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(54) Title: VANADIUM COMPOSITIONS

(57) Abstract

There are provided vanadium compositions for use in the treatment of hypertension, obesity and diabetes, in particular improved oral compositions comprising oxovanadium (IV) chelates of monoprotic, bidentate oxygen coordinating ligands especially kojic acid and maltol.

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VANADIUM COMPOSITIONS

This invention relates to pharmaceutical compositions useful for lowering blood sugar, lowering blood pressure and suppressing appetite in mammals, and to a method of lowering blood glucose, treating hypertension and suppressing appetite.

Diabetes mellitus is a mammalian condition in which the amount of glucose in the blood plasma is abnormally high. This condition can be life-threatening and high glucose levels in the blood plasma (hyperglycemia) can lead to a number of chronic diabetes syndromes, for example, atherosclerosis, microangiopathy, kidney disorders, renal failure, cardiac disease, diabetic retinopathy and other ocular disorders including blindness.

It is a disease of some complexity, as indicated by its effect on a large number of important functions of the body. There are large numbers of sufferers. For example, in the late 1980's over 2.6 million people in the United States were diabetics who were taking insulin by injection daily. Approximately an equal number of diabetics were taking oral hypoglycemic agents and another 2 to 3 million people were controlling the disease by dietary methods alone. It is estimated there are several million people in the U.S. who are undiagnosed diabetics.

In non-diabetics, plasma glucose level is maintained automatically in a complex procedure that involves, inter alia, the hormone insulin. In diabetes, external intervention is needed. Treatment of diabetics is now carried out using several drugs. Insulin is the mainstay of treatment; it replaces the natural hormone produced in the pancreas. In diabetics, insulin is not produced in sufficient quantities, or the body becomes

tolerant to insulin and requires more than normal amounts to produce the necessary effect.

Insulin must be given by injection. Insulin cannot be administered orally as it is decomposed before or during passage through the gastrointestinal tract. It is difficult to determine the exact amount required. This can result in overdoses, leading to hypoglycemia, and in inadequate doses, leading to poor control of the disease and the development of secondary complications.

Oral diabetes medications (such as sulphonylureas and biguanides) are available. As with insulin it can be difficult to obtain a correct dose. There has been shown to be a difficulty with the sulphonylureas in correctly regulating blood glucose levels. Hypoglycemic episodes often occur in elderly patients. The biguanides lower blood glucose, but can produce side effects, such as lactic acidosis, which can be fatal.

Sodium orthovanadate has been found to be a potent inhibitor of Na^+-K^+ATPase. It has also been found that vanadate (vanadium (V)) taken up by the red blood cells was reduced to vanadium (IV) in the form of vanadyl ion VO^{2+} in the cytoplasm. Since this work, there has been interest in the effects of vanadium, mostly as vanadate, on glucose metabolism and uptake into cells. A natural outgrowth of this work has been the study of vanadium and diabetes. (See J. Biol. Chem. 252: 7421-7423 (1977), J. Biol. Chem. 254: 1781-1784 (1979) and Diabetes 39: 1-5 (1990)).

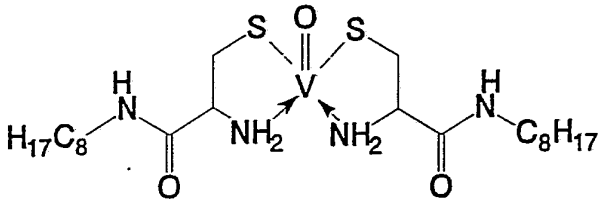
The insulin-like effect of the vanadate ion (VO_4^{3-}) *in vitro* has been known since 1980 (see Nature 284: 556-558 (1980)) when it was shown that the insulin-like stimulation of glucose oxidation in rat adipocytes was due to the vanadyl ion. In 1985, McNeill et al. (Science 227: 1474-1477 (1985)) reported that vanadate, when administered in drinking water, decreased the elevated blood glucose and prevented the depression of cardiac performance in rats made diabetic with

streptozotocin (STZ). Subsequently, there has been interest in the insulin-mimetic effects of both vanadate and vanadyl since Sakurai et al. showed that vanadate is reduced in vivo to vanadyl (Biochem. Biophys. Res. Comm. 96: 293-298 (1980)).

Vanadate however has the drawbacks that it is poorly absorbed from the gastrointestinal tract to the blood and that it is toxic. Administered concentrations must be close to the toxic level if the insulin-mimetic effects in mammals are to be achieved.

Work by McNeill et al. (see Am. J. Physiol 257: H904-H911 (1989), Metabolism 38: 1022-1028 (1989), Diabetes 38: 1390-1395 (1989) and Can. J. Physiol & Pharmacol. 68: 486-491 (1990)) has shown that vanadyl administered orally as vanadyl sulfate also lowers blood glucose and blood lipids in STZ diabetic rats and prevents secondary complications of diabetes such as cataracts and cardiac dysfunction. Vanadyl sulfate is less toxic than the vanadate form of vanadium but is also poorly absorbed. There have been attempts to modify the biological uptake of vanadium by changing the chemical form in which it is supplied from either vanadate (VO_4^{3-}) or vanadyl sulfate ($VOSO_4.(H_2O)_x$). In this regard, the use of various vanadate:peroxide reaction products as insulin mimics has been suggested by Posner et al. (See US-A-4882171, US-A-5069913 Biochem. Biophys. Res. Comm. 147: 259-266 (1987) and J. Biol. Chem. 262: 8252-8256 (1987)).

The use, for the oral treatment of diabetes, of a cysteine complex of vanadium of the formula



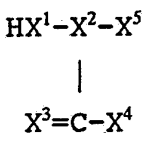
is described in EP-A-305264. The use of vanadium cysteine complexes was also reported in JP-A-2/292217 by Komatsu et al. However these cysteine complexes are only poorly water soluble and thus are not suited to oral administration. Moreover their utility is further diminished by the relatively long delay between administration and the onset of a blood sugar lowering effect. Komatsu et al. (supra) also proposed the use of a pentane-2,4-dione complex of the vanadyl ion. This however is hydrophobic and insoluble in water and thus too is unsuited to oral administration.

Accordingly, there remains a need for compositions useful in the treatment of diabetes with vanadium and we have found that certain bidentate, monoprotic chelants, with vanadium-co-ordinating oxygen, sulphur or nitrogen atoms form vanadium complexes particularly suitable for therapeutic use, especially for oral administration.

Thus viewed from one aspect the invention provides a pharmaceutical composition, preferably in a form adapted for administration into the gastrointestinal tract, comprising at least one physiologically acceptable carrier together with a physiologically tolerable vanadium complex of a bidentate monoprotic chelant capable of chelating vanadium to form a five-membered, unsaturated, vanadium-containing ring, said ring containing at least two further heteroatoms besides vanadium.

The five-membered rings formed by chelation of vanadium by the bidentate monoprotic chelant used according to the invention preferably involve oxygen, sulphur or nitrogen coordination of the vanadium, particularly oxygen and/or nitrogen coordination, and preferably oxygen coordination alone.

Particularly suitably the chelants useful according to the invention may be represented by the formula I



(I)

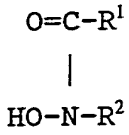
(where X^1 and X^3 are independently oxygen, sulphur or NX^6, preferably oxygen or NX^6;
X^2 is nitrogen or CX^7;
X^4, X^5, X^6 and X^7 are independently non-labile protons or optionally substituted alkyl, aryl, aralkyl or alkaryl or at least one pair of X^4 to X^7, preferably X^4 and X^5, together with the intervening atoms represents an optionally substituted, saturated or unsaturated homo or heterocyclic ring, or where X^1 represents a NX^6 group X^4 may represent a group X^8H where X^8 is oxygen or sulphur; and one X^1 or X^8 attached proton, preferably an X^1 attached proton, is labile).

Thus, for example, the chelant used according to the invention may be an α-amino acid (other than cysteine), a hydroxamate or thiohydroxamate an α-hydroxy-carbonyl, such as for example an α-hydroxy-pyridinone or an α-hydroxypyrone, eg. maltol or kojic acid.

Where the chelant comprises a homo or heterocyclic ring, this will preferably be a 5, 6 or 7 membered ring containing 1, 2 or 3, especially 1, heteroatom, selected from O, N and S, preferably O or N. Each aryl group is preferably phenyl or naphthyl, especially phenyl; each alkyl group or moiety preferably contains 1 to 6 carbon

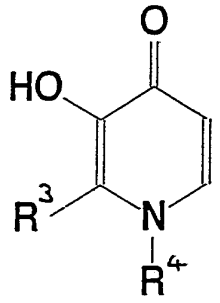
atoms, especially 1 to 4; and the optional substituents, which do not include thiol groups, are preferably selected from hydroxy, alkoxy, oxo, amide and amine groups, and alkyl groups carrying such substituents. Such groups may be selected for their ability to enhance the hydrophilicity or lipophilicity of the complex or to enable the complex to be conjugated to a further species, eg. a biomolecule, protein, polymer, etc.

Particularly suitable chelants of formula I include the hydroxamates of formula II



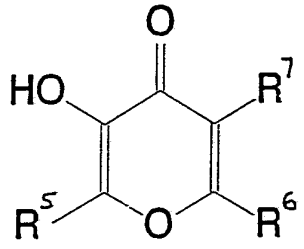
(II)

the α-hydroxy-pyridinones of formula III



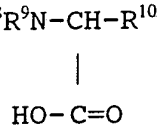
(III)

the α-hydroxypyrones of formula IV



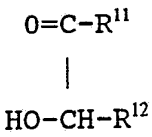
(IV)

the α-amino acids of formula V

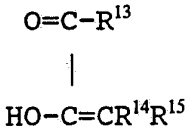


(V)

the hydroxy carbonyls of formulae VI and VII

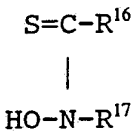


(VI)

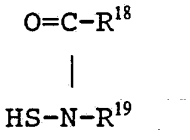


(VII)

and the thiohydroxamates of formulae VIII and IX



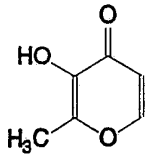
(VIII)



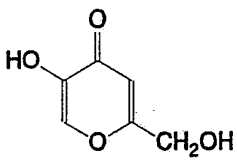
(IX)

(where R^1 to R^{19} are hydrogen or optionally substituted, e.g. hydroxylated, C_{1-4} alkyl).

Especially preferred as chelants of formula I are maltol and kojic acid



Maltol



Kojic Acid

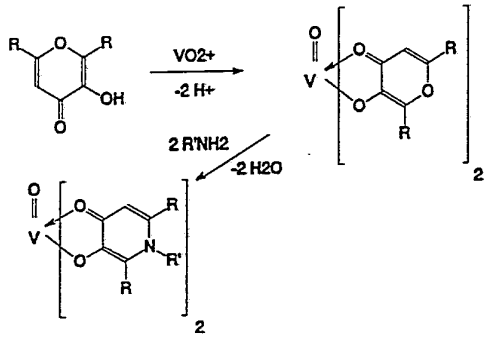
Chelants of this type are known from the literature (see for example Matsuba et al Inorg. Chem. 27: 3935-3939 (1988)) or may be produced from known starting materials by conventional techniques.

Examples of hydroxamate chelants may be found in J. Ind. Chem. Soc. 44:369-376 (1967) and Inorg. Chem. 28:4399-4406 (1989).

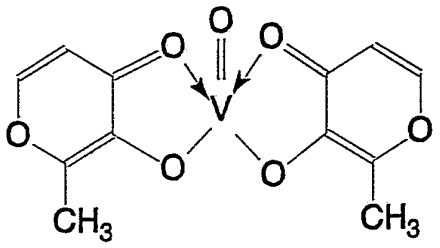
Thus for example many hydroxamate chelants are known. These can readily be prepared by reaction of an acyl halide with a substituted hydroxylamine

$$RCOX + R'NHOH \text{ -------> } RCON(OH)R'$$

Many of the vanadium chelates with ligands of formula I might be prepared from maltol or maltol derivatives in a one-pot synthesis analogous to that described for gallium, aluminium and indium complexes by Zhang et al in Can. J. Chem. 67: 1708-1710 (1989). Thus



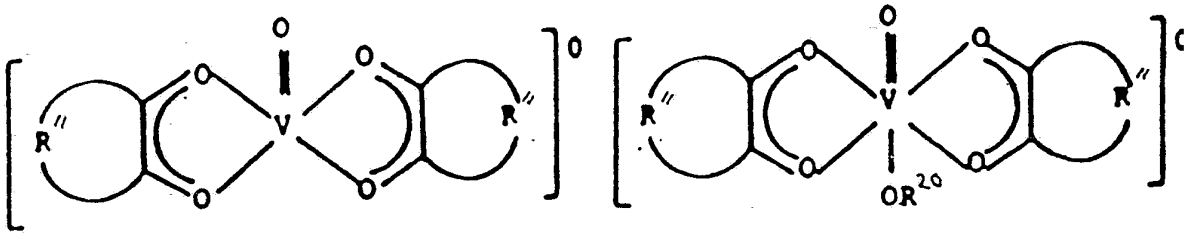
The direct electrochemical preparation of a vanadyl maltol compound was reported in J. Coord. Chem. 8: 27-33 (1978). The biological activity was not examined and the structural formula was not determined but the data suggested it to be:



Alkoxo-oxovanadium (V) derivatives of the maltolate anion have been known since the 1960's, but again the biological activity was not examined (see Z. Anal. Chem. 203: 257-260 (1964)).

The vanadium complex used according to the invention is preferably a complex of vanadium (IV) or (V) with a ligand as described above, especially preferably a complex VOL_2 or $VOL_2(OR^{20})$ where LH is a chelate as described above and R^{20} is an optionally substituted alkyl, aryl, aralkyl or alkenyl group (where such groups are as defined above), preferably such a group is substituted by at least one hydroxyl group.

Where LH is a chelant which forms a five membered $\overbrace{C{=}O{\rightarrow}V{-}O{-}C}$ ring, these VOL_2 and $VOL_2(OR^{20})$ complexes, can schematically be represented by the formulae



where R" is the remainder of the chelating group.

In general, backbone and ring substituents on the vanadium coordinating ligands are selected for their solubilizing effect. Desirably the vanadium complexes used according to the invention will be neutrally charged overall, water soluble (eg. capable of forming an at least 0.1 mM solution, preferably an at least 0.2 mM solution), orally absorbable (preferably with significant gastro-intestinal absorption), and have moderate to high complex stability (eg. with ligand binding constants log $\beta_2 \leq 2$ to 30, preferably 6 to 20).

Particularly preferred compounds include the maltol and kojic acid complexes bis(maltolato)oxovanadium(IV) and bis(kojato)oxovanadium(IV).

We have surprisingly found that, besides being effective in the treatment of diabetes by virtue of its blood sugar lowering effect, vanadium has appetite suppressing and antihypertensive effects.

Accordingly, viewed from a further aspect, the invention provides the use of a physiologically

tolerable vanadium compound, preferably a V(V) or V(IV) compound, for the manufacture of an antihypertensive or blood pressure lowering agent.

Viewed from a further aspect the invention provides the use of a physiologically tolerable vanadium compound, preferably a V(V) or V(IV) compound, for the manufacture of an appetite suppressing agent.

Viewed from a yet further aspect the invention provides a method of treatment of the human or non-human mammalian body to combat elevated blood pressure which method comprises administering to said body, for example parenterally or into the gastrointestinal tract, a physiologically tolerable vanadium compound, preferably a water soluble compound.

Viewed from a still further aspect the invention provides a method of treatment of the human or non-human mammalian body to suppress appetite, eg. for cosmetic purposes or in the treatment of illness, dysfunction or obesity, which method comprises administering to said body, for example parenterally or into the gastrointestinal tract, a physiologically tolerable vanadium compound, preferably a water soluble compound.

The vanadium compounds useful according to the invention are conveniently oxovanadium chelates but the sulphur and nitrogen analogues, i.e. VS and VNR^{21} (where R^{21} is as defined for R^1 to R^{20}), may also be used according to the invention in treatment to lower blood sugar, to suppress appetite or to combat hypertension.

The vanadium compounds can be given by conventional administration routes, eg. oral, rectal, intravenous, subcutaneous, intraperitoneal, transdermal, etc. However oral administration is preferred.

In the treatment of hypertension and in the appetite suppression treatment it is especially preferred that the vanadium compound be one which can be taken up by the body following administration into a body orifice, particularly oral or rectal

administration, and it is especially preferred to use water-soluble vanadium (IV) or vanadium (V) compounds. While the use of the compounds described earlier for the treatment of diabetes are of course especially preferred, other physiologically tolerable inorganic salts or organic salts or complexes of vandium can be used. In this regard reference may be made to the vanadium peroxide compositions of Posner (see US-A-4882171 and US-A-5069913), the vanadyl complexes of Lazaro (such as the cysteine complex Naglivan, see US-A-5023358), vanadyl sulphate, sodium orthovanadate, vanadyl complexes with monoprotic bidentate 2,4-diones, and the vanadyl complexes of Komatsu (see JP-A-2/292217).

The invention thus also provides pharmaceutical compositions for use in the treatment of diabetes and hypertension and pharmaceutical compositions for use in suppressing appetite containing at least one physiologically acceptable vanadium source as discussed above together with at least one physiologically acceptable carrier or excipient.

The compositions for use according to the invention may be in conventional administration forms, eg. capsule, tablet, coated tablet, solution, suspension, syrup or suppository, and conventional formulation aids and excipients may be used, eg. viscosity modifiers, buffers, flavouring agents, suspension agents, stabilizers, excess ligand, and other additives.

The compositions of the invention may also contain other therapeutically active agents, eg. antidiabetic, antihypertensive and appetite suppressing agents. In this regard, special mention may be made of the metal compounds considered to be useful in the treatment of diabetes, e.g. $MgCl_2$. Vanadium may of course be used in combination therapy with insulin, e.g. in the treatment of Type I diabetes.

Depending on the animal and condition being treated

and on the administration route the vanadium compounds will generally be administered in dosages of 0.00035 to 600 mg V/kg bodyweight per day. The range is broad since in general the efficacy of a therapeutic effect for different mammals varies widely with doses typically being 20, 30 or even 40 times smaller (per unit bodyweight) in man than in the rat. Similarly the mode of administration can have a large effect on dosage. Thus for example oral dosages in the rat may be ten times the injection dose. As a result, the preferred range for rats is 0.1 to 300 mg V/kg/day while for man it may be 0.0007 to 2.0 mg V/kg/day.

The compositions, if in solution preferably contain the vanadium compounds at concentrations which provide the effective dose either in normal daily water intake or as a daily solution supplement(s) or tablet that provides the correct dose.

The vanadium compounds will generally be administered in dosages of 10^{-5} to 1000 mg V/kg/day bodyweight, especially 10^{-4} to 500 mg V/kg/day, particularly up to 400 mg V/kg/day and most especially up to 300 mg V/kg/day. Dosages for humans by oral administration will generally lie in the range 10^{-4} to 200 mg V/kg/day, e.g. 10^{-4} to 100 mg V/kg/day and by injection in the range 10^{-4} to 100 mg V/kg/day, e.g. 10^{-4} to 50 mg V/kg/day. For rats the minimum effective doses lie generally in the ranges 10 to 30 mg V/kg for oral administration and 6 to 20 mg V/kg for injection. Thus, for example, appropriate injection dosages for rats bis(maltolato)oxovanadium(IV) may be about 15 mg V/kg and appropriate oral dosages 150-300 mg/kg.

The compositions, if in solution form preferably contain the vanadium compounds at concentrations of 0.1 mM to 10 M, especially 0.2 mM to 1 M, particularly 1.6 to 3.2 mM.

Where the compositions are to be injected, the carrier for the vanadium compound may conveniently be

aqueous methyl cellulose containing about 1% methyl cellulose.

The following non-limiting examples are provided to illustrate the invention further.

Example 1:
Bis(maltolato)oxovanadium(IV) (BMOV)

The compound bis(maltolato)oxovanadium(IV) (hereinafter BMOV) was prepared nearly quantitatively by combining maltol (3-hydroxy-2-methyl-4-pyrone) and vanadyl sulfate in hot or boiling water at a ratio of 2 to 1. The pH of the solution was raised to 8.5 and the solution was refluxed overnight. The product was a deep purple-green compound that precipitated and was filtered after cooling the reaction mixture to ambient temperature. The compound is birefringent.

The compound is characterised as follows:

Its elemental analysis is correct for $C_{12}H_{10}O_7V$; % calculated (found): C 45.45 (45.60): H 3.18 (3.30) and its electron impact mess spectrum is also consistent: m/e = 317 (M^+). The infrared spectrum shows absorptions (KBr disk) characteristic of the maltolato anion bound to a metal cation (1610, 1570, 1560, 1465 cm^{-1}) and characteristic of the vanadyl group V=O (995 cm^{-1}). It has a magnetic moment in the solid state of 1.76 B.M. indicating one unpaired electron; however, it gives a clear ^1H NMR spectrum in D_2O or d_4-methanol (δ 2.5 (s,6H), 6.55(d,2H), 8.15(d,2H)) and a clear ^{51}V NMR spectrum in D_2O(-496 ppm). The compound is quite water-soluble (about 7 mM, 2mg mL^{-1}). Its log p value is 0.12.

Example 2
Bis(kojato)oxovanadium(IV) (vanadyl kojate)

$VOSO_4.5H_2O$ (Aldrich, 2.50g, 9.88mmol) was dissolved in 10 mL hot water, and the solution was degassed with

Ar for 10 minutes and added to 10 mL of an aqueous solution of kojic acid (2-hydroxymethyl-5-hydroxy-γ-pyrone) (Sigma, 2.88g, 20.3 mmol) and $NaOAc.3H_2O$ (Fisher, 2.97g, 21.8 mmol), which had also been degassed. After refluxing under Ar overnight, a blue precipitate was isolated by vacuum flitration using a Schlenk filtering funnel and dried overnight in vacuo. The yield was 2.69g (78% based on V).

The compound is characterised as follows:

Its elemental analysis is correct for $C_{12}H_{10}O_9V$; % Calculated (found): C, 41.28 (41.06); H, 2.89 (2.89) and its fast atom bombardment mass spectrum is also consistent: m/e = 350 (MH^+). The infrared spectrum shows absorptions (KBr Disk) characteristic of the kojato anion bound to a metal cation (1610, 1550, 1500, 1470 ($\nu_{c=o}$, $\nu_{c=c}$) cm^{-1}) and characteristic of the vanadyl group V=O (980 cm^{-1}). It has a magnetic moment in the solid state of 1.76B.M., indicating one unpaired electron. No ^1H NMR signal was observed in a chemical shift range from -15 to +20ppm in a freshly prepared D_2O solution. The compound is quite water-soluble (about 45 mM, 16mg/mL). Its log p value is 0.012.

The following experiments were conducted in rats with BMOV, vanadyl kojate and vanadyl sulphate, the first two as prepared above.

The results are presented in part in the accompanying drawings in which:

Figure 1 relates change in weight with time;

Figure 2 shows average blood glucose values in mmol/l (over time);

Figure 3 shows the daily food consumption;

Figure 4 shows daily fluid consumption;

Figures 5 and 6 shows the effects of vanadyl sulphate on blood pressure; and

Figure 7 shows the effect of BMOV on blood

pressure.

Example 3

Initial experiments were carried out to determine pharmacological effectiveness of BMOV. Using male rats, made diabetic by the injection of streptozoticin (STZ) at a dose of 60 mg/kg i.v., BMOV was initially given by intraperitoneal (i.p.) injection as a suspension in 1% methyl cellulose.

1. Injection

Nine out of the twelve rats given 15 mg/kg i.p. (0.05 mmol/kg) responded to the compound with a decrease in blood glucose. Two animals developed hypoglycemia.

2. Oral Administration

a. Drinking. Administration of the vanadyl compound in the drinking water at doses of 0.46-0.92 mmol/kg (150-300 mg/kg, using concentrations of 0.5-1.3 mg/mL) reduced blood glucose in four diabetic rats into the normal range. Fluid intake was also decreased to normal in these animals.

b. Gavage. Six out of six diabetic rats responded to the vanadyl compound with a decrease in blood glucose when BMOV was given by gavage in a dose of 160 μmol/kg over 40 days. The peak fall in blood glucose occurred 5 hours after administering the drug. The blood glucose returned to diabetic levels usually within 12 hours.

Based on these preliminary studies, more detailed studies were undertaken to determine the minimum effective dose of BMOV required to maintain normal blood glucose in diabetic animals.

Example 4
Prolonged administration

BMOV was administered to normal and diabetic rats in drinking water.

The study was composed of 4 groups of animals. Control (8 animals), Control-Treated (11 animals), Diabetic (11 animals) and Diabetic-Treated (12 animals). The diabetic state was induced by injecting STZ at 60 mg/kg dissolved in 0.9% NaCl I.V. via the tail vein to anaesthetised rats. The two control groups were injected with 0.9% NaCl I.V. through the tail vein. The diabetic state was determined with a "Testape"™ test at 3 days post-injection and later confirmed with a glucometer test. Blood glucose and insulin assays were carried out post-injection over the course of the study. Treatment according to the invention was started 1 week after determination of the diabetic state.

Treated diabetic animals received between 0.3 and 0.5 mmol/kg of the compound/day in drinking water over a 77 day period. Treated control animals received a slightly lower dose (0.2-0.3 mmol/day) over the same period. The concentration of BMOV in the drinking water was varied between 1.6 and 3.2 mM. The reason for the differing doses in the two sets of animals is that the two groups drank different amounts of water daily; it was difficult to attain the same dose in both groups.

With the above treatment regimen, the following was noted:

1. The weight gain over the 77 day period is shown in Figure 1. As indicated above, 4 groups of animals were studied, but the diabetic-treated group was sub-divided to diabetic-treated responders (8 animals) and diabetic-treated non-responders (4 animals). Curve 1 is the control group, curve 2 the control-treated group, curve 3 the diabetic group, curve 4 is the diabetic-treated responders and curve 5 is the diabetic-treated non-

responders. Initially, there was a significant difference only between the two control groups with respect to the three diabetic groups. However, by day 7 there was a significant difference between the two diabetic-treated groups with respect to all other groups. By day 28, the diabetic-treated non-responder group was significantly different from the other 4 groups and there was no longer a significant difference between the diabetic group and the diabetic responder group. By day 56, there was a significant difference between the control group and the control treated group. Treatment began with a 3.17 mM solution of the compound. On day 6, the concentration was reduced to 1.58 mM (0.5 mg/ml). On day 24, the concentration was increased to 2.37 mM (0.7 mg/ml). At this point 8 out of the 12 animals were responding to the compound.

The control treated animals showed a significantly decreased weight gain beginning at day 56. The decrease was correlated with the decrease in food intake.

2. Figure 2 shows average blood glucose values (mmol/l) for the 5 groups based on weekly blood glucose determinations. The 5 groups were as in Figure 1. Initially, there was a significant difference between the two control groups with respect to all three of the diabetic groups. By day 7, there was a significant difference between the diabetic group with respect to both diabetic-treated groups. By day 14, the diabetic-treated responder group was euglycemic; however, on day 18 there was an increase in blood glucose levels for this group due to the necessity to withhold treatment for a few days to treat hypoglycemia, which developed in several animals. The diabetic-treated non-responder group consists of rats which exhibited marked fluctuations in glucose values.

Thus, eight of the twelve treated diabetic rats had their blood glucose values lowered from 20+mM to less

than 10 mM from day 7 onwards. Four of the twelve rats had blood glucose readings ranging from 6-20mM on any given day. These rats are the non-responders. By day 24, diabetic rats that responded to the compound had normal blood glucose.

3. Figure 3 demonstrates the daily food consumption per rat. The rats were allowed free access to food with 2-3 rats per cage for the four treatment groups. The control group is curve 1, the diabetic group is curve 2, the control-treated group is curve 3 and the diabetic-treated group is curve 4. For the first approximately 50 days of treatment, the only significant difference occurred between the diabetic group with respect to all of the other groups. However, from day 63 on, there was also a significant difference between the control-treated group and all the other groups. There was no significant difference between the control and the diabetic-treated groups at any time.

Figure 4 shows the daily fluid consumption per rat. The rats had free access to fluids with 2-3 rats per cage for the four treatment groups. The control group is curve 1, the control-treated group is curve 2, the diabetic group is curve 3 and the diabetic-treated group is curve 4. Initially, the only significant difference occurred between the diabetic group with respect to all of the other treatment groups. However by day 63, there was also a significant difference between the control treated group and all the other treatment groups. There was no significant difference in fluid consumption between the control and the diabetic-treated groups throughout.

Figures 3 and 4 illustrate that control of blood glucose was accompanied by a reduction in food intake in the diabetic rats from greater than 50 grams per day to about 30 grams per day (28±1.6 on day 77). There was a slight reduction in food intake (from 29± 0.1 to 25.5±

0.3) in control rats treated with the compound. Fluid intake fell from about 275 ml/rat in the diabetic group to about 60 ml/rat in the diabetic treated group. There was also a decrease in fluid intake in the control treated group compared with control rats (62.7±7.4 vs 33.6±7.2 ml on day 77). As stated above, the decrease in food and water intake correlates with the decrease in weight gain in control animals.

4. Treatment with BMOV decreased weight gain in control animals (200g vs 250g for control over the 77 day period). Diabetic-treated animals gained almost exactly the same weight (140g) as diabetics, despite the decrease in food intake; thus weight gain in diabetic-treated animals lagged behind that of control groups animals.

5. Insulin values in control treated rats decreased to the same value as those for diabetic animals (approximately 22 μU/ml) and were significantly lower than those of control (35.8±1.2 μU/ml) as shown in Table I:

Table I

Insulin Values, μU/ml (Day 28 of BMOV study)

Control	Control-Treated	Diabetic	Diabetic-Treated
35.8±1.2	21.6±1.2	21.4±2.6	22.0±1.6

6. Plasma triglyceride and cholesterol values in diabetic animals were restored to control values by treatment with BMOV as shown in Table II:

Table II
Lipid Values in BMOV Study

Cholesterol mmol/l Means ± S.E.M.

	Control	Control Treated	Diabetic-Treated Responders	Diabetic-Treated Non-Responders	Diabetic
Pretreatment (Day before treatment started)	1.40±0.03 (8)	1.64±0.08 (8)	1.67±0.10 (7)	1.51±0.07 (4)	1.58±0.10 (11)
Week 6	1.37±0.06 (8)	1.60±0.06 (10)	1.58±0.11 (8)	1.48±0.09 (4)	2.65±0.28* (11)

* Diabetic untreated is significantly different from all other groups by ANOVA followed by either Fishers', Newman-Keuls' or Duncans' test.

Table II cont.

Triglyceride mmol/l Means ± S.E.M.

	Control	Control Treated	Diabetic-Treated Responders	Diabetic-Treated Non-Responders	Diabetic
Pretreatment (Day before treatment started)	1.27±0.08 (8)	1.21±0.14 (8)	1.10±0.09 (8)	1.85±0.41* (4)	1.21±0.08 (11)

* Diabetic-treated nonresponders are significantly different from all other groups by ANOVA followed by either Fishers', Newman-Keuls' or Duncans' test.

Week 6	1.81±0.11 (8)	2.04±0.22 (10)	1.70±0.18 (8)	2.31±0.19 (4)	4.14±0.98* (11)

* Diabetic-untreated are significantly different from all groups except diabetic-treated nonresponders by ANOVA followed by Fishers' and Duncans' test, but not by ANOVA followed by Newman-Keuls' test. One rat in this group had a very high triglyceride value - 13.1 mmol/l.

7. At the end of 77 days of treatment, 0/8 of the treated diabetic rats, which were controlled by the drug, had cataracts. 5/11 Of the untreated diabetic animals showed cataracts. One of the four treated animals, not controlled by the drug, also had a cataract. The first cataract developed at 60 days in the untreated diabetic group.

Example 5
Comparison of BMOV and vanadyl kojate

Using BMOV and Vanadyl Kojate as prepared in Examples 1 and 2 above, the relative efficacy of the two compounds was assessed using rats made diabetic with STZ. Again the animals were divided into responder and non-responder groups. For a 0.063 mmol V/kg ip injection, BMOV gave a 50:50 split between responder and non-responder groups whereas for vanadyl kojate the ratio was 60:40. On oral gavage of 0.55 mmol V/kg the responder:non-responder ratios were 50:50 and 57:43 respectively (n=10). Results are presented in Table III. However over a 12 day test period, there was a 100% response in terms of blood glucose reduction for diabetic rats receiving vanadyl kojate orally. Likewise the food and fluid demand by the rats was significantly reduced.

Example 6
Blood pressure reduction - Vanadyl sulphate

The insulin resistant spontaneously hypertensive rat was used as the experimental model and the vanadyl form of vanadium as the experimental intervention. The spontaneously hypertensive rats (SHR), have been shown to be insulin resistant and hyperinsulinemic as compared to their genetic controls, the Wistar Kyoto (WKY) strain. Rats, procured at 4 weeks of age, were divided

TABLE III: Comparison of the plasma glucose levels between the acute time course experiments for BMOV (n=8) and vanadyl kojate (N=10)

	BMOV				Vanadyl Kojate			
	I.P. (Injection (0.063 mmol/kg)		oral Gavage) (0.55 mmol/kg)		I.P. Injection (0.063 mmol/kg)		Oral Gavage (0.55 mmol/kg)	
TIME (Hrs)	DTR (50%)	DTN (50%)	DTR (50%)	DTN (50%)	DTR (60%)	DTN (40%)	DTR (57%)	DTN (43%)
0	17.51 ±0.8	19.42 ±0.15	18.09 ±0.72	19.31 ±0.35	17.94 ±0.41	20.86 ±0.28	15.75 ±0.55	23.08 ±1.27
1	14.77 ±2.0	18.82 ±0.8	14.55 ±1.96	18.05 ±0.46	14.75 ±0.44	18.55 ±0.42	12.5 ±1.46	21.18 ±1.45
2	12.65 ±2.33	18.51 ±0.79	13.13 ±2.04	17.57 ±0.5	10.17 ±0.47	16.62 ±0.65	10.1 ±0.99	21.51 ±1.72
4	10.6 ±2.39	17.94 ±0.85	11.68 ±2.07	16.92 ±0.35	9.26 ±0.32	17.37 ±0.23	7.96 ±0.26	20.57 ±1.07
6	10.28 ±1.76	18.08 ±0.48	9.47 ±2.58	16.43 ±0.43	8.73 ±0.17	16.62 ±0.35	6.6 ±0.35	18.2 ±0.89
8	9.08 ±1.06	17.77 ±0.59	9.27 ±2.27	15.02 ±1.13	7.4 ±0.12	15.49 ±0.65	6.87 ±0.33	19.06 ±0.96
12	9.38 ±0.96	19.07 ±0.37	7.01 ±0.57	17.17 ±0.60	10.37 ±0.46	18.24 ±0.45	6.01 ±0.25	18.81 ±0.33
24	7.15 ±1.51	19.01 ±0.21	6.31 ±0.57	18.07 ±0.74	13.28 ±0.83	18.77 ±0.49	6.74 ±0.05	25.0 ±0.4

DTR - Diabetic Treated Responders
DTN - Diabetic Treated Non-responders

into 4 groups: SHR (untreated), SHRV (treated), WKY (untreated), WKYV (treated). Chronic vanadyl sulfate treatment (0.75 mg/ml ad libitum in the drinking water) was started on the SHRV and WKYV groups at 5 weeks of age. After week 8, when hypertension becomes fully manifest in the SHR, weekly measurements of plasma insulin and systolic blood pressure (tail cuff method) were done on all four groups. Vanadyl, in doses of 75-125 mg/kg/day, lowered both plasma insulin and systolic blood pressure in the SHRV group at all time points from weeks 8-12 (Figure 5), without causing any change in the plasma glucose levels. No changes were seen in the control, normotensive WKYV animals.

At week 11, the untreated control SHR and WKY groups from the prevention study were further subdivided and half the animals in each group were started on oral vanadyl sulfate treatment. This was done to observe the reversal effects of vanadyl, if any, after the SHR had become hypertensive. Vanadyl reversed hypertension in the SHR treated group ($SHRV_1$) as well as lowering their plasma insulin to control values, but had no effect in the Wistar Kyoto rats ($WKYV_1$) (see Figure 6). A direct blood pressure reading done in all groups at termination validated the earlier indirect measurements. Vanadyl sulfate prevented as well as reversed hypertension in the hyperinsulinemic spontaneously hypertensive rat, a model that closely resembles human essential hypertension. Besides establishing further the role that insulin resistance/hyperinsulinemia may play in the development of high blood pressure, this study also indicated a possible therapeutic role for vanadyl sulfate in hypertensive, hyperinsulinemic subjects. No gastrointestinal, renal or hepatic toxicity of vanadyl was observed in the treated animals (at doses of 75-125 mg/kg/day) during the entire experimental period.

In summary, Figure 5 shows that vanadyl treatment prevents the onset of hypertension in rats that normally

become hypertensive during the first months of life. No change is observed in the Wistar rats which do not normally become hypertensive.

Figure 6 shows that once SHR have been allowed to become hypertensive that the vanadyl treatment will reduce the blood pressure. Again, no effect is observed in the control Wistar rats.

The conclusion is that vanadyl is useful as a prophylactic and as a therapeutic in hypertension. Another advantage is that vanadyl has no adverse effect on normal animals (i.e. it did not produce hypotension in normal animals).

Example 7

Blood pressure reduction - BMOV

11 SHR and 12 WKY rats (all male) were procured at 4 weeks of age from Charles River, Canada. The animals were divided into four experimental groups: SHR (untreated n=5), SHRO (BMOV treated n=6), WKY (untreated n=8) and WKYO (BMOV treated n=4). Chronic BMOV treatment was commenced on the SHRO and WKYO groups at 5 weeks of age. The rats were given BMOV (0.75 mg/ml) ad libitum in the drinking water. A concentration of 0.75 mg/ml was chosen since previous laboratory studies indicated that this concentration was sufficient to lower plasma insulin levels in non diabetic rats, while allowing them to gain weight at rates that were similar to untreated control animals.

Once at week 5 and then starting at week 8, weekly measurements of blood pressure were done on all groups for the next 3 weeks. Blood samples were also collected via the tail vein during the weeks mentioned above. These were centrifuged and plasma was collected for subsequent glucose and insulin analyses. Chronic blood pressure measurements were done on conscious rats, using the tail cuff method without external preheating. Initial experiments to validate the tail cuff method by comparing the readings obtained with it to those obtained by direct arterial cannulation were conducted. It was found that systolic blood pressure measured with this technique was similar to that obtained by direct arterial cannulation.

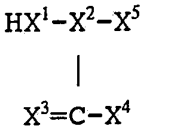
BMOV caused a reduction in systolic blood pressure in the treated SHRO rats, without having any effect on the normotensive WKYO controls (Figure 7). The decrease in systolic blood pressure after BMOV treatment was marked (25-30 mmHg) and persisted throughout the entire experimental period. Thus, BMOV was able to prevent the development of high blood pressure in the SHR.

The present invention provides a pharmaceutical composition useful for the treatment of diabetes (especially type II diabetes), as an appetite suppressant, or as an antihypertensive. The active compounds are absorbed across the gastrointestinal barrier and deliver the vanadyl ion to the bloodstream, where the insulin-mimetic properties of vanadium can be expressed. In contrast to insulin, the compositions are active when taken by mouth, and represent a significant advance in diabetes therapy. The compositions are also useful as orally active appetite suppressants and would be effective in treating obesity. The majority of diabetics are overweight, but obesity in general is a significant problem in western society, leading to an increase in morbidity and mortality. Morbid obesity is a health endangering condition. A drug that will suppress appetite, leading to weight loss, is of significant value. The active ingredients of the present invention are simple, monomeric species in the solid state. They are easily prepared, easily administered, relatively stable, and highly effective in lowering blood glucose, in suppressing appetite and in combatting hypertension.

Claims

1. A pharmaceutical composition comprising at least one physiologically acceptable carrier together with a physiologically tolerable vanadium complex of a bidentate monoprotic chelant capable of chelating vanadium to form a five membered, unsaturated, vanadium-containing ring, said ring containing at least two further heteroatoms.

2. A pharmaceutical composition comprising a physiologically tolerable vanadium complex of a bidentate monoprotic chelant of formula I



(I)

(where X^1 and X^3 are independently oxygen, sulphur or NX^6; X^2 is nitrogen or CX^7;
X^4, X^5, X^6 and X^7 are independently non-labile protons or optionally substituted alkyl, aryl, aralkyl or alkaryl groups, or at least one pair of X^4 to X^7 together with the intervening atoms represents an optionally substituted, saturated or unsaturated homo- or heterocyclic ring, or where X^1 is NX^6 X^4 may represent a group X^8H where X^8 is oxygen or sulphur; and one X^1 or X^8 attached proton is labile), together with at least one physiologically acceptable carrier.

3. A composition as claimed in either of claims 1 and 2 wherein said chelant is selected from hydroxamates, α-hydroxycarbonyl compounds, and α-amino acids other than cysteine.

4. A composition as claimed in claim 3 wherein said chelant is an α-hydroxypyrone or α-hydroxypyridinone.

5. A composition as claimed in claim 4 wherein said chelant is maltol or kojic acid.

6. A composition as claimed in any one of claims 1 to 5 wherein said complex is vanadium (IV) or (V) complex.

7. A composition as claimed in any one of claims 1 to 5 wherein said complex is an oxovanadium (IV) complex.

8. A pharmaceutical composition for use in the treatment of obesity or hypertension comprising a physiologically tolerable vanadium compound together with at least one carrier or excipient.

9. A composition as claimed in claim 8 wherein said vanadium compound is an organic vanadium salt or a vanadium complex.

10. A composition as claimed in claim 9 wherein said vanadium compound is a complex as defined in any one of claims 1 to 7.

11. A composition as claimed in any one of claims 1 to 10 in a form adapted for parenteral administration by injection.

12. A composition as claimed in any one of claims 1 to 9 in a form adapted for oral administration.

13. A composition as claimed in any one of claims 1 to 10 in a form adapted for subcutaneous, intraperitoneal or transdermal administration.

14. Bis(kojato)oxovanadium(IV) for use in therapy.

15. Bis(maltolato)oxovanadium(IV) for use in therapy.

16. The use of a vanadium complex as defined in any one of claims 1 to 7 for the manufacture of a therapeutic agent for lowering blood sugar levels.

17. The use of a vanadium compound as defined in any one of claims 1 to 15 for the manufacture of an agent for use as an antihypertensive.

18. The use of a vanadium compound as defined in any one of claims 1 to 15 for the manufacture of an agent for use as an appetite suppressant.

19. A method of treatment of the human or non-human mammalian body to combat elevated blood sugar levels, said method comprising administering to said body a vanadium complex as defined in any one of claims 1 to 6.

20. A method of treatment of the human or non-human mammalian body to combat elevated blood pressure, said method comprising administering to said body a vanadium compound as defined in any one of claims 1 to 15.

21. A method of treatment of the human or non-human mammalian body to suppress appetite, said method comprising administering to said body a vanadium compound as defined in any one of claims 1 to 15.

22. A method as claimed in any one of claims 19 to 21 wherein administration is effected orally.

FIG. 1

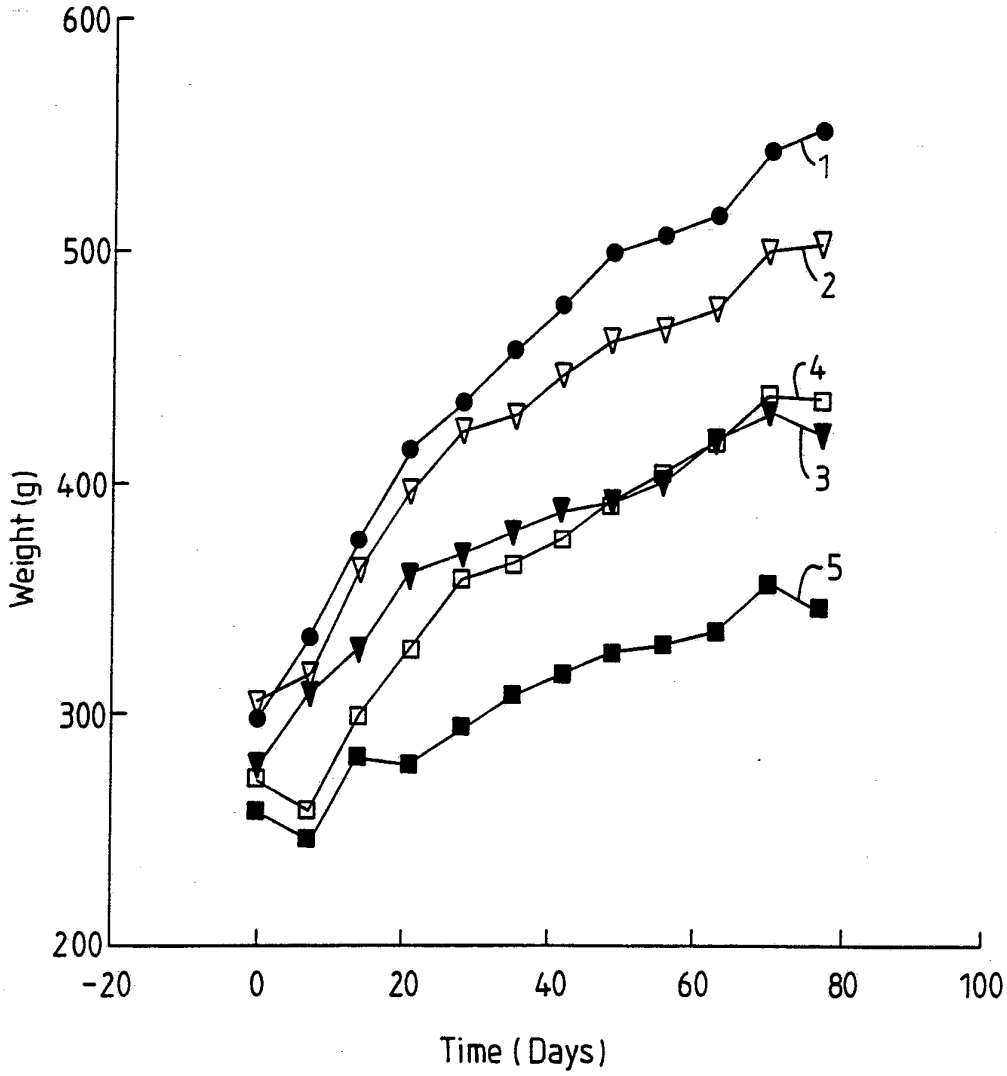


FIG. 2

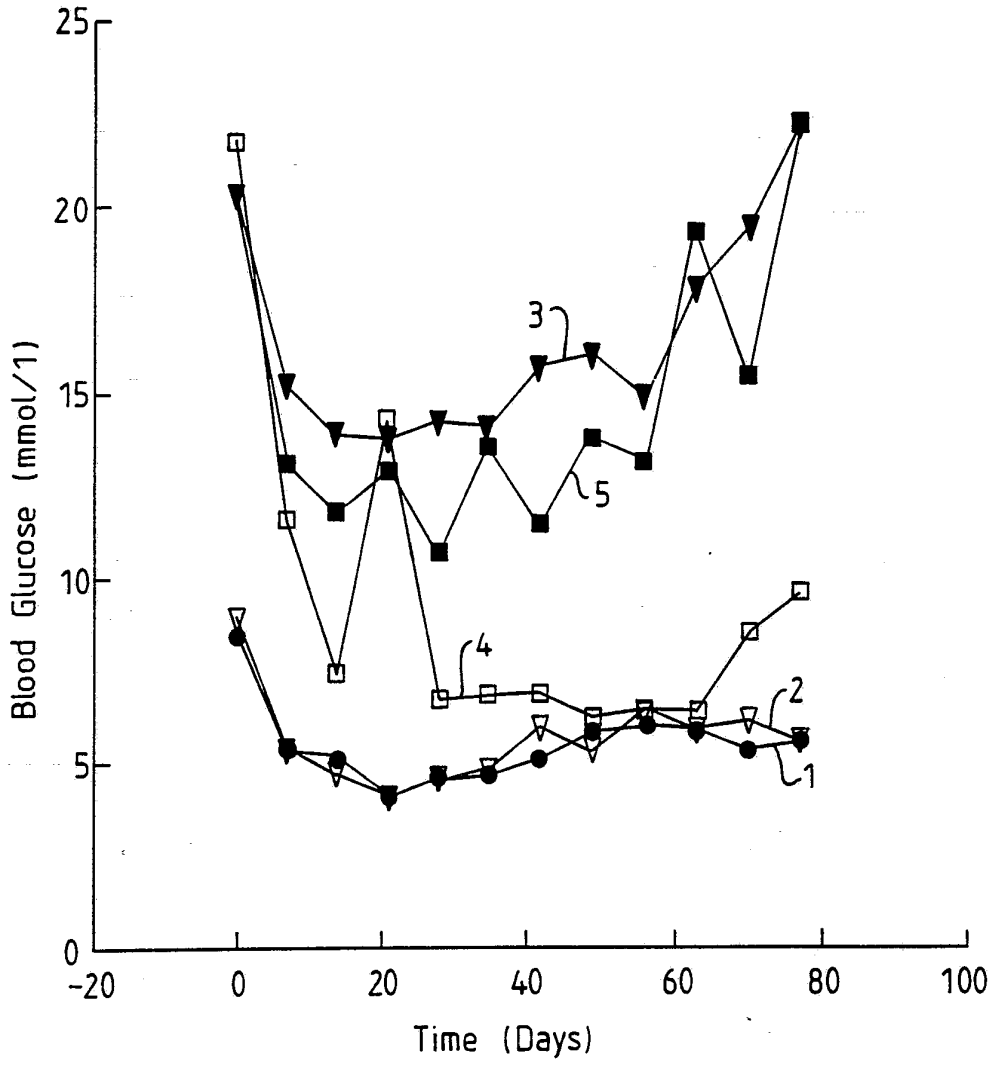

Blood Glucose (mmol/1)
25
20
15
10
5
0
-20
0
20
40
60
80
100
Time (Days)
1
2
3
4
5

FIG. 3

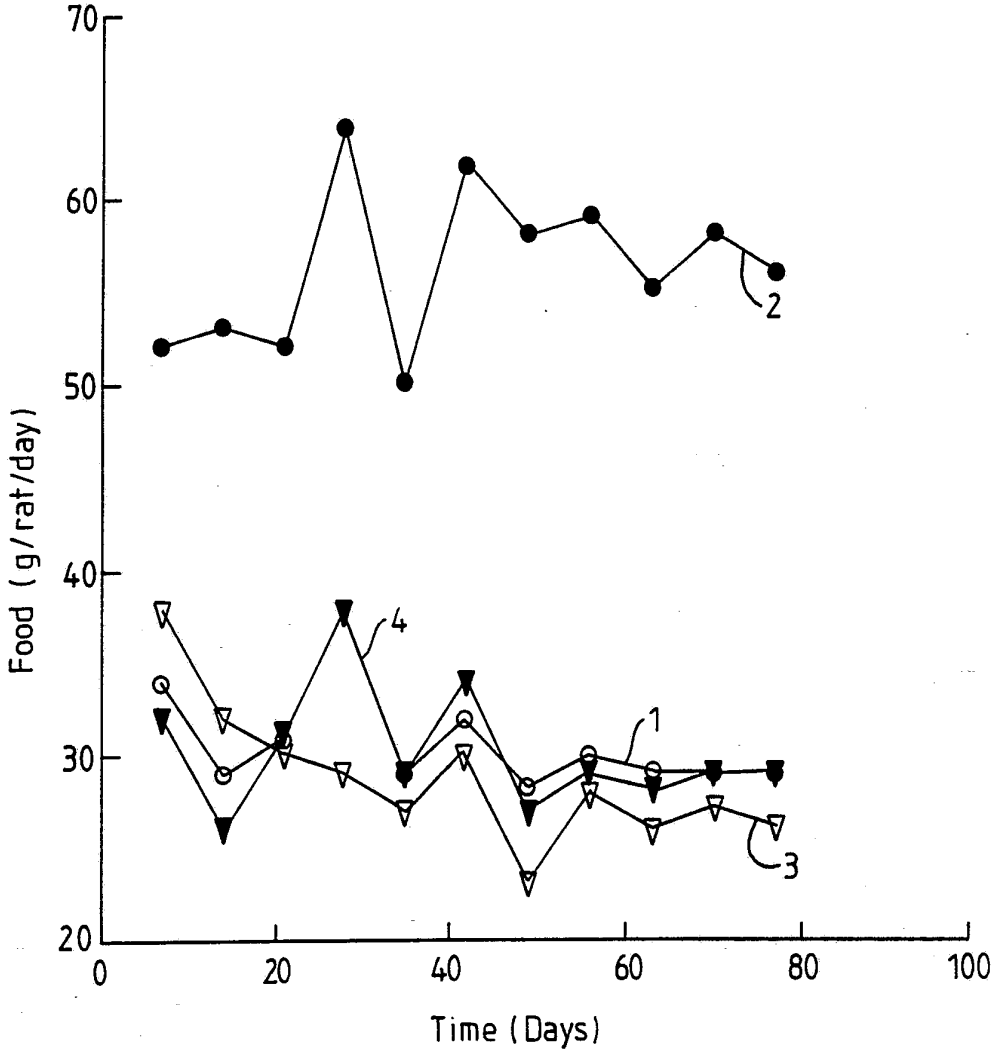


FIG. 4

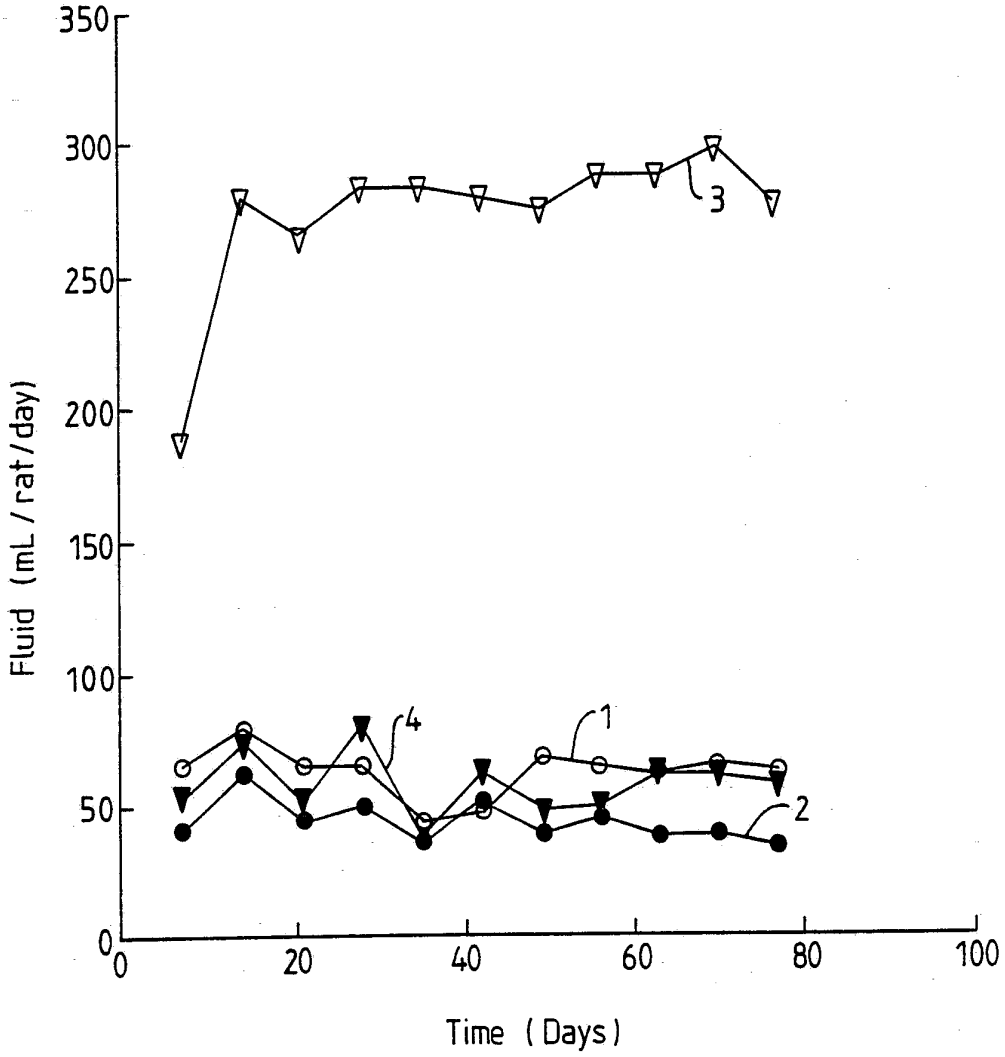

350
300
250
200
150
100
50
0
0
20
40
60
80
100
Fluid (mL / rat / day)
Time (Days)
1
2
3
4

FIG. 5

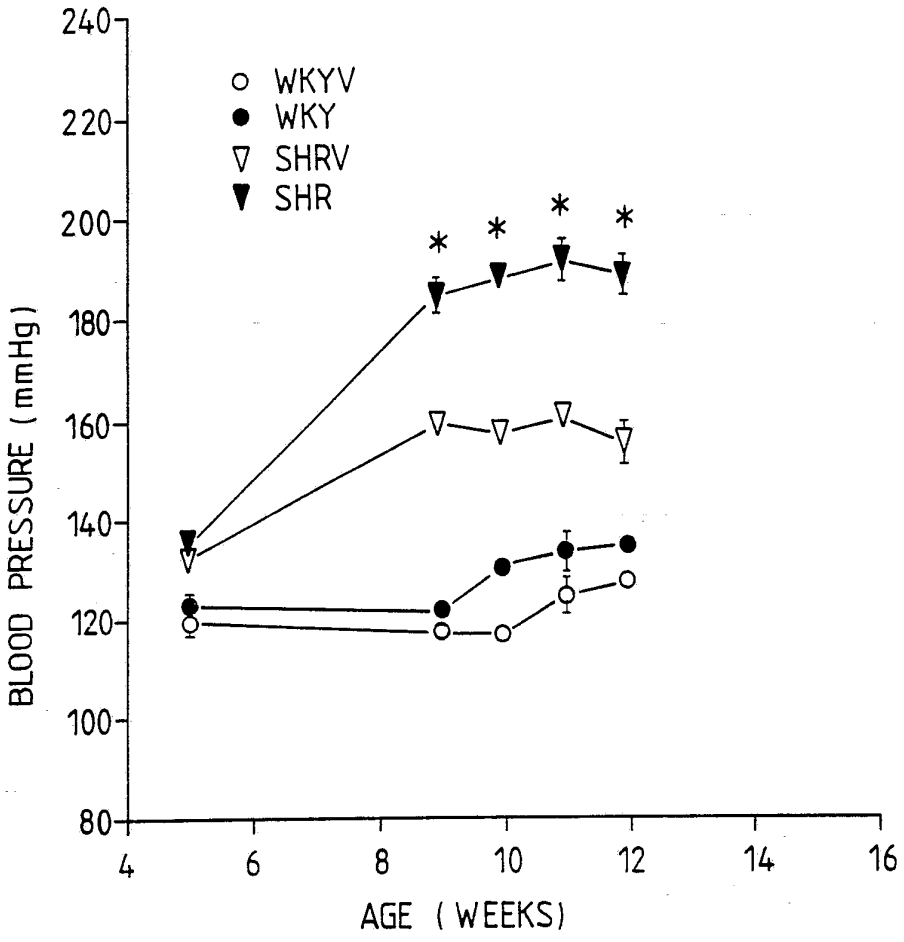

240
220
200
180
160
140
120
100
80
BLOOD PRESSURE (mmHg)
WKYV
WKY
SHRV
SHR
*
*
*
*
4
6
8
10
12
14
16
AGE (WEEKS)

FIG.6

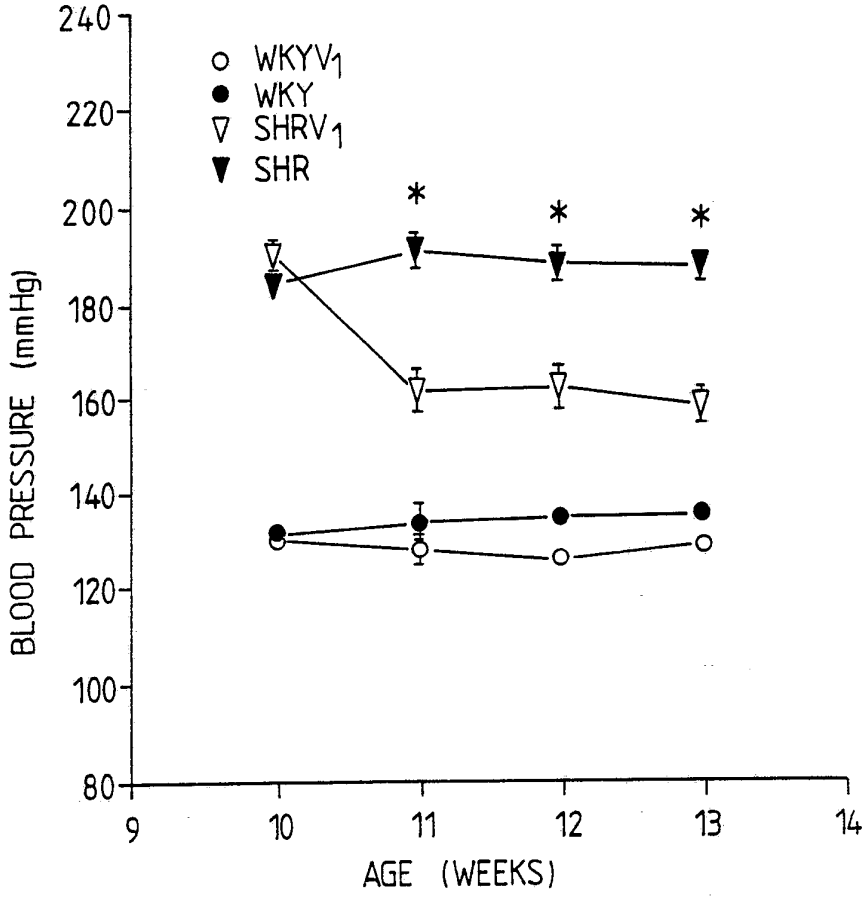
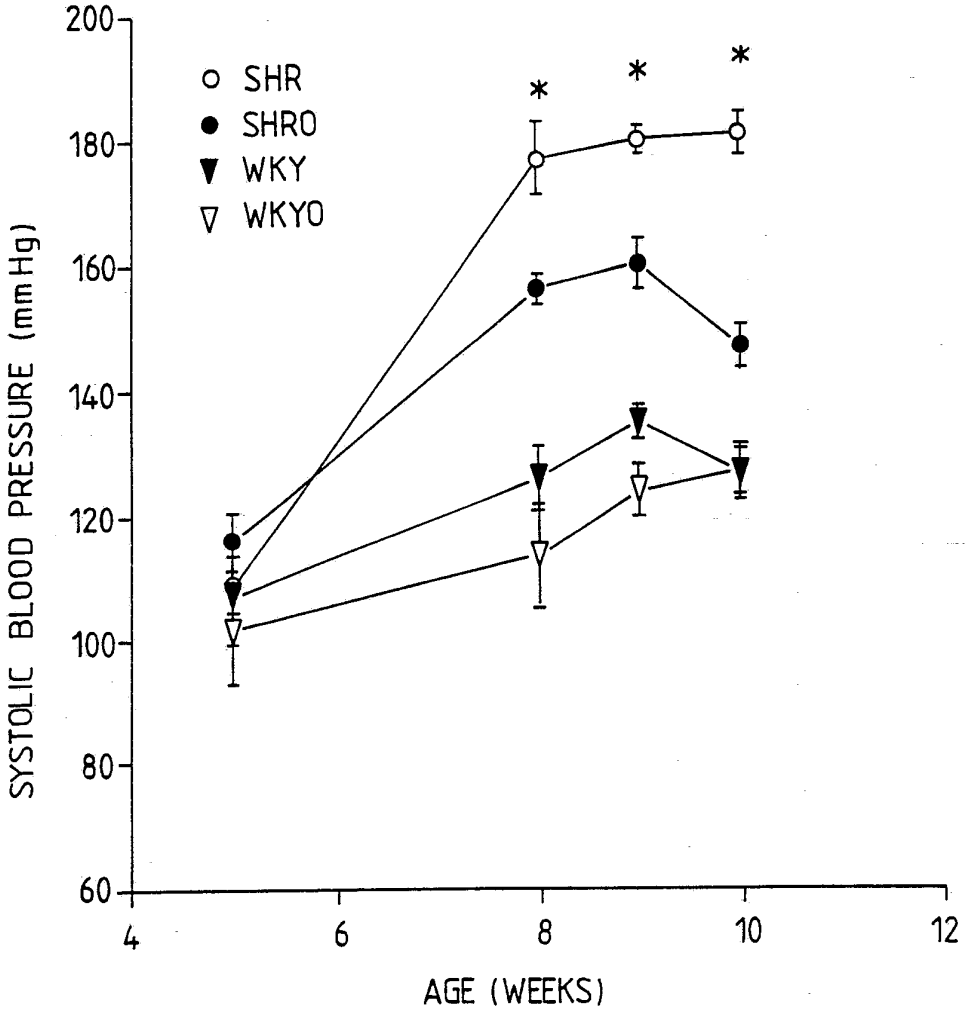

240
220
200
180
160
140
120
100
80
BLOOD PRESSURE (mmHg)
WKYV1
WKY
SHRV1
SHR
*
*
*
9
10
11
12
13
14
AGE (WEEKS)

FIG. 7


SYSTOLIC BLOOD PRESSURE (mmHg)
200
180
160
140
120
100
80
60
4
6
8
10
12
AGE (WEEKS)
SHR
SHRO
WKY
WKYO
*
*
*