



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2074166 C 2003/11/25

(11)(21) **2 074 166**

(12) **BREVET CANADIEN
CANADIAN PATENT**

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 1991/01/30
(87) Date publication PCT/PCT Publication Date: 1991/08/22
(45) Date de délivrance/Issue Date: 2003/11/25
(85) Entrée phase nationale/National Entry: 1992/07/17
(86) N° demande PCT/PCT Application No.: AU 1991/000031
(87) N° publication PCT/PCT Publication No.: 1991/012018
(30) Priorité/Priority: 1990/02/13 (PJ 8586) AU

(51) Cl.Int.⁶/Int.Cl.⁶ A61K 38/30, A61K 38/27

(72) Inventeurs/Inventors:
BALLARD, FRANCIS J., AU;
READ, LEANNA C., AU

(73) Propriétaire/Owner:
GROPEP LIMITED, AU

(74) Agent: SIM & MCBURNEY

(54) Titre : METHODE DE TRAITEMENT DE MALADIES INTESTINALES
(54) Title: METHOD FOR TREATING INTESTINAL DISEASES

(57) **Abrégé/Abstract:**

A method for the treatment of disorders in gut function in animals including humans, which method includes administering to a patient to be treated an effective amount of a mammalian insulin-like growth factor-I (IGF-I) or a peptide analogue thereof.



PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ :**A61K 37/36****A1****(11) International Publication Number:****WO 91/12018****(43) International Publication Date:****22 August 1991 (22.08.91)****(21) International Application Number:** PCT/AU91/00031**(22) International Filing Date:** 30 January 1991 (30.01.91)**(30) Priority data:**

PJ 8586

13 February 1990 (13.02.90) AU

(71) Applicant (for all designated States except US): GROPEP
PTY. LTD. [AU/AU]; 39 Winwood Street, Thebarton,
S.A. 5031 (AU).**(72) Inventors; and****(75) Inventors/Applicants (for US only) :** BALLARD, Francis,
John [AU/AU]; 23 Lowan Avenue, Glenalta, S.A. 5052
(AU). READ, Leanna, Christine [AU/AU]; 4/46 High
Street, Kensington, S.A. 5068 (AU).**(74) Agent:** PHILLIPS ORMONDE & FITZPATRICK; 367
Collins Street, Melbourne, VIC 3000 (AU).**(81) Designated States:** AT (European patent), AU, BE (Euro-
pean patent), CA, CH (European patent), DE (Euro-
pean patent), DK (European patent), ES (European pa-
tent), FR (European patent), GB (European patent), GR
(European patent), IT (European patent), JP, LU (Euro-
pean patent), NL (European patent), SE (European pa-
tent), US.**Published***With international search report.***2074166****(54) Title:** METHOD FOR TREATING INTESTINAL DISEASES**(57) Abstract**

A method for the treatment of disorders in gut function in animals including humans, which method includes administering to a patient to be treated an effective amount of a mammalian insulin-like growth factor-I (IGF-I) or a peptide analogue thereof.

METHOD FOR TREATING INTESTINAL DISEASES

This invention relates to insulin-like growth factor-I (IGF-I) and its analogues. More particularly, the invention relates to the use of IGF-I and its analogues to treat disorders in gut function, e.g. relating to the effects of intestinal diseases.

IGF-I is a small protein that has been shown to stimulate the growth of cells in culture. Animal growth is also stimulated in pituitary-deficient, normal and catabolic states. Kidney function is also improved. These studies have led to the interpretation that IGF-I may be usefully applied in humans:

- (1) to treat growth hormone deficiencies;
- (2) to suppress the loss of body protein in catabolic states following burns, infection or other trauma; and
- (3) as a treatment for patients suffering from renal diseases.

A number of human diseases result either in the subject having a lesser amount of gut tissue than required for normal digestion or absorption, or alternatively a diseased but normal-length gut in which digestion or absorption is impaired. Examples of human diseases that fit these two categories include short-gut syndrome, chronic ulcerative gut diseases, inflammatory gut diseases such as colitis and Crohn's disease, and necrotising enterocolitis in infants.

IGF-I is not known in the prior art to increase the growth or function of the cell types that comprise the abdominal gut, viz the stomach, duodenum, jejunum, ileum, cecum and colon. It is these regions of the gut that are affected in the diseases described above. Gut tissues do contain receptors for IGF-I (for example see: M. Laburthe et al, Am. J. Physiol. 254, G457, 1988; D.J. Pillion et al., Am. J. Physiol. 257, E27, 1989; H. Werner et al, Proc. Natl. Acad. Sci. USA 86, 7451, 1989), and are known under certain conditions to synthesise IGF-I or IGF-I messenger RNA (for example see: P.K. Lund et al., J. Biol. Chem. 261, 14539, 1986; A.J. D'Ercole et al.,

Pediatr. Res. 20, 253, 1986; H.-A. Hansson et al.,
Histochemistry 89, 403, 1988; W.L. Lowe, Jr., J. Clin. Invest.
84, 619, 1989).

IGF-I has previously been administered to dwarf mice,
5 hypophysectomized rats, diabetic rats, starved mice and rats,
normal rats, mini poodles and normal human subjects. The
levels of IGF-I have also been increased by insertion of an
IGF-I-expressing transgene in mice. In most of these studies
IGF-I treatment has led to an increase in body growth. It has
10 been noted that an undesirable side effect of high doses of
IGF-I is hypoglycemia. However, in none of the reported
studies was there any indication of an IGF-I effect on the
abdominal gut. For example in one investigation where over
expression of IGF-I was produced by transgenesis and led to
15 substantial body growth in transgenic mice, the fractional
weight of the duodenum was the same as in IGF-deficient
animals (R.R. Behringer et al., Endocrinology 127, 1033,
1990). In another study the administration of low doses of
IGF-I to suckling rats did not alter the weights of
20 gastrointestinal organs (G.P. Young et al., Digestion 46S2,
240, 1990). In particular, there exists no prior report on the
increase of gut weight following IGF-I administration.

It is accordingly an object of an aspect of the present
invention to overcome or at least alleviate, one or more of
25 the difficulties related to the prior art.

The first aspect of the present method concerns the
treatment of animals, including humans, with disorders in gut
function by using mammalian, preferably human IGF-I.

Accordingly in a first aspect of the present invention
30 there is provided a method for the treatment of disorders in
gut function in animals, including humans, which method
includes administering to a patient to be treated an effective
amount of mammalian insulin-like growth factor-1 (IGF-I) or a
peptide analogue thereof.

35 By the term "disorders in gut function", as used herein,
we mean disorders in one or more of the stomach, duodenum,
jejunum plus ileum, and colon. Such disorders

may relate to intestinal diseases and/or surgical treatments. Thus where reference is made herein in general terms to the treatment of intestinal diseases, it is to be understood that we include the treatment of non-diseased intestines, part of which have been surgically removed and where increased growth of the residual intestine may be advantageous.

The term "peptide analogue", as used herein, includes one or more of the peptide analogues referred to in applicant's International Patent WO 87/01038 and WO 89/05822, or fusion proteins derived therefrom as described in International Patent WO 90/15142, referred to above.

An effective amount of mammalian IGF-1 or an analogue of IGF-1 is defined as that needed to increase the weight of gut tissue by more than approximately 20% above the weight of gut tissue prior to treatment.

Surprisingly IGF-1 and its analogues have now been found by the applicants to increase the weight of the stomach, duodenum, jejunum plus ileum, and colon as well as the total gut weight. Of particular significance is the fact that these effects on growth have been found to occur in animals under a range of conditions, including

- (a) in animals in which gut function had previously been markedly compromised by the surgical removal of a substantial portion of the jejunum plus ileum
- (b) animals to which glucocorticoids have been administered in order to produce a catabolic state

-4-

(c) animals in which diabetes had been induced by the drug streptozotocin, a condition that led to gut growth so that the effects of IGF-1 were above an already-stimulated growth state, and

5 (d) animals which had compromised kidney function.

Since glucocorticoids are administered to humans as a current treatment for the inflammation that characterizes several gut diseases, the present discovery that gut growth can be partially restored by IGF-1 or its analogues even in the presence of glucocorticoids is both
10 surprising and advantageous.

In accordance with an aspect of the invention use of a mammalian insulin-like growth factor-I (IGF) or an analogue thereof capable of increasing the growth of gut
15 tissue as an agent for the treatment of a disorder of gut function in a mammalian subject, wherein the disorder in gut function results in the subject having a lesser amount of gut tissue than required for normal digestion or absorption.

In accordance with another aspect of the
20 invention a pharmaceutical or veterinary composition for the treatment of a disorder of gut function results in the subject having a lesser amount of gut tissue than required for normal digestion or absorption, the pharmaceutical or veterinary composition comprising:

25 a) and effective amount of mammalian insulin-like growth factor-I (IGF-1) or an analogue thereof capable of increasing the growth of gut tissue; and

b) a pharmaceutically or veterinarily acceptable diluent carrier or excipient therefore.

30 In a preferred aspect of the present invention a peptide analogue to mammalian, preferably human, IGF-1 is administered. Preferably the peptide analogue is an analogue wherein from 1 to 5 amino acid residues are absent from the N-terminal of mammalian IGF-1. Preferably

-5-

3 amino acid residues are absent therefrom. Such peptide analogue has been designated des(1-3)IGF-1.

Alternatively the peptide analogue may be an analogue wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of mammalian IGF-1, and optionally replaced by a different amino acid residue. Preferably the analogue is one wherein the glutamic acid residue is replaced by an arginine residue.

More preferably the peptide analogue has an N-terminal sequence selected from

Val-Leu-Cys-
Arg-Leu-Cys-
Gly-Leu-Cys-
Gly-Thr-Leu-Cys-
15 Gly-Pro-Arg-Thr-Leu-Cys-
Gly-Pro-Gly-Arg-Leu-Cys-
Gly-Pro-Gly-Gly-Leu-Cys-
Gly-Pro-Gly-Thr-Leu-Cys-
Gly-Pro-Gln-Thr-Leu-Cys-
20 Gly-Pro-Lys-Thr-Leu-Cys-
Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 6 from the N-terminal.

In a further alternative aspect, the peptide analogue is a fusion protein including

a first amino acid sequence including approximately the first 100 N-terminal amino acids of methionine porcine growth hormone, or a fragment thereof; and

30 a second amino acid sequence of mammalian insulin-like growth factor-1, or an analogue thereof, joined to the C-terminal of the first amino acid sequence.

-5a-

Preferably the first amino acid sequence includes approximately the first 46, more preferably the first 11, N-terminal amino acids of methionine porcine growth hormone, or fragment thereof.

5 The IGF-1 analogues include des(1-3)IGF-1, specified in the co-owned International application PCT/AU86/00246 had been shown to increase the growth of cultured cells at lower dose rates than required for IGF-1. In the development of the present invention, des
10 (1-3)IGF-1 has surprisingly been shown to show this increased potency in vivo, in the treatment of disorders in gut function, since lower concentrations of the analogue than of IGF-1 itself produce comparable increases in the growth of gut tissue.

15 The IGF-1 analogues also include a fusion protein MpGH(11)VN/R³IGF-1 (abbreviated to LR³), specified as Example 13 in the co-owned International Application PCT/AU90/00210, had been shown to increase the growth of cultured cells at lower dose rates than required for IGF-
20 1. In the present invention, LR³ has also surprisingly been shown to show increased potency in the treatment of disorders in gut function, since lower concentrations of the analogue than of IGF-1 itself produce comparable increases in the growth of gut tissue.

25 Although the method in particular applies to the treatment of human subjects with IGF-I or an analogue of IGF-1, it can also be applied veterinarily to animals with intestinal diseases.

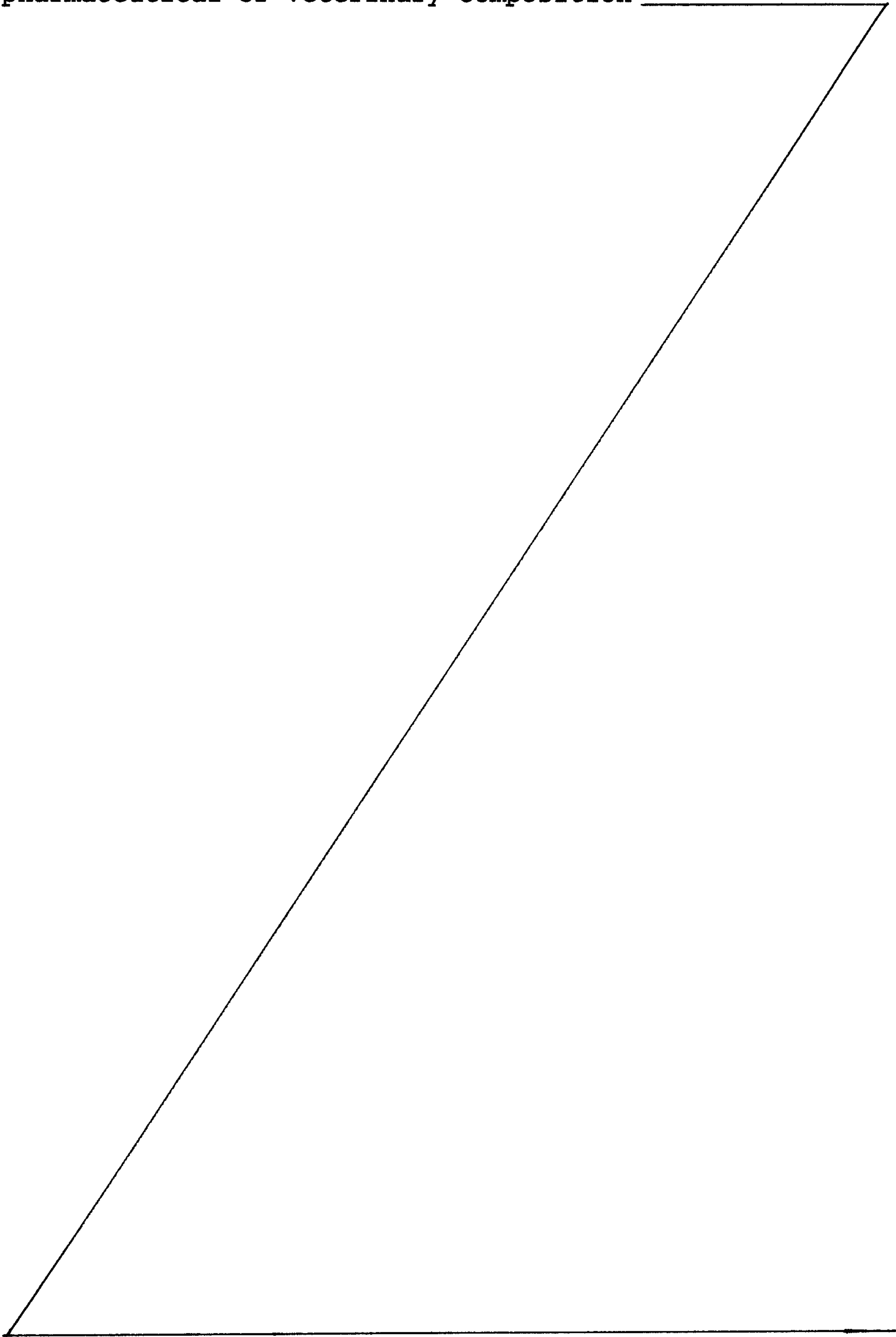
 Accordingly, in a further aspect, the present
30 invention provides a pharmaceutical or veterinary composition for the treatment of disorders in gut function including:

- (a) an effective amount of a mammalian, preferably human, IGF-1 or peptide analogue thereof; and

-5b-

(b) a pharmaceutically or veterinarily acceptable
diluent, carrier or excipient therefor.

In a preferred form, the present invention a
pharmaceutical or veterinary composition _____



wherein the IGF-I or the analogue of IGF-I is present in amounts sufficient to provide a dose rate of approximately 10 to 2000, preferably 100 to 1000 micrograms/kg body weight/day.

5 In a further preferred aspect of the present invention, there is provided a method for the treatment of disorders in gut function which method includes administering to a patient to be treated a pharmaceutical or veterinary composition including

- 10 (a) an effective amount of a mammalian, preferably human, IGF-I or peptide analogue thereof, and
(b) a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor;
intravenously, subcutaneously, intramuscularly or
15 enterally at a dose rate of approximately 10 to 2000, preferably 100 to 1000 micrograms/kg body weight/day. Treatment may continue for a period of approximately 1 to 60 days, preferably approximately 5 to 30 days.

The dose rate, dose intervals and treatment
20 period may be adjusted to the degree of intestinal disease and the route of administration. Caution should be taken that blood glucose is monitored so that hypoglycemia can be prevented.

The dose rates and intervals for the
25 administration of des(1-3)IGF-I and related analogues may be set at levels proportionally adjusted to the relative potency of the analogue to that of IGF-I itself. For example the levels for des(1-3)IGF-I or LR³ will be proportionally less than for the full IGF-I peptide in
30 accordance with the increased potency of des(1-3)IGF-I or LR³. Dose rates of 50 to 500 micrograms of des(1-3)IGF-I LR³ /kg body weight/day are preferred.

In a still further aspect of the present invention there is provided the use of a mammalian
35 insulin-like growth factor-1 (IGF-I) or a peptide analogue thereof for the manufacture of a pharmaceutical or veterinary preparation for the treatment of disorders in gut function.

The pharmaceutical or veterinary preparations may

be prepared utilising conventional techniques.

The benefits and parameters of the present invention will now be more fully described with reference to the accompanying examples. It should be understood, however, that the following description is illustrative only and should not be taken in any way as a restriction of the generality of the foregoing description.

EXAMPLE 1

Effects of IGF-I and des(1-3)IGF-I on stomach growth in the growth hormone-deficient lit/lit mouse

At 6 weeks of age lit/lit mice were housed individually and weighed on a daily basis, and by 8 weeks of age it had been established that body growth had virtually ceased.

Mice were then randomised into 5 groups according to body weight and sex and were injected daily for 20 days with

- (a) 120 μ l of a sterile solution containing one part of HCl (10 mmol/l) and nine parts of potassium phosphate (50 mmol/l), NaCl (150 mmol/l) and 0.1% human serum albumin at pH 7.4 (diluent),
- (b) 120 μ l of diluent containing 3 μ g IGF-I,
- (c) 120 μ l of diluent containing 3 μ g des(1-3)IGF-I,
- (d) 120 μ l of diluent containing 30 μ g IGF-I, or
- (e) 120 μ l of diluent containing 30 μ g des(1-3)IGF-I.

Each dose was administered subcutaneously as two injections, one at 9-10 am, the other at 4-5 pm. The animals were weighed and their lengths measured at 7 day intervals.

On day 21 the animals were killed by an anaesthetic overdose and tissues removed for weighing. The body weights, animal lengths (including tail) and stomach weights are shown in the following Table 1. Values are means $\pm$ standard errors with statistical significance from diluent-treated control animals shown as *P<0.05; **P<0.01. The numbers of animals are given in parentheses.

Since the initial body weights were 10 grams for each group, the daily dose rates were approximately

-8-

equivalent to 300 µg and 3000 µg of each peptide per kg body weight.

Stomach weights, expressed as a percentage of the diluent group, were 105% and 123% with 300 µg and 3000 µg/kg body weight/day of IGF-I respectively, and 110% and 123% with 300 µg and 3000 µg/kg body weight/day of des(1-3)IGF-1 respectively.

EXAMPLE 2

Effects of IGF-I and des(1-3)IGF-I LR³ on gut weights in dexamethasone-treated rats

Male Hooded Wistar rats, weighing on average 152 g (range 138-164 g) and maintained in metabolism cages, had AlzetTM model 2001 osmotic pumps inserted subcutaneously within the scapular region under ether anaesthesia. One pump delivered dexamethasone at 20 micrograms/d and the other either

- (a) 0.1M acetic acid as diluent;
- (b) IGF-I at 111 µg/d;
- (c) IGF-I at 278 µg/d;
- (d) IGF-I at 695 µg/d;
- (e) des(1-3) GF- at 44 µg/d;
- (f) des(1-3)IGF-I at 111 µg/d;
- (g) des(1-3)IGF-I at 278 µg/d;
- (h) LR³ at 44 µg/d;
- (i) LR³ at 111 µg/d;
- (j) LR³ at 278 µg/d.

Animals were maintained in the metabolism cages for 7 days with daily measurements of body weight, food and nitrogen intake and nitrogen excretion. After this period the animals were killed by exsanguination under anaesthesia and the gastro-intestinal tract from stomach to colon was removed and separated into stomach, duodenum, jejunum plus ileum, cecum and colon. All regions were cleared of food or fecal contents and weighed.

The body weights and the weights of the different regions of the gastrointestinal tract are given as means ± standard errors in Table 2. Statistical significance from the diluent-treated group is shown as *P<0.05; **P<0.01; ***P<0.001. There were six animals in each

group.

The weights of the total gut from stomach through colon is depicted in Figure 1 as a fraction of total body weight in the format of dose-response curves for IGF-I, des(1-3)IGF-I and LR³.

For a midpoint region of the ileum and the colon a portion was cut longitudinally and scraped to remove the mucosal layer. The weight of this layer was expressed as a percentage of the total mucosa plus muscularis. The protein contents of the same regions of the ileum were measured and expressed as mg per gram wet weight of tissue. These values are given as means $\pm$ standard errors in Table 3. Statistical significance from the diluent-treated animals was not achieved using ANOVA ($P > 0.05$).

This example demonstrates marked increases in the weights of different regions of the gut of dexamethasone-treated rats following the administration OF IGF-I, des(1-3)IGF-I or LR³. The effects are dose dependent and are greater for des(1-3)IGF-I or LR³ at doses equivalent to IGF-I.

The increased growth occurs predominantly through an expansion in the cross-sectional area of the gut because the length of each region is either not increased or increased only slightly.

It is evident from Figure 1 that the increase in gut weight is proportionally above that occurring for body weight.

Both mucosal and muscularis regions of the jejunum and colon show increased growth because the percent by weight accounted for by the mucosa is not affected by IGF treatment.

Growth occurs by an increase in tissue protein since the percent protein content of the jejunum and colon is not changed by IGF treatment.

EXAMPLE 3

Quantitative histology of the duodenum from rats in Example 2

The mid region of the duodenum from certain animals in Example 1 was fixed in Bouin's fixative,

-10-

dehydrated, embedded, transverse sections cut and stained with haematoxylin and eosin for quantitative histological analysis. All animals in groups (a), (c), (g) and (j) of Example 2 were processed and measurements of villus height, crypt depth, mucosal area, submucosal area, muscularis externa area and total cross-sectional area were obtained. For each duodenum, 30 villus heights, 30 crypt depths and 8 area measurements were averaged to obtain representative values. Means $\pm$ SEM (N=6) are shown in Table 4.

This example establishes that the growth of the duodenum produced by IGF-I, des(1-3)IGF-I or LR³ at dose rates of 278 μ g/d is accompanied by statistically significant ($P < 0.01$) increases in cross-sectional area. The increase is predominantly in the mucosal area, although the muscularis layer is also increased. The villi that make up much of the mucosa and play a key role in digestion and absorption, are also increased in height.

EXAMPLE 4

Effects of IGF-I and des(1-3)IGF-I on gut weights in rats treated following partial resection of the jejunum plus ileum

Male Sprague Dawley rats, weighing on average 175 g (range 160-193 g) and maintained in metabolism cages, had Alzet model 2001 osmotic pumps inserted as in Example 2. The dose ranges of growth factors were 170 μ g/d for IGF-I and for des(1-3)IGF-I as well as a higher dose rate of 425 μ g IGF-I/d. At the same time as the pumps were inserted subcutaneously, and using tribromethanol in amylene hydrate anaesthesia and aseptic techniques, the jejunum plus ileum was exposed through a mid-line incision. The mid 80% of these parts of the intestine was removed starting 10 cm from the ligament of Treitz and finishing 10 cm from the ileo-cecal valve. The intestine and abdominal cavity were bathed frequently in sterile saline containing penicillin (1000 U/ml). To further guard against possible infection, the animals were injected with 0.6 ml of procaine penicillin before surgery and again 4 days later. The animals were returned to

their metabolic cages and allowed free access to food and water. Body weights, food intakes, nitrogen intakes and nitrogen outputs were measured daily.

After 7 days of treatment, the animals were
5 killed by exsanguination under anaesthesia and the
gastro-intestinal tract from stomach to colon was removed
and separated into stomach, duodenum, residual jejunum
plus ileum, colon and cecum. All collected regions of the
gut were cleared of food or fecal contents and weighed.
10 The body weights and the weights of the different regions
of the gut are given as means $\pm$ standard errors in Table
5. Statistical significance from the diluent-treated
group is shown as * $P < 0.05$; ** $P < 0.01$. There were 7 animals
in the diluent and des(1-3)IGF-I groups, six in the group
15 with the low dose of IGF-I and five in the group with the
high dose of IGF-I.

The example shows that des(1-3)IGF-I and a 2.5
fold higher dose of IGF-I produce pronounced growth
effects on the gut. A dose of IGF-I equal
20 to that of des(1-3)IGF-I gave no statistically-significant
effects.

The total gut weight less the residual jejunum
and ileum, expressed as a percentage of the diluent group,
was 113% with 170 μ g IGF-I/d, 131% with 425 μ g IGF-I/d,
25 and 125% with 170 μ g des(1-3)IGF-I/d.

EXAMPLE 5

Effects of IGF-I, des(1-3)IGF-I and MpGH(11)VN/R³IGF-I
(LR³) on gut growth in diabetic rats

Male Hooded-Wistar rats weighing approximately
30 150 g were injected with streptozotocin intra-peritoneally
at a dose of 70 mg/kg and transferred to metabolic cages.
Diabetes was confirmed by blood glucose measurements.
After 7 days (average body weight 162 g) the animals were
implanted with osmotic pumps in exactly the same way and to
35 deliver exactly the same doses as in Example 1. After 7
days treatment the animals were killed and the weights of
the following gut organs measured: stomach, duodenum,
ileum plus jejunum and colon. These values are shown in
Table 6 and the total gut weights in Figure 2. Each value

is the mean $\pm$ SEM for six animals. Statistical significance (ANOVA; least significant difference) is shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus diluent-treated diabetic rats.

5 These results demonstrate that IGF-I and lower doses of des(1-3)IGF-I or LR³ produce substantial growth effects on the stomach, ileum plus jejunum, colon and total gut weights. Effects on the duodenum are somewhat less.

10 The response of IGFs in the diabetic rats is especially important since even untreated animals already have heavier gut weights as a result of the hyperphagia associated with this condition. For example the weight of ileum plus jejunum was 5.08 ± 0.29 g in another group of
15 animals in which the diabetes was treated by insulin administration. This is lower than the diluent group in Table 5, notwithstanding the fact that the body weight of the insulin-treated animals was much heavier (231 ± 8 g).

EXAMPLE 6

20 Effects of IGF-I and des(1-3)IGF-I on gut weights in rats following partial nephrectomy

 Partial renal failure was produced in Sprague Dawley male rats (95-125 g) by a 2-stage sub-total nephrectomy. This was performed via flank incisions, by
25 ligating terminal branches of the left renal artery to give ischemia of at least half of the left kidney (Day 0), with a right nephrectomy being undertaken one week later, at which time the right jugular vein was also cannulated (Day 7). Partially nephrectomized rats were selected on
30 day 14 for inclusion in the treatment period of the study on the basis of detectable proteinuria, and of increased urine volume and serum urea levels at least twice those of the sham-operated control animals. On Day 16 treatment was commenced by means of mini-osmotic pumps (Alzet Model
35 2001, Alza Co., Palo Alto, California) which were implanted subcutaneously in the dorsal thoracic region under halothane anesthesia. Nephrectomized rats were randomly allocated to 4 treatment groups: diluent treated (0.1M acetic acid), low dose IGF-I treated ($170 \mu\text{g/d}$),

high dose IGF-I treated (425 µg/d), or des(1-3)IGF-I treated (170 µg/d). The average body weight at time of pump insertion was 193 g. Animals were killed on Day 23 and gut weights measured as in Example 2. These are shown in Table 7. Statistical significance from diluent-treated animals is indicated by *P<0.05, **P<0.01.

This example demonstrates that gut weights are increased by 7 days treatment with IGF-I or des(1-3)IGF-I in rats that are compromised by partial nephrectomy.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

15

20

25

30

35

2971166
- 14 -

TABLE 1
Growth effects of IGF-I and des(1-3)IGF-I over a 20-day treatment period of lit/lit mice

Treatment Group	Body Weight (g)	Animal Length (mm)	Stomach Weight (mg)
Diluent (8)	10.20±0.19	124±2	64.3±3.0
IGF-I, 3 µg/d (8)	10.75±0.28	127±1	67.8±2.1
30 µg/d (6)	11.25±0.41	132±1*	79.0±3.0**
des(1-3) IGF-I, 3 µg/d (8)	10.76±0.31	127±2	70.6±2.5
30 µg/d (6)	11.33±0.42	132±1	79.3±4.4**

TABLE 2
Gut weights (g), lengths (cm) and body weights (g) of dexamethasone-treated rats
also treated with IGF-I, des(1-3)IGF-I or LR³

Treatment	Body Weight	Stomach Weight	Duodenum	
			Weight	Length
Diluent	131±3	0.87±0.03	0.50±0.02	7.2±0.4
IGF-I, 111 µg/d 278 µg/d 695 µg/d	130±4	0.93±0.03*	0.55±0.03	6.9±0.3
	143±4*	1.09±0.02***	0.70±0.02***	7.6±0.2
	158±2***	1.17±0.02***	0.84±0.02***	8.0±0.2*
des(1-3) IGF-I, 44 µg/d 111 µg/d 278 µg/d	135±3	0.98±0.02**	0.63±0.03**	7.6±0.3
	144±5*	1.06±0.02***	0.68±0.02***	7.6±0.2
	151±2***	1.14±0.02***	0.86±0.03***	8.7±0.3***
LR ³ 44 µg/d 111 µg/d 278 µg/d	135±1	0.95±0.01*	0.61±0.02**	7.3±0.3
	140±4	1.07±0.02***	0.75±0.03***	7.6±0.4
	150±3***	1.16±0.02***	0.86±0.03***	8.3±0.2**

2074166

- 16 -

TABLE 2 - CONTINUED

Ileum + Jejunum		Colon	
Weight	Length	Weight	Length
3.07±0.22	70.8±4.1	0.68±0.05	11.0±0.4
3.60±0.19	71.8±2.6	0.64±0.02	12.0±0.3
4.30±0.21***	71.7±3.1	0.92±0.04***	13.3±0.6
5.22±0.23***	86.1±3.6	0.93±0.05***	12.7±0.6
3.80±0.25*	72.1±3.5	0.72±0.05	11.3±0.8
4.44±0.24***	71.8±2.6	0.86±0.04**	13.4±0.5
5.09±0.21***	73.0±4.1	0.91±0.04***	11.3±0.8
3.93±0.24**	72.4±3.8	0.75±0.03	12.3±0.2
4.67±0.18***	75.8±3.3	0.89±0.05**	12.5±0.8
5.20±0.33***	79.5±4.1	0.98±0.05***	12.9±0.6

TABLE 3

Fractional weights and protein contents of the jejunum in the mucosal layers in the jejunum and in the colon dexamethasone-treated rats treated with IGFs

Treatment	Jejunum			Colon	
	Mucosa (% of total)	Mucosa Protein (mg/g weight)	Muscularis Protein (mg/g weight)	Mucosa (% of total)	
Diluent	55.1±3.3	124±5	169±4	30.5±2.5	
IGF-I, 111 µg/d	58.8±2.2	117±6	151±4	29.6±1.7	
278 µg/d	62.7±1.5	135±5	161±5	31.7±1.7	
695 µg/d	61.1±2.8	136±5	159±2	28.8±1.2	
des(1-3)IGF-I, 44 µg/d	53.3±3.9	134±5	161±5	31.1±1.7	
111 µg/d	59.6±2.3	140±5	162±4	27.5±2.2	
278 µg/d	55.3±3.6	140±4	154±6	32.5±2.5	
LR ³ , 44 µg/d	56.8±4.2	119±10	147±4	30.2±2.8	
111 µg/d	60.7±3.3	139±4	168±6	29.6±2.1	
278 µg/d	54.2±3.2	135±6	159±5	30.6±2.2	

TABLE 4
Sections from rats treated with IGF-I des(1-3)IGF-I, LR³ or diluent

Measurement	Treatment Group			
	Diluent	IGF-I	des(1-3)IGF-I	LR ³
Villus height (mm)	0.65±0.02	0.76±0.03	0.73±0.03	0.84±0.03**
Crypt depth (mm)	0.20±0.01	0.23±0.01	0.23±0.00	0.23±0.01
Villus : crypt ratio	3.3 ±0.2	3.4 ±0.2	3.3 ±0.1	3.7 ±0.2
Mucosal area (mm ²)	5.67±0.44	7.72±0.56**	8.03±0.58**	8.29±0.56**
Sub-mucosal area (mm ²)	0.42±0.04	0.51±0.04	0.52±0.03	0.51±0.04
Musc. externa area (mm ²)	1.20±0.15	1.51±0.05*	1.54±0.06**	1.41±0.12
Total area (mm ²)	7.29±0.61	9.74±0.59**	10.10±0.62**	10.21±0.66**

*P<0.05, **P<0.01, ***P<0.001 versus diluent

TABLE 5

Duodenum weight (mg)	Diluent	IGF-I 170µg/d	IGF-I 425 µg/d	des(1-3) IGF-I 170 µg/d
Body weight (g)	171.6 ± 4.5	173.4 ± 5.9	190.9 ± 4.2	184.1 ± 5.6
Stomach weight (mg)	980 ± 75	1089 ± 47	1236** ± 26	1136* ± 52
Duodenum weight (mg)	1067 ± 75	1179 ± 88	1572** ± 81	1542** ± 102
Residual Jejunum + Ileum weight (mg)	1984 ± 118	2217 ± 244	2288 ± 60	2259 ± 150
Colon weight (g)	1067 ± 162	1305 ± 164	1330 ± 65	1250 ± 127
Total gut weight less residual jejunum & ileum (g)	4.28 ± 0.27	4.83 ± 0.34	5.59** ± 0.13	5.37** ± 0.24

2071166

- 20 -

TABLE 6
Gut weights (g) of diabetic rats and body weights (g)

	Body Weight	Stomach	Duodenum	Ileum + Jejunum	Colon
Diluent	172±6	1.01±0.04	0.72±0.09	6.10±0.49	0.95±0.02
IGF-I, 111 µg/d	186±4	1.13±0.04	0.74±0.08	6.21±0.37	1.05±0.07
278 µg/d	198±8*	1.19±0.06*	0.91±0.05	7.41±0.32*	1.09±0.05
695 µg/d	210±8***	1.44±0.08***	0.94±0.06*	8.17±0.32***	1.18±0.05**
des(1-3) IGF-I, 44 µg/d	185±6	1.23±0.05**	0.82±0.11	6.83±0.38	1.02±0.05
111 µg/d	194±7*	1.27±0.06**	0.89±0.07	7.97±0.20**	1.16±0.08**
278 µg/d	212±8***	1.49±0.07***	1.02±0.09**	8.69±0.40***	1.41±0.03***
LR ³ 44 µg/d	190±7	1.14±0.06	0.85±0.07	7.58±0.48*	1.05±0.04
111 µg/d	204±6**	1.38±0.04***	0.88±0.11	7.83±0.53**	1.25±0.07***
278 µg/d	213±8***	1.48±0.08***	0.91±0.06	8.47±0.46***	1.50±0.04***

TABLE 7

Gut weights (g) in partially-nephrectomised rats treated with IGF-I or des(1-3)IGF-I

Treatment	Stomach	Duodenum	Ileum + Jejunum	Colon	Total
Diluent	1.08±0.04	0.51±0.02	3.78±0.19	0.83±0.03	6.21±0.22
IGF-I, 170 µg/d	1.19±0.03	0.63±0.03*	4.70±0.27**	0.97±0.03*	7.50±0.30**
425 µg/d	1.25±0.06*	0.75±0.07**	5.11±0.22**	1.05±0.07**	8.16±0.27**
des(1-3)IGF-I					
170 µg/d	1.16±0.06	0.66±0.03*	4.48±0.15*	0.95±0.06	7.24±0.10**

2074166

CLAIMS:

1. Use of a mammalian insulin-like growth factor-I (IGF-1) or an analogue thereof capable of increasing the growth of gut tissue as an agent for the treatment of a disorder of gut function in a mammalian subject, wherein the disorder in gut function results in the subject having a lesser amount of gut tissue than required for normal digestion or absorption.
2. A use, according to claim 1, wherein the disorder in the gut function is selected from the group consisting of short gut syndrome, chronic ulcerative gut disease, inflammatory gut disease and neorotizing enterocolitis.
3. A use, according to claim 2, wherein the inflammatory gut disease is colitis or Crohn's disease.
4. A use, according to claim 1, wherein the use increases gut weight relative to the weight of the subject.
5. A use, according to claim 4 wherein the increase in gut weight is due to an increase in the weight of one or more of stomach, small intestine, duodenum, jejunum, ileum, cecum or colon.
6. A use, according to claim 4 wherein the increase in gut weight is due to an increase in cross-sectional area, muscularis layer, mucosal area, or villus height in gut tissue.
7. A use, according to claim 2, wherein the disorder in gut function is selected from the group including

disorders of the stomach, small intestine, duodenum, jejunum, ileum, cecum, colon, or combinations thereof.

8. A use according to claim 1, wherein the mammalian
5 insulin-like growth factor-I (IGF-1) or analogue thereof
is for the manufacture of a composition for the treatment
of a gut disorder in a mammalian subject where the
composition is delivered to said mammalian subject
intravenously, subcutaneously, intramuscularly or
10 enterally.

9. A use, according to claim 1, wherein the mammalian
insulin-like growth factor-I (IGF-1) or analogue thereof
is for the manufacture of a composition for the treatment
15 of a gut disorder in a mammalian subject where the
composition is delivered to said mammalian subject in a
amount of from 10 to 5000 microgram/kg body weight/day
for a period of 1 to 60 days.

20 10. A use, according to claim 1 wherein the mammalian
IGF-1 is human IGF-1.

11. A use, according to claim 1, wherein the IGF-1
analogue is selected from the group consisting of
25 mammalian des (1-3) IGF-1 and LR³.

12. A pharmaceutical or veterinary composition for the
treatment of a disorder of gut function in a mammalian
subject, wherein the disorder in gut function results in
30 the subject having a lesser amount of gut tissue than
required for normal digestion or absorption, the
pharmaceutical or veterinary composition comprising:

- a) an effective amount of a mammalian insulin-like
growth factor-I (IGF-1) or an analogue thereof

capable of increasing the growth of gut tissue;
and

- b) a pharmaceutically or veterinarily acceptable
diluent carrier or excipient therefore.

5

13. A pharmaceutical or veterinary composition according
to claim 12 wherein the analogue is selected from the
group consisting of mammalian des (1-3) IGF-1 and
MpGH(11)VM/R3IGF-1.

10

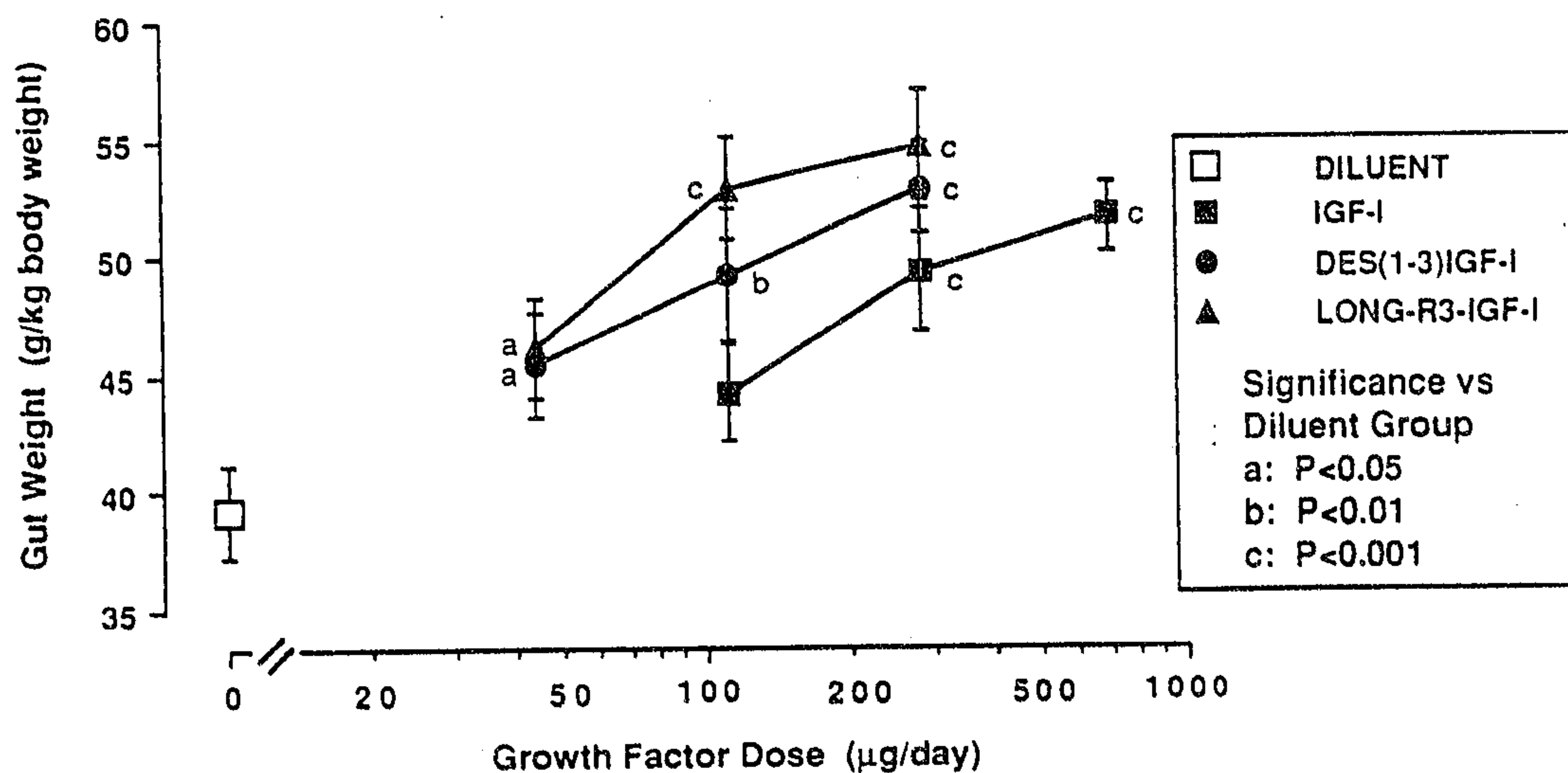
14. A pharmaceutical or veterinary composition according
to claim 12 wherein the IGF-1 or analogue thereof is
present in amounts sufficient to provide a dose rate of
approximately 10 to 5000 microgram/kg body weight/day for
15 a period of 1 to 60 days.

FIGURE 1

Effects of IGF-I, des(1-3)IGF-I and LR³ on total gut weight in dexamethasone-treated rats. Letters indicate statistical significance from diluent group (ANOVA): (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$.

FIG. 1. GLUCOCORTICOID-TREATED RATS:
TOTAL GUT WEIGHT AS A PROPORTION OF BODY WEIGHT

Dose Responses for IGF-I, Des(1-3)IGF-I and Long-R3-IGF-I



2/2

FIGURE 2

Total gut weights (stomach to colon) of diabetic rats treated with IGFs for 7 days. Letters indicate statistical significance (ANOVA) from diluent group : (a) $P < 0.05$; (b) $P < 0.01$; (c) < 0.001 .

**FIG. 2. DIABETIC RATS:
TOTAL GUT WEIGHT AS A PROPORTION OF BODY WEIGHT**

Dose Responses for IGF-I, Des(1-3)IGF-I and Long-R3-IGF-I

