SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

6354

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the convention application made for a patent for an invention entitled:

Method of Weight Control by Low Level Administration of Cobalt Protoporphyrin or Cobalt Mesoporphyrin

I/~~We~~, William H. Griesar
[full name of declarant(s)]

of 1230 York Avenue, New York, New York 10021 U.S.A.
[full address of declarant(s) - not post office box]

do solemnly and sincerely declare as follows:

1. I am/~~We~~ are authorised by The Rockefeller University, the applicant for the patent to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was made in United States of America on 14 February 1989 by Attallah Kappas and Richard A. Galbraith.

3. Attallah Kappas and Richard A. Galbraith, of 1161 York Avenue, Apt. 4L, New York, New York 10021 and 450 East 63rd Street, Apt. 3G, New York, New York 10021 both in the United States of America, respectively, are the actual inventors of the invention and the facts upon which the applicant is entitled to make the application are as follows:-

   The applicant is the assignee of the invention from the actual inventors.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at New York this 19th day of December 1990

Signature of Declarant(s)

William H. Griesar
Vice President

TO: THE COMMISSIONER OF PATENTS
AUSTRALIA
S&F REF: 144981
KRS/3122D

(12) PATENT ABRIDGMENT (11) Document No. AU-B-51546/90
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 613554

(54) Title
METHOD OF WEIGHT CONTROL BY LOW LEVEL ADMINISTRATION OF COBALT PROTOPORPHYRIN OR COBALT MESOPORPHYRIN

International Patent Classification(s)
(51)[5] A61K 031/40 A61K 031/555

(21) Application No. : 51546/90 (22) Application Date : 12.02.90

(87) PCT Publication Number : WO90/09173

(30) Priority Data

(31) Number (32) Date (33) Country
310854 14.02.89 US UNITED STATES OF AMERICA

(43) Publication Date : 05.09.90

(44) Publication Date of Accepted Application : 01.08.91

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(57) Claim

1. A method of controlling weight gain in animals in need of such control without concurrent decrease in endocrine activity which comprises parenteral administration of from 0.1 to about 4 µm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

6. A method of controlling the protein to fat ratio in animals in need of such control without concurrent decrease in endocrine activity which comprises parenteral administration of from 0.1 to about 4 µm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

11. A method of controlling weight gain in animals in need of such control without concurrent decrease in endocrine activity which comprises intraventricular administration of from 0.1 to 0.4 µm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

OPI DATE 05/09/90 APPLN. ID 51546 / 90

PCT AOJP DATE 11/10/90 PCT NUMBER PCT/US90/00790

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/40 — A1

(11) International Publication Number: WO 90/09173

(43) International Publication Date: 23 August 1990 (23.08.90)

(21) International Application Number: PCT/US90/00790

(22) International Filing Date: 12 February 1990 (12.02.90)

(30) Priority data:
310,854 14 February 1989 (14.02.89) US

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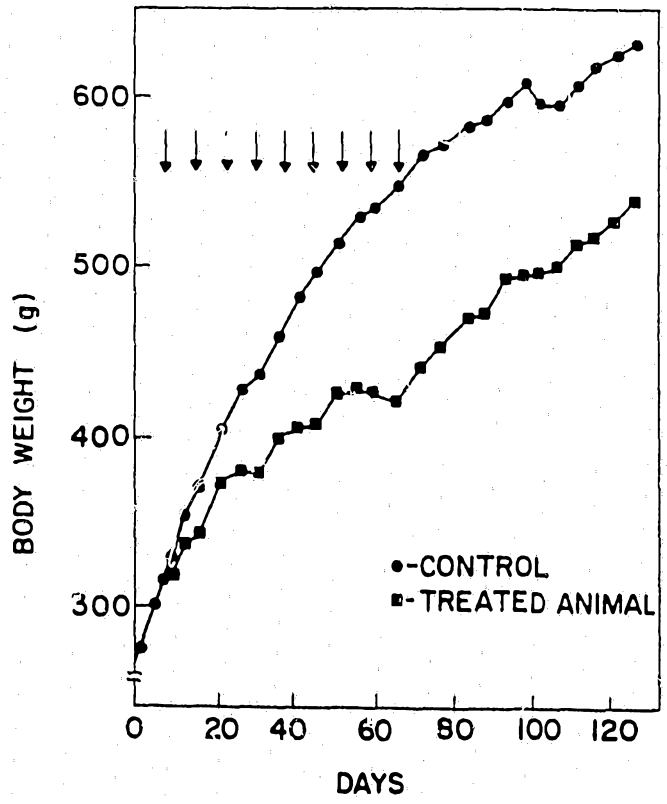
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: METHOD OF WEIGHT CONTROL BY LOW LEVEL ADMINISTRATION OF COBALT PROTOPORPHYRIN OR COBALT MESOPORPHYRIN

(57) Abstract

Cobalt protoporphyrin and cobalt mesoporphyrin are administered to animals to achieve weight loss and improved protein to fat ratio without decrease in hormone concentration.



METHOD OF WEIGHT CONTROL BY LOW LEVEL ADMINISTRATION OF COBALT PROTOPORPHYRIN OR COBALT MESOPORPHYRIN

This invention is concerned with methods of controlling the weight of animals, i.e., living beings including mammals such as man; bovines, particularly beef cattle; sheep, goats; poultry, especially chickens, ducks and turkeys; as well as fish, particularly those raised in fish farms such as salmon and trout; and, in general, all animals of economic importance in addition to pets by treatment with cobalt protoporphyrin (CoPP) or cobalt mesoporphyrin (CoMP). Both of these compounds are known. It is also known to treat animals with CoPP or CoMP.

United States Patent 4,393,071 relates to methods of treating malignant tumors with porphyrins including mesoporphyrin and protoporphyrin. Neither CoPP or CoMP are specifically named or illustrated. Moreover, if CoPP or CoMP were administered at the levels described for those porphyrins which are specifically named and illustrated there would be a serious question of toxicity.

Drummond and Kappas in Proc. Natl. Acad. Sci. USA 79:2284 (1982) describe the administration of CoPP to animals as a method to deplete the cytochrome P-450 content of the liver. The lowest level of administration described by the coauthors is 25 um/kg body weight.

Sako and coworkers have published the results of their studies of administration of CoPP to mice and rats to control the growth of Sarcoma 180 (ascites form), Sarcoma 180 (nodular form), Yoshida sarcoma (nodular

form) and Ehrlich's carcinoma (ascites form). See Juzen Igakukai Zasshi 67: 384 (1966), 67; 390 (1961) and 67: 395 (1961).

Kappas and Drummond in copending and commonly assigned patent application (Docket 17693E), describe parenteral administration of isotonic compositions containing CoPP and/or CoMP to animals to achieve reduced activity of the endocrine system, weight control and improved (P/F) ratio. More specifically, they describe the administration of the active compounds to limit the production of gonadal hormones and to suppress the production of thyroid hormones. This patent application also describes the administration of CoPP and CoMP to animals, including humans, to achieve weight control and to improve the P/F ratio. At the levels of administration described in the application endocrine suppression, weight control and improved P/F ratio all take place simultaneously.

The invention defined in the copending patent application is extremely valuable for all of the reasons described therein. However, in many instances it would be useful, in fact highly desirable, to control weight and P/F ratio without endocrine involvement. For example, the use of the active agents to control the weight of individuals in their reproductive years might be contraindicated because of the concurrent limited production of gonadal hormones.

It has now been discovered that the endocrine suppression activity of CoPP and CoMP declines as the dosage is decreased so that at a dosage of about 10

μm/kg b.w. (body weight), say 9.8 μm/kg b.w. it becomes negligible and at 4 μm/kg b.w. it has effectively disappeared. However, the weight control activity and the improved P/F ratio continues even down to as low as 0.1 μm/kg b.w. It is, therefore, possible to control the weight and P/F ratio of animals by treatment of animals in need of such control or improved P/F ratio with from about 0.1 μm/kg b.w. to about 4 μm/kg b.w. The preferred range is 0.1 to 0.3 μm/kg b.w. because at this low dosage there is substantially no endocrine suppression activity.

Figure 1 graphically illustrates the results observed in Example 1. Taken together with the results reported in Table I, it shows a decrease in body weight without decrease in hormone concentration on administration of CoPP to adult male rats.

Figure 2 graphically illustrates the results observed in Example 2. Taken in conjunction with the results reported in Table II, it shows a decrease in body weight with no decrease in testosterone levels on administration of CoPP to adult male dogs.

Figure 3 shows weight loss achieved in adult male rats on intraventricular administration of CoPP as described in Example 3.

Figure 4 shows the results reported in Example 4. The example describes the intraventricular administration of CoPP to adult male rats to achieve weight loss without significant changes in testosterone concentration.

Figure 5 illustrates the results reported in Example 5 and shows prolonged weight loss following a single intraventricular administration of CoPP.

Comparable results to those illustrated in the figures for CoPP are obtained with CoMP. Similar administration of either CoPP or CoMP results in improved P/F ratios.

The CoPP and CoMP utilized in this invention appear to achieve their desired effect of weight control by two mechanisms. One is appetite suppression. The other is actual weight loss by a metabolic mechanism which is not fully understood at this time.

The methods of the invention are especially useful for the treatment of diabetes mellitus, Type II, the so called adult type. This type of diabetes is normally treated by diet control. For this utility, the two pronged attack of appetite suppression coupled with actual weight loss is ideal.

The compounds of the invention will normally be administered parenterally, i.e. intravenously, subcutaneously or intramuscularly in sterile, isotonic parenteral solutions. For such solutions, any of a wide variety of pharmaceutically acceptable carriers currently in use for the preparation of parenteral solutions may be employed. The solutions may be buffered, for example with a phosphate buffer to a pH of about 7 to 8, preferably 7.4 to 7.5, and contain such solutes as saline or glucose. The solutions may also contain a polyhydroxy alcohol such as ethylene or

propylene glycol. The active compounds may also be administered in solution or suspension in a sterile inert oil such as sesame or safflower oil. A typical dosage regimen for humans will be from about 0.5 to 2 µm/kg b.w. per week.

Typically, isotonic solutions for use in this invention can be prepared by dissolving the selected amount of CoPP or CoMP in 0.1M aqueous sodium hydroxide solution, adjusting to the selected pH with 1M hydrochloric acid, and making up to volume with 0.9 aqueous sodium chloride solution. For the low levels of active agent utilized in the practice of this invention, parenteral compositions will normally be prepared to contain from about 1 to 15 mg/ml.

The physician or veterinarian will determine the specific dosage, and it will depend upon such well understood factors as the age, weight and general health of the patient. Typically, treatment will be initiated at a dosage level of about 0.5 to 1 µm/kg b.w. and the patient will be observed so that the decline in weight is not too precipitous. Too rapid a decline in weight could elicit toxic effects similar to those observed in starvation, i.e., kidney damage, ketosis, electrolyte imbalance, etc. Therefore, the object will be to decrease weigh gradually, in effect to titrate the patient so that the weight is brought under control without attendant undesirable effects.

The active compounds of the invention may also be administered intraventricularly. For most patients this method of administration is neither necessary nor

practical. It does have the advantage that desired weight control can be achieved at dosage levels at the low end of the dosage range utilized for parenteral administration. A typical range for intraventricular administration is from about 0.1 to 0.5 µm/kg body weight. Additionally the rate of weight loss is much higher than with parenteral administration. The procedure is useful with bedridden individuals and with the morbidly obese.

The following examples are given by way of illustration only and should not be understood as limitations of this invention since many apparent variations are possible without departing from its spirit or scope. In the examples, the vehicle is isotonic aqueous saline at a pH of about 7.4.

EXAMPLE 1

Adult male Sprague-Dawley rats (≈250g) were injected subcutaneously with CoPP 1 µmol/kg b.w. (12 rats) or vehicle (12 rats) on day 0. Thereafter, injections (Vertical arrows in Figure 1) were repeated weekly until 10 doses had been given. Animals were allowed Purina rat chow and water ad libitum and animal weights were recorded thrice weekly; means are presented in Figure 1 (● is control and ■ is treated animals). On day 71, 6 control rats and 6 CoPP-treated rats were sacrificed, blood collected for hormone determinations and hepatic mitochondrial and microsomal fractions prepared for biochemical assays. These procedures were repeated at day 124 for the remainder of the animals. The results are listed in Table 1. Although differences

in weights of control and CoPP-treated rats were highly significant, there were no significant differences in naso-anal length, hormone concentrations or heme pathway and cytochrome P-450-dependent enzyme activities.

EXAMPLE 2

Adult male Beagle dogs (≈1 year) were housed in single runs with free access to Purina dog chow and water. On day 0, 3 animals were injected intramuscularly in the upper postero-lateral aspect of the hindlimb with CoPP 2.5 µmol/kg b.w., and 3 animals with control vehicle. Injections (Vertical arrows in Figure 2) were repeated weekly, in alternating hindlimbs, until a total of 9 doses had been given. Animal weights were recorded thrice weekly and means are presented in Figure 2 (● is control and ■ is treated animals). Mean weights were unchanged during the first 3 weeks of treatment, but progressively decreased thereafter to a nadir at day 74, when CoPP-treated dogs had lost approximately 25% of their body weight. By day 84, animals had returned to their pre-treatment weights. Blood was collected for testosterone determinations before the start of the experiment and on days 62 and 112; as can be seen in Table II, there was no effect of CoPP on testosterone concentrations.

EXAMPLE 3

Adult male rats were anesthetized during stereotaxic placement of chronic indwelling catheters into the third ventricle of the brain. After 3-4 days

post-operative recovery, animals were injected intraventricularly (i/vt) with small volumes (<10µl) of vehicle (●) or CoPP 0.1 (■), 0.2 (○) or 0.4 (■) µmol/kg b.w. Daily weights were recorded and the means ± SEM of 4-6 animals per group are displayed in Figure 3. The effects of I/vt CoPP were dose responsive but i/vt dosages were approximately 50-100 fold less than those required to elicit the same changes by s/c administration.

EXAMPLE 4

Figure 4 depicts the means ± SEM of the body weights of 5 adult male rats treated i/vt with vehicle (●) and 5 animals with CoPP 0.4 µmol/kg b.w. (■, lower panel). Although CoPP-treated animals displayed typical weight loss, testosterone concentrations of CoPP-treated rats were not significantly different from vehicle-treated rats (means ± SEM indicated, respectively, by solid and broken lines).

EXAMPLE 5

The prolonged loss of body weight following single i/vt treatments with CoPP 0.2 (▼) or 0.4 (■) umol/kg b.w. are contrasted with that of vehicle-treated rats (●) in Figure 5 (n=4). It will be seen that treatment with a low level of active compound resulted in a prompt decrease in weight. The differential between treated and untreated animals was maintained for the duration of the treatment.

WHAT IS CLAIMED IS:

1. A method of controlling weight gain in animals in need of such control without concurrent decrease in endocrine activity which comprises parenteral administration of from 0.1 to about 4 μm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

2. A method as in claim 1 wherein the animal is a human.

3. A method as in claim 1 wherein the amount of cobalt protoporphyrin or cobalt mesoporphyrin administered is from 0.1 to 3 μm/kg body weight.

4. A method as in claim 1, 2 or 3 wherein cobalt protoporphyrin is administered.

5. A method as in claim 1, 2 or 3 wherein cobalt mesoporphyrin is administered.

6. A method of controlling the protein to fat ratio in animals in need of such control without concurrent decrease in endocrine activity which comprises parenteral administration of from 0.1 to about 4 μm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

7. A method as in claim 6 wherein the animal is a human.

8. A method as in claim 6 wherein the amount of cobalt protoporphyrin or cobalt mesoporphyrin administered is from 0.1 to 3 μm/kg body weight.

9. A method as in claim 6, 7 or 8 wherein cobalt protoporphyrin is administered.

10. A method as in claim 6, 7 or 8 wherein cobalt mesoporphyrin is administered.

11. A method of controlling weight gain in animals in need of such control without concurrent decrease in endocrine activity which comprises intraventricular administration of from 0.1 to 0.4 μm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

12. A method as in claim 11 wherein the animal is a human.

13. A method as in claim 11 or 12 wherein cobalt protoporphyrin is administered.

14. A method as in claim 11 or 12 wherein cobalt mesoporphyrin is administered.

15. A method of controlling the protein to fat ratio weight gain in animals in need of such control without concurrent decrease in endocrine activity which comprises intraventricular administration of from 0.1 to 0.4 μm/kg body weight of cobalt protoporphyrin or cobalt mesoporphyrin.

16. A method as in claim 15 wherein the animal is a human.

17. A method as in claim 15 or 16 wherein cobalt protoporphyrin is administered.

18. A method as in claim 15 or 16 wherein cobalt mesoporphyrin is administered.

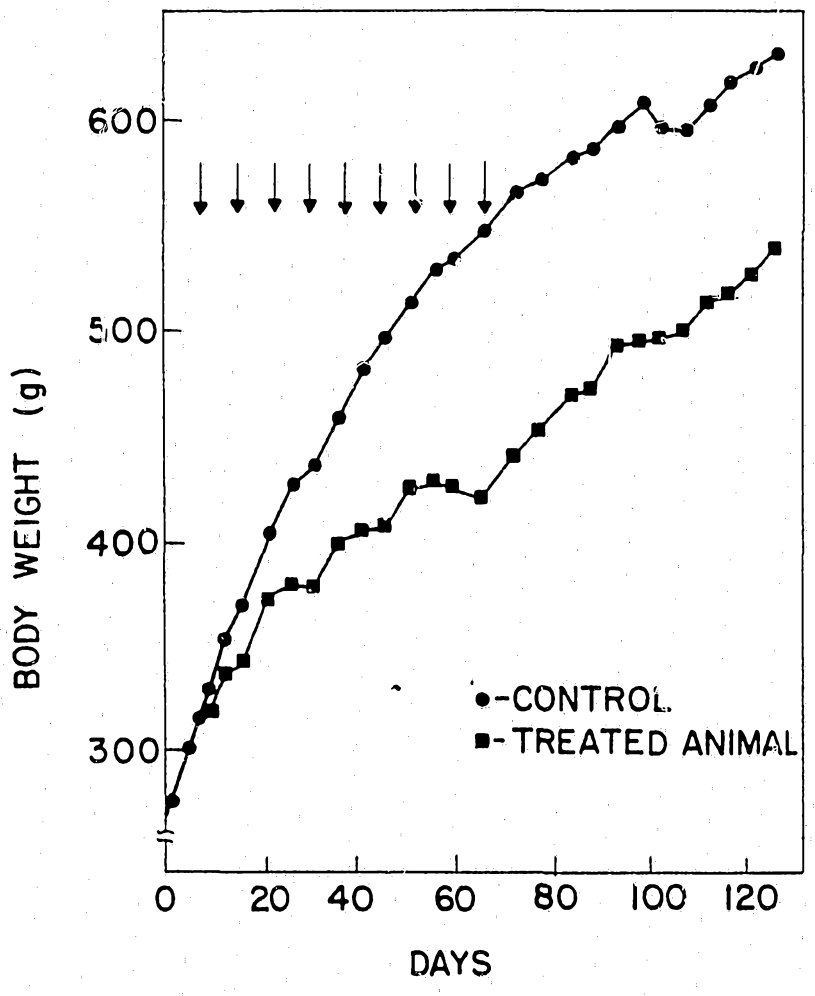

600
500
400
300
BODY WEIGHT (g)
0
20
40
60
80
100
120
DAYS
●-CONTROL
■-TREATED ANIMAL

FIG.1

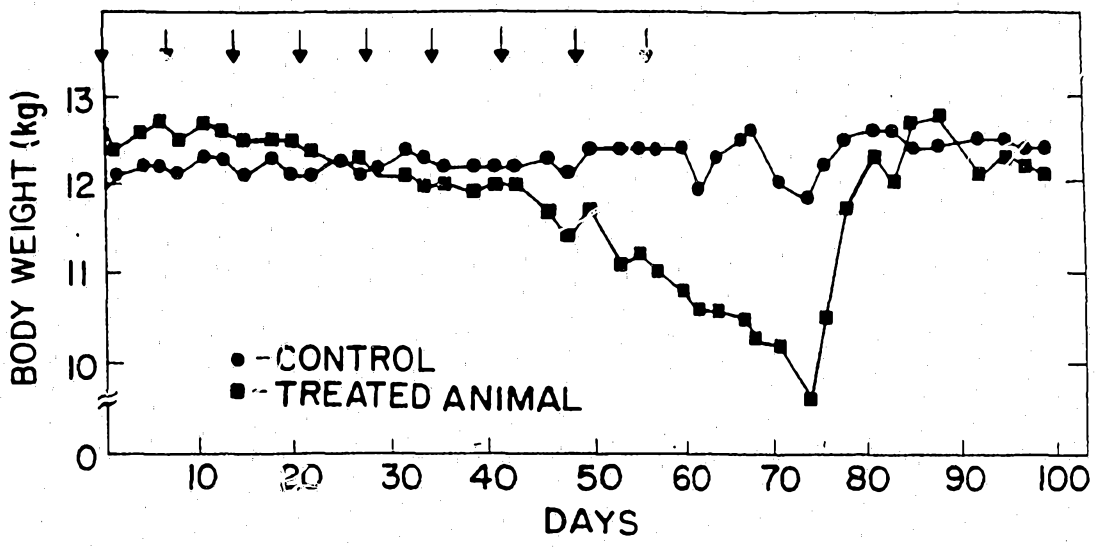

BODY WEIGHT (kg)
13
12
11
10
0
● - CONTROL
■ - TREATED ANIMAL
10
20
30
40
50
60
70
80
90
100
DAYS

FIG.2

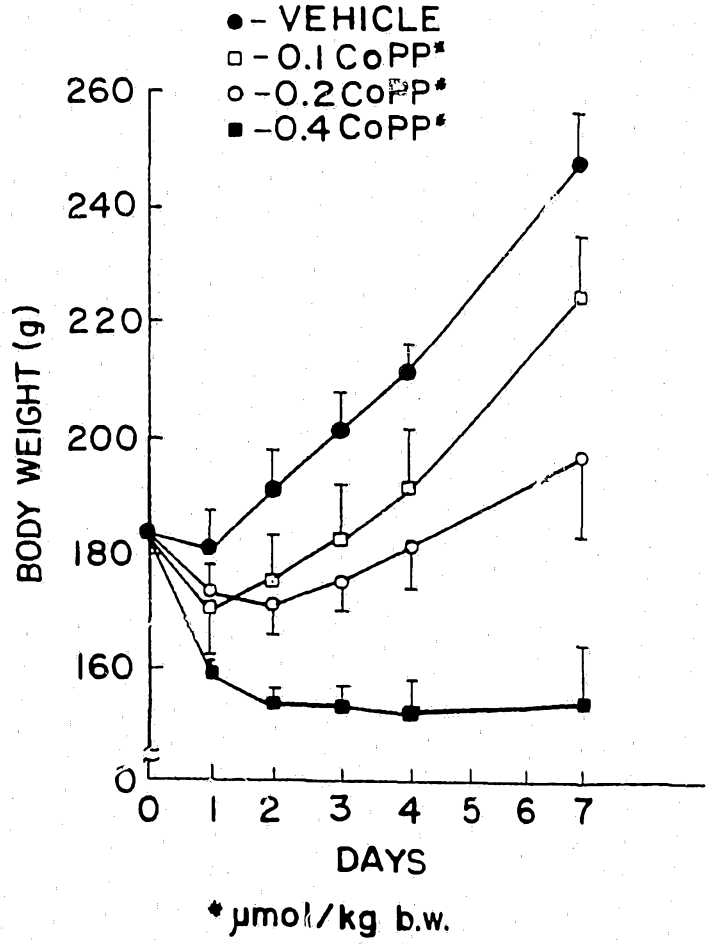

● - VEHICLE
□ - 0.1 CoPP*
○ - 0.2 CoPP*
■ - 0.4 CoPP*
BODY WEIGHT (g)
260
240
220
200
180
160
0
0 1 2 3 4 5 6 7
DAYS
* μmol/kg b.w.

FIG.3

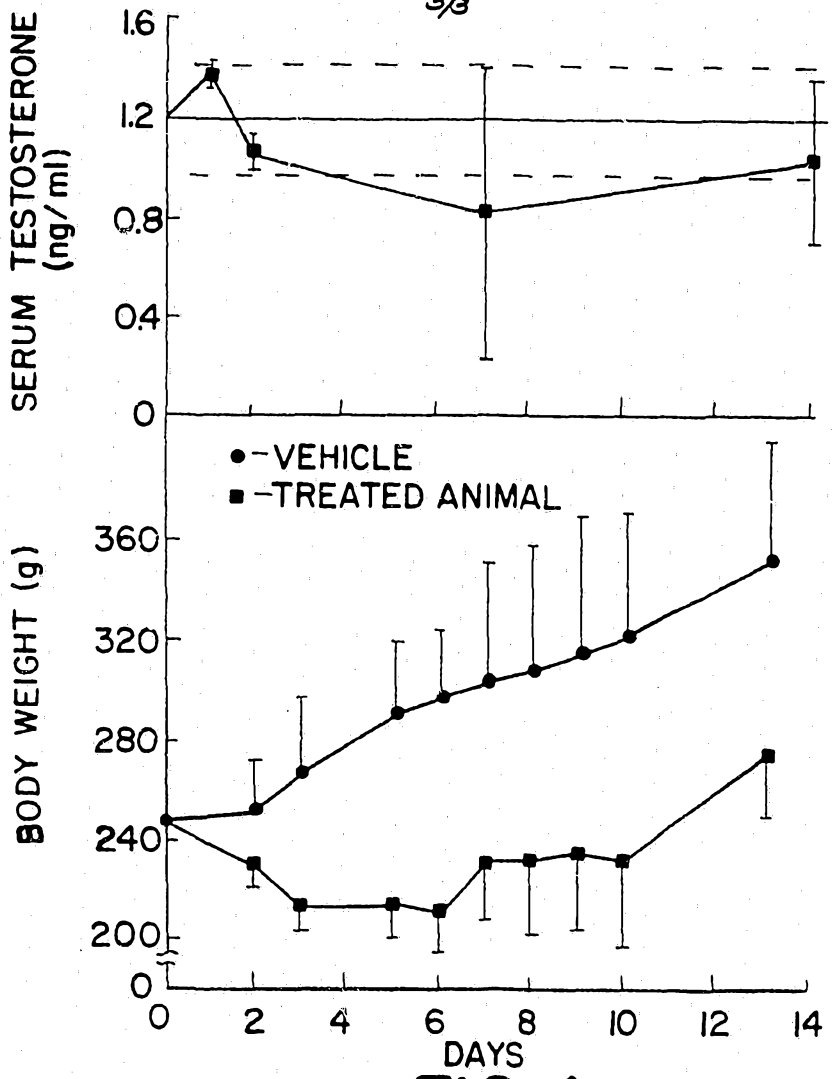

SERUM TESTOSTERONE (ng/ml)
1.6
1.2
0.8
0.4
0
●-VEHICLE
■-TREATED ANIMAL
BODY WEIGHT (g)
360
320
280
240
200
0
0
2
4
6
8
10
12
14
DAYS

FIG.4

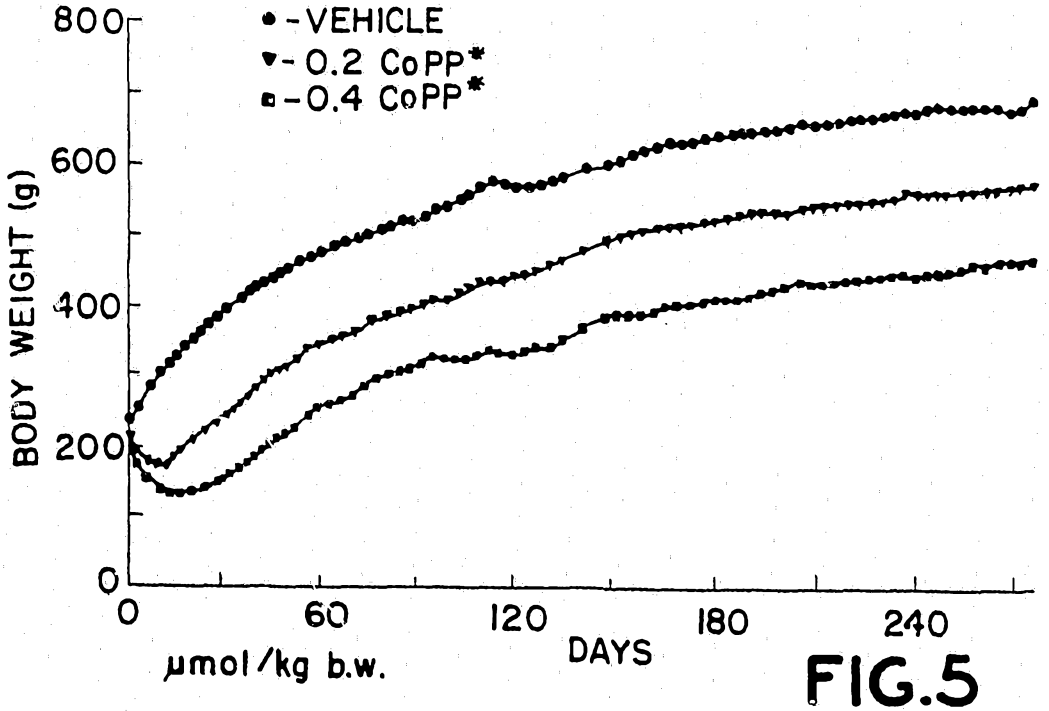

●-VEHICLE
▼-0.2 CoPP*
■-0.4 CoPP*
BODY WEIGHT (g)
800
600
400
200
0
0
60
120
180
240
µmol/kg b.w.
DAYS

FIG.5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/00790

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

514/185,410 A61K 31/40

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/185,410

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Galbraith et al Pharmacology 1987 34(5)241-9 Chemical Abstracts Vol. 108. 1987 Abstract 188705N	Claims 1 to 4, 6 to 9 11 to 13 15 to 17
A	US,A, 4,519,923 Kappas et al 28 October 1986 [See entire document]	Claims 1 to 15
A	Drummond et al Proc. Nat. Acad. Sci. USA 79 April 1982, pp. 2384-8 [See entire document]	1 to 15

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

16 March 1990

Date of Mailing of this International Search Report

03 MAY 1990

International Searching Authority

ISA/US

Signature of Authorized Officer

Edward C. Ward

Form PCT/ISA/210 (second sheet) (Rev.11-87)