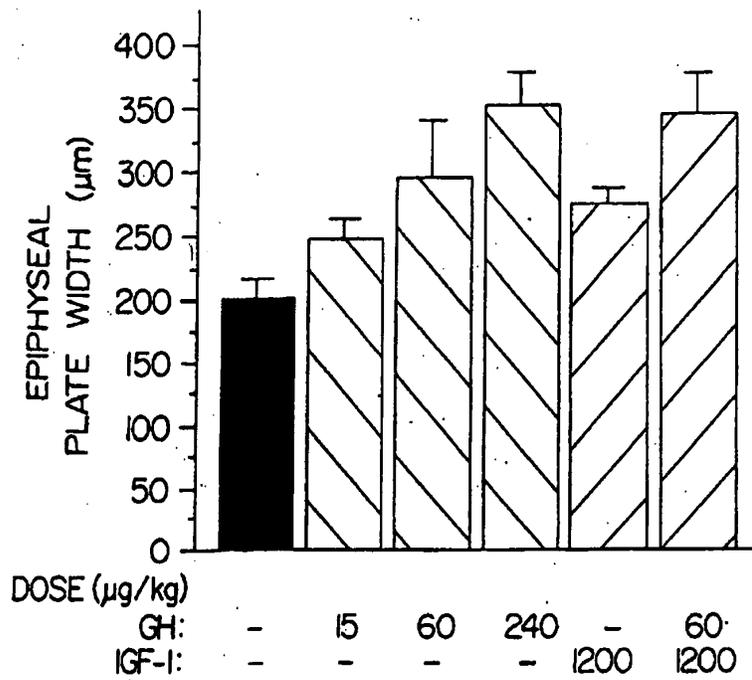


FIG. 2

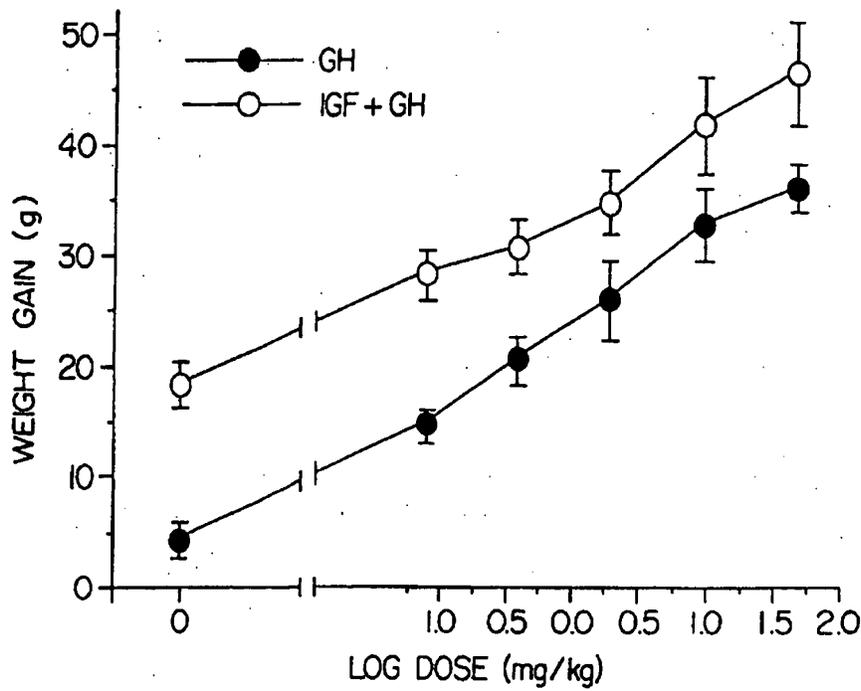


FIG. 4

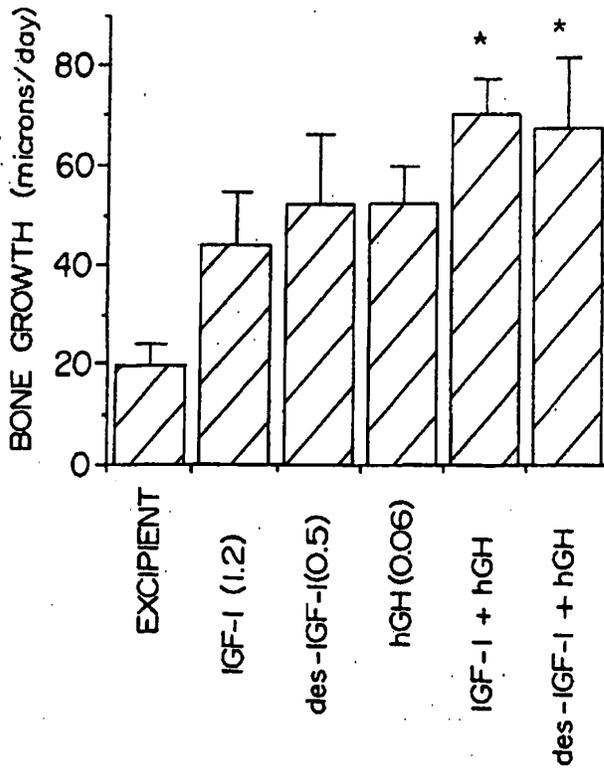


FIG. 3A

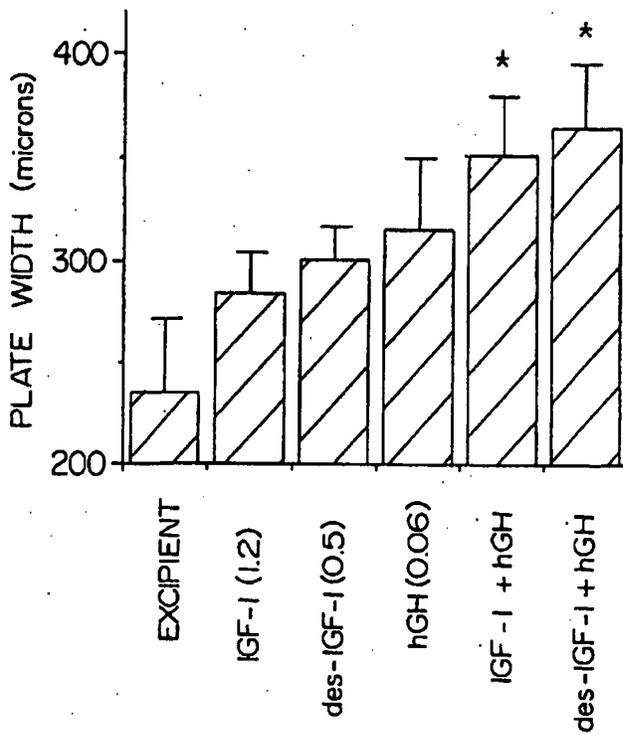


FIG. 3B

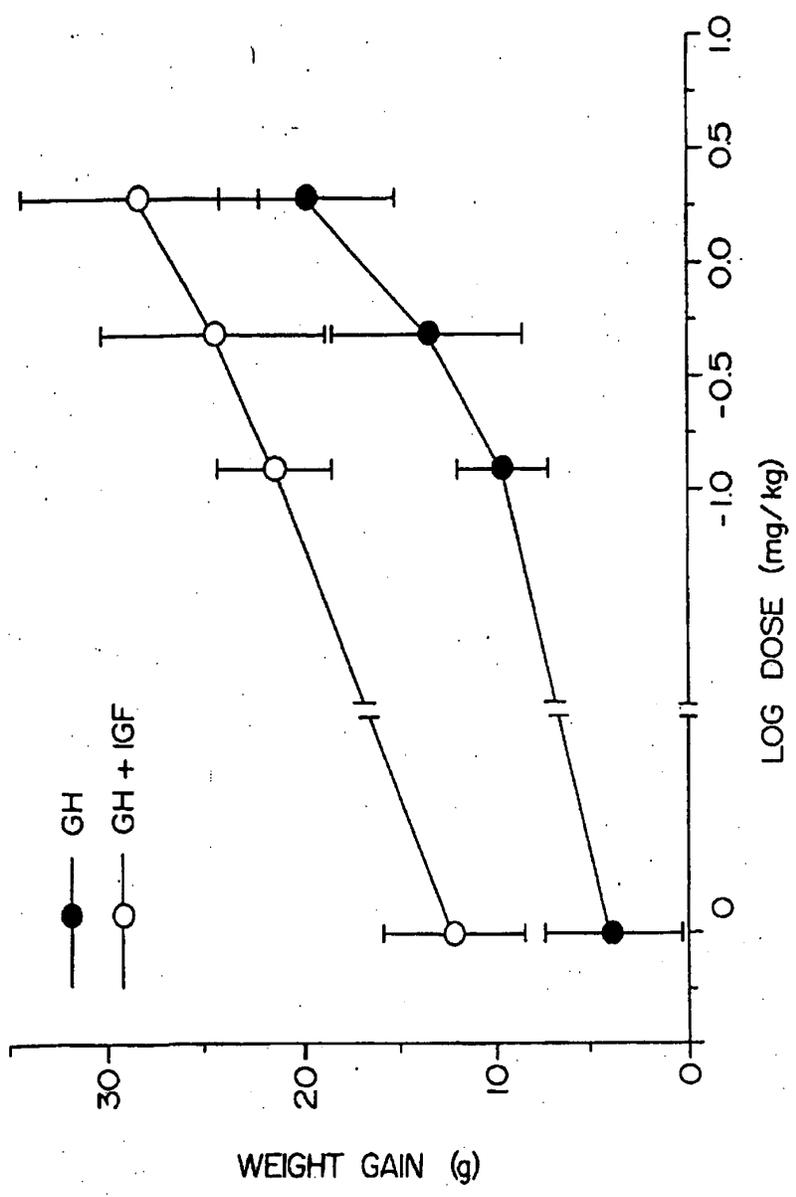


FIG. 5

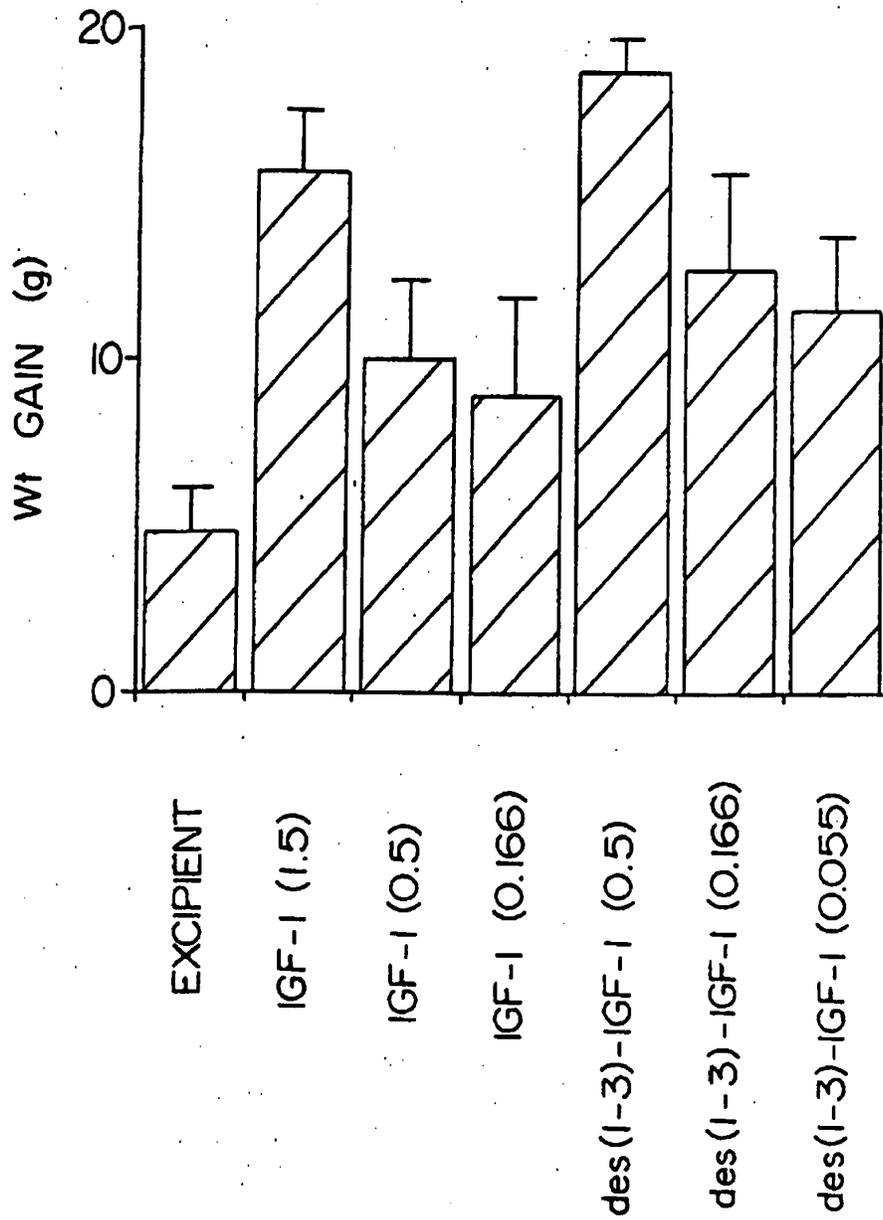


FIG. 6

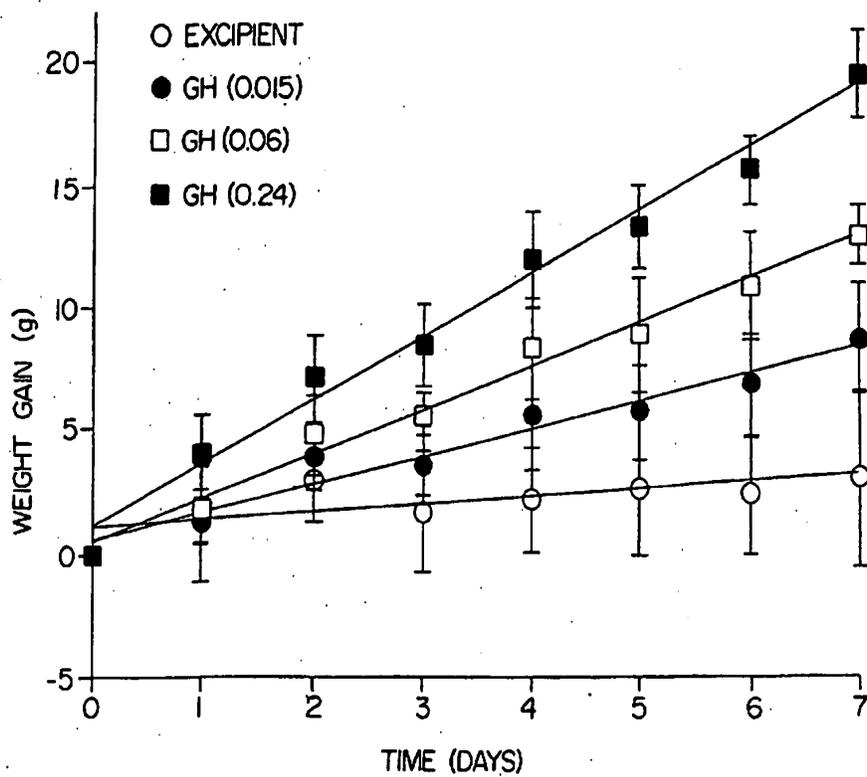


FIG. 7

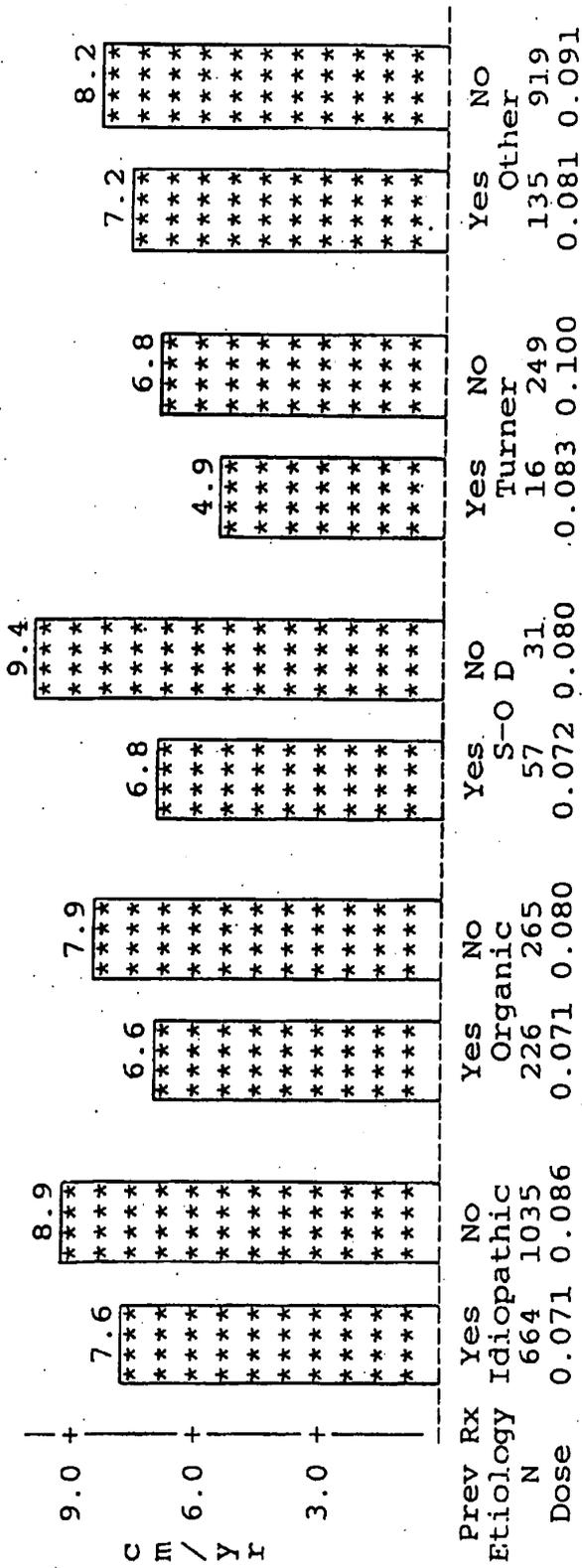


FIG. 8A

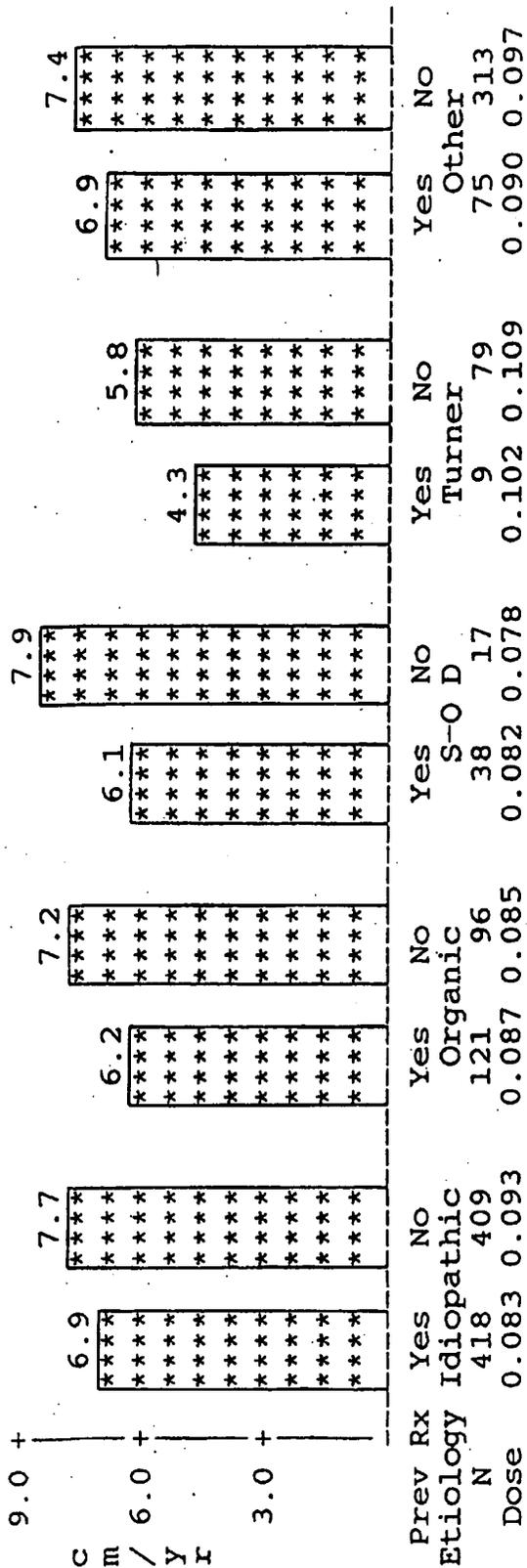


FIG. 8B

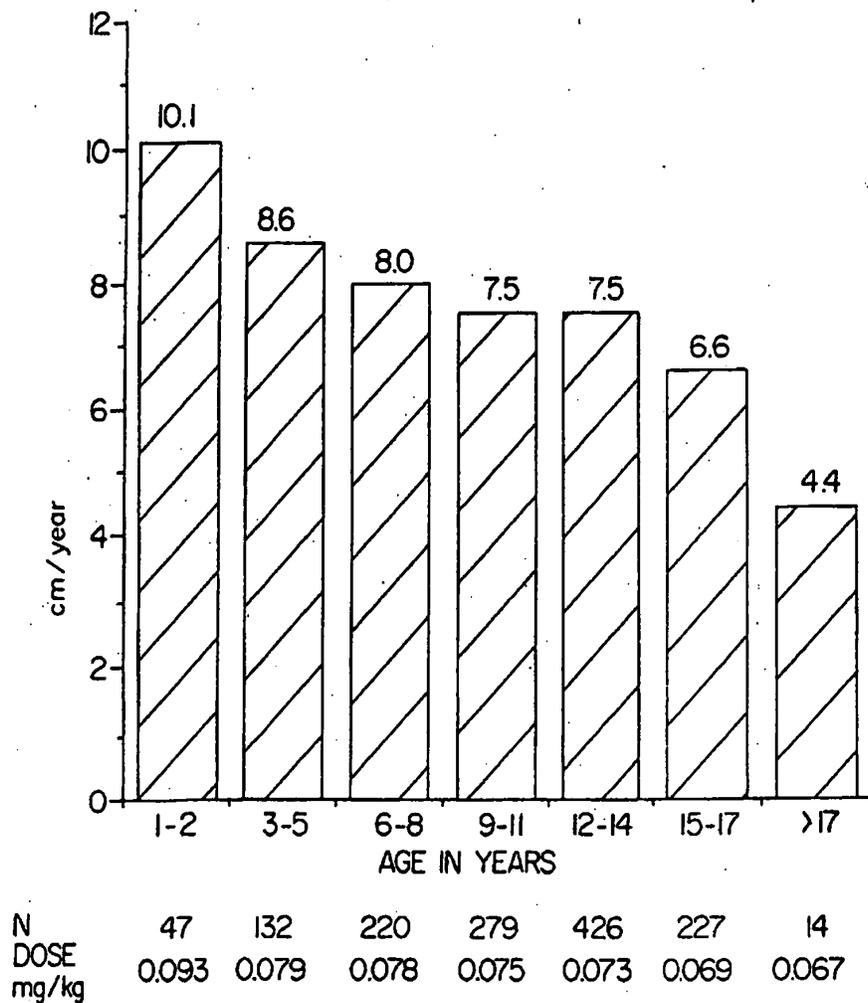


FIG. 9

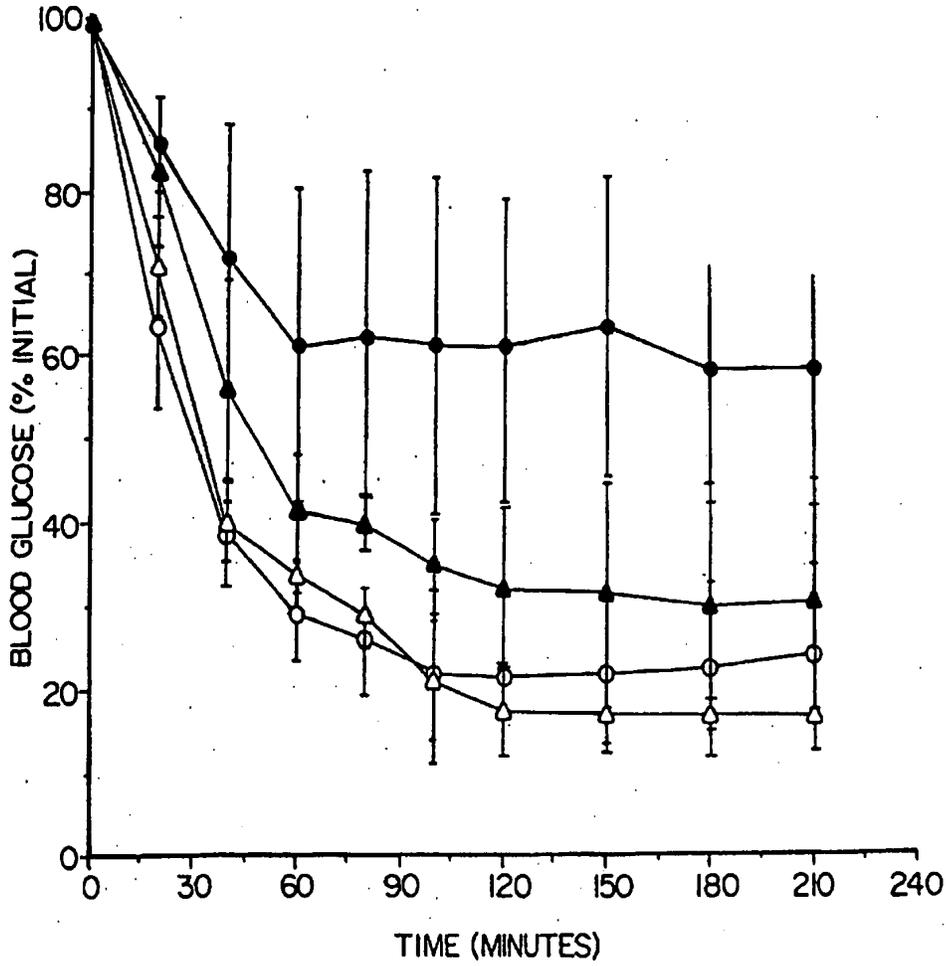


FIG. 10

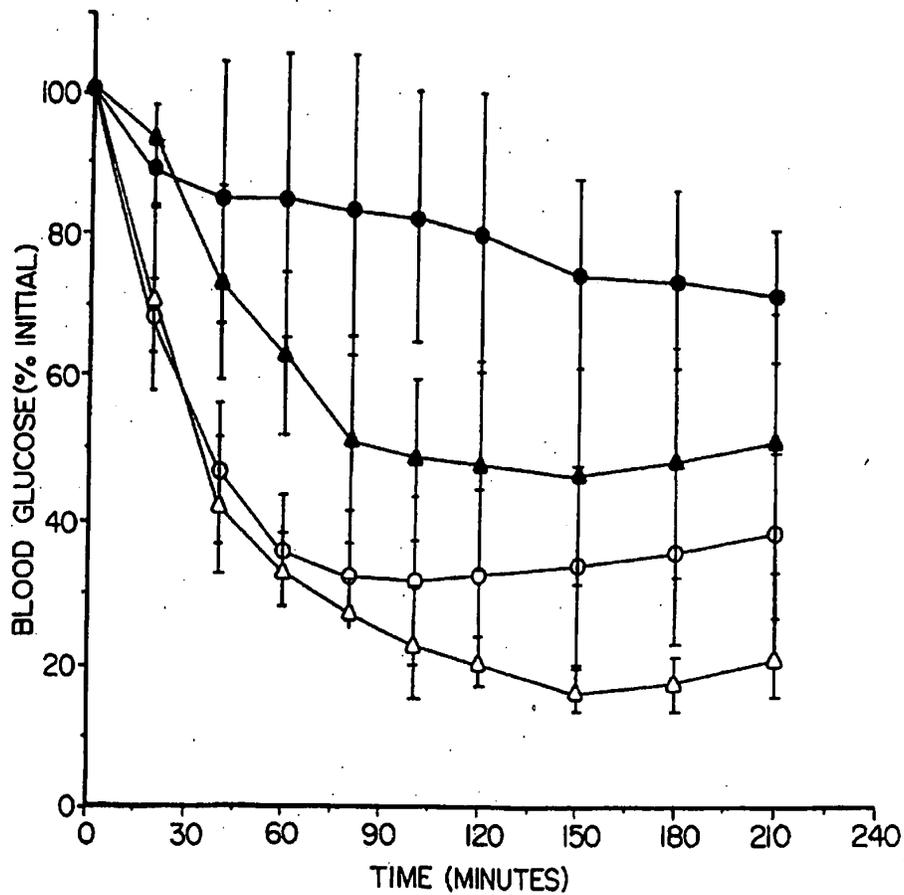


FIG. II

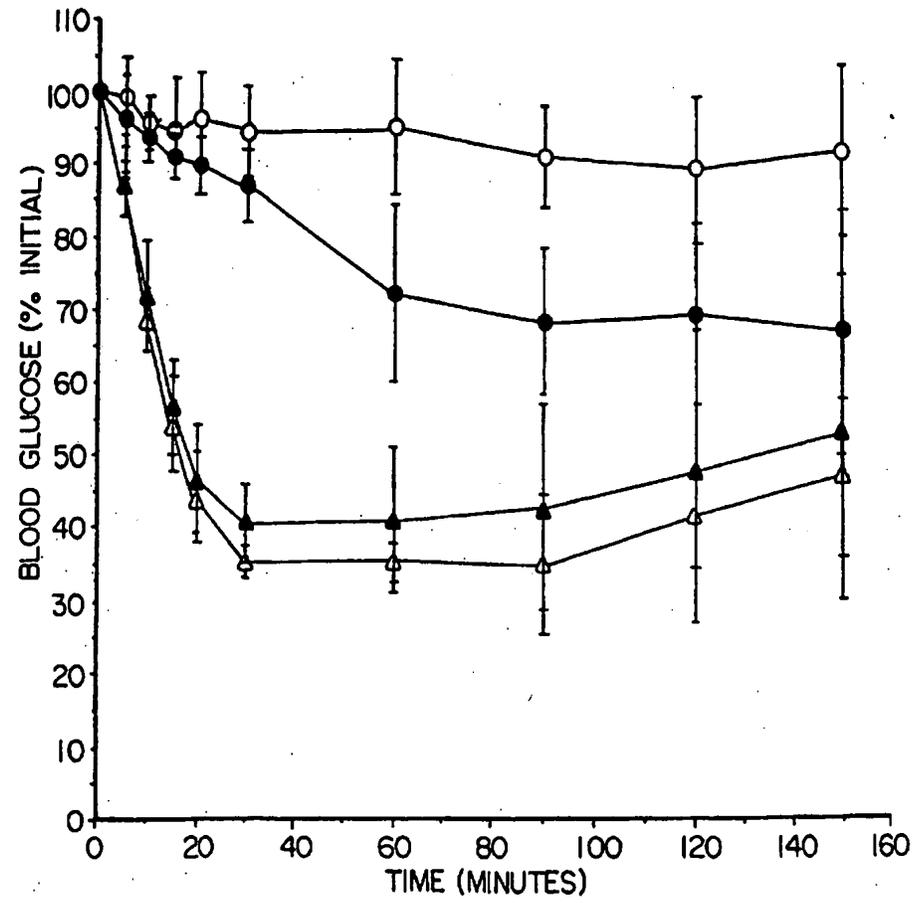


FIG. 12

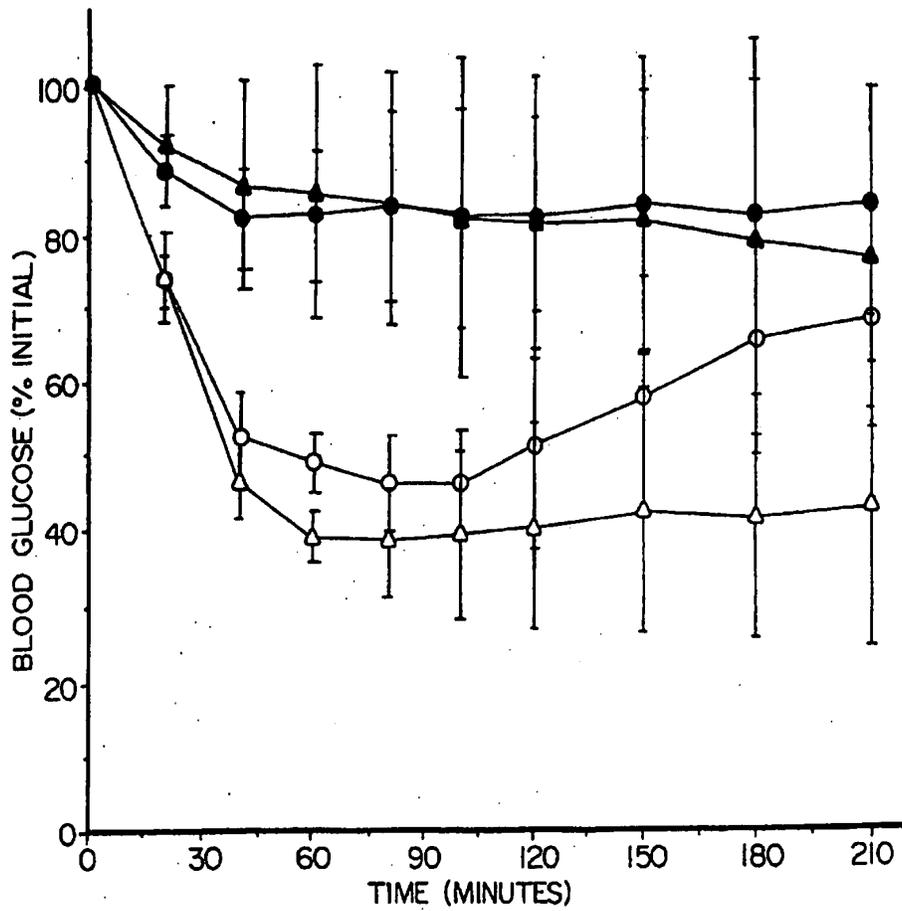


FIG. 13

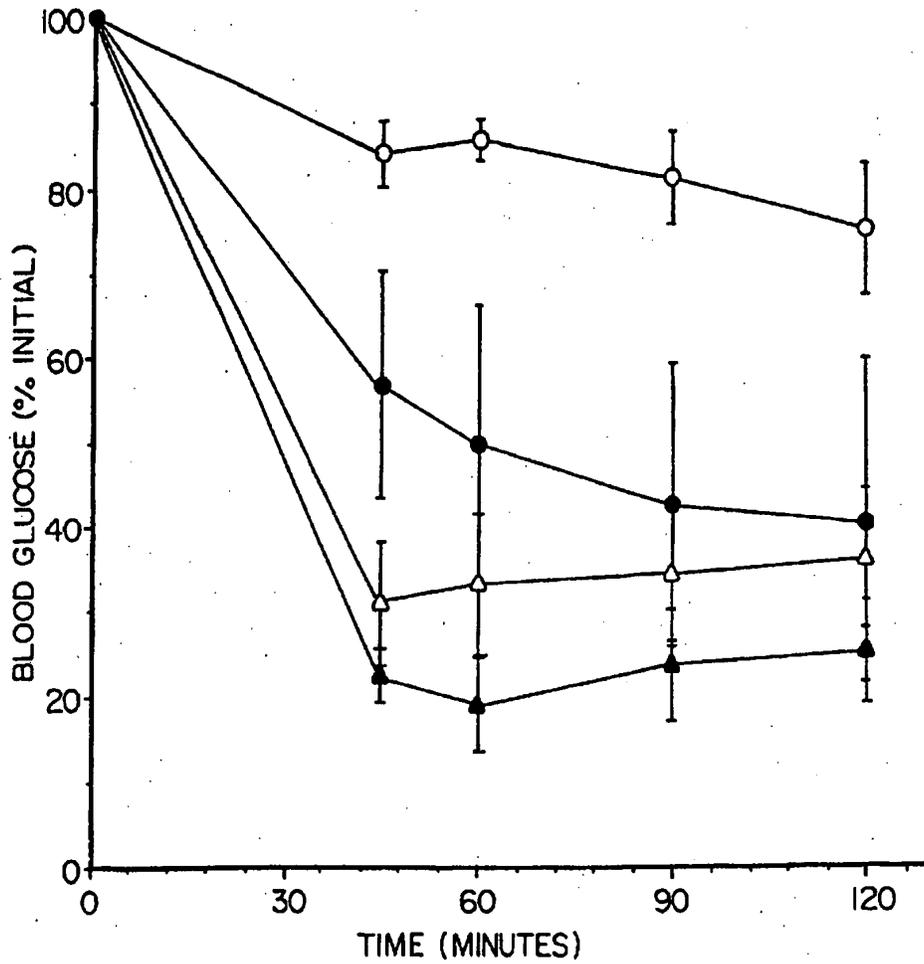


FIG. 14

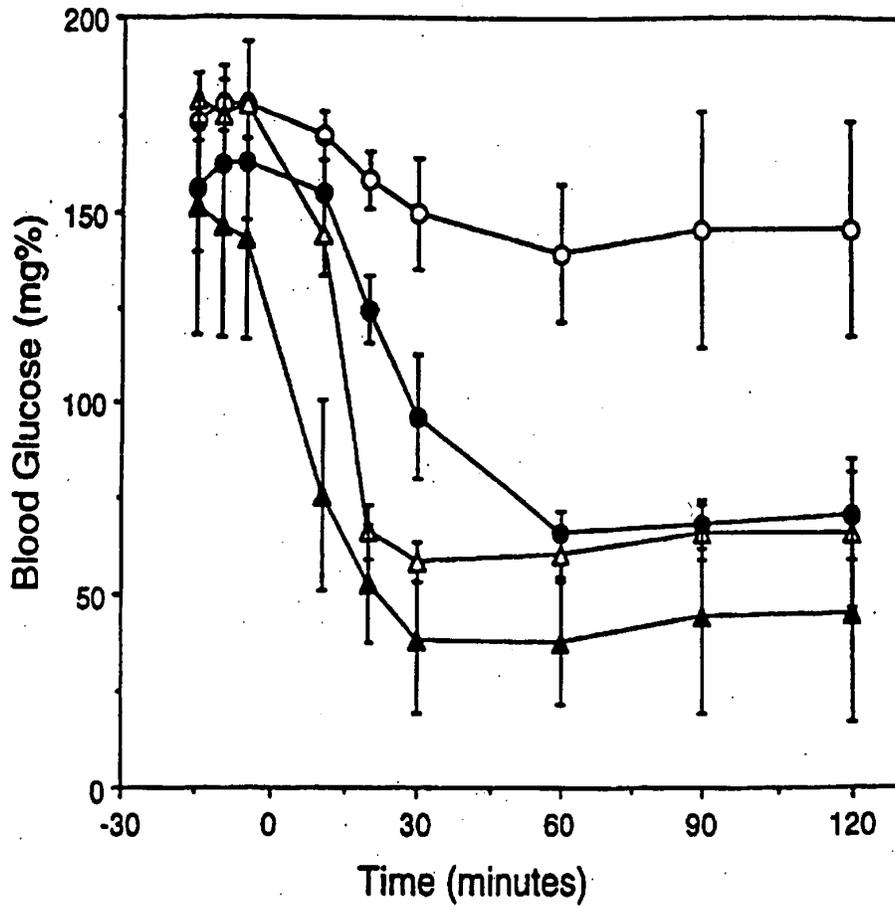


FIG. 15

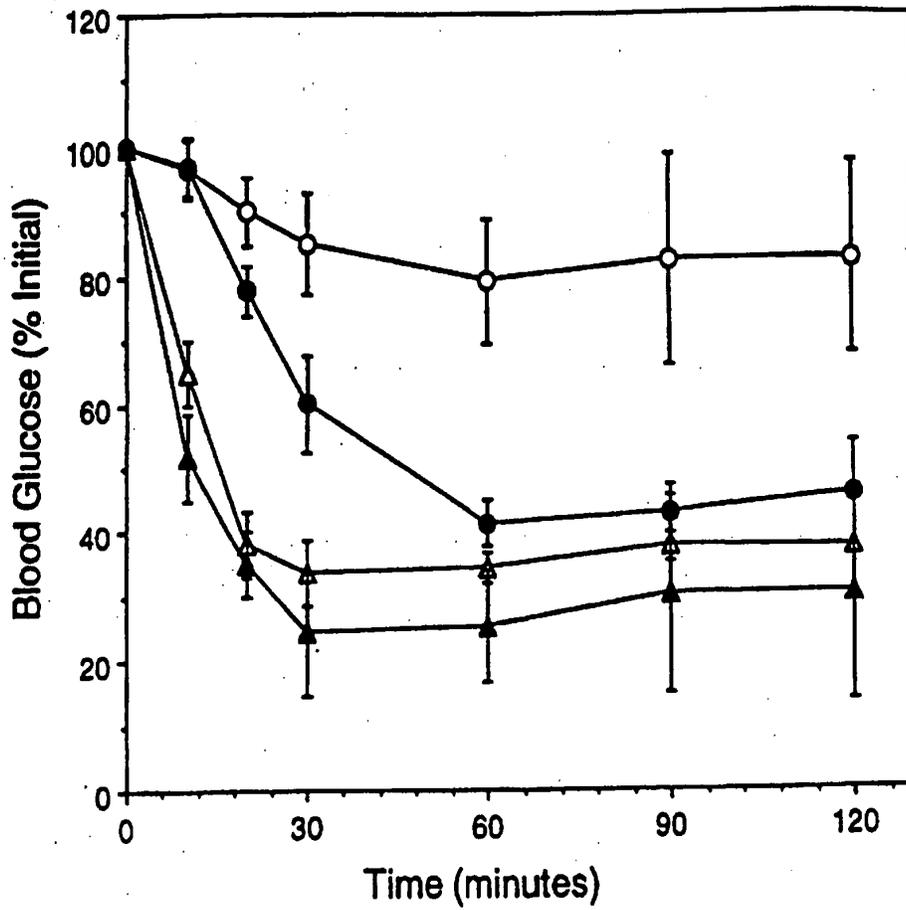


FIG. 16

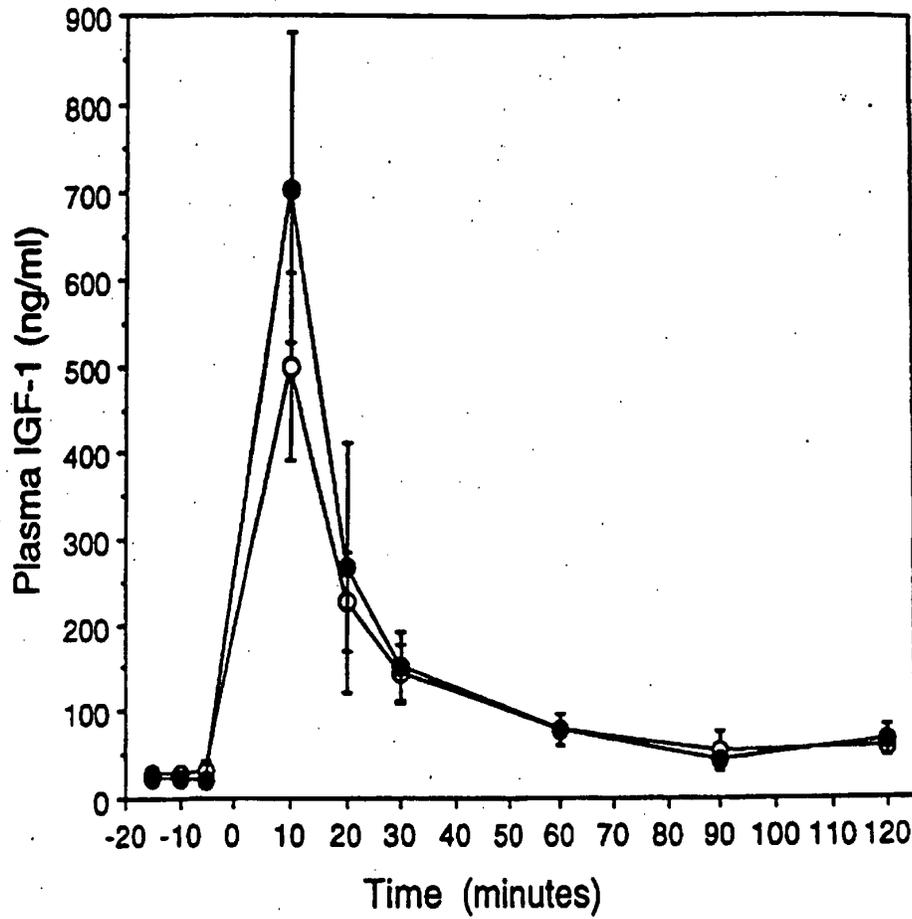


FIG. 17

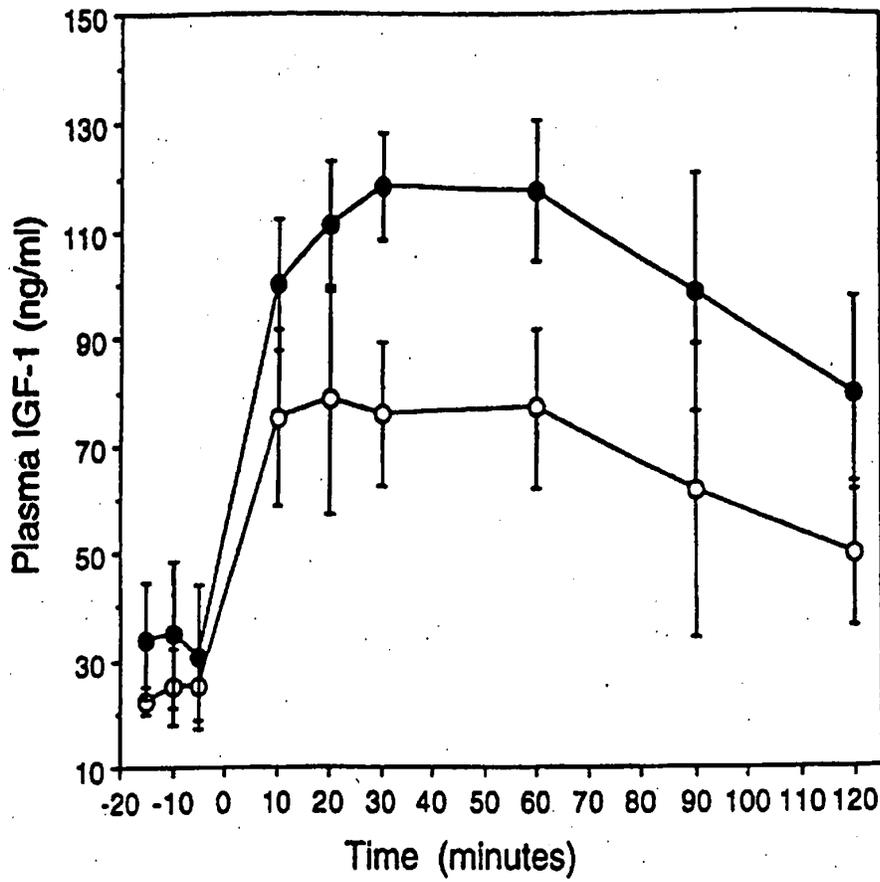


FIG. 18

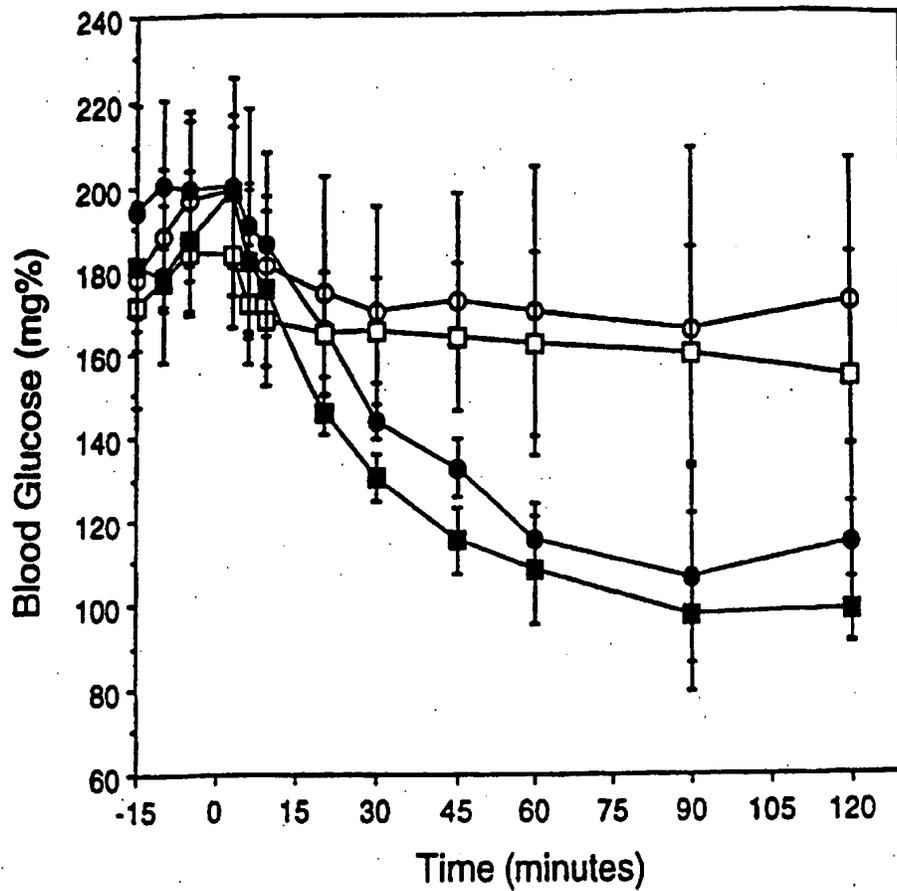


FIG. 19

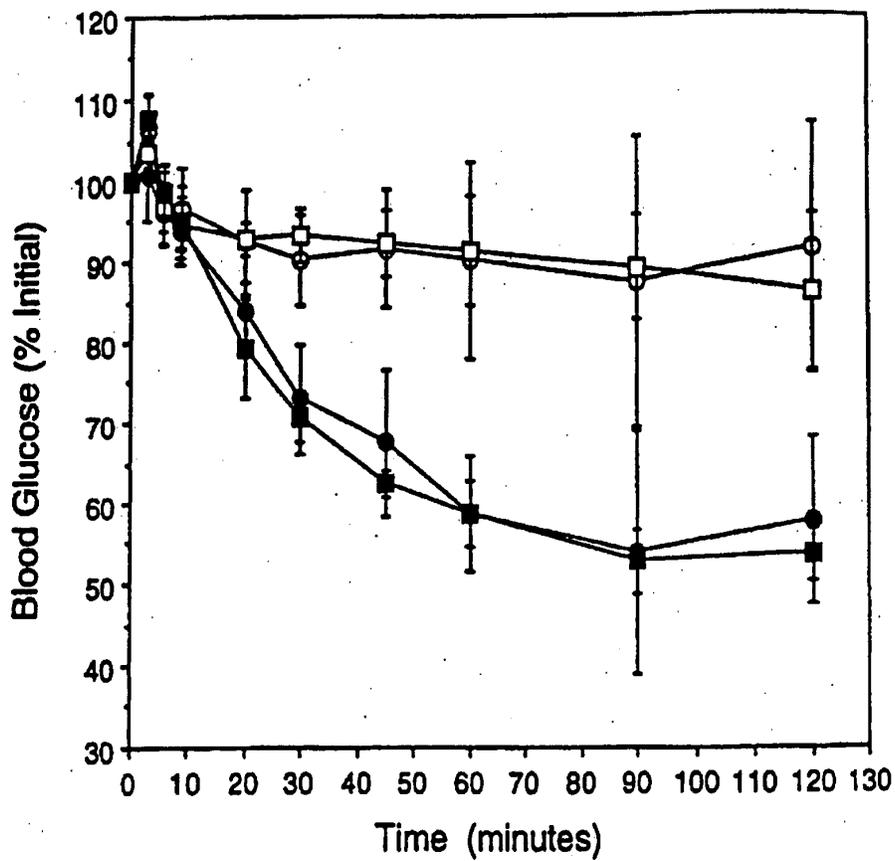


FIG. 20

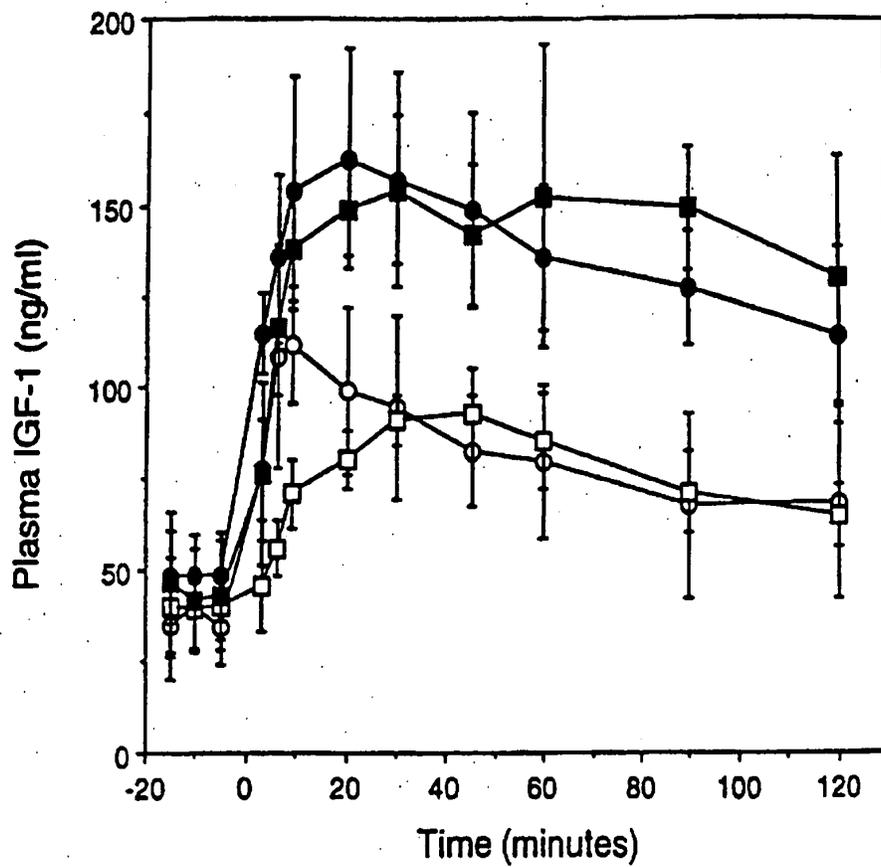


FIG. 21

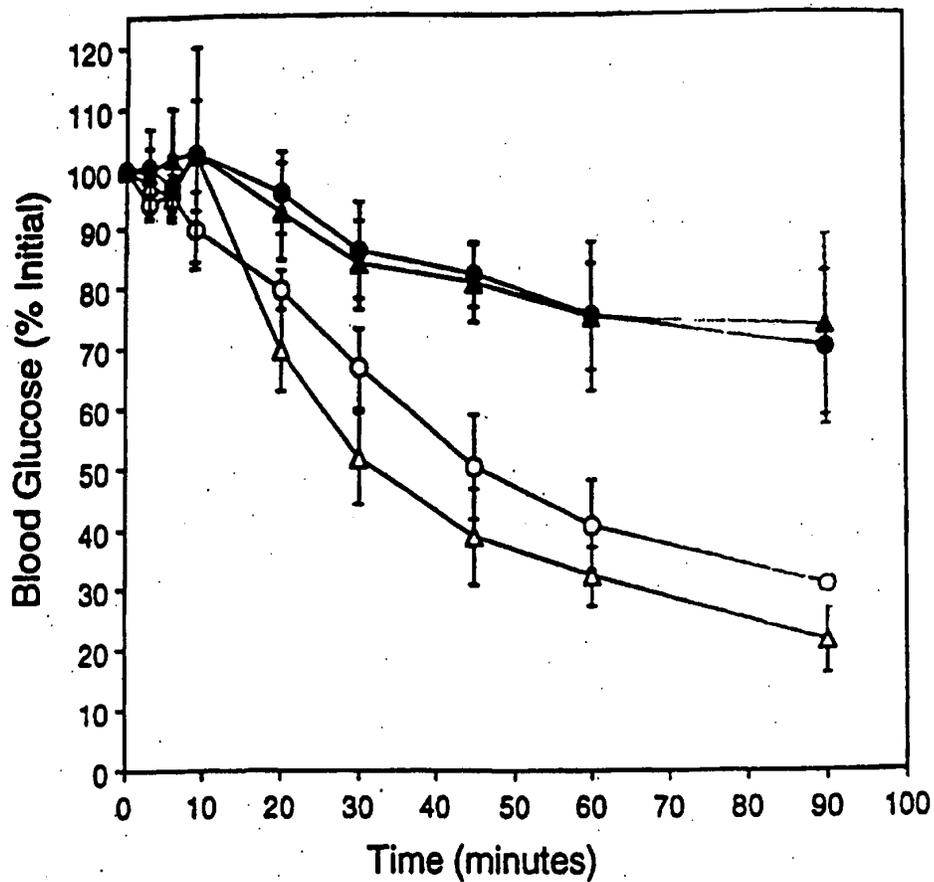


FIG. 22

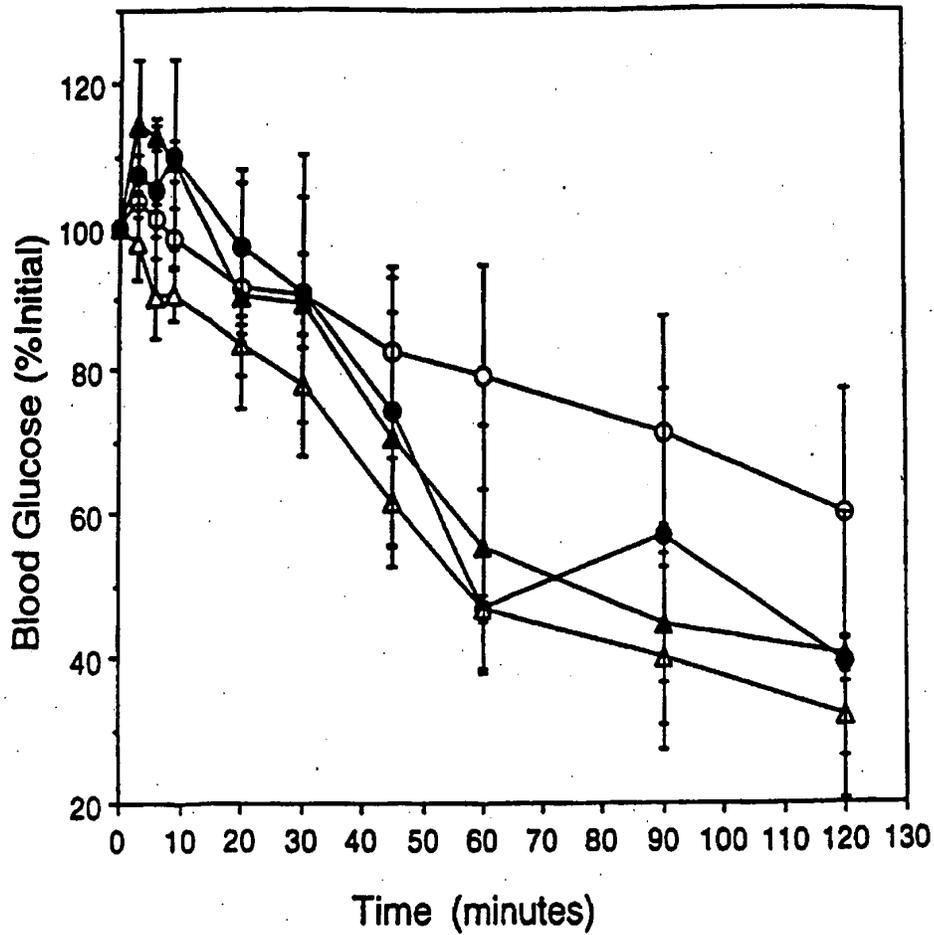


FIG. 23

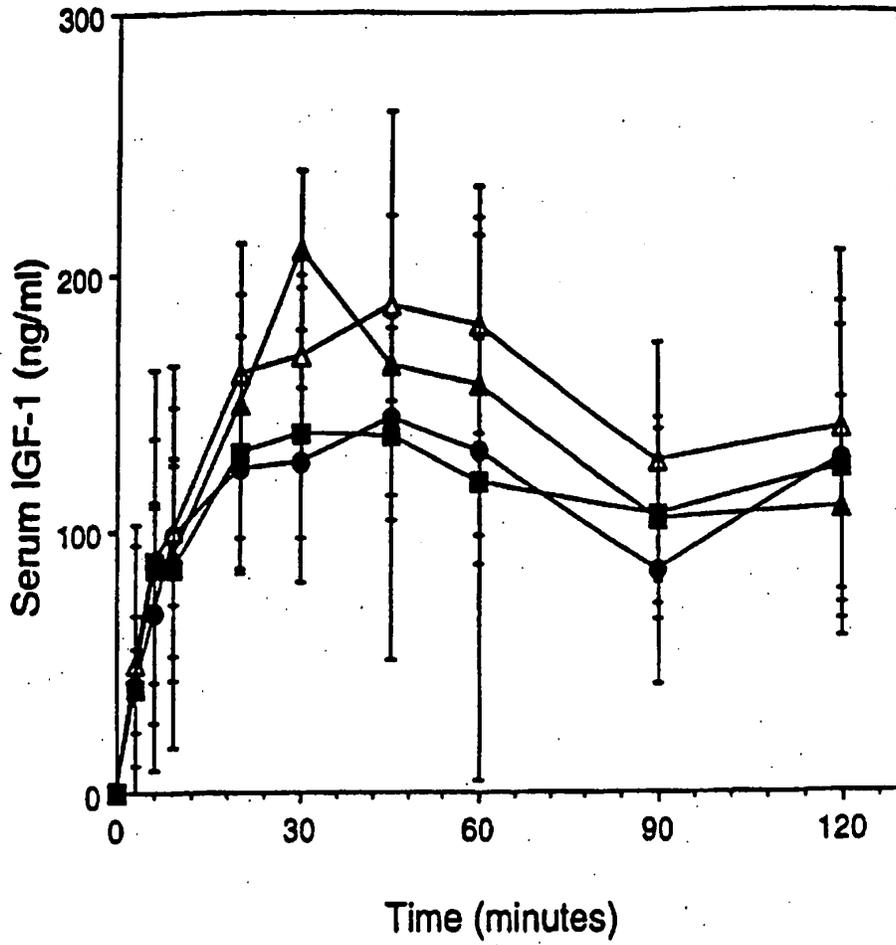


FIG. 24

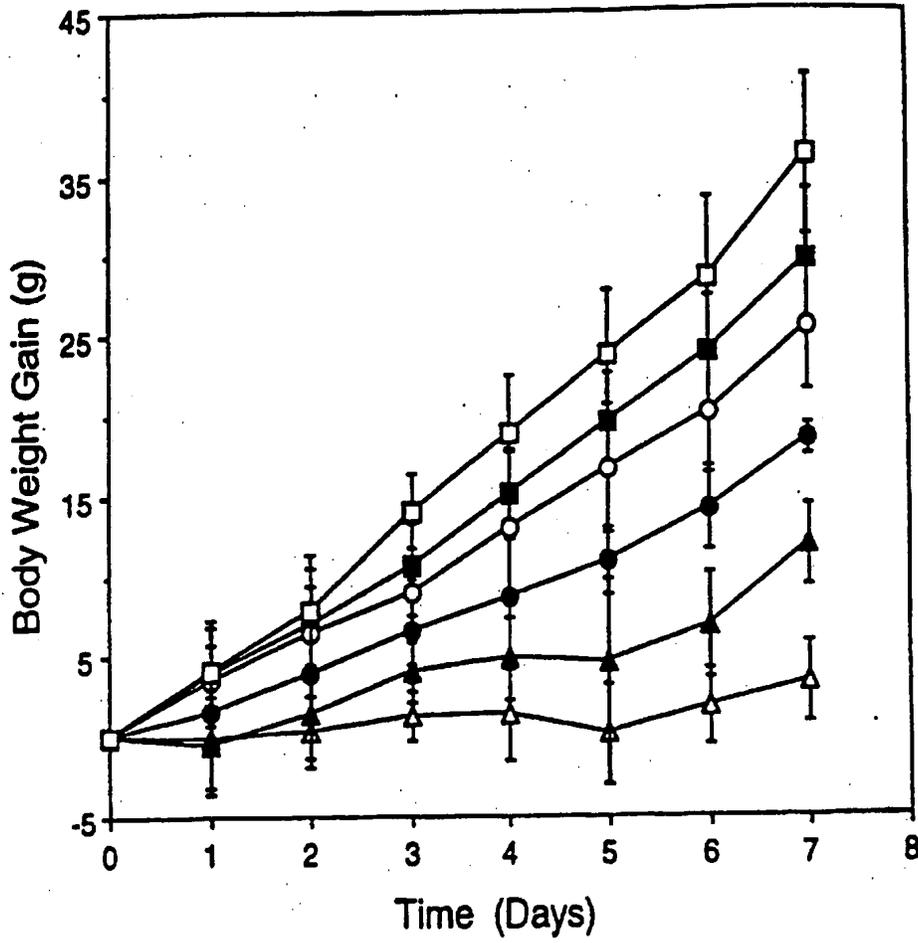


FIG. 25

FORMULATED IGF-I COMPOSITION

This application is a continuation-in-part application of U.S. Ser. No. 07/806,748 filed Dec. 13, 1991, now abandoned, which is a divisional application of U.S. Ser. No. 07/535,005 filed Jun. 7, 1990, now issued as U.S. Pat. No. 5,126,324.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to formulations containing IGF-I useful in treating all pathological or other conditions that can be ameliorated or improved using IGF-I, including treating hyperglycemia and enhancing growth (anabolism) in patients, particularly those exhibiting a retarded growth rate or weight loss using a combination of natural hormones.

2. Description of Related Art

Insulin-like growth factor I (IGF-I) is a polypeptide naturally occurring in human body fluids, for example, blood and human cerebral spinal fluid. Most tissues and especially the liver produce IGF-I together with specific IGF-binding proteins. These molecules are under the control of growth hormone (GH). Like GH, IGF-I is a potent anabolic protein. See Tanner et al., *Acta Endocrinol.*, 84: 681-696 (1977); Uihne et al., *J. Clin. Endocrinol. Metab.*, 39: 548-554 (1974). IGF-I has been isolated from human serum and produced recombinantly. See, e.g., EP 123,228 and 128,733.

Various biological activities of IGF-I have been identified. Researchers have found that an intravenous bolus injection of IGF-I lowers blood glucose levels in humans. See Guler et al., *N. Engl. J. Med.*, 317: 137-140 (1987). Additionally, IGF-I promotes growth in several metabolic conditions characterized by low IGF-I levels, such as hypophysectomized rats [Guler et al., *Endocrinology*, 118: Supp 129 abstract, Skottner et al., *J. Endocr.*, 112: 123-132 (1987); Guler et al., *Proc. Natl. Acad. Sci. USA*, 85: 4889-4893 (1988); Froesch et al., in *Endocrinology, Intl. Congress Series 655*, ed. by Labrie and Proulx (Amsterdam: Excerpta Medica, 1984), p. 475-479], diabetic rats [Scheiwiller et al., *Nature*, 323: 169-171 (1986)], and dwarf rats [Skottner et al., *Endocrinology*, 124: 2519-2526 (1989)]. The kidney weight of hypophysectomized rats increases substantially upon prolonged infusions of IGF-I subcutaneously. Guler et al., *Proceedings of the 1st European Congress of Endocrinology*, 103: abstract 12-390 (Copenhagen, 1987). The kidneys of Snell dwarf mice and dwarf rats behaved similarly. van Bunt-Offers et al., *Pediatr. Res.*, 20: 825-827 (1986); Skottner et al., *Endocrinology*, supra. An additional use for IGF-I is its administration to improve glomerular filtration and renal plasma flow in human patients. See EP 327,503 published Aug. 9, 1989; Guler et al., *Proc. Natl. Acad. Sci. USA*, 86: 2868-2872 (1989).

Human growth hormone (hGH) is a single-chain polypeptide consisting of 191 amino acids (molecular weight 21,500). Disulfide bonds link positions 53 and 165 and positions 182 and 189. Niall, *Nature, New Biology*, 230: 90 (1971). Human GH is a potent anabolic agent, especially due to retention of nitrogen, phosphorus, potassium, and calcium. Treatment of hypophysectomized rats with GH can restore at least a portion of the growth rate of an intact animal. Moore et al., *Endocrinology*, 122: 2920-2926 (1988). Among its most striking effects in hypopituitary (GH-deficient) subjects is accelerated linear growth of bone growth plate cartilage resulting in increased stature. Kaplan, *Growth Disorders in Children and Adolescents* (Springfield, Ill.: Charles C. Thomas, 1964).

In 1957, the mechanism of GH action was postulated as being due to GH inducing production of somatomedins (subsequently identified and named IGF-I) in the liver, which travel via the circulation to produce all the effects of GH. Salmon and Daughaday, *J. Lab. Clin. Med.*, 49: 825-836 (1957). Many studies investigating the relationships among GH, IGF-I, cartilage, cultured human fibroblasts, skeletal muscle, and growth have supported this somatomedin hypothesis. See, e.g., Phillips and Vassilopoulou-Sellin, *N. Engl. J. Med.*, 302: 372-380; 438-446 (1980); Vetter et al., *J. Clin. Invest.*, 7: 1903-1908 (1986); Cook et al., *J. Clin. Invest.*, 81: 206-212 (1988); Isgaard et al., *Endocrinology*, 123: 2605-2610 (1988); Schoenle et al., *Acta Endocrin.*, 108: 167-174 (1985).

Another theory holds that GH has a direct effect on chondrocytes that is not dependent on circulating IGF-I. For example, several *in vivo* studies have demonstrated longitudinal long bone growth in rats receiving hGH injected directly into the tibial growth plate [Isaksson et al., *Science*, 216: 1237-1239 (1982); Russell and Spencer, *Endocrinology*, 116: 2563-2567 (1985)] or the arterial supply to a limb [Schlechter et al., *Am. J. Physiol.*, 250: E231-235 (1986)]. Additionally it was found that proliferation of cultured lapine ear and rib chondrocytes in culture is stimulated by hGH [Madsen et al., *Nature*, 304: 545-547 (1983)], this being consistent with a direct GH effect or with an indirect effect of GH mediated by local GH-dependent IGF-I production. Such an autocrine or paracrine model for stimulation of growth has been supported by various lines of experimental evidence. Schlechter et al., *Proc. Natl. Acad. Sci. USA*, 83: 7932-7934 (1986); Nilsson et al., *Calcif. Tissue Int.*, 40: 91-96 (1987). Nilsson et al. showed that while unilateral arterial infusion of IGF-I did not produce a tibial longitudinal bone growth response in hypophysectomized rats, infusion of hGH did induce such growth. Moreover, the influence of GH on the functional maturation of human fetal islet cells *in vitro* could not be reproduced by adding IGF-I, suggesting a direct rather than a somatomedin-mediated action of GH for these particular cells. Otonkoski et al., *Diabetes*, 37: 1678-1683 (1988).

A third theory for GH and IGF-I actions is that GH promotes differentiation of stem cells, rendering them responsive to stimulation of proliferation by IGF-I. Green et al., *Differentiation*, 29: 195-198 (1985). Although support for this model of GH acting to produce IGF-I locally, called the dual effector theory, has been obtained for certain cell types [Zezulak and Green, *Science*, 233: 551-553 (1986)], its application to skeletal growth has not been established. It has been found that both GH and testosterone could stimulate skeletal growth in the hypophysectomized prepubertal lamb without alteration of circulating IGF-I concentrations, the results not precluding the possibility that the growth-promoting effect of GH was affected by local actions at the site of osteogenesis. Young et al., *J. Endocrin.*, 121: 563-570 (1989). Also, GH has been reported to stimulate tibial epiphyseal plate width in the hypophysectomized rat without increasing circulating IGF-I concentrations. Orłowski and Chernausk, *Endocrinol.*, 123: 44-49 (1988).

More recently, a study was undertaken to reproduce the "direct" *in vitro* GH effect on epiphyseal and articular chondrocytes to determine whether this effect is mediated by IGF-I in a local autocrine or paracrine fashion. Trippel et al., *Pediatr. Res.*, 25: 76-82 (1989). Human GH was found not to stimulate rabbit articular or epiphyseal chondrocytes or bovine epiphyseal chondrocytes, whereas IGF-I stimulated both mitotic and differentiated cell functions in both epiphyseal and articular chondrocytes. The authors state that

the data suggest that the role of IGF-I in skeletal development is complex and may be diverse both in the cellular functions it regulates and the cell populations regulated, requiring further investigation to define the relationship of IGF-I to GH.

It has been reported that the growth response to co-addition of GH and IGF-I was not statistically different from that of GH alone when body weight gain, bone length, or tibial epiphyseal cartilage width was measured. Skottner et al., *J. Endocr.*, supra [iv infusion of bGH (10 mu/day) for 8 days and met-IGF-I (with specific activity of 3400 U/mg, 120 µg/day) for the last 4 days]; Isgaard et al., *Am. J. Physiol.*, 250: E367-E372 (1986) [5 µg of IGF-I and 1 µg of hGH injected locally daily for 5 days]. It was also found that IGF-I, when injected or infused subcutaneously or infused intravenously, is a weak growth promoter in hypophysectomized rats compared with hGH, even when infused in combination with small amounts of hGH. Robinson and Clark, *Acta Paediatr. Scand. Supp.*, 347: 93-103 (1988).

As regards osteoblast-like cells in culture, direct stimulation of their proliferation by hGH is at least partially mediated by IGF-I-like immunoreactivity [Ernst and Froesch, *Biochem. Biophys. Res. Commun.*, 151: 142-147 (1988)]; the authors found that IGF-I and hGH had additive effects on osteoblast proliferation only when the exogenous IGF-I concentration exceeded that of endogenously produced IGF-I by a large margin. Another *in vitro* study showed that purified human and synthetic IGF-I stimulated adult articular chondrocyte DNA and proteoglycan synthesis; GH had no effect on either process; and GH added in combination with IGF-I increased proteoglycan, cell-associated proteoglycan, and keratan sulfate synthesis over levels observed with IGF-I alone. Smith et al., *J. Orthop. Res.*, 7: 198-207 (1989). Separate administration of hGH and IGF-I was found to enhance human granulopoiesis, with the effect of hGH on marrow myeloid progenitors apparently mediated by paracrine IGF-I. Merchav et al., *J. Clin. Invest.*, 81: 791-797 (1988). Merchav et al. also noted that myeloid colony formation was significantly enhanced in cultures stimulated with combined limiting concentrations of both IGF-I and hGH, whereas combined maximal concentrations of both peptides did not exert an additive effect.

Also, based on recent immunohistochemical data regarding the GH receptor, it has been suggested that GH may act independently of or synergistically with hepatic IGF-I in carrying out its growth-promoting role in the gastrointestinal tract. Lobie et al., *Endocrinol.*, 126: 299-306 (1990). It has been shown that pretreatment of hypophysectomized rats with GH, but not with IGF-I, promotes the formation of chondrocyte colonies and makes the chondrocytes susceptible to IGF-I *in vitro*. Lindahl et al., *Endocrinol.*, 121: 1070-1075 (1987). The authors suggest that GH induces colony formation by IGF-I-independent mechanisms and that IGF-I is a second effector in GH action. Further, treatment of hypophysectomized animals with a single dose of hGH restored IGF-I mRNA in parenchymal and in non-parenchymal cells to the extent found in intact animals. van Neste et al., *J. Endocr.*, 119: 69-74 (1988).

However, it has also been reported that IGF-I directly suppresses GH gene transcription and GH secretion at the pituitary level in an inhibitory feedback control mechanism. Namba et al., *Endocrinol.*, 124: 1794-1799 (1989); Yamashita et al., *J. Biol. Chem.*, 262: 13254-13257 (1987). Additionally, it was reported that the maximum stimulation of glucose metabolism in 3T3 adipocytes achieved by hGH is only a fraction of that produced by various IGFs, indicating that extracellular IGFs do not mimic the effects of

hGH on glucose metabolism in these adipocytes. Schwartz et al., *Proc. Natl. Acad. Sci. USA*, 82: 8724-8728 (1985). Moreover, human GH was found not to enhance further the IGF-I-stimulated Leydig cell steroidogenesis. Horikawa et al., *Eur. J. Pharmacol.*, 166: 87-94 (1989). Another negative finding was that the combination of chick growth hormone and human IGF-I did not stimulate cell proliferation and metabolic activity of cultured epiphyseal growth plate chondrocytes above human IGF-I alone. Rosselot et al., *The Endocrine Society 72nd Annual Meeting*, abstract 202, p. 75, of Program and Abstracts released prior to the meeting in Atlanta, Ga. on Jun. 20-23, 1990. It has also been reported that both hGH and hIGF-I can promote growth in the mutant dwarf rat, but they differ both quantitatively and qualitatively in their pattern of actions. Skottner et al., *Endocrinology*, supra. Additionally, a loss of IGF-I receptors in cultured bovine articular chondrocytes was found after pre-exposure of the cells to pharmacological doses of either hGH or bGH. Watanabe et al., *J. Endocr.*, 107: 275-283 (1985). The necessity for large amounts of GH is attributed to extremely low affinity of GH binding sites on these cells. The authors speculate that living organisms have a protection mechanism to avoid unnecessary overgrowth of the body resulting in down-regulation of the IGF-I receptors.

U.S. Pat. No. 4,857,505 issued Aug. 15, 1989 discloses use of an adduct of a growth hormone, growth factor, IGF-I, or fragment thereof covalently bonded to an activated polysaccharide for increased half-life, increased weight gain in animals, and increased milk production.

Known side effects of hGH treatment include sodium retention and expansion of extracellular volume [Ikko et al., *Acta Endocrinol.* (Copenhagen), 32: 341-361 (1959); Biglieri et al., *J. Clin. Endocrinol. Metab.*, 21: 361-370 (1961)], as well as hyperinsulinemia and hyperglycemia. The major apparent side effect of IGF-I is hypoglycemia. Guler et al., *Proc. Natl. Acad. Sci. USA*, 1989, supra.

Various methods for formulating proteins or polypeptides have been described. These include EP 267,015 published May 11, 1988; EP 308,238 published Mar. 22, 1989; and EP 312,208 published Apr. 19, 1989, which disclose formulation of a polypeptide growth factor having mitogenic activity, such as transforming growth factor-β (TGF-β), in a polysaccharide such as methylcellulose; EP 261,599 published Mar. 30, 1988 disclosing human topical applications containing growth factors such as TGF-β; EP 193,917 published Sep. 10, 1986, which discloses a slow-release composition of a carbohydrate polymer such as a cellulose and a protein such as a growth factor; GB Pat. No. 2,160,528 granted Mar. 9, 1988, describing a formulation of a bioactive protein and a polysaccharide; and EP 193,372 published Sep. 3, 1986, disclosing an intranasally applicable powdery pharmaceutical composition containing an active polypeptide, a quaternary ammonium compound, and a lower alkyl ether of cellulose. See also U.S. Pat. No. 4,609,640 issued Sep. 2, 1986 disclosing a therapeutic agent and a water-soluble chelating agent selected from polysaccharides, celluloses, starches, dextroses, polypeptides, and synthetic polymers able to chelate Ca and Mg; and JP 57/026625 published Feb. 12, 1982 disclosing a preparation of a protein and water-soluble polymer such as soluble cellulose.

EP 123,304 published 31 Oct. 1984 discloses mixing tissue plasminogen activator with gelatin or Polysorbate 80, and JP 58/224,687 published 27 Dec. 1983 [Toryo, *Chem. Abs.*, 100: 197765r (1984)] discloses formulation of plasminogen-activating enzyme with PEG-3-sorbitan monooleate, dextrin, gelatin, mannitol, dextran, glycine, and hydrolyzed gelatin for stability.

Furthermore, preservatives containing a quaternary ammonium salt have been added to chemical drug formulations to prevent growth of bacteria. See, e.g., Remington's *Pharmaceutical Sciences*, 18th edition (definition of benzethonium chloride), Martindale, *The Extra Pharmacopoeia*, 28th edition (p.550, entry on benzethonium chloride), *United States Pharmacopoeia*, 22nd edition (pp. 146-147, entries on benzethonium chloride topical solution and tincture), *Handbook on Injectable Drugs*, 5th edition (p. 246, entry on diphenhydramine HCl, which contains 0.1% benzethonium chloride; pp. 396-397, entry on ketamine HCl, which contains 0.1 mg/ml of benzethonium chloride; and pp. 695-696, entry on Vidarabine, which contains 0.1 mg benzethonium chloride). Another example is the formulation of octreotide in benzalkonium chloride for nasal application as described in GB Appln. 2,193,891 published Feb. 24, 1988. The preservatives have been used in parenteral formulations at low concentrations, and in antiseptic washes for wound care at higher concentrations. In addition, a mixture of a physiologically active polypeptide with a quaternary ammonium compound and a lower alkyl ether of cellulose is disclosed, wherein the quaternary ammonium compound is added to improve stability and preservability. EP 193,372.

It is an object of the present invention to provide a formulation of IGF-I that is more potent as a hypoglycemic agent and better absorbed when administered subcutaneously than an existing IGF-I formulation.

It is another object to provide an IGF-I formulation useful along with a GH formulation in the treatment of patients.

It is yet another object to provide an IGF-I formulation useful for preparing a formulation of both IGF-I and GH, and methods for such preparation.

These and other objects will be apparent to those of ordinary skill in the art.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides an IGF-I-containing composition comprising about 2-20 mg/ml of IGF-I, about 2-50 mg/ml of an osmolyte, about 1-15 mg/ml of a stabilizer, and a buffered solution at about pH 5-5.5. This composition has been found, preferably at pH 5.4, to be more potent as a hypoglycemic agent than a pH 6.0 citrate-buffered IGF-I formulation when administered subcutaneously, and to be better absorbed than the pH 6.0 formulation when administered subcutaneously.

Therefore, in a further aspect, the invention supplies a method for treating hyperglycemic disorders comprising administering to a mammal having such disorder an effective amount of the above IGF-I formulation, preferably subcutaneously.

In a still further aspect, the invention provides a process for preparing a formulation comprising mixing the above composition with a buffered solution comprising GH at pH about 6 in a dose (mg) ratio of from about 2:1 to 100:1 IGF-I:GH up to a dose no greater than about 5 mg/ml GH.

The literature shows that the role of IGF-I in skeletal development in conjunction with GH is complex, and evidence supporting various theories of GH action is contradictory and inconclusive. If GH acts via production of circulating IGF-I (the somatomedin hypothesis), then a maximal dose of GH would not be expected to be enhanced by administering IGF-I systemically. If GH acts locally to produce IGF-I, then it is unlikely that the high local concentrations of IGF-I predicted by this second theory could be reproduced by administering IGF-I systemically. If some

actions of GH do not involve IGF-I generation, then adding GH might enhance the effect of IGF-I. However, in view of the confusion surrounding which of these three unresolved theories is correct, there was no clear basis to predict the outcome on body and bone growth of administering to a mammal a combination of GH and IGF-I.

Unexpectedly, a significantly greater daily body weight gain, increased longitudinal bone growth, and enhanced epiphyseal width of the tibia were achieved after combination treatment with IGF-I and GH as compared with the same doses of each of IGF-I and GH alone. Further, the additive effect of IGF-I and GH was not seen for all tissues, indicating a selectivity for whole body growth, bone, and cartilage. Moreover, IGF-I enhanced the growth-promoting effect of GH even at the maximum effective dose of GH, and can further enhance a low dose of GH to produce a maximal growth response. Thus, IGF-I may be used in combination with lower doses of GH to increase growth of those immature patients that have reached their maximum growth rate after treatment with maximal doses of GH alone and then experienced a fall in their annualized growth rate. This is an effect that is widespread in all growth-deficient patients after several months of treatment. The combination could also be used to maximize the growth response in patients who present late in development with growth retardation, and only have a few years of therapeutic intervention potential. Additionally, the combination can be used to treat those patients who exhibit side effects such as diabetogenic symptoms with maximum doses of GH or hypoglycemia with maximum doses of IGF-I.

In addition, the IGF-I formulation herein can be used by itself in the treatment of hyperglycemic disorders as noted above.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1(A and B) represent graphs of cumulative body weight gain over seven days for each group of treated hypophysectomized adult male rats for two replicate studies 1 and 2, respectively, performed one month apart (mean±SD).

FIG. 2 shows a bar graph of the increase in width of epiphyseal bone growth plate after seven days of hGH and/or IGF-I treatment of hypophysectomized rats (mean±SD).

FIGS. 3(A and B) represent graphs of longitudinal bone growth and epiphyseal plate width (a separate study from FIG. 2), respectively, for each group of hypophysectomized rats treated with hGH alone, or IGF-I or des(1-3)-IGF-I alone or in combination with hGH (mean±SD).

FIG. 4 illustrates a graph of weight gain in hypophysectomized rats over one week as a function of hGH concentration (log dose), where rats were treated with IGF-I (2.4 mg/kg/day) using minipumps and with hGH daily injections (mean±SD).

FIG. 5 illustrates a graph of weight gain in dwarf rats over one week as a function of hGH concentration (log dose), where rats were treated with IGF-I (1.2 mg/kg/day) using minipumps and with hGH daily injections (mean±SD).

FIG. 6 depicts a graph of weight gain in hypophysectomized rats using three different doses of IGF-I or des(1-3)-IGF-I infused subcutaneously by minipumps for seven days (mean±SD).

FIG. 7 depicts a graph of weight gain in hypophysectomized rats using three different doses of hGH injected daily subcutaneously for seven days (mean±SD).

FIG. 8A and 8B illustrate bar graphs of the growth rate in cm/year of patients of various growth inhibition etiologies having had either no previous treatment (Prev Rx No) or previous treatment (Prev Rx Yes) with hGH. N indicates the number of patients at the indicated dose level of hGH given in units of mg/kg. FIG. 8A is the data for the first year of hGH treatment and FIG. 8B is for the second year of hGH treatment.

FIG. 9 illustrates bar graphs of the annualized (12-month) growth rate in cm/year of patients treated with the indicated dose of hGH in the 1-2, 3-5, 6-8, 9-11, 12-14, 15-17, and more than 17 year ranges. N indicates the number of patients in each age group.

FIG. 10 illustrates the percent change in blood glucose levels with time when either 750 (open) or 250 (filled) µg/rat of IGF-I is given subcutaneously in two different formulations: pH 6.0 citrate (circles) and pH 5.0 acetate (triangles).

FIG. 11 illustrates the percent change in blood glucose levels with time when either 450 (open) or 150 (filled) µg/rat of IGF-I is given subcutaneously in two different formulations: pH 6.0 citrate (circles) and pH 5.0 acetate (triangles).

FIG. 12 illustrates the percent change in blood glucose levels with time when 150 µg/rat of IGF-I in either the pH 6.0 citrate formulation (open) or pH 5.0 acetate formulation (filled) is given subcutaneously (circles) or intravenously (triangles).

FIG. 13 illustrates the percent change in blood glucose levels with time when either 450 (open) or 150 (filled) µg/rat of IGF-I is given subcutaneously in two different formulations: pH 6.0 citrate (circles) and pH 5.0 citrate (triangles).

FIG. 14 illustrates the percent change in blood glucose levels with time when 150 µg/rat of IGF-I in either the pH 6.0 citrate formulation (open) or pH 5.4 acetate formulation (filled) is given subcutaneously (circles) or intravenously (triangles).

FIG. 15 shows the absolute glucose levels (mg %) when 150 µg/rat of IGF-I is given in four different formulations: pH 6.0 citrate, administered subcutaneously (open circles), pH 5.4 acetate, administered subcutaneously (filled circles), pH 6.0 citrate, administered iv (open triangles), and pH 5.4 acetate, administered iv (filled triangles).

FIG. 16 shows the percent change in blood glucose levels with time using the four different formulations, with the symbols being the same as for FIG. 15.

FIG. 17 shows the level of plasma IGF-I (ng/ml) versus time after injection of IGF-I (150 µg/rat iv) using either pH 6.0 citrate formulation (open circles) or pH 5.4 acetate formulation (filled circles).

FIG. 18 is the same as FIG. 17 except that the IGF-I was administered subcutaneously instead of intravenously.

FIG. 19 shows the absolute glucose levels (mg %) when a pH 6.0 citrate IGF-I formulation (circles) was administered subcutaneously in doses of 150 µg (open) and 450 µg (filled) and when a pH 5.4 acetate IGF-I formulation (squares) was administered subcutaneously in doses of 50 µg (open) and 150 µg (filled).

FIG. 20 shows the percent change in blood glucose levels with time using the four different formulations, with the symbols being the same as for FIG. 19.

FIG. 21 shows the level of plasma IGF-I (ng/ml) versus time after subcutaneous injection of the four different formulations, with the symbols being the same as for FIG. 19.

FIG. 22 shows the percent change in blood glucose levels with time in normal rats when a pH 6.0 citrate IGF-I

formulation (triangles) was administered subcutaneously in doses of 900 µg (open) and 300 µg (filled) and when a pH 5.4 acetate IGF-I formulation (circles) was administered subcutaneously in doses of 300 µg (open) and 100 µg (filled).

FIG. 23 shows the percent change in blood glucose levels with time in normal rats when a pH 6.0 citrate IGF-I formulation (open circles) was administered subcutaneously in a dose of 300 µg, when a pH 5.4 acetate IGF-I formulation (filled circles) was administered subcutaneously in a dose of 300 µg, and when a pH 5.4 acetate IGF-I and GH co-mixed formulation (triangles) was administered subcutaneously in doses of 100 µg (open) and 10 µg (filled) of hGH and 300 µg IGF-I.

FIG. 24 shows the level of plasma IGF-I (ng/ml) versus time after subcutaneous injection of the four different formulations, with the symbols being the same as for FIG. 22 for the comix, and with the filled circles being the pH 6.0 IGF-I formulation and the filled squares being the pH 5.4 IGF-I formulation alone.

FIG. 25 shows the body weight gain (g) in dw/dw rats administered by subcutaneous injection excipient (open triangles), IGF-I at a dose of 600 µg (filled triangles), rhGH at a dose of 30 µg (filled circles), rhGH at a dose of 120 µg (filled squares), IGF-I+hGH at doses of 600 µg and 30 µg IGF-I and hGH, respectively (open circles), and IGF-I +hGH at doses of 600 µg and 120 µg IGF-I and hGH, respectively (open squares).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. Definitions

As used herein, "mammal" signifies humans as well as animals, and includes animals of economic importance such as bovine, ovine, and porcine animals. The preferred mammal herein is a human. The term "non-adult" refers to mammals that are from perinatal age (such as low-birth-weight infants) up to the age of puberty, the latter being those that have not yet reached full growth potential.

As used herein, "IGF-I" refers to insulin-like growth factor from any species, including bovine, ovine, porcine, equine, and preferably human, in native-sequence or in variant form, and from any source, whether natural, synthetic, or recombinant. Preferred herein for animal use is that form of IGF-I from the particular species being treated, such as porcine IGF-I to treat pigs, ovine IGF-I to treat sheep, bovine IGF-I to treat cattle, etc. Preferred herein for human use is human native-sequence, mature IGF-I, more preferably without a N-terminal methionine, prepared, e.g., by the process described in EP 230,869 published Aug. 5, 1987; EP 128,733 published Dec. 19, 1984; or EP 288,451 published Oct. 26, 1988. More preferably, this native-sequence IGF-I is recombinantly produced and is available from Genentech, Inc., South San Francisco, Calif. for clinical investigations. Also preferred for use is IGF-I that has a specific activity greater than about 14,000 units/mg as determined by radioreceptor assay using placenta membranes, such as that available from KabiGen AB, Stockholm, Sweden.

The most preferred IGF-I variants are those described in PCT WO 87/01038 published Feb. 26, 1987 and in PCT WO 89/05822 published Jun. 29, 1989, i.e., those wherein at least the glutamic acid residue is absent at position 3 from the N-terminus of the mature molecule or those having a deletion of up to five amino acids at the N-terminus. The most preferred variant has the first three amino acids from the

N-terminus deleted (variously designated as brain IGF, tIGF-I, des(1-3)-IGF-I, or des-IGF-I).

As used herein, "GH" refers to growth hormone from any species, including bovine, ovine, porcine, equine, and preferably human, in native-sequence or in variant form, and from any source, whether natural, synthetic, or recombinant. Preferred herein for animal use is that form of GH from the particular species being treated, such as porcine GH to treat pigs, ovine GH to treat sheep, bovine GH to treat cattle, etc. Preferred herein for human use is human native-sequence, mature GH with or without a methionine at its N-terminus. Also preferred is recombinant hGH, i.e., that produced by means of recombinant DNA technology. More preferred is methionyl human growth hormone (met-hGH) produced in *E. coli*, e.g., by the process described in U.S. Pat. No. 4,755,465 issued Jul. 5, 1988 and Goeddel et al., *Nature*, 282: 544 (1979). Met-hGH, which is sold under the trademark PROTROPIN® by Genentech, Inc., is identical to the natural polypeptide, with the exception of the presence of an N-terminal methionine residue. This added amino acid is a result of the bacterial protein synthesis process.

Another preferred hGH for human use is a recombinant hGH (rhGH), available to clinical and research investigators from Genentech, Inc. under the trademark Nutropin®, and commercially available from Eli Lilly, that lacks this methionine residue and has an amino acid sequence identical to that of the natural hormone. See Gray et al., *Biotechnology*, 2: 161 (1984). Both met-hGH and rhGH have equivalent potencies and pharmacokinetic values. Moore et al., supra.

As used herein, the term "growth" refers to the dynamics of statural growth experienced by an individual during infancy, childhood, and adolescence as depicted by a normal growth curve. Thus, growth herein refers to the growth of linear-producing bone plate driven by chondrocytes, as distinguished from the growth of osteoblast cells, derived from a different part of the bone. Restoration of normal growth patterns would allow the patient to approach a more satisfactory growth curve. Examples of patients that are relatively resistant to GH but require treatment to induce an anabolic effect include those with Turner's Syndrome, GH-deficient children who grow poorly in response to GH treatment, children who experience a slowing or retardation in their normal growth curve about 2-3 years before their growth plate closes, so that GH administered alone would no longer increase growth of the children, so-called short normal children, and patients where the IGF-I response to GH has been blocked chemically (i.e., by glucocorticoid treatment) or by a natural condition such as in adult patients or in catabolic patients where the IGF-I response to GH is naturally reduced.

B. Modes for Carrying Out the Invention

The IGF-I and GH are directly administered to the mammal by any suitable technique, including parenterally, intranasally, intrapulmonarily, or orally. They need not be administered by the same route and can be administered locally or systemically. The specific route of administration will depend, e.g., on the medical history of the patient, including any perceived or anticipated side or reduced anabolic effects using hGH or IGF-I alone, and the growth defect to be corrected. Examples of parenteral administration include subcutaneous, intramuscular, intravenous, intraarterial, and intraperitoneal administration. Most preferably, the administration is by continuous infusion (using, e.g., minipumps such as osmotic pumps), or by injection (using, e.g., intravenous or subcutaneous means). Preferably, the administration is subcutaneous for both

IGF-I and GH. The administration may also be as a single bolus or by slow-release depot formulation.

Most preferably, the IGF-I is administered by injection, most preferably subcutaneously, at a frequency of, preferably, one-half, once, twice, or three times daily, most preferably daily. Most preferably, the GH is administered daily subcutaneously by injection. Co-injection of the IGF-I and GH is an optimal drug delivery system to ensure normal growth of the mammal, i.e., no overgrowth. Hence, delivery of hGH and IGF-I by injection will be the preferred form of administration for body growth/anabolism, as it will preserve normal body proportions.

In addition, the IGF-I is suitably administered together with its binding protein, for example, BP53, which is described in WO 89/09268 published Oct. 5, 1989, which is equivalent to U.S. Ser. No. 07/171,623 filed Mar. 22, 1988, now U.S. Pat. No. 5,258,287, and by Martin and Baxter, *J. Biol. Chem.*, 261: 8754-8760 (1986), the disclosures of which are incorporated herein by reference. This administration may be by the method described in U.S. Pat. No. 5,187,151. This protein is an acid-stable component of about 53 Kd on a non-reducing SDS-PAGE gel of a 125-150 Kd glycoprotein complex found in human plasma that carries most of the endogenous IGFs and is also regulated by GH. The IGF-I is also suitably coupled to a receptor or antibody or antibody fragment for administration. Similarly, the GH can be delivered coupled to another agent such as an antibody, an antibody fragment, or one of its binding proteins.

The IGF-I and GH composition(s) to be used in the therapy will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with hGH or IGF-I alone or growth retardation after continuous GH treatment), the site of delivery of the IGF-I and GH composition(s), the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amounts" of each component for purposes herein are thus determined by such considerations and must be amounts that enhance growth of the treated patient over growth enhancement that is obtained using the same amount of IGF-I or GH individually.

As a general proposition, the total pharmaceutically effective amount of each of the IGF-I and GH administered parenterally per dose will be in the range of about 1 µg/kg/day to 100 mg/kg/day of patient body weight, although, as noted above, this will be subject to a great deal of therapeutic discretion. More preferably, this dose is at least 0.1 mg/kg/day, and most preferably at least 1 mg/kg/day for each hormone. If given continuously, the IGF-I and GH are each typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a minipump. An intravenous bag solution may also be employed. The key factor in selecting an appropriate dose is the result obtained, as measured by increases in body weight gain, lean body mass, or statutory growth approximating the normal range, or by other criteria for measuring growth as defined herein as are deemed appropriate by the practitioner.

The IGF-I and GH are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include poly(lactides) (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (U. Sidman

et al., *Biopolymers*, 22, 547-556 (1983)), poly(2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.*, 15: 167-277 (1981), and R. Langer, *Chem. Tech.*, 12: 98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D(-)-3-hydroxybutyric acid (EP 133, 988). Sustained-release IGF-I compositions also include liposomally entrapped IGF-I. Liposomes containing IGF-I are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. U.S.A.*, 82: 3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. U.S.A.*, 77: 4030-4034 (1980); EP 36,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appln. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal IGF-I and GH therapy.

For parenteral administration, in one embodiment, the IGF-I and GH are formulated generally by mixing each at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the IGF-I and GH each uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; nonionic surfactants such as polysorbates, poloxamers, or PEG; and/or neutral salts, e.g., NaCl, KCl, MgCl₂, CaCl₂, etc.

The IGF-I and GH are each typically formulated individually in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 4.5 to 8. Full-length IGF-I is generally stable at a pH of no more than about 6.5; des(1-3)-IGF-I is stable at about 3.2 to 5; hGH is stable at a higher pH of about 5.5-9. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of IGF-I or GH salts.

In addition, the IGF-I and GH, preferably the full-length IGF-I, are suitably formulated together in a suitable carrier

vehicle to form a pharmaceutical composition that does not contain cells. In one embodiment, the buffer used for formulation will depend on whether the composition will be employed immediately upon mixing or stored for later use. If employed immediately after mixing, a mixture of full-length IGF-I and GH can be formulated in mannitol, glycine, and phosphate, pH 4-6. If this mixture is to be stored, it is formulated in a buffer at a pH of about 5-6, such as acetate or citrate, with a surfactant that increases the solubility of the GH at this pH, such as 0.1-0.2% polysorbate 20 or poloxamer 188. The final preparation may be a stable liquid or lyophilized solid.

In one particularly preferred embodiment, the composition comprises IGF-I and GH in a weight ratio of IGF-I:GH of between about 2:1 and 100:1 (w/w), about 0.05-0.3 mM of an osmolyte, preferably an inorganic salt and/or sugar alcohol, about 0.1-10 mg/ml of at least one stabilizer, about 1-5 mg/ml of a surfactant, and about 5 to 100 mM of a buffer at about pH 5-6. The more preferred amounts of IGF-I and GH in this composition are about 2-20 mg/ml IGF-I and about 0.2-10 mg/ml GH. The more preferred weight ratio of IGF-I:GH is about 3:1 to 50:1, more preferably about 3:1 to 30:1, and still more preferably about 3:1 to 25:1, and most preferably about 5:1 to 20:1.

An "osmolyte" refers to an isotonic modifier or osmotic adjuster that lends osmolality to the buffered solution. Osmolality refers to the total osmotic activity contributed by ions and nonionized molecules to a solution. Examples include inorganic salts such as sodium chloride and potassium chloride, mannitol, polyethylene glycols (PEGs), polypropylene glycol, glycine, sucrose, glycerol, amino acids, and sugar alcohols such as mannitol known to the art that are generally regarded as safe (GRAS). The preferred osmolyte herein is sodium chloride or potassium chloride.

The "stabilizer" is any compound that functions to preserve the active ingredients in the formulation, i.e., GH and IGF-I, so that they do not degrade or otherwise become inactive over a reasonable period of time or develop pathogens or toxins that prevent their use. Examples of stabilizers include preservatives that prevent bacteria, viruses, and fungi from proliferating in the formulation, anti-oxidants, or other compounds that function in various ways to preserve the stability of the formulation.

For example, quaternary ammonium salts are useful stabilizers in which the molecular structure includes a central nitrogen atom joined to four organic (usually alkyl or aryl) groups and a negatively charged acid radical. These salts are useful as surface-active germicides for many pathogenic non-sporulating bacteria and fungi and as stabilizers. Examples include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyl dimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of stabilizers include aromatic alcohols such as phenol and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, and m-cresol. The most preferred stabilizer herein is phenol or benzyl alcohol.

The stabilizer is included in a stable liquid form of the GH and IGF-I formulation, but not in a lyophilized form of the formulation. In the latter case, the stabilizer is present in the bacteriostatic water for injection (BWFI) used for reconstitution. The surfactant is also optionally present in the reconstitution diluent.

The "inorganic salt" is a salt that does not have a hydrocarbon-based cation or anion. Examples include sodium chloride, ammonium chloride, potassium chloride,

magnesium chloride, calcium chloride, sodium phosphate, calcium phosphate, magnesium phosphate, potassium phosphate, ammonium phosphate, sodium sulfate, ammonium sulfate, potassium sulfate, magnesium sulfate, calcium sulfate, etc. Preferably, the cation is sodium and the anion is chloride or sulfate, and the most preferred inorganic salt is potassium chloride or sodium chloride.

The "surfactant" acts to increase the solubility of the IGF-I and GH at a pH about 4-7. It is preferably a nonionic surfactant such as a polysorbate, e.g., polysorbates 20, 60, or 80, a poloxamer, e.g., poloxamer 184 or 188, or any others known to the art that are GRAS. More preferably, the surfactant is a polysorbate or poloxamer, more preferably a polysorbate, and most preferably polysorbate 20.

The "buffer" may be any suitable buffer that is GRAS and confers a pH of 5-6 on the GH+IGF-I formulation and a pH of about 5-5.5 on the IGF-I formulation. Examples include acetic acid salt buffer, which is any salt of acetic acid, including sodium acetate and potassium acetate, succinate buffer, phosphate buffer, citrate buffer, or any others known to the art to have the desired effect. The most preferred buffer is sodium acetate, optionally in combination with sodium citrate.

The most preferred composition containing both IGF-I and GH is the following: about 7-10 mg/ml of IGF-I, about 0.2-1.5 mg/ml of GH at a weight ratio of IGF-I:GH of about 3:1 to 20:1, about 5-7 mg/ml of sodium chloride, about 0.1-3 mg/ml of phenol and/or about 6-10 mg/ml of benzyl alcohol, about 1-3 mg/ml of polysorbate, about 2.5-4 mg/ml of sodium acetate, and about 0.1-1 mg/ml of sodium citrate, pH about 5.4.

The final formulation, if a liquid, is preferably stored at a temperature of about 2°-8° C. for up to about four weeks. Alternatively, the formulation can be lyophilized and provided as a powder for reconstitution with water for injection that is stored as described for the liquid formulation.

IGF-I and GH to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes).

Therapeutic IGF-I and GH compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

IGF-I and GH ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous GH solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized GH using bacteriostatic Water-for-Injection.

It was found that when whole body weight is to be increased without concomitant increases in kidney or thymus weights, the GH+IGF-I formulation is preferably injected. If, however, the object is to affect the body composition of the patient or to increase not only whole body weight but also selected organs such as the thymus and kidney, for example, in patients that are immunodeficient (such as AIDS patients) or in patients with kidney disorders (such as ischemic or nephrotoxic dysfunction or chronic or acute renal insufficiency), the GH+IGF-I formulation is preferably infused to the patient.

The formulation containing both the IGF-I and GH can be made by many different methods. One method comprises mixing an IGF-I-containing composition (having osmolyte, stabilizer, and buffer as described below) with a buffered

solution comprising GH at a pH about 6 in a dose (mg) ratio of from about 2:1 to 100:1 IGF-I:GH up to a dose no greater than about 5 mg/ml of GH. Preferably, this buffered solution contains about 1-10 mg/ml of GH in about 5-15 mg/ml of an inorganic salt, about 1-5 mg/ml of a stabilizer, about 1-5 mg/ml of a surfactant, and sodium citrate buffer at pH about 6. More preferably, the liquid GH formulation contains about 3-5 mg/ml GH, about 8-9 mg/ml sodium chloride, about 1-3 mg/ml phenol, about 1-3 mg/ml polysorbate 20, and about 10 mM sodium citrate, pH about 6.

The IGF-I-containing solution useful for administering IGF-I separately from GH and for admixing with the GH solution as described above is as follows: about 2-20 mg/ml of IGF-I, about 2-50 mg/ml of an osmolyte, about 1-15 mg/ml of at least one stabilizer, and a buffer (preferably an acetic acid salt buffer, and most preferably sodium acetate) in an amount such that the composition has a pH of about 5-5.5. The osmolyte, stabilizer, and buffer, and the preferred compounds within these categories are defined above. Optionally, the formulation may also contain a surfactant selected from the types described above, preferably in an amount of about 1-5 mg/ml, more preferably about 1-3 mg/ml.

In a preferred embodiment, the osmolyte is an inorganic salt at a concentration of about 2-10 mg/ml or a sugar alcohol at a concentration of about 40-50 mg/ml, the stabilizer is benzyl alcohol, phenol, or both, and the buffered solution is an acetic acid salt buffered solution. More preferably, the osmolyte is an inorganic salt, most preferably sodium chloride.

In an even more preferred formulation, the amount of IGF-I is about 8-12 mg/ml, the amount of sodium chloride is about 5-6 mg/ml, the stabilizers are benzyl alcohol in an amount of about 8-10 mg/ml and/or phenol in an amount of about 2-3 mg/ml, and the buffer is about 50 mM sodium acetate so that the pH is about 5.4. Optionally, the formulation contains polysorbate as a surfactant in an amount of about 1-3 mg/ml. A 50-mM acetate concentration in the starting IGF-I solution before mixing with GH ensures that the final pH will not vary significantly from 5.4 in the final IGF-I/GH mixture to maintain good solubility of both proteins over a wide mixing ratio range. However, a broader pH range in terms of stability of both proteins is from about 5 to about 6.

The IGF-I formulation of this invention can be used to treat any condition that would benefit from treatment with IGF-I, including, for example, diabetes, chronic and acute renal disorders, such as chronic renal insufficiency, necrosis, etc., obesity, hyperinsulinemia, GH-insufficiency, Turner's syndrome, short stature, undesirable symptoms associated with aging such as increasing lean mass to fat ratios, immuno-deficiencies including increasing CD4 counts and increasing immune tolerance, catabolic states associated with wasting, etc., Laron dwarfism, insulin resistance, and so forth.

This IGF-I formulation especially was found by itself to have increased potency in treating mammals, especially humans, with hyperglycemic disorders by reducing their glucose levels. It was also found to increase the mammal's absorbance of the IGF-I if administered subcutaneously. For purposes herein, "hyperglycemic disorders" refers to all forms of diabetes, such as type I and type II diabetes, as well as hyperinsulinemia and hyperlipidemia, e.g., obese subjects. The preferred disorder is diabetes, especially type II diabetes:

For treating these various conditions, IGF-I is administered by any suitable means, including intravenously,

intraperitoneally, subcutaneously, or intramuscularly. The effective amount of IGF-I for this purpose is generally adjusted in accordance with many factors, including the patient's specific disease, the route of administration, the individual weight and general condition of the patient to be treated, and the judgment of the medical practitioner. Caution must be taken to monitor blood glucose periodically to avoid hypoglycemia.

Generally, the dosing will range between about 1 µg/kg/day up to about 100 mg/kg/day, preferably 10 µg/kg/day up to about 10 mg/kg/day. If given continuously, the IGF-I is generally administered in doses of about 1 µg/kg/hour up to about 100 µg/kg/hour, either by two daily injections or by subcutaneous infusions, e.g., via minipump or a portable infusion pump. Preferably, the IGF-I is given subcutaneously or intravenously, and most preferably subcutaneously.

If the IGF-I is administered together with insulin, the latter is used in lower amounts than if used alone, down to amounts which by themselves have little effect on blood glucose, i.e., in amounts of between about 0.1 IU/kg/24 hour to about 0.5 IU/kg/24 hour.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature and patent citations are expressly incorporated by reference.

EXAMPLE I

I. Protocol

Hypophysectomized adult male rats weighing 85 to 105 grams (Taconic, N.Y.) were received 7 days after surgery and then weighed every 2-3 days for ten days to meet entry criteria of a weight gain of less than 7 grams and no overall body weight loss. The rats were maintained on Purina rat chow ad libitum. Each lot of animals was divided into a control (excipient), a IGF-I-supplemented group, a des(1-3)-IGF-I-supplemented group, a GH-supplemented group, a IGF-I/GH-supplemented group, and a des(1-3)-IGF-I/GH-supplemented group.

Alzet osmotic pumps (Alza, Palo Alto, Calif.) were implanted to deliver continuously either excipient (10mM citrate buffer and 126 mM NaCl, pH 6.0) or recombinant human IGF-I (produced in *E. coli* as a Z-Z fusion polypeptide by the process generally described in EP 230,869 published Aug. 5, 1987, or available commercially from KabiGen AB, Stockholm, Sweden (specific activity >14,000 U/mg by radioreceptor assay using placental membranes), or available for clinical investigations from Genentech, Inc., South San Francisco). The IGF-I was dissolved at 5 mg/ml in 10 mM citrate buffer and 126 mM NaCl, pH 6.0 and delivered to the rats at a rate of 120 µg/rat per day (equivalent to 1.2 mg/kg/day assuming that the rats weigh 100 g each). This rate represents a submaximal dose that gives a consistent body weight gain in this model.

Alternatively, the pumps were implanted to deliver continuously recombinant human des(1-3)-IGF-I (produced in *E. coli* as generally described by PCT WO 87/01038 published Feb. 26, 1987 and expected to have a specific activity of >about 14,000 U/mg by radioreceptor assay using placenta membranes, or available as brain IGF from KabiGen AB, Stockholm, Sweden, >14,000 U/mg by radioreceptor assay using placenta membranes). It was then formulated at 2 mg/ml in 20 mM acetic acid, pH 3.2, and delivered at a rate of 0.055, 0.166, or 0.5 mg/kg/day.

To the GH-supplemented groups was delivered recombinant methionyl human growth hormone (Protropin® brand,

Genentech, Inc., South San Francisco, Calif.) dissolved at 2 mg/ml in 16 mg/ml mannitol and 5 mM phosphate, pH 7.8, as excipient. The hGH was injected subcutaneously each day, also at submaximal doses (15, 60, and 240 µg/kg per day) for the weight gain response. Moore et al., supra.

Alternatively, recombinant (metless) human growth hormone (Nutropin® brand, Genentech, Inc.) may be employed that is formulated at 2 mg/ml in 18 mg/ml mannitol, 0.68 mg/ml glycine, and 5 mM phosphate, pH 7.4.

At pump implant the animals received oxytetracycline in a single intraperitoneal injection as an intravital marker of longitudinal bone growth.

The growth rates of the hypophysectomized animals were determined by following daily body weights, organ weights at sacrifice, and tibial bone fixed for subsequent assessment of the growth plate. The bone was decalcified, bisected longitudinally, and embedded in paraffin for sectioning and staining with toluidine blue. The distance between the germinal cell layer and the transition from active chondrocytes to new bone deposits was measured microscopically with the aid of a calibrated ocular micrometer. In addition, undecalcified sections were prepared from the proximal tibia and the distance between the growth plate and the tetracycline line, laid down in calcified bone, was determined to assess cumulative longitudinal bone growth.

The remaining solution was removed from all osmotic pumps, and verified by immunoassay to contain either excipient, IGF-I, or des(1-3)-IGF-I. Furthermore, the amount of hormones remaining in the pump of each rat was that expected for continuous delivery over seven days at the rate of delivery specified by the manufacturer.

Independent replicate studies are designated as Study 1 and Study 2, performed a month apart. Statistical comparisons were made by an analysis of variance with follow-up comparisons made by Duncan's Multiple Range Test. A p value of less than 0.05 was considered significant. All data are represented as the mean±SD of 6-8 animals per group. Two other independent studies confirmed these data.

II. Results

FIGS. 1A and 1B represent the cumulative daily body weight increments for the hypophysectomized rats treated with either excipient, 60 µg/kg/day hGH, 1.2 mg/kg/day IGF-I, or the hGH/IGF-I combination for seven days for Studies 1 and 2, respectively. The mean±SD of 7-9 animals/group is shown in the graphs; statistical significance was assumed if p<0.05. The excipient control group did not gain or lose a significant amount of weight during the week, confirming the completeness of the hypophysectomy and the health of the animals in both studies. The mean body weight was increased by hGH in a dose-dependent manner such that on days 3-7 the responses to all hGH doses were significantly different from each other (see FIG. 7). Likewise, IGF-I produced a significant body weight gain that was first recognized on day 2 of dosing, and by day 7 was highly significantly different from excipient (2.9±3.5 g vs. 16.6±2.5 g, t=16.86, p<0.001).

The combination of hGH plus IGF-I yielded a body weight gain that was greater than either hormone alone and appeared to be at least additive. By day 7, the body weight increments for the excipient control, IGF-I, hGH, and combination treatments were, respectively: Study 1: 2.9±3.51 g, 16.6±2.5 g, 12.9±1.2 g, and 22.2±2.7 g; Study 2: -0.04±2.41 g, 10.8±3 g, 9.04±0.92 g, and 19.3±1.6 g. The weight increment of the combination group was statistically different from the means of the other three groups. For example, in Study 1 the mean weight gain at day 7 for the

combination (22.2±2.7 g) was greater than that for GH alone (12.9±1.2 g, $t=10.80$, $p<0.001$) or for IGF-I alone (16.6±2.5 g, $t=6.710$, $p<0.001$). In the same experiment (data not shown on this FIG. 1), des-(1-3)-IGF-I also increased weight gain (to 19.9±2.6 g), which on the addition of GH was increased to 24.7±1.3 g ($t=5.75$, $p<0.001$).

In contrast, it was reported earlier that when native bovine GH (bGH) was delivered intravenously for four days to hypophysectomized rats, and then bGH plus methionine-IGF-I for four more days, there was no greater weight gain than that measured with bGH alone. Skottner, *J. Endocrin.*, supra. Beyond the different delivery routes and dosing regimens of these two studies, the methionyl-IGF-I itself produced no incremental weight gain in this earlier report. To the contrary, this experiment shows repeatedly that IGF-I and des(1-3)-IGF-I promote body weight gain in hypophysectomized rats and that there was an additive effect when GH was co-delivered.

In the hypophysectomized rat weight gain assay, there is an excellent correlation between the weight gain and the bone growth responses to GH. Therefore, an enhanced weight gain is likely to be accompanied by enhanced bone growth, as is the case below.

FIG. 2 illustrates a bar graph of the increase in width of the epiphyseal bone growth plate after seven days of hGH and/or IGF-I treatment in hypophysectomized rats. The mean±SD for 7-9 rats per group is illustrated for Study 1. Statistically significant differences were assumed if $p<0.05$.

In Study 2, shown in FIG. 3B, the groups treated with 60 µg/kg/day of GH (315±35 µm) or with 120 µg/rat of IGF-I (284±20 µm) were significantly different ($t=6.859$, $p<0.001$; $t=4.00$, $p<0.01$, respectively) from the excipient group (235±36 µm); the plate width for GH plus IGF-I group (351±29 µm) differed from both the GH alone ($t=3.069$, $p<0.05$) and IGF-I alone ($t=5.535$, $p<0.001$). Thus, in both studies GH and IGF-I alone induced a significant widening of the tibial epiphysis as compared to the control group, whereas co-treatment with both hormones produced a greater width than treatment with either GH or IGF-I by itself, except at the high-dose GH level. In addition (FIG. 3B), des(1-3)-IGF-I also stimulated growth plate width to 300±17 µm compared to excipient ($t=5.545$, $p<0.001$), and once again co-administration of GH resulted in a further increase in plate width to 364±31 µm, which was greater than for des(1-3)-IGF-I alone ($t=5.507$, $p<0.001$) and GH alone ($t=4.193$, $p<0.01$). The epiphyseal cartilage widening

in response to these hormone treatments was similar in pattern to the body weight changes (FIG. 1).

As with body weight gain, other investigators have tested the effects of such combination treatments on tibial bone growth. GH and IGF-I, delivered intravenously to rats by Skottner et al., *J. Endocrin.*, supra, induced no significantly greater response on tibial bone growth or epiphyseal cartilage width than that resulting from treatment with either hormone alone. The IGF-I did induce widening of the epiphyseal cartilage and lengthening of the bone, while having no effect on body weight, as noted above. In another experiment, direct administration of either of these hormones to the tibial epiphysis stimulated longitudinal bone growth. Isgaard et al., supra. However, the combination of IGF-I and GH yielded no greater growth than that achieved with GH alone.

FIG. 3 illustrates two measures of bone growth, longitudinal bone growth (FIG. 3A) and epiphyseal plate width (FIG. 3B, Study 2 as opposed to Study 1 shown in FIG. 2, where only epiphyseal plate width is shown), obtained in hypophysectomized rats treated for 7 days with IGF-I or des(1-3)-IGF-I alone or in combination with hGH. For both full-length IGF-I and des(1-3)-IGF-I, the results show that their combination with hGH yielded bone growth or cartilage expansion that was greater than the effect using either hormone alone and was additive.

The relevant changes in the weights of the five organs measured are as follows (Table 1). While GH inconsistently increased heart, thymus, and spleen, IGF-I and the combination of IGF-I and GH clearly increased all organ weights relative to the excipient group. The preferential effect of IGF-I on kidney, spleen, and thymus has been shown by others. Guler et al., *Proc. Natl. Acad. Sci. USA*, 85: 4889-4893 (1988). A significantly greater effect of the combination treatment was measured only in Study 2, for all organs except the thymus. Correcting for the body weight increment, the organ-to-body weight ratios were increased by IGF-I for kidneys, spleen, and thymus; the hormone combination did not amplify this effect in these three responsive tissues. In contrast, GH treatment did not alter the organ-to-body weight ratios.

These data indicate that at least a fraction of the hormone combination response can be attributed to weight increases in

TABLE 1

GH AND IGF-I ELICIT DIFFERENT ORGAN WEIGHT RESPONSES				
	Excipient	GH 60 µg/kg	IGF-I 1.2 mg/kg	GH + IGF-I
A. Absolute Wet Weights				
<u>Study 1</u>				
Heart (mg)	291 ± 20	324 ± 13 ^a	341 ± 24 ^a	344 ± 16 ^a
Kidneys (mg)	630 ± 46	686 ± 60 ^{ab}	849 ± 50 ^{ab}	869 ± 31 ^{ab}
Liver (g)	3.80 ± 0.17	4.00 ± 0.23 ^{ab}	4.43 ± 0.27 ^{ab}	4.44 ± 0.39 ^{ab}
Spleen (mg)	234 ± 56	244 ± 26 ^{ab}	369 ± 50 ^{ab}	389 ± 54 ^{ab}
Thymus (mg)	233 ± 24	317 ± 82 ^{ab}	391 ± 49 ^a	414 ± 110 ^{ab}
<u>Study 2</u>				
Heart (mg)	355 ± 22	374 ± 43 ^a	376 ± 24 ^b	440 ± 65 ^{ab}
Kidneys (mg)	688 ± 37	736 ± 44 ^{ab}	871 ± 62 ^{ab}	973 ± 45 ^{ab}
Liver (g)	3.77 ± 0.25	4.04 ± 0.30 ^a	4.42 ± 0.41 ^a	4.58 ± 0.13 ^{ab}
Spleen (mg)	197 ± 16	260 ± 24 ^{ab}	297 ± 30 ^{ab}	342 ± 23 ^{ab}
Thymus (mg)	257 ± 42	336 ± 50	436 ± 154 ^a	450 ± 113 ^a

TABLE 1-continued

GH AND IGF-I ELICIT DIFFERENT ORGAN WEIGHT RESPONSES				
Excipient	GH 60 ug/kg	IGF-I 1.2 mg/kg	GH + IGF-I	
B. Organ to Body Weight (BW) Ratio ($\times 10^{-3}$)				
Study 1				
Heart/BW	3.00 \pm 0.19	3.08 \pm 0.17	3.10 \pm 0.21	2.95 \pm 0.15
Kidneys/BW	6.71 \pm 0.52	6.51 \pm 0.57 ^{ab}	7.70 \pm 0.38 ^{ab}	7.45 \pm 0.38 ^{ab}
Liver/BW	39.2 \pm 1.8	37.9 \pm 1.7	40.2 \pm 2.1	38.0 \pm 2.2
Spleen/BW	2.42 \pm 0.65	2.31 \pm 0.27 ^{ab}	3.35 \pm 0.47 ^{ab}	3.33 \pm 0.42 ^{ab}
Thymus/BW	2.41 \pm 0.28	3.00 \pm 0.72	3.55 \pm 0.97 ^a	3.55 \pm 0.97 ^a
Study 2				
Heart/BW	3.91 \pm 0.22	3.72 \pm 0.34	3.69 \pm 0.25	3.98 \pm 0.51
Kidneys/BW	7.57 \pm 0.27	7.33 \pm 0.30 ^{ab}	8.56 \pm 0.64 ^{ab}	8.80 \pm 0.37 ^{ab}
Liver/BW	41.4 \pm 2.2	40.2 \pm 1.9	43.4 \pm 4.2	41.4 \pm 1.0
Spleen/BW	2.16 \pm 0.13	2.69 \pm 0.52 ^{ab}	2.92 \pm 0.28 ^a	3.09 \pm 0.21 ^{ab}
Thymus/BW	2.83 \pm 0.43	3.35 \pm 0.48	4.28 \pm 1.48 ^a	4.06 \pm 0.99 ^a

Mean \pm SD (7-9 rats/group); the # denotes statistically different from excipient and similar letter superscripts denote group differences by Duncan's test after analysis of variance (ANOVA) at $p < 0.05$

specific organs. In addition, they indicate that the additive effect of IGF-I and GH was not seen on all tissues, for example, for the absolute weight of thymus (Table 1), or for all the organ/body weight ratios. This varying sensitivity of different tissues to the combination of GH and IGF-I was unexpected. In some tissues, notably in whole body growth and on bone and cartilage, IGF-I and GH are both effective and additive. In other tissues, i.e., thymus, IGF-I and GH are both effective but not additive, indicating a selective effect.

EXAMPLE II

A. Combination Studies

In the two experiments described below, hypophysectomized rats as described in Example I (Study 3) or female dwarf rats (60-70 days of age, 100-140 g, Study 4) were anesthetized with ketamine/xylazine. Then 2 (for the dwarf rats) or 2 (for the hypophysectomized rats) osmotic minipumps (Alza 2001, delivery rate 1 μ l/hour/pump) were placed subcutaneously. The pumps contained either the excipient (10 mM citrate buffer and 126 mM NaCl, pH 6) or IGF-I (5 mg/ml) so that the approximate dose administered was 240 μ g/rat/day (2.4 mg/kg assuming a 100 g rat) for both types of rats. The hGH formulation employed was that described in Example I. The IGF-I was prepared by direct secretion of the IGF-I gene from *E. coli* as in accordance with EP 128,733 published Dec. 19, 1984 or EP 288,451 published Oct. 26, 1988, and expected to have a specific activity of $>$ about 14,000 U/mg by radioreceptor assay using placental membranes, or was obtained from KabiGen AB (specific activity $>$ 14,000 U/mg) or from Genentech, Inc. as described in Example I. It was formulated as described in Example I. In Study 3 the solubility of hGH was increased by adding 0.1% Tween 20 to the 5 mM phosphate buffer (pH 7.8). The hGH in both studies was given daily as a single 0.1-ml subcutaneous injection.

In Study 3 (hypophysectomized rats) the experimental groups were:

- 1) Excipient pump, excipient injections
- 2) IGF-I pump (2.4 mg/kg), excipient injections
- 3) Excipient pump, hGH injections (50.0 mg/kg)
- 4) Excipient pump, hGH injections (10.0 mg/kg)
- 5) Excipient pump, hGH injections (2 mg/kg)

- 6) Excipient pump, hGH injections (0.4 mg/kg)
- 7) Excipient pump, hGH injections (0.08 mg/kg)
- 8) IGF-I pump (2.4 mg/kg), hGH injections (50.0 mg/kg)
- 9) IGF-I pump (2.4 mg/kg), hGH injections (10.0 mg/kg)
- 10) IGF-I pump (2.4 mg/kg), hGH injections (2.0 mg/kg)
- 11) IGF-I pump (2.4 mg/kg), hGH injections (0.4 mg/kg)
- 12) IGF-I pump (2.4 mg/kg), hGH injections (0.08 mg/kg).

In Study 4 (dwarf rats) the experimental groups were:

- 1) Excipient pump, excipient injections
- 2) IGF-I pump (2.4 mg/kg), excipient injections
- 3) Excipient pump, hGH injections (2.0 mg/kg)
- 4) Excipient pump, hGH injections (0.5 mg/kg)
- 5) Excipient pump, hGH injections (0.125 mg/kg)
- 6) IGF-I pump (2.4 mg/kg), hGH injections (2.0 mg/kg)
- 7) IGF-I pump (2.4 mg/kg), hGH injections (0.5 mg/kg)
- 8) IGF-I pump (2.4 mg/kg), hGH injections (0.125 mg/kg).

FIG. 4 shows the results from Study 3 for the 7-day weight gains in the hypophysectomized rat. The excipient gave a weight gain of 4.46 ± 1.66 g and IGF-I at 240 μ g/day gave a weight gain of 8.23 ± 1.98 g. Once more, the inclusion of IGF-I in the minipumps greatly enhanced the potency of daily injections of hGH in promoting weight gain. The weight gain responses to hGH or hGH plus IGF-I were analyzed as a parallel line bioassay against log dose of hGH. The two dose-response lines fulfilled the criteria for a bioassay, as they were statistically proved to be linear and parallel. The potency of hGH plus IGF-I was 26.6 times that of hGH alone (95% confidence, 14.8 to 51.7), with the difference between the two dose-response lines being highly significant (1,49 degrees of freedom (d.f.), $F=169.4$, $p < 0.0001$).

FIG. 5 shows the weight gains over 7 days from Study 4. The excipient gave a weight gain of 3.95 ± 3.56 g and IGF-I at 240 μ g/day gave a weight gain of 12.15 ± 3.76 g. The weight gain responses to hGH or hGH plus IGF-I were analyzed as a parallel line bioassay against log dose of hGH. The two dose-response lines fulfilled the criteria for a bioassay, as they were statistically proved to be linear and parallel. Individually, IGF-I and hGH gave substantial

weight gains in the dwarf rat. The relative potency of the hGH plus IGF-I was 28.9 times that of the hGH alone (95% confidence limits, 7.7 to 514.6), with the difference between the two dose-response lines being highly significant (1,30 d.f., $F=45.75$, $p<0.0001$).

B. Dose Response Curve of IGF-I Alone

FIG. 6 illustrates the weight gain of hypophysectomized rats treated with excipient (citrate buffer as described above), or the IGF-I or des(1-3)-IGF-I used in Example I at three different doses subcutaneously using minipumps for seven days, following the general protocol described in Example I. This figure illustrates the minimal doses of IGF-I and des(1-3)-IGF-I for bioactivity in the rat.

C. Dose Response Curve of hGH Alone

FIG. 7 illustrates the weight gains of hypophysectomized rats treated with excipient or three different doses of the hGH of Examples I and II daily subcutaneously for seven days, following the general protocol described in Example I. This figure illustrates the minimal doses of GH for bioactivity in the rat. At day 7, low-dose GH showed a greater weight gain than excipient (2.9 ± 3.5 g vs. 8.6 ± 2.3 g, $t=7.03$, $p<0.001$), which was in turn less than medium-dose GH (12.9 ± 1.2 g, $t=4.91$, $p<0.01$).

In the two animal models of GH deficiency (Studies 3 and 4), the potency of hGH administered as a daily subcutaneous injection was increased over 25 fold by co-treatment with IGF-I. This result in the hypophysectomized rat might be explained by the relative lack of hormones (thyroid and glucocorticoids) known to be permissive for hGH action leading to a poor IGF-I generation. However, the result in the dwarf rat, where only hGH appears to be lacking, with all the other hormone systems (especially the thyroid and adrenal hormones) being normal, indicates that the additive effect of hGH and IGF-I occurs independent of the status of thyroid or adrenal hormones. However, the close agreement in the two models of the enhanced potency of hGH due to IGF-I and the magnitude of the effect (about 25 \times) is surprising.

The doses of hGH that were used in Study 3 have rarely been used in the hypophysectomized rat, and the literature is unclear as to the dose of hGH that gives a maximal growth response. Doses of 10 and 50 mg/kg/day given as single daily subcutaneous injections for one week produce a maximal growth response. But the dose responses for the two regimes (hGH and hGH plus IGF-I) were parallel, even over this 625-fold dose range of five doses of hGH, including the two maximal doses of hGH. Therefore, the maximal growth response to hGH can clearly be increased if IGF-I is co-administered. This is surprising, as the maximal weight gain response to IGF-I in the hypophysectomized rat appears to be less than the weight gain in response to hGH.

The range of doses of hGH over which IGF-I would be predicted to have an additive effect on weight gain is clearly the full range of effective GH dose, in the hypophysectomized rat from 0.01 to 50 mg/kg. In the dwarf rat the maximal effective doses of hGH are not known, but 50 mg/kg would also be assumed to be an effective maximal dose of hGH. The previous work in the hypophysectomized rat has shown 2.4 mg/kg of IGF-I delivered as a subcutaneous infusion for one week to be near to maximal, as higher doses of IGF-I cause fatal hypoglycemia. The minimal effective dose of IGF-I in the hypophysectomized rat is around 0.1 mg/kg per day.

In the dwarf rat, 2.4 mg/kg of IGF-I was used, while in the hypophysectomized rat both 1.2 mg/kg and 2.4 mg/kg doses of IGF-I were used (Examples I and II), yet an additive effect of IGF-I and GH was observed despite different doses of

IGF-I being used. The full dose-response curves for GH alone and GH plus IGF-I were parallel, which implies that at any dose of hGH, even at a very small dose of hGH that by itself might not give a measurable response, the effects of IGF-I and GH would be additive. It would therefore be expected that at any daily dose of GH (from 0.01 to 50 mg/kg) or IGF-I (from 0.1 to 2.4 mg/kg) the two molecules would have additive effects on body growth.

EXAMPLE III

Two Clinical Scenarios for the Combination Treatment

Two examples of pertinent clinical scenarios are described below that will undoubtedly benefit from concomitant administration of GH and IGF-I.

1. Patients who exhibit a slowing in growth rate after at least twelve months of GH administration.

It is well recognized by pediatric endocrinologists that either naive (no previous treatment) or previously treated patients (following a break in GH administration) exhibit a second-year fall in growth rate. This phenomenon is independent of the etiology of the type of short stature or GH deficiency (e.g., whether idiopathic, organic, septo-optic dysplasia (S—O D), Turner, or other). See FIG. 8.

Thus, during the period where the growth rate is slowing, IGF-I treatment together with GH treatment would increase the annualized rate to compensate for this second-year loss in response.

2. Patients who have little time for GH administration to be maximally effective.

If patients are older when they are diagnosed with GH deficiency, less time is available to correct their resultant short stature. This is illustrated in FIG. 9, where the annualized growth rate is reported for patients in seven age groups. Older patients have only, for example, 2-3 years left before their growth plates close, making further linear growth unlikely. These patients could be treated with the combination of IGF-I and hGH to allow optimization of their growth rates.

DISCUSSION AND SUMMARY

The results shown herein have significance in medicine and agriculture in any situation where GH or IGF-I treatment is used. This regime of combined IGF-I and GH treatment would allow smaller doses of GH (approximately 25-fold less) to be given to produce equivalent responses to treatment with GH alone. This would be of particular importance in situations where the side effects of GH treatment (i.e., hyperinsulinemia, hyperglycemia) should be minimized. In diabetes, combined GH and IGF-I treatment, with smaller GH doses being possible, would minimize the insulin-resistant effect of the administered GH. In patients where the anabolic effect of GH is reduced, possibly by a reduced ability to produce an IGF-I response to the administered GH, co-treatment with GH and IGF-I would also be expected to give a larger anabolic response.

A broad class of patients where the regime of combined GH and IGF-I treatment would be beneficial is in adult patients where the IGF-I response to GH is naturally reduced. In adults, the unwanted effects of GH (insulin resistance) may be a direct consequence of a reduced IGF-I response to administered GH. In adults, the co-administration of GH and IGF-I might be viewed as restoring the situation in a younger animal where there is a more vigorous IGF-I response to GH treatment.

The mode of administration of the GH in the present studies was intermittent, by daily subcutaneous injection. However, at the largest doses used (50 mg/kg), considerable concentrations of hGH would have persisted in the blood at physiologically effective concentrations, making the blood concentrations of hGH always at a level that would provide a stimulus to GH receptors. Therefore, at the highest dose the tissue exposure to hGH was in essence one of continuous exposure, so that the growth response to administering hGH as a continuous infusion would likely be enhanced by the co-administration of IGF-I. The potency of hGH delivered in any manner that would stimulate body growth or be anabolic would be expected to increase if IGF-I were co-administered. Also, it is likely that the improved potency of co-administered hGH and IGF-I would allow less frequent injections of hGH or IGF-I than for hGH alone.

IGF-I was delivered as a continuous infusion, because previous studies showed that IGF-I given alone as injections is less effective at enhancing body growth. However, the combination of GH plus IGF-I would allow the use of sub-optimal regimes of IGF-I administration, such as injections, when combined with GH treatment.

In conclusion, cotreatment of hypophysectomized or dwarf rats with GH and IGF-I or des(1-3)-IGF-I amplifies the body weight gain, longitudinal bone growth, and tibial epiphyseal widening relative to the response to either hormone alone. This finding indicates for the first time that exogenous IGF-I can increase some growth responses initiated by GH in a manner that is at least additive. Thus, the IGF-I is effective at increasing the responses to GH treatment or at decreasing the amount of GH needed to produce a significant response.

EXAMPLE IV

Preparation of IGF-I Formulation and Combination of IGF-I and GH

It was desired to produce a formulation of IGF-I that could be mixed with hGH in dose ratios of IGF-I:hGH of greater than about 2:1 to provide a stable co-mix of both proteins. In this example, the IGF-I formulation used to achieve this was:

- 10 mg/ml IGF-I
- 5.84 mg/ml NaCl
- 9.0 mg/ml benzyl alcohol
- 2.0 mg/ml polysorbate 20
- 50 mM sodium acetate pH 5.4.

The intended final product configuration contained 7 ml (70 mg) of the above solution in a 10-ml glass vial, which is generally stored refrigerated (2°-8° C.) to maximize its lifetime. This product is designed to be a ready-to-use liquid for subcutaneous or intravenous administration using a conventional needle and syringe.

For administration of GH and IGF-I together rather than separately, the above formulation (70 mg IGF-I vial) was mixed with a liquid formulation of hGH (5 mg/ml hGH, 8.77 mg/ml NaCl, 2.5 mg/ml phenol, 2.0 mg/ml polysorbate 20, and 10 mM sodium citrate, pH 6.0), available from Genentech, Inc. The hGH was added up to about 10 mg (2 ml) hGH. The formulations were mixed in dose ratios of 7:1, 14:1, and 28:1 IGF-I:hGH. The resulting formulations were generally stored at 2°-8° C. and used within a two-week period. These final formulations had concentration ranges as follows:

- IGF-I 7.1-9.6 mg/ml
- hGH 0.2-1.4 mg/ml
- NaCl 6.0-6.7 mg/ml
- Phenol 0.1-0.7 mg/ml
- Benzyl alcohol 6.4-8.7 mg/ml
- Polysorbate 20 2.0 mg/ml
- Sodium citrate 0.1-0.7 mg/ml
- Sodium acetate 2.8-3.8 mg/ml

The aim of the metabolic studies reported in Examples V-XIII below was to investigate the hypoglycemic effects of different formulations of recombinant human IGF-I and their pharmacokinetics, alone or in combination with GH, on plasma glucose levels in the anesthetized dwarf (dw/dw) rat or the normal rat. The aim of the growth study reported in Example XIV below was to investigate the anabolic effect of a formulation containing the combination of GH and IGF-I in the dw/dw rat and to compare this effect with that of formulations with either agent alone.

EXAMPLE V

The fall in blood glucose caused by an injection of IGF-I is a rapid response that can be easily measured and can serve as a reasonable bioassay for the "insulin-like" activity, or bioactivity in vivo, of IGF-I.

It had been found in the dw/dw rat that doses of 750 and 250 µg of IGF-I formulated in a citrate buffer at pH 6.0 and given subcutaneously reduced blood glucose by a moderate and maximal amount, respectively. Therefore, in this Example these doses of IGF-I were given.

Experimental Design

Sixteen 11-week-old female dw/dw rats (138-162 g; Simonsen Labs, Gilroy Calif.) were anesthetized using Ketamine (62.5 mg/kg/Rompun™ Xylazine (12.5 mg/kg) anesthesia, intraperitoneally. An additional dose was given as needed to maintain anesthesia throughout the study. The right jugular vein was cannulated using Microrenathane™ 0.033 OD×0.014 ID (Braintree Scientific, Braintree, Ma.) inserted 23 mm into the jugular. The free end of the cannula was attached to an automated blood sampling machine to collect blood samples at various timepoints.

Four treatment groups, four rats per group, were dosed subcutaneously with one of the four IGF-I formulations below:

- A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):
 - 1) 750 µg in 150 µl of solution
 - 2) 250 µg in 150 µl of solution
- B. pH 5.0 formulation of IGF-I (10 mg/ml in 20 mM sodium acetate buffer, 2.5 mg/ml (0.25%) phenol, 45 mg/ml mannitol, pH 5.0):
 - 3) 750 µg in 150 µl of solution
 - 4) 250 µg in 150 µl of solution

Three blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 20, 40, 60, 80, 100, 120, 150, 180, and 210 minutes post-injection. The plasma was immediately separated by centrifugation. The glucose concentration in the plasma was subsequently determined by a coupled hexokinase procedure using a Monarch 2000 chemical systems instrument. Statistical comparisons were made by an analysis of variance with a Duncan's Multiple Range test. A p value of less than 0.05 was considered as being statistically significant. All data are represented as the mean±standard deviation with four animals per treatment group.

Results

There was considerable variation between animals in their basal blood glucose, but much less variation within an animal for the three initial blood glucose measurements. Therefore, it was decided to express the blood glucose measurements for each individual animal as a percentage of the mean of the three initial pre-injection basal blood glucose values of that animal.

FIG. 10 shows the mean percentage changes in blood glucose with time after a subcutaneous injection of IGF-I given at time zero. There was a clear dose-related reduction in blood glucose. The 750- μ g dose of IGF-I gave equal decreases in blood glucose for both formulations of IGF-I. However, at the lower dose of IGF-I (250 μ g) there was a clear difference between the two preparations of IGF-I that remained statistically significant at each time point from 60 to 210 minutes post-injection. The formulation in sodium acetate at pH 5.0 therefore was more potent.

EXAMPLE VI

In Example V it was found that the re-formulated IGF-I at pH 5.0 appeared to be more potent, as it had a greater effect on blood glucose than the citrate-buffered formulation of pH 6.0. As the response to the 750- μ g dose appeared to be maximal, and because the re-formulated IGF-I was more potent, the doses of IGF-I were reduced in this example to 450 and 150 μ g/rat.

Experimental Design

Female dw/dw rats (122-138 g) were anesthetized with ketamine/RompunTM anesthesia, a jugular catheter was inserted, and blood samples were taken as described for Example V.

Four treatment groups, four rats per group, were dosed subcutaneously with one of the four solutions of two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 450 μ g in 150 μ l of solution
- 2) 150 μ g in 150 μ l of solution

B. pH 5.0 formulation of IGF-I (10 mg/ml in 20 mM sodium acetate buffer, 2.5 mg/ml phenol, 45 mg/ml mannitol, pH 5.0):

- 3) 450 μ g in 150 μ l of solution
- 4) 150 μ g in 150 μ l of solution

Three blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 20, 40, 60, 80, 100, 120, 150, 180, and 210 minutes post-injection. The experiment was otherwise conducted in an identical manner to Example V.

Results

The blood glucose measurements for each individual animal were expressed as a percentage of the mean of the three initial pre-injection basal blood glucose values of that animal.

FIG. 11 shows the mean percentage changes in blood glucose with time after a subcutaneous injection of IGF-I given at time zero. As in Example V, there was a clear dose-related reduction in blood glucose. The 450- μ g dose of IGF-I gave similar initial reductions in blood glucose for both formulations of IGF-I. However, at later time points the blood glucose values for the pH 6.0 formulation at 450 μ g rose above those for the pH 5.0 re-formulated IGF-I, although this difference approached but did not reach statistical significance ($p < 0.1$). However, at the lower dose of IGF-I (150 μ g) there was once more a clear difference between the two preparations of IGF-I that remained statistically significant at each time point from 60 to 210 minutes post-injection.

EXAMPLE VII

In Examples V and VI it was established that the re-formulated pH 5.0 IGF-I had an increased potency over the pH 6.0 formulation. The present example was performed to determine if this increase in potency was due to the IGF-I being better absorbed from the subcutaneous injection site or whether the IGF-I was in some way inherently more bioactive. Both formulations of IGF-I were given as a 150- μ l bolus at 150 μ g per dose either subcutaneously or intravenously. The intravenous injection was given via the jugular catheter and blood samples were taken as described in Example V.

Experimental Design

Four treatment groups of female dw/dw rats, four rats per group, were dosed as indicated with one of the four solutions of two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 150 μ g in 150 μ l of solution subcutaneously
- 2) 150 μ g in 150 μ l of solution intravenously

B. pH 5.0 formulation of IGF-I (10 mg/ml in 20 mM sodium acetate buffer, 2.5 mg/ml phenol, 45 mg/ml mannitol, pH 5.0):

- 3) 150 μ g in 150 μ l of solution subcutaneously
- 4) 150 μ g in 150 μ l of solution intravenously

Three blood samples were taken before the injections at -15, -10, and -5 minutes. Then samples were taken at 5, 10, 15, 20, 30, 60, 90, 120, and 150 minutes post-injection, as the instant exposure to an intravenous injection gives more rapid responses than to a subcutaneous injection. The experiment was otherwise conducted in an identical manner to Example V.

Results

The blood glucose measurements for each individual animal were again expressed as a percentage of the mean of the three initial pre-injection basal blood glucose values of that animal.

FIG. 12 shows the mean percentage changes in blood glucose with time after a subcutaneous or an intravenous injection of IGF-I given at time zero. In this experiment the results in Example VI were confirmed, as there was a clear difference between the blood glucose responses to the two formulations given subcutaneously at the 150- μ g dose of IGF-I. However, the blood glucose values for the two formulations of IGF-I were nearly identical if they were delivered intravenously.

Therefore, a clear difference was seen in the bioactivity of the IGF-I when given subcutaneously, but little or no difference when it was given intravenously. These data suggest that the difference between the two formulations was primarily an effect on the amount, or nature, of the IGF-I absorbed into the blood from the site of subcutaneous injection.

EXAMPLE VIII

In Examples V-VII it was established that the pH 5.0 re-formulated IGF-I had increased potency when given by subcutaneous injection, and that this was probably related to an increased absorption of the IGF-I in the pH 5.0 formulation. The present example questions whether this increase in potency was due to the IGF-I being better absorbed from the subcutaneous injection site at the lower pH (6 versus 5). Therefore, the pH 6.0 formulation of IGF-I in citrate buffer, but at low pHs (dose 150 μ g), was given subcutaneously. Blood samples were taken as described in Example V.

Experimental Design

Four treatment groups of female *dw/dw* rats, four rats per group, were dosed subcutaneously with one of the four solutions of two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 450 µg in 150 µl of solution
- 2) 150 µg in 150 µl of solution

B. pH 5.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 5.0):

- 3) 450 µg in 150 µl of solution
- 4) 150 µg in 150 µl of solution

Three blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 20, 40, 60, 80, 100, 120, 150, 180, and 210 minutes. The experiment was otherwise conducted in an identical manner to Example V.

Results

The blood glucose measurements for each individual animal were again expressed as a percentage of the mean of the three initial pre-injection basal blood glucose values of that animal.

FIG. 13 shows the mean percentage changes in blood glucose with time after a subcutaneous injection of IGF-I given at time zero. In this experiment there was no clear difference between the blood glucose responses to the two formulations given at the 150-µg dose of IGF-I. However, the blood glucose values for the two formulations of IGF-I given at the 450-µg dose appeared to be different at later time points: this difference approached statistical significance ($p < 0.1$).

Thus, some evidence was obtained showing that a difference in pH between IGF-I formulations could affect the absorption of IGF-I. However, changing the pH of the citrate-buffered formulation from pH 6 to pH 5 did not produce the large difference in potency that was seen between the two formulations of IGF-I in Examples V-VII. Therefore, the absorption of IGF-I from the pH 6.0 formulation can unexpectedly be improved by a combination of pH and formulation changes.

EXAMPLE IX

In Examples V-VIII it was established that a re-formulation of IGF-I could change its bioactivity. In this example, a new formulation of IGF-I is devised that can be co-mixed with hGH. The bioactivity of this new IGF-I formulation is tested to assess the effects of a different pH (5.4) and different additives and ions.

The design of Example VII was repeated to discover if there was an increased potency of the pH 5.4 formulation given subcutaneously and if this was due to the IGF-I being better absorbed from the subcutaneous injection site or whether the IGF-I was in some way inherently more bioactive (tested by intravenous injection). Both formulations of IGF-I were therefore given at one dose (150 µg) either subcutaneously or intravenously. The intravenous injection was given via the jugular catheter and blood samples were taken as described in Example V.

Experimental Design

Four treatment groups of female *dw/dw* rats, four rats per group, were dosed as indicated with one of the two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 150 µg in 150 µl of solution subcutaneously
- 2) 150 µg in 150 µl of solution intravenously

B. pH 5.4 formulation of IGF-I (10 mg/ml in 50 mM sodium acetate buffer, 2.5 mg/ml phenol, 5.84 mg/ml NaCl, and 9 mg/ml benzyl alcohol, pH 5.4):

3) 150 µg in 150 µl of solution subcutaneously

4) 150 µg in 150 µl of solution intravenously

Blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 10, 20, 30, 45, 60, 90, and 120 minutes. The experiment was otherwise conducted in an identical manner to Example V.

Results

The blood glucose levels for each individual animal were expressed as a percentage of the mean of the three initial pre-injection basal blood glucose values of that animal.

FIG. 14 shows the mean percentage changes in blood glucose with time after a subcutaneous injection of IGF-I given at time zero. In this experiment samples at 10, 20, and 30 minutes were lost, due to overdilution, so only the data from 45 minutes onward is shown. In this example, there was a clear difference between the blood glucose responses to the two formulations given subcutaneously at the 150-µg dose of IGF-I. However, the blood glucose values for the two formulations of IGF-I given intravenously at the 150-µg dose were not significantly different. It can also be seen that the response at 120 minutes for the subcutaneously delivered pH 5.4 formulation IGF-I approached that of the intravenously delivered dose.

Therefore, the pH 5.4 IGF-I formulation also was very well absorbed compared to the pH 6.0 formulation of IGF-I. A comparison of FIGS. 12 and 14 indicates that the pH 5.4 formulation used in the present study was probably superior to the pH 5.0 formulation used in Example VII. Changing the pH of the formulation from pH 6 to pH 5.4 and changing the components of the formulation unexpectedly led to marked increases in biopotency.

EXAMPLE X

This example repeats the design of Example IX to attempt to duplicate the results showing an increased potency of the pH 5.4 formulation given subcutaneously but a similar effectiveness when given intravenously. Therefore, both formulations of IGF-I were given at one dose (150 µg) either subcutaneously or intravenously. The intravenous injection was given via the jugular catheter and blood samples were taken as described in Example V.

Experimental Design

Four treatment groups of female *dw/dw* rats, four rats per group, were dosed with one of the two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 150 µg in 150 µl of solution subcutaneously
- 2) 150 µg in 150 µl of solution intravenously

B. pH 5.4 formulation of IGF-I (10 mg/ml in 50 mM sodium acetate buffer, 2.5 mg/ml phenol, 5.84 mg/ml NaCl, and 9 mg/ml benzyl alcohol, pH 5.4):

- 3) 150 µg in 150 µl of solution subcutaneously
- 4) 150 µg in 150 µl of solution intravenously

Blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 10, 20, 30, 60, 90, and 120 minutes. The experiment was otherwise conducted in an identical manner to Example V. However, in addition to blood glucose levels, the plasma IGF-I concentration was measured to determine directly its absorption and clearance from the blood. The IGF-I concentration in the plasma samples was measured after acid-ethanol extraction to remove the IGF binding proteins) by radioimmunoassay.

Results

This example shows the absolute (FIG. 15) and the mean percentage (FIG. 16) changes in blood glucose with time after subcutaneous or intravenous injections of IGF-I given

at time zero. A clear difference existed between the blood glucose responses to the two formulations given subcutaneously at the 150- μ g dose of IGF-I. However, the blood glucose values for the two formulations of IGF-I given intravenously at the 150- μ g dose were not significantly different. It can also be seen that the response at 60 minutes for the subcutaneously delivered pH 5.4 IGF-I formulation approached that of the intravenous dosing.

Also shown are the plasma IGF-I concentrations after intravenous (FIG. 17) or subcutaneous (FIG. 18) injections of IGF-I given at time zero. There was a clear difference between the plasma IGF-I concentrations for the two formulations given subcutaneously. The concentration of IGF-I was increased by about 40 ng/ml (from about 30 to about 70 ng/ml) by the pH 6.0 formulation, but was increased by about 80 ng/ml (from about 30 to about 110 ng/ml) by the pH 5.4 formulation. However, the plasma IGF-I concentrations for the two formulations of IGF-I (FIG. 17) given intravenously were not significantly different.

This experiment confirmed and extended the results of Example IX. Once again, the pH 5.4 IGF-I formulation was very well absorbed compared to the pH 6.0 formulation of IGF-I. The improved absorption is now directly shown, as the plasma IGF-I concentrations were doubled when the same dose of IGF-I was given subcutaneously in the pH 5.4 formulation. The pH 5.4 formulation of IGF-I gave a hypoglycemic response that was nearly identical to that of the same dose of IGF-I given intravenously. These data suggest that the IGF-I delivered in the pH 5.4 formulation is nearly 100% bioavailable to the rat. These data confirm that the absorption of IGF-I from the pH 5.4 formulation is unexpectedly improved over that from the pH 6.0 formulation by a combination of pH and formulation changes.

Summary

By measuring the hypoglycemic response to intravenous and subcutaneous dosing of the two formulations of the IGF-I, it may be concluded that:

1. The pH 5.4 formulation is more potent and better absorbed than the pH 6.0 formulation when given subcutaneously.
2. The two formulations give statistically equivalent hypoglycemic responses when dosing is intravenous.
3. There is a suggestion that the mean fall in blood glucose is greater with the pH 5.4 formulation even with intravenous dosing, although this does not reach statistical significance.

EXAMPLE XI

In Example X it was established that the re-formulated pH 5.4 IGF-I had an increased potency when given by subcutaneous injection. This example was designed to determine the relative potency of the two preparations of IGF-I by giving two doses of IGF-I by subcutaneous injection and measuring hypoglycemia and the serum IGF-I concentrations. It appeared that the pH 5.4 formulation was about 3-fold more effective as a hypoglycemic agent than the pH 6.0 formulation. Therefore, the pH 6.0 formulation of IGF-I was given by subcutaneous injection at two doses (150 μ g and 450 μ g) and the pH 5.4 formulation of IGF-I at two doses (50 μ g and 150 μ g), such that matching effects on blood glucose and serum IGF-I might be expected. In addition, the rapid absorption of IGF-I was measured by taking samples at very frequent intervals immediately following the injection of the respective IGF-I formulations.

Experimental Design

Four treatment groups of female dw/dw rats, four rats per group, were injected subcutaneously with one of the four solutions of the two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 150 μ g in 100 μ l of solution
- 2) 450 μ g in 100 μ l of solution

B. pH 5.4 formulation of IGF-I (10 mg/ml in 50 mM sodium acetate buffer, 2.5 mg/ml phenol, 5.84 mg/ml NaCl, and 9 mg/ml benzyl alcohol, pH 5.4):

- 3) 50 μ g in 100 μ l of solution
- 4) 150 μ g in 100 μ l of solution

Three blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 3, 6, 9, 20, 30, 45, 60, 90, and 120 minutes. The experiment was otherwise conducted in an identical manner to Example V.

Results

In this example the absolute (FIG. 19) and the mean percentage (FIG. 20) changes in blood glucose with time after subcutaneous injections of IGF-I given at time zero are shown. There was a clear difference between the blood glucose responses to the two formulations given subcutaneously. At three-fold different doses of IGF-I, equivalent hypoglycemic responses were obtained, as the 150- μ g dose of the pH 6.0 formulation and the 50- μ g dose of the pH 5.4 formulation were equivalent, as were the 450- μ g dose of the pH 6.0 formulation and the 150- μ g dose of the pH 5.4 formulation.

FIG. 21 shows the plasma IGF-I concentrations after the subcutaneous injections of IGF-I given at time zero. The pre-injection IGF-I concentrations were not different for the four groups. The IGF-I concentrations achieved were dose related and directly mirrored the blood glucose concentrations. At three-fold different doses of IGF-I, equivalent plasma IGF-I concentrations were obtained for the 150- μ g dose of the pH 6.0 formulation and the 50- μ g dose of the pH 5.4 formulation and for the 450- μ g dose of the pH 6.0 formulation and the 150- μ g dose of the pH 5.4 formulation.

In conclusion, following a subcutaneous injection, the absorption and therefore the efficacy of the IGF-I was improved about 3-fold using the pH 5.4 formulation of IGF-I.

EXAMPLE XII

The previous examples established that the re-formulated pH 5.4 IGF-I had an increased potency in terms of a hypoglycemic response when given by subcutaneous injection. In addition, the examples showed that after a subcutaneous injection a three-fold increase in the absorption and efficacy of the IGF-I was measured using the re-formulated pH 5.4 IGF-I.

All these experiments were conducted in the dw/dw rat. It is possible that such rats, which are GH-deficient and IGF-I-deficient compared to a normal GH-sufficient and IGF-I-sufficient rat, might in some way allow IGF-I to be absorbed much better in the pH 5.4 formulation. In the present example, IGF-I was given by subcutaneous injection in a normal rat, and blood glucose and serum IGF-I concentrations were measured. As these normal rats weighed twice as much (230-250 grams) as the dw/dw rats, and might be expected to have higher concentrations of plasma IGF binding proteins, the doses of IGF-I were doubled, compared to those used in the earlier examples in the dw/dw rat.

Experimental Design

Four groups of normal male Sprague-Dawley rats (230-250 g), four rats per group, were dosed subcutaneously with one of the four solutions of the two IGF-I formulations below:

A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):

- 1) 300 µg in 200 µl of solution
- 2) 900 µg in 200 µl of solution
- B. pH 5.4 formulation of IGF-I (10 mg/ml in 50 mM sodium acetate buffer, 2.5 mg/ml phenol, 5.84 mg/ml NaCl, and 9 mg/ml benzyl alcohol, pH 5.4):
- 3) 100 µg in 200 µl of solution
- 4) 300 µg in 200 µl of solution

Three blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 3, 6, 9, 20, 30, 45, 60, and 90 minutes. The experiment was otherwise conducted in an identical manner to Example V. Results

FIG. 22 shows the mean percentage changes in blood glucose with time after a subcutaneous injection of IGF-I given at time zero. There was a clear difference between the blood glucose responses to the two formulations given subcutaneously at the 300 µg dose of IGF-I. The hypoglycemic response induced by 300 µg of the pH 6.0 formulation of IGF-I was nearly identical to that induced by 100 µg of the pH 5.4 formulation of IGF-I. The response to 900 µg of the pH 6.0 formulation was also similar to the response to 300 µg of the pH 5.4 formulation of IGF-I.

Therefore, the pH 5.4 formulation of IGF-I showed improved efficacy compared to the pH 6.0 formulation when injected subcutaneously in a normal rat. Data obtained in GH- and IGF-I-deficient dwarf animals could thus be extrapolated to a GH- and IGF-I-sufficient normal animal.

EXAMPLE XIII

The previous examples established that the re-formulated pH 5.4 IGF-I had an increased potency in terms of a hypoglycemic response when given by subcutaneous injection in normal rats. After a subcutaneous injection a three-fold increase in the absorption and efficacy of the IGF-I was measured using the re-formulated pH 5.4 IGF-I.

In these normal male rats weighing 230-250 grams it was established that a dose of 300 µg of IGF-I caused a small reduction in blood glucose when given in the pH 6.0 formulation or a large reduction when given in the pH 5.4 formulation. An object of this example was to develop a formulation in which IGF-I was stable, and which also could be co-mixed with hGH.

In this example, therefore, IGF-I was co-delivered with hGH, and the effect of the hGH and its formulation on the absorption of the IGF-I was studied. The efficacy of the co-mix was tested when given by subcutaneous injection, the usual route of injection for these therapeutic drugs. This example tested the acute hypoglycemic efficacy of the co-mix. In the next example, the long-term anabolic efficacy of the co-mix on body growth was tested.

Experimental Design

Four groups of normal male Sprague-Dawley rats (230-250 g), four rats per group, were dosed subcutaneously with one of the four IGF-I or IGF-I/hGH formulations below:

- A. pH 6.0 formulation of IGF-I (5 mg/ml in 10 mM sodium citrate buffer and 126 mM NaCl, pH 6.0):
- 1) 300 µg IGF-I in 200 µl of solution
- B. pH 5.4 formulation of IGF-I (10 mg/ml in 50 mM sodium acetate buffer, 2.5 mg/ml phenol, 5.84 mg/ml NaCl, and 9 mg/ml benzyl alcohol, pH 5.4) and/or pH 6.0 formulation of hGH (5 mg/ml in 10 mM sodium citrate buffer, 2.5 mg/ml phenol, 8.77 mg/ml NaCl, and 2.0 mg/ml polysorbate 20, pH 6.0):
- 2) 300 µg IGF-I in 200 µl of solution
- 3) 300 µg IGF-I+100 µg hGH in 200 µl of solution
- 4) 300 µg IGF-I+10 µg hGH in 200 µl of solution

Three blood samples were taken before the injections at -15, -10, and -5 minutes; then samples were taken at 3, 6, 9, 20, 30, 45, 60, 90, and 120 minutes. The experiment was otherwise conducted in an identical manner to Example V. Results

FIG. 23 shows the mean percentage changes in blood glucose with time after a subcutaneous injection of IGF-I given at time zero. There was a small reduction in blood glucose when the pH 6.0 formulation was given but a much larger fall when the pH 5.4 formulation was given. The hypoglycemia induced by the co-mixes of hGH and IGF-I formulation was similar to that induced by the pH 5.4 IGF-I formulation alone. At the 60-minute timepoint all three groups receiving the pH 5.4 formulation had significantly lower blood glucose levels than the group that received the pH 6.0 formulation of IGF-I.

FIG. 24 shows the plasma IGF-I concentrations after the subcutaneous injections of IGF-I given at time zero. The pre-injection IGF-I concentrations were not different for the four groups. The IGF-I concentrations tended to mirror the blood glucose concentrations, with the pH 6.0 formulation of IGF-I tending to be more poorly absorbed compared to the pH 5.4 IGF-I formulation. The co-mix of hGH and IGF-I led to similar blood IGF-I concentrations to that of the pH 5.4 IGF-I formulation alone.

Changing the formulation to one that was mixable with hGH led to the best IGF-I formulation in terms of hypoglycemic activity and IGF-I absorption, even when co-mixed with hGH.

EXAMPLE XIV

The previous examples have shown that the re-formulated pH 5-5.5 IGF-I formulations enhance the absorption and bioactivity of IGF-I. In addition, these re-formulations of IGF-I can be co-mixed with hGH while maintaining IGF-I absorption. In this example, the anabolic activity of the co-mixed combination is studied using two doses of hGH and a fixed dose of IGF-I.

In Examples I and II it was established that subcutaneous injections of hGH and infusions of IGF-I gave additive growth responses in the rat. Now it was desired to know if the co-formulation of IGF-I and hGH could be delivered to an animal with the additive anabolic effects of IGF-I and hGH being retained. The options were to deliver the mixture either as an injection (as with hGH) or as an infusion (as with IGF-I).

First, the co-formulation could be delivered as a co-mix by injection. It was shown in Examples I and II that infused IGF-I and injected GH had an additive effect. Since it was found that infused GH did not have such an effect with IGF-I, injections were the obvious method of delivering the hGH. However, IGF-I had been given by infusion in these examples to show additive effects. It had not been shown that the pH 6.0 IGF-I formulation given by injection was efficacious in terms of inducing an additive anabolic response with co-injected hGH.

Nevertheless, when it was attempted to induce growth responses in GH-deficient rats by delivering the pH 6.0 formulation alone by injection, it was found that IGF-I injections were very poor at inducing an anabolic effect, but very effective at inducing a hypoglycemic response. U.S. Pat. No. 5,187,151. The poor anabolic effect of IGF-I injections had also been shown in the mouse. Woodall et al., *Horm. Metab. Res.*, 23: 581-584 (1991). Four daily injections of IGF-I (two daily injections had marginal effects) were needed to induce anabolic effects approaching those seen with IGF-I infusions.

Therefore, it was unclear whether the improved glycemic potency of injections of the pH 5.4 formulation of IGF-I also would be translated into an improved anabolic efficacy of injections of IGF-I. In this example, hGH and IGF-I were co-injected twice daily to determine the results.

Methods

1. Compounds

All solutions of IGF-I and GH alone or together were prepared on day 0 so that sufficient drug was made for the entire example and stored at 4° C. during the seven-day experiment. The separate hGH and IGF-I formulations employed were the pH 5.4 IGF-I formulation and the pH 6.0 hGH formulation described in Example XIII.

2. Animals

Sixty female dw/dw rats of 60-70 days of age were obtained from Simonsen (Gilroy, Calif.). They were group housed in a room with controlled lighting and temperature and fed a grain diet and water ad libitum. They were weighed twice and the largest and the smallest animals were discarded to leave 48 rats ranging in body weight from 105 to 135 grams at their first injection.

3. Experimental Design

The rats were randomly assigned to six groups of eight rats per group based on their body weight, so that the average weight per group (119 g) was not different.

The six treatment groups were:

- 1) two excipient injections/day
 - 2) pH 5.4 IGF-I formulation alone, 300 µg twice daily=600 µg/day
 - 3) pH 6.0 hGH formulation alone, 15 µg twice daily=30 µg/day
 - 4) pH 6.0 hGH formulation alone, 60 µg twice daily=120 µg/day
 - 5) co-mix of pH 5.4 IGF-I formulation, 300 µg twice daily, plus pH 6.0 hGH formulation, 15 µg twice daily
 - 6) co-mix of pH 5.4 IGF-I formulation, 300 µg twice daily, plus pH 6.0 hGH formulation, 60 µg twice daily
- 100-µl injections of each formulation were given subcutaneously in the nape of the neck twice daily either at the time the animals were weighed (8-9AM) or in the late afternoon (4-5PM).

The last injection was a PM injection, with all the animals being sacrificed approximately 18 hours later. At sacrifice, a large blood sample was taken, and the liver, heart, spleen, thymus, and kidneys were dissected and weighed. Serum was assayed for IGF-I after extraction with acid-ethanol and a subsequent conventional RIA for IGF-I.

All data shown are Mean±SD with eight rats per group. Treatment groups were compared by ANOVA and then by Duncan's Multiple Range Test.

Results

1. Body Weight Gain

The dose of IGF-I (300 µg/injection) was chosen as a near maximally effective hypoglycemic dose of IGF-I (based on the earlier examples) when given in the pH 5.4 formulation. These earlier examples used only one dose, so it was

uncertain if a second or third dose would be more or less effective at inducing hypoglycemia. However, it was assumed that the 300 µg/injection dose would be nearly maximal as a hypoglycemic dose when given repeatedly, and that it also would be nearly maximal as an anabolic dose. The doses of hGH were based on effective doses established in other experiments of twice daily hGH dosing in the dw/dw rat.

In addition, the doses of hGH and of IGF-I were chosen to attempt to obtain growth responses similar to those found with daily hGH dosing in the dw/dw rat in Example II. Finally, the doses were also chosen to give a broad range of the hGH to IGF-I weight ratio (1:5 to 1:20) in the range where the hGH and IGF-I in the co-mix were shown to be chemically stable.

A comparison of the results in the present example with the results in Example II show that comparable effects of IGF-I and hGH were found with the dosing regimes chosen.

The animals appeared to tolerate the repeated injections of IGF-I with no overt signs of hypoglycemia. The anabolic or growth-promoting activity of hGH and IGF-I was gauged primarily by measuring the weight gain in the rats. FIG. 25 and Table 2 show the weight gains of the dw/dw rats over the seven-day study. The control excipient-injected rats showed a very small weight gain (3.4±2.5 g over the 7-day study). In other words, their growth reflected the GH- and IGF-deficient state expected of dwarf rats.

Twice daily IGF-I injections induced a surprisingly large, and statistically significant ($p < 0.001$), amount of body weight gain (11.9±2.5 g). These data are very similar to the data in FIG. 14, where 240 µg/day of rhIGF-I was infused for seven days in dw/dw rats and resulted in a weight gain of 12.15±3.75 g.

The two doses of rhGH both induced significant ($p < 0.001$) weight gain (18.5±2.9 and 29.6±4.4 g for the 30 and 120 µg/day doses, respectively). The weight gain in Example II in the dw/dw rat with 200 µg/day of hGH given once daily was 19.9±4.5 g, which was similar to the response to 30 µg/day of hGH in the present example.

There was an additive effect of the co-mixed IGF-I and hGH treatments. Thus, the weight gains for the co-mixed formulations of hGH and IGF-I were 25.4±3.9 and 36.1±4.9 g, for 30 and 120 µg hGH/day groups, respectively, compared to the 18.5±2.9 and 29.6±4.4 g gains for these doses given alone. The comparison of hGH alone to hGH+IGF-I was statistically significant ($p < 0.01$) for both hGH doses.

In Example II, the weight gain with the 200 µg/day dose of hGH injected once daily along with an IGF-I infusion was 28.4±6.0 g, again similar to the response to the 30 µg/day dosing of the hGH+IGF-I formulation in the present example. These data therefore indicate that with equivalent growth responses induced by hGH injection (whether given once or twice a day) the co-administration of IGF-I gives an additive growth response, irrespective of whether the IGF-I is given by infusion or by injection.

TABLE 2

Group	Wt Gain (g)	Serum			
		IGF-I (ng/ml)	Kidney Wt (% Bwt)	Thymus Wt (% Bwt)	Spleen Wt (% Bwt)
Excipient	3.4 ± 2.5	129 ± 49	0.86 ± .10	0.16 ± .02	0.23 ± .02
IGF-I (600 µg)	11.9 ± 2.5*	140 ± 42	0.83 ± .05	0.17 ± .03	0.25 ± .02
hGH (30 µg)	18.5 ± 2.9*	163 ± 47	0.81 ± .07	0.17 ± .04	0.25 ± .02
hGH (120 µg)	29.6 ± 4.4*	189 ± 50*	0.84 ± .10	0.18 ± .03	0.27 ± .03*

TABLE 2-continued

Dw/dw rats treated with IGF-I + hGH; twice daily injection					
Group	Wt Gain (g)	Serum IGF-I (ng/ml)	Kidney Wt (% Bwt)	Thymus Wt (% Bwt)	Spleen Wt (% Bwt)
IGF-I (600 µg) + hGH (30 µg)	25.4 ± 3.9*	120 ± 29	0.77 ± .04	0.16 ± .04	0.27 ± .02*
IGF-I (600 µg) + hGH (120 µg)	36.1 ± 4.9*	152 ± 41	0.77 ± .05	0.17 ± .03	0.31 ± .04*

Wt = weight
Bwt = body weight
* = statistically significant
(n = 8, p < 0.05 vs Excipient)

2. Serum IGF-I Concentrations

The serum samples obtained at sacrifice 18 hours after the last injection were extracted and IGF-I was measured (Table 2). There was a small but statistically significant rise in IGF-I after the injection of hGH at the high dose. However, there was no maintained rise in serum IGF-I after the injection of IGF-I or after the injection of the co-mix, since IGF-I when given by injection is quite rapidly cleared in the rat. Sampling at times closer to the time of injection would be expected to show the higher blood IGF-I concentrations seen in the earlier examples. The greater efficacy of the co-mix therefore could not be directly tied to a higher serum concentration of IGF-I.

3. Organ Growth

The data in Table 2 for the growth of the different body organs gave surprising results. In animals infused with IGF-I clear overgrowth of the spleen, thymus, and kidney has been shown in many studies, in hypophysectomized, dwarf, and normal animals. For example, see the data in Examples I and II above. However, in the present example, injections of IGF-I at doses that induced additive anabolic effects with hGH on whole body growth gave surprisingly little organ overgrowth. These data are quite different from the data with IGF-I infusions in the earlier examples.

For instance, with IGF-I infusions in dw/dw rats in Example II, the relative size of the kidney increased significantly as a percent of body weight from 0.79±0.05 in excipient-treated controls to 1.0±0.09 in IGF-I-treated rats. However, in the present example (Table 2) when IGF-I was given by injection, with or without hGH, there was a tendency for the relative size of the kidney to show, in fact, a reduction from 0.86±0.10 in excipient-treated controls to 0.83±0.05 in IGF-I-treated rats.

In Example II the thymus showed a vigorous growth response to IGF-I infusions, increasing well out of proportion to the rest of the body its relative size as a percent of body weight from 0.14±0.04 in excipient-treated controls to 0.18±0.09 in IGF-I-treated rats. However, in the present Example (Table 2) IGF-I injections had no significant effect on relative thymus weight whether IGF-I was given alone or with hGH.

The relative size of the spleen did show a significant increase with IGF-I treatment, but only when given along with high-dose hGH treatment. In comparison, in Example II the relative size of the spleen increased dramatically as a percent of body weight from 0.23±0.01 in excipient-treated controls to 0.47±0.12 in rats treated with IGF-I alone. In the present example, when IGF-I injections were given alone there was no significant effect on absolute or relative spleen size, yet there was a clear effect of IGF-I increasing spleen size when the IGF-I was given along with the hGH. This evidence suggests that the IGF-I injections in some way synergized with the hGH to induce spleen growth.

This relative lack of an effect of the IGF-I injections on the growth of the different tissues, compared to the effect of IGF-I infusions, made the additive whole body effect of hGH and IGF-I even more surprising. Without being limited to any one theory, it appears that injections and infusions of IGF-I cause a different spectrum of growth responses in different tissues. All the tissues measured seemed to respond to the IGF-I injections with a growth response more like that of GH in that they grew in proportion to the increase in whole body size. In the earlier examples, it might have been reasoned that GH and IGF-I might synergize to cause a whole body anabolic response because they caused selective and differential organ growth. However, when the IGF-I is given by injection there is much less evidence of selective or differential organ growth. It was surprising that the overgrowth of some organs was lost when IGF-I was injected, yet when hGH was added to the formulation to be injected, the additive whole body anabolic activity was retained.

Conclusion

Injections of the pH 5.4 formulation of IGF-I induced a very significant whole body growth response with little organ overgrowth. In addition, when the IGF-I was co-injected with GH there was an additive anabolic or growth-promoting effect very similar to that seen previously with IGF-I infusions.

What is claimed is:

1. An IGF-I-containing composition for subcutaneous administration comprising about 8-12 mg/ml of IGF-I, about 5-6 mg/ml of sodium chloride, a stabilizer consisting of about 8-10 mg/ml of benzyl alcohol or about 2-3 mg/ml of phenol, or both about 8-10 mg/ml of benzyl alcohol and about 2-3 mg/ml of phenol, and an about 50 mM sodium acetate buffered solution at a pH of about 5.4.

2. The composition of claim 1 additionally comprising about 1-5 mg/ml of a surfactant.

3. The composition of claim 2 wherein the surfactant is polysorbate or poloxamer in an amount of about 1-3 mg/ml.

4. The composition of claim 3 wherein the surfactant is polysorbate.

5. A method for treating diabetes comprising administering subcutaneously to a mammal having diabetes an amount of the composition of claim 1 effective to treat diabetes.

6. A method for treating diabetes comprising administering subcutaneously to a mammal having diabetes an amount of the composition of claim 2 effective to treat diabetes.

7. A method for treating diabetes comprising administering subcutaneously to a mammal having diabetes an amount of the composition of claim 3 effective to treat diabetes.

8. A method for treating diabetes comprising administering subcutaneously to a mammal having diabetes an amount of the composition of claim 4 effective to treat diabetes.

* * * * *

Exhibit E

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Maintenance Fee Statement

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PATENT NUMBER	FEE AMT	SUR-CHARGE	U.S. PATENT APPLICATION NUMBER	U.S. PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?
5,681,814	\$2,300.00	\$0.00	08/071,819	10/28/97	06/04/93	08	NO

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PATENT NUMBER	FEE AMT	SUR-CHARGE	U.S. PATENT APPLICATION NUMBER	U.S. PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ST
5,681,814	\$850.00	\$0.00	08/071,819	10/28/97	06/04/93	04	NO	PA

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Patent Number:	5681814	Application Number:	08071819
Issue Date:	10/28/1997	Filing Date:	06/04/1993
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Patent Number: 5681814

Application Number: 08071819

	4th Year	8th Year	12th Year
Open Date	10/30/2000	10/28/2004	10/28/2008
Surcharge Date	05/01/2001	04/29/2005	04/29/2009
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Patent #: 5681814 **Issue Dt:** 10/28/1997 **Application #:** 08071819 **Filing Dt:** 06/04/1993

Inventors: ROSS G. CLARK, DOUGLAS A. YEUNG, JAMES Q. OESWEIN

Title: FORMULATED IGF-I COMPOSITION

Assignment: 1

Reel/Frame: 007020/0348

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Exec Dt: 06/06/1994

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EXHIBIT F

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: CLARK, Ross G., et al. Docket No.: 39766-0190
Serial No.: 08/071,819 Group Art Unit: 1642
Filing Date: June 4, 1993 Examiner: Huff, Sheela Jitendra
Patent No.: U. S. Patent No. 5,681,814 Issued: October 28, 1997
For: **FORMULATED IGF-I COMPOSITION**

POWER OF ATTORNEY AND GENERAL AUTHORITY FROM ASSIGNEE;
CERTIFICATE UNDER 37 C.F.R. §3.73 (b)

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Dear Sir:

Genentech, Inc. hereby certifies that it is the owner of the entire right, title and interest in U.S. Patent No. 5,681,814, as evidenced by the Assignment recorded on June 13, 1994, at REEL 007020, FRAME 0348.

The undersigned (whose title is supplied below) is empowered to act on behalf of Genentech, Inc.

The undersigned has reviewed all of the documents in the chain of title of U.S. Patent No. 5,681,814 and, to the best of the undersigned's knowledge and belief, title is in Genentech, Inc.

Genentech, Inc. hereby appoints Ginger R. Dreger (Registration No. 33,055), registered to practice before the U. S. Patent and Trademark Office, as its attorney with full power of substitution and revocation to transact all business in the U. S. Patent and Trademark Office in connection with U. S. Patent No. 5,681,814, including, but not limited to, filing for patent term extensions under 35 U.S.C. § 156. Genentech, Inc. requests that all correspondence and telephone communications be directed to the following person at the mailing address and telephone numbers hereinafter given:

U. S. Patent No. 5,681,814 issued October 28, 1997
Docket No. 39766-0190

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Genentech, Inc. gives general authority to Ginger R. Dreger to act on its behalf in patent matters.

Executed this the 25th day of October, 2005.

By: Janet E. Hasak
Janet E. Hasak
Title: Associate General Counsel

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10/24/05 10:41 AM (39766.0190)