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(54) Titre : UTILISATION D'ANALOGUES DE CREATINE POUR LE TRAITEMENT DE TROUBLES DU METABOLISME DU GLUCOSE

(54) Title: USE OF CREATINE ANALOGUES FOR THE TREATMENT OF DISORDERS OF GLUCOSE METABOLISM

(57) **Abrégé/Abstract:**

The present invention relates to the use of creatine compounds including cyclocreatine and creatine phosphate for treating or preventing a metabolic disorder consisting of hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinsulinemia, insulin insensitivity, diabetes related diseases in a patient experiencing said disorder. The creatine compounds which can be used in the present method include: (1) analogues of creatine which can act as substrates or substrate analogues for the enzyme creatine kinase; (2) compounds which can act as activators or inhibitors of creatine kinase; (3) compounds which can modulate the creatine transporter; (4) N-phosphocreatine analogues bearing transferable or non-transferable moieties which mimic the N-phosphoryl group; (5) compounds which modify the association of creatine kinase with other cellular components.



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(21) International Application Number: PCT/US96/16365 (22) International Filing Date: 11 October 1996 (11.10.96) (30) Priority Data: 08/540,894 11 October 1995 (11.10.95) US (71) Applicants: AVICENA GROUP, INC. [US/US]; Building 200, Suite 2200, One Kendall Square, Cambridge, MA 02139 (US). DANA-FARBER CANCER INSTITUTE [US/US]; 44 Binney Street, Boston, MA 02115 (US). (72) Inventors: KADDURAH-DAOUK, Rima; 4 Ross Road, Belmont, MA 02178 (US). TEICHER, Beverly, A.; 135 Hunting Road, Needham, MA 02194 (US). (74) Agent: CAMPBELL, Paula, A.; Testa, Hurwitz & Thibault, L.L.P., High Street Tower, 125 High Street, Boston, MA 02110 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: USE OF CREATINE ANALOGUES FOR THE TREATMENT OF DISORDERS OF GLUCOSE METABOLISM (57) Abstract The present invention relates to the use of creatine compounds including cyclocreatine and creatine phosphate for treating or preventing a metabolic disorder consisting of hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinsulinemia, insulin insensitivity, diabetes related diseases in a patient experiencing said disorder. The creatine compounds which can be used in the present method include: (1) analogues of creatine which can act as substrates or substrate analogues for the enzyme creatine kinase; (2) compounds which can act as activators or inhibitors of creatine kinase; (3) compounds which can modulate the creatine transporter; (4) N-phosphocreatine analogues bearing transferable or non-transferable moieties which mimic the N-phosphoryl group; (5) compounds which modify the association of creatine kinase with other cellular components.		

USE OF CREATINE ANALOGUES FOR THE TREATMENT OF DISORDERS OF GLUCOSE METABOLISM

Field of invention

The present invention provides for new use for creatine compounds (compounds which modulate one or more of the structural or functional components of the creatine kinase/creatine phosphate system) as therapeutic agents. More particularly, the present invention provides a method of treating or preventing certain metabolic disorders of human and animal metabolism, e.g., hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, insulin insensitivity, hyperinsulinemia and related diseases secondary to diabetes.

Background of the invention

There are several metabolic diseases of human and animal glucose metabolism, eg., hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinsulinemia, and insulin insensitivity, such as in non-insulin dependent diabetes mellitus (NIDDM). Hyperglycemia is a condition where the blood glucose level is above the normal level in the fasting state, following ingestion of a meal or during a glucose tolerance test. It can occur in NIDDM as well as in obesity. Hyperglycemia can occur without a diagnosis of NIDDM. This condition is called impaired glucose tolerance or pre-diabetes. Impaired glucose tolerance occurs when the rate of metabolic clearance of glucose from the blood is less than that commonly occurring in the general population after a standard dose of glucose has been orally or parenterally administered. It can occur in NIDDM as well as obesity, pre-diabetes and gestational diabetes. Hyperinsulinemia is defined as having a blood insulin level that is above normal level in fasting state or following ingestion of a meal. It can be associated with or causative of hypertension or atherosclerosis. Insulin insensitivity, or insulin resistance occurs when the insulin-dependent glucose clearance rate is less than that commonly occurring in the general population during diagnostic procedures.

A number of compounds have been tried to alleviate symptoms associated with glucose metabolism disorders. For example, guanidine, monoguanidine and diguanidine compounds have been shown to produce hypoglycemia. Watanabe, C., *J. Biol. Chem.*, 33: 253-265 (1918);

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Bischoff, F. et al., *Guanidine Structures and Hypoglycemia*, 81: 325-349 (1929). However these compounds were shown to be toxic. Biguanide derivatives, e.g., phenformin and metformin, have been used clinically as antidiabetic agents. Some members of this class continue to be used today, while others have been withdrawn from the market. Schafer, G., *Diabetes Metabol. (Paris)* 9:148-163 (1983). Gamma-guanidinobutyramide, also known as Tyformin, and its salt derivative, Augmentin, were investigated as potential anti-diabetic agents from the mid 1960's to mid 1970's. While Augmentin produced hypoglycemia, it was reported to have major undesirable side effects such as hypertension and circulatory collapse. Malaisse, W. et al., *Horm. Metab. Res.*, 1:258-265 (1969); *ibid*, 3:76-81 (1971).

British patent 1,153,424 discloses the use of certain esters and amides of guanidino-aliphatic acids in the treatment of diabetes mellitus where hyperuremia is present. The patent does not disclose that these compounds have an effect on hyperglycemia or any other symptom or pathological state related to disease. Canadian patent 891509 discloses the use of esters and amides of guanidinoaliphatic acids were disclosed for treating hyperuremia and hyperglycemia in diabetes mellitus.

British patents 1,195,199, and 1,195,200 disclose the use of guanidino alkanoic acids or their amides or esters for the treatment of hyperglycemia occurring in diabetes. A variety of British patents (1,552,179/ 1,195,199/ 1,195,200/1,552,179) describe the low potency of the guanidino alkanoic acid derivatives as single agents but describe their use in combination with other modalities.

Aynsley-Green and Alberti injected rats intravenously with beta guanidino propionic acid, arginine, guanidine, 4 guanidinobutyramide and 4 guanidinobutyric acid. Arginine and beta guanidino propionic acid stimulated insulin release but did not affect glucose levels. Also the treatment of animals with large amounts of beta guanidino propionic acid for several weeks was shown not to affect glucose levels. Moerland, T. et al., *Am. J. Physiol.*, 257:C810-C816 (1989). The two other compounds did stimulate insulin release but increased glucose levels. Aynsley-Green, A. et al., *Horm. Metab.*, 6:115-120 (1974).

It is an object of the present invention to provide methods for treatment of metabolic diseases that relate to glucose level regulation by administering to an afflicted individual an amount of a compound or compounds which modulate one or more of the structural or functional components of the creatine kinase/creatine phosphate system sufficient to prevent, reduce or

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ameliorate the symptoms of the disease. These compounds are collectively referred to as "creatine compounds." The experiments described herein demonstrate that the creatine kinase system is directly related to control of blood glucose levels in animals. Creatine analogues are shown herein to be effective hypoglycemic agents for treatment of glucose metabolic diseases.

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Summary of the Invention

The present invention provides a method of treating or preventing a glucose metabolic disorder using creatine, creatine phosphate, or a compound or compounds which modulates one or more of the structural or functional components of the creatine kinase/creatine phosphate system. Disorders which may be treated using the present invention include, for example, those selected from the group consisting of hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinsulinemia and diabetes related complications. The method of the invention comprises administering to a subject afflicted with or susceptible to said disorder an amount of a creatine compound (compounds which modulate one or more of the structural or functional components of the creatine kinase/creatine phosphate system) sufficient to alleviate or prevent the symptoms of the disorder. The creatine compound may be in the form of a pharmacologically acceptable salt or combined with an adjuvant or other pharmaceutical agent effective to treat or prevent the disease or condition.

Prior to the present invention, the creatine kinase system had not been implicated in glucose metabolic disorders. The substrates for the creatine kinase enzyme, i.e., creatine and creatine phosphate, are both guanidino compounds. The present inventors have discovered that the creatine kinase (CK) enzyme modifies key events involved in glucose regulation by potentially regulating energy (ATP) involved in the release of insulin or the uptake of glucose in tissue. It is now possible to modify the CK system and design compounds that can prevent or ameliorate these diseases. The present invention demonstrates that at least two creatine compounds, creatine phosphate and cyclocreatine, are hypoglycemic agents. That is, these compounds cause glucose levels to drop significantly in a subject.

As stated hereinabove, a variety of guanidino compounds have been shown to act as hypoglycemic agents including the compound beta guanidino propionic acid (see, for example, PCT Publication Number WO 91/12799). The target for these compounds and their mode of action is not fully understood. However, beta guanidino propionic acid was shown not to affect glucose levels in normal animals, but had an effect on glucose levels in a model for non-insulin dependent diabetes mellitus. This compound has some structural similarity to creatine, but the compound and its mechanism of action does not modulate the creatine kinase system, and therefore do not form a part of this invention. Compounds useful in the present invention are creatine compounds which modulate the creatine kinase system.

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The present invention also provides pharmaceutical compositions containing creatine compounds in combination with a pharmaceutically acceptable carrier. The present compositions may be used in combination with effective amounts of standard chemotherapeutic agents which act on regulating glucose levels, such as insulin or sulphonylureas, to prophylactically and/or therapeutically treat a subject with a disease related to glucose levels.

Packaged drugs for treating subjects having a disease relating to glucose level regulation also are the subject of the present invention. The packaged drugs include a container holding the creatine compound, in combination with a pharmaceutically acceptable carrier, along with instructions for administering the same for the purpose of preventing, ameliorating, arresting or eliminating a disease related to glucose level regulation.

By treatment is meant the amelioration of one or more symptoms of, or total avoidance of, the metabolic disorder as described herein. By prevention is meant the avoidance of a currently recognized disease state, as described herein, in a patient evidencing some or all of the glucose metabolic disorders described above. The present compositions may be administered in a sustained release formulation. By sustained release is meant a formulation in which the drug becomes biologically available to the patient at a measured rate over a prolonged period. Such compositions are well known in the art.

Brief Description of the Figures

Figure 1 graphically illustrates the effect of selected creatine compounds on glucose levels in rats: Panel (A): glucose levels in control (unmanipulated animals); Panel(B): glucose levels in cyclocreatine treated animals; Panel (C): glucose levels in beta-guanidino propionic acid treated
5 animals; and Panel (D): glucose levels in creatine phosphate treated animals.

Figure 2 graphically illustrates the effect of the selected compounds on glucose levels in rats: Panel (A): control (unmanipulated animals); Panel (B): cyclocreatine treated; Panel (C): beta-guanidino propionic acid treated; Panel (D): creatine phosphate treated animals.

10 Figure 3 graphically illustrates the effect of selected creatine compounds on glucose levels in rats over time: Panel (A): cyclocreatine treated; Panel (B): beta-guanidino propionic acid treated; Panel (C): creatine phosphate treated animals.

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Detailed Description of the Invention

The method of the present invention generally comprises administering to an individual afflicted with a disease or susceptible to a disease involving glucose level regulation, an amount of a compound or compounds which modulate one or more of the structural or functional components of the creatine kinase/phosphocreatine (CK/CrP) system sufficient to prevent, reduce or ameliorate symptoms of the disease. Components of the CK/CrP system which can be modulated include the enzyme creatine kinase (CK), the substrates creatine, creatine phosphate, ADP, ATP, and the transporter of creatine. As used herein, the term "modulate" means to change, affect or interfere with the functioning of the component in the CK/CrP enzyme system.

The CK/CrP is an energy generating system operative predominantly in the brain, muscle, heart, retina, and the pancreas. Walliman et al., *Biochem. J.*, 281, 21-401 (1992). The components of the system include the enzyme creatine kinase (CK), the substrates creatine (Cr), creatine phosphate (CrP), ATP, ADP, and the creatine transporter. The enzyme reversibly catalyzes the transfer of a phosphoryl group from CrP to ADP to generate ATP. It is found to be localized at sites where rapid rate of ATP replenishment is needed. Some of the functions associated with this system include efficient regeneration of energy in the form of ATP in cells with fluctuating and high energy demand, energy transport to different parts of the cell, phosphoryl transfer activity, ion transport regulation, and involvement in signal transduction pathways.

The substrate creatine is a compound which is naturally occurring and is found in mammalian brain, skeletal muscle, retina and the heart. Its phosphorylated form CrP is also found in the same organs and is the product of the CK reaction. Both compounds can be easily synthesized and are believed to be non-toxic to man. A series of creatine analogues have also been synthesized and used as probes to study the active site of the enzyme. Kaddurah-Daouk et al. (WO 92/08456 published May 29, 1992 and WO 90/09192, published August 23, 1990, U.S. 5,321,030; and U.S. 5,324,731) described methods for inhibiting growth, transformation, or metastasis of mammalian cells using related compounds. Examples of such compounds include cyclocreatine, homocyclocreatine and beta guanidino propionic acid. These same inventors have also demonstrated the efficacy of such compounds for combating viral infections (U.S. 5,321,030). Elgebaly in U.S. Patent 5,091,404 discloses the use of cyclocreatine for restoring functionality in

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muscle tissue. Cohn in PCT publication No. WO94/16687 describes a method for inhibiting the growth of several tumors using creatine and related compounds. No prior work has established a direct link between the creatine kinase system and diseases related to glucose level regulation such as hyperglycemia, insulin dependent or independent diabetes and related diseases secondary to diabetes.

Compounds which are particularly effective for use in the present invention include cyclocreatine, creatine phosphate and analogues thereof which are described below. The term "creatine compound" will be used herein to include Cr, CrP, cyclocreatine, compounds which are structurally similar to Cr, CrP, and cyclocreatine, and analogues of Cr, CrP, and cyclocreatine. The term "creatine compound" also includes compounds which "mimic" the activity of cyclocreatine and creatine phosphate or creatine analogues i.e., compounds which modulate the creatine kinase system. The term "mimics" is intended to include compounds which may not be structurally similar to creatine but mimic the therapeutic activity of the creatine analogues cyclocreatine and creatine phosphate or structurally similar compounds. The term creatine compounds will also include inhibitors of creatine kinase, ie. compounds which inhibit the activity of the enzyme creatine kinase, molecules that inhibit the creatine transporter or molecules that inhibit the binding of the enzyme to other structural proteins or enzymes or lipids. The term "modulators" of the creatine kinase system are compounds which modulate the activity of the enzyme, or the activity of the transporter of creatine, or the ability of the enzyme to associate with other cellular components. These could be substrates for the enzyme and they would have the ability to build in their phosphorylated state intracellularly. These types of molecules are also included in our term creatine compounds. The term creatine "analogue" is intended to include compounds which are structurally similar to creatine such as cyclocreatine and creatine phosphate, compounds which are art-recognized as being analogues of creatine, and/or compounds which share the same function as cyclocreatine and creatine phosphate.

Creatine (also known as N-(aminoiminomethyl)-N-methyl glycine; methylglycosamine or N-methyl-guanidino acetic acid) is a well-known substance. *See, The Merck Index*, Eleventh Edition No. 2570 (1989). Creatine is phosphorylated chemically or enzymatically to creatine kinase to generate creatine phosphate, which is also well known (*see, The Merck Index*, Eleventh Edition No. 7315 (1989)). Both creatine and creatine phosphate (phosphocreatine) can be extracted from animals or tissue or synthesized chemically. Both are commercially available.

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Cyclocreatine is an essentially planer cyclic analogue of creatine. Although cyclocreatine is structurally similar to creatine, the two compounds are distinguishable both kinetically and thermodynamically. Cyclocreatine is phosphorylated efficiently by the enzyme creatine kinase in the forward reaction, both *in vitro* and *in vivo*. Rowley, G.L., *J.AM. Chem.Soc.*, 93:5542-5551 (1971); McLaughlin, A.C. et. al., *J. Biol. Chem.*, 247, 4382-4388 (1972). It represents a class of substrate analogues of creatine kinase and which are believed to be active.

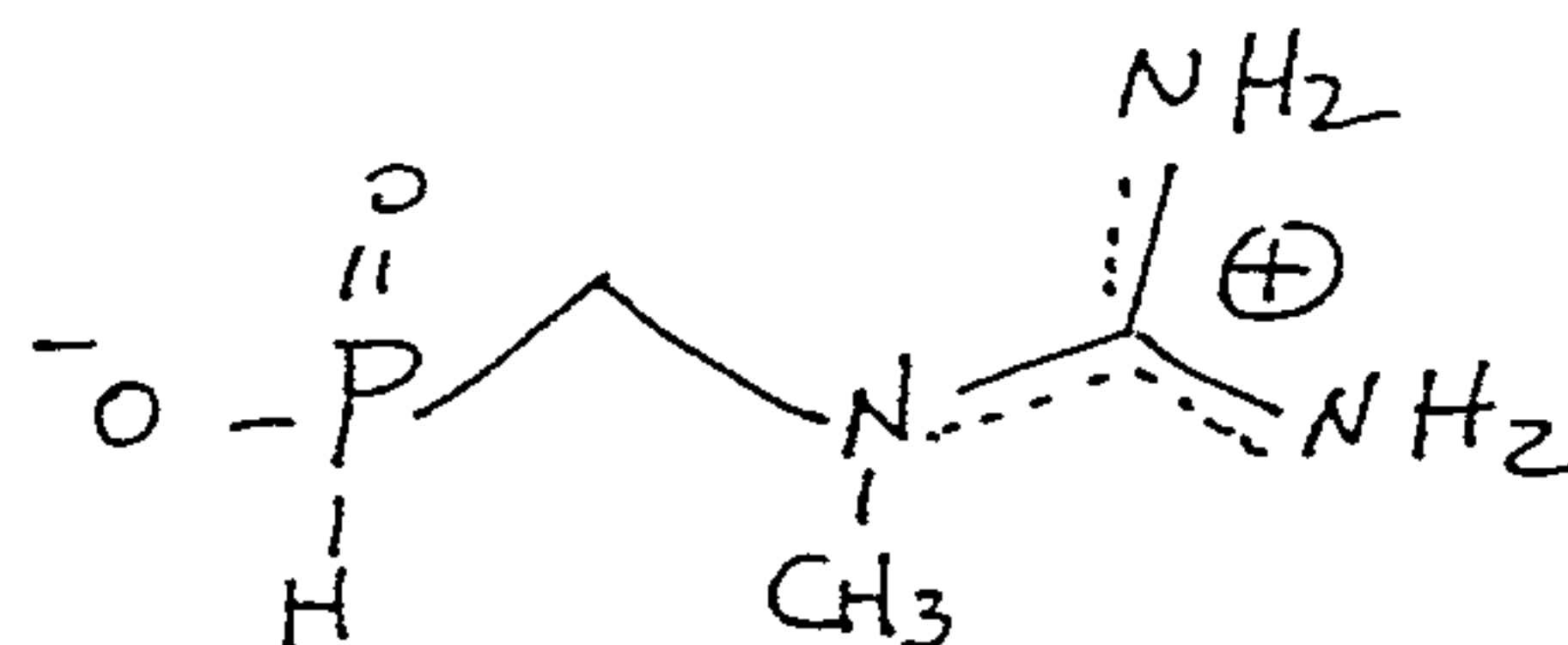
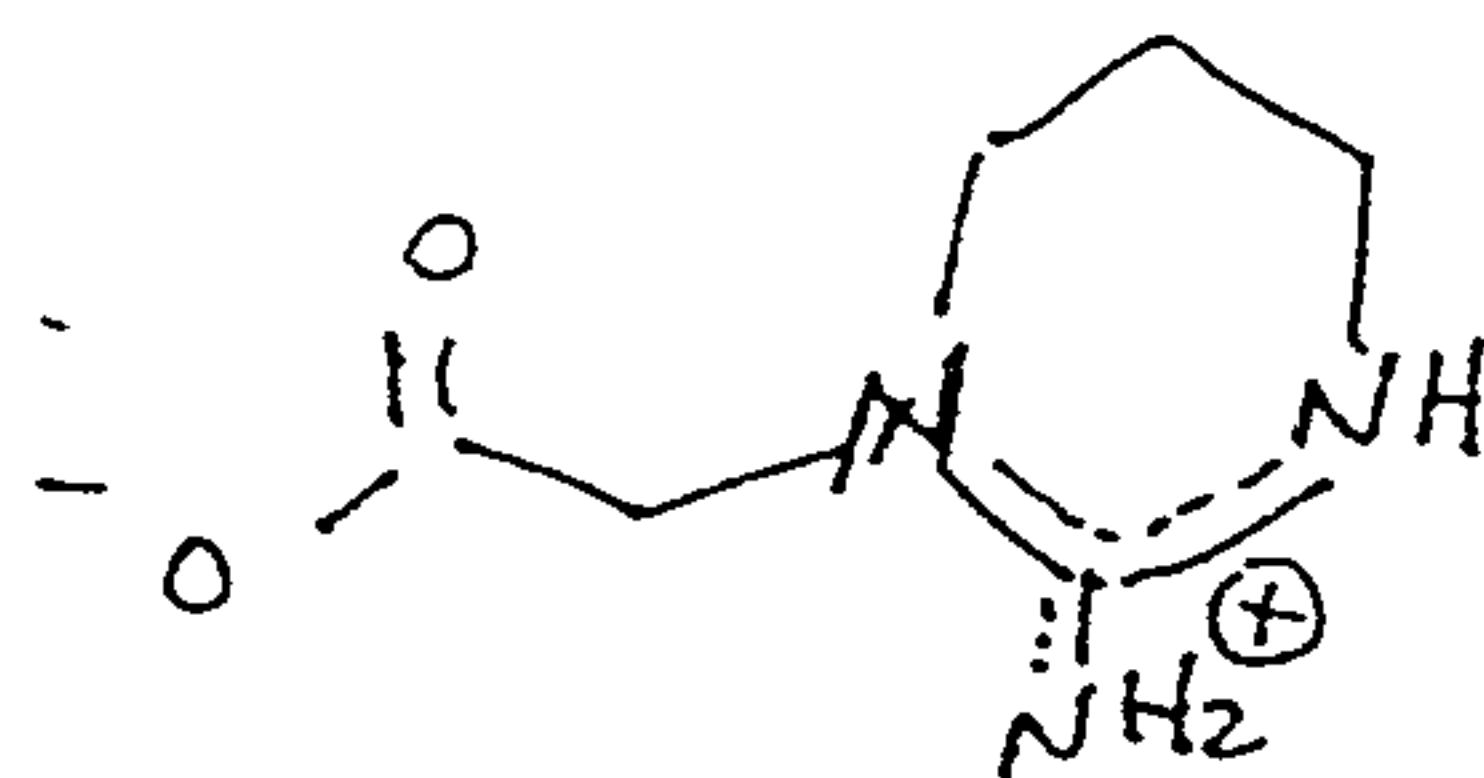
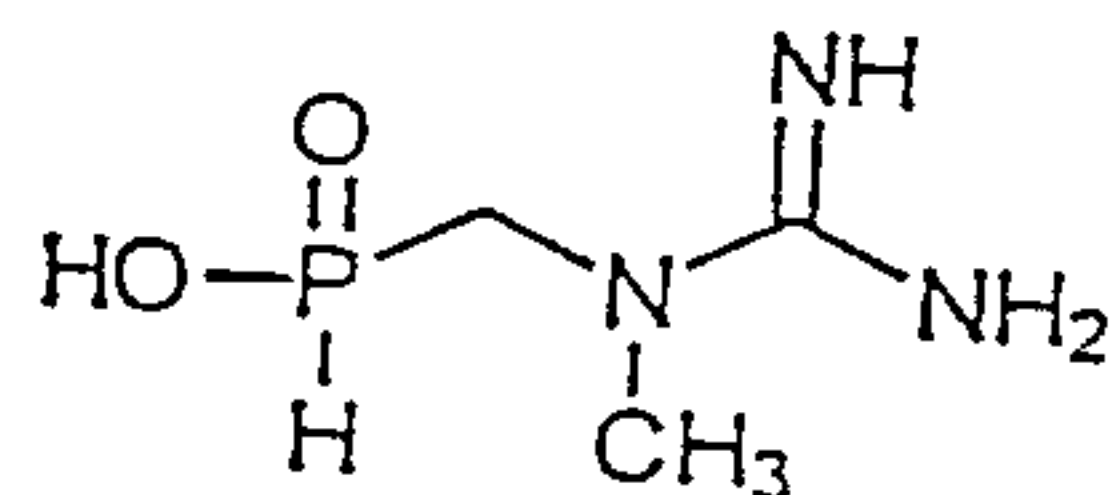
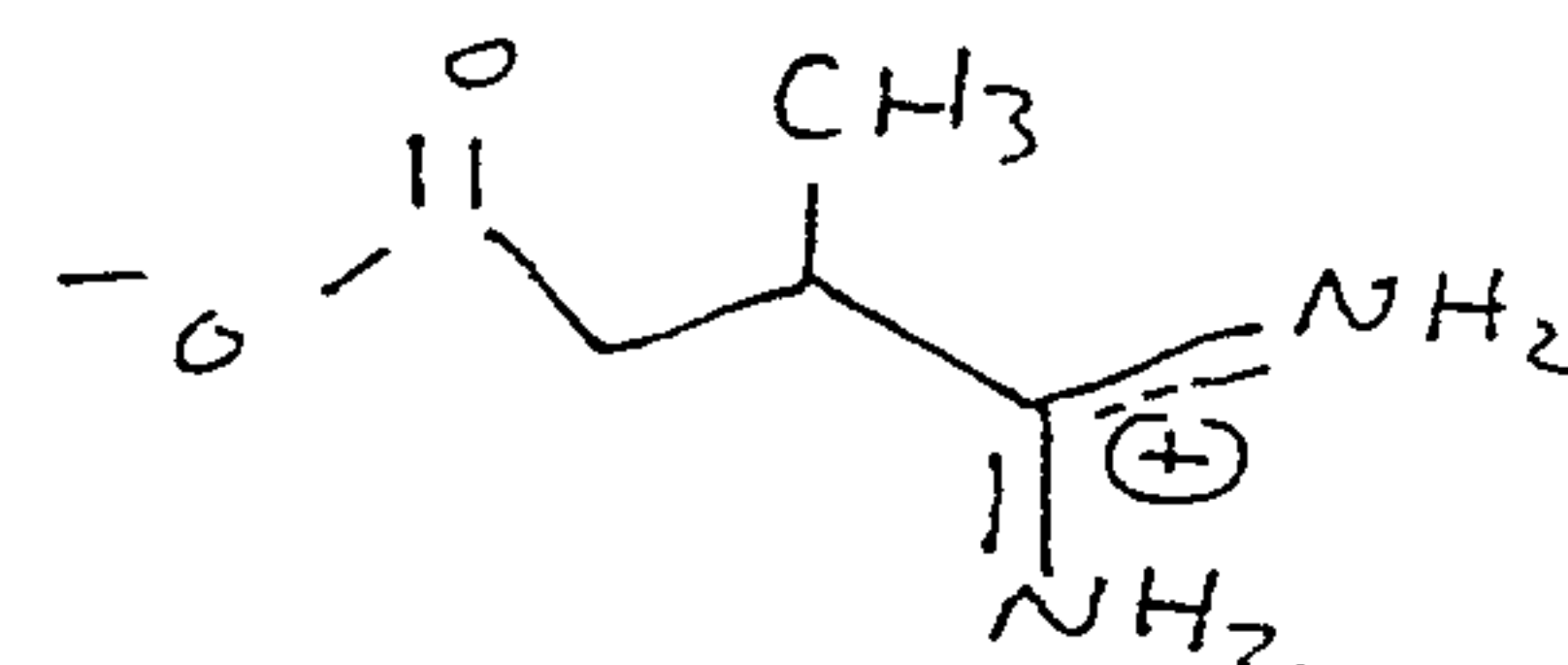
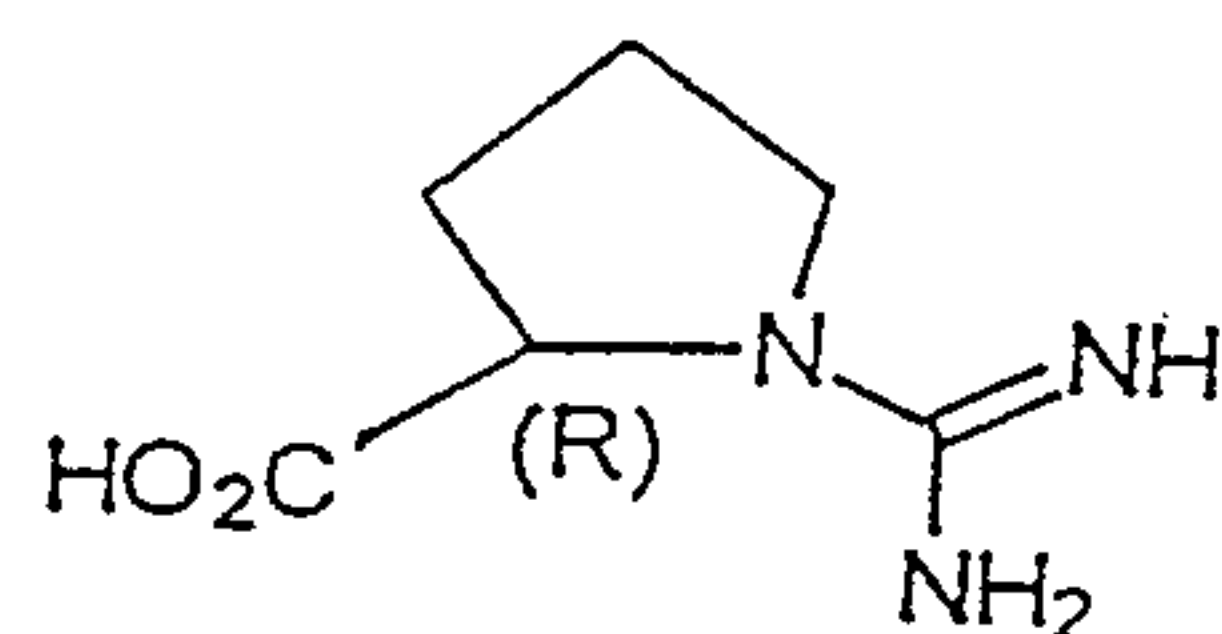
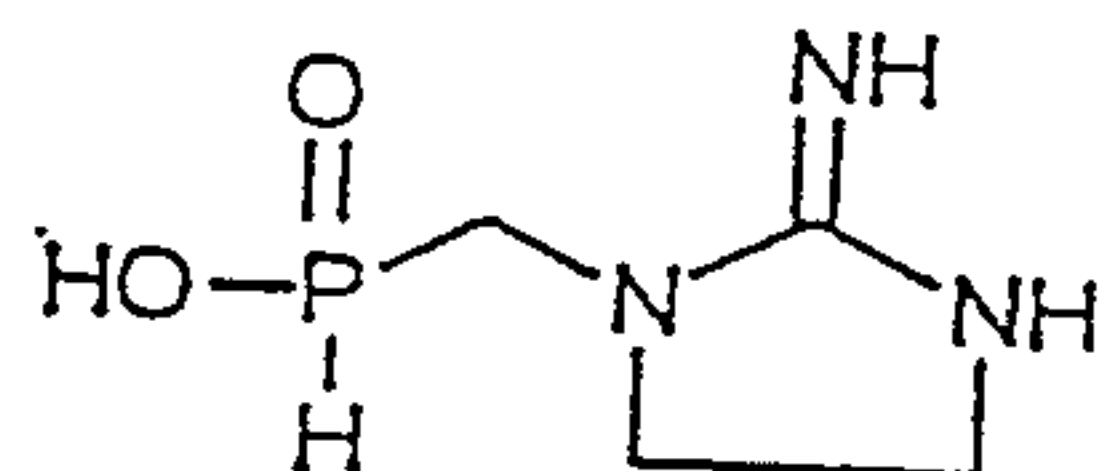
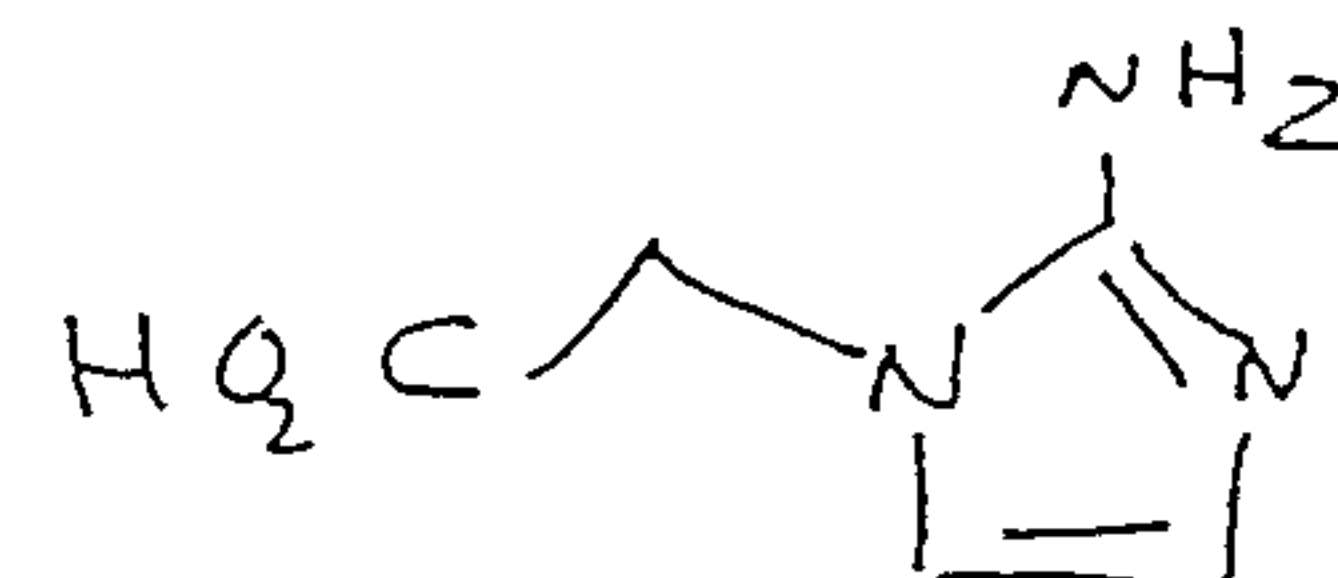
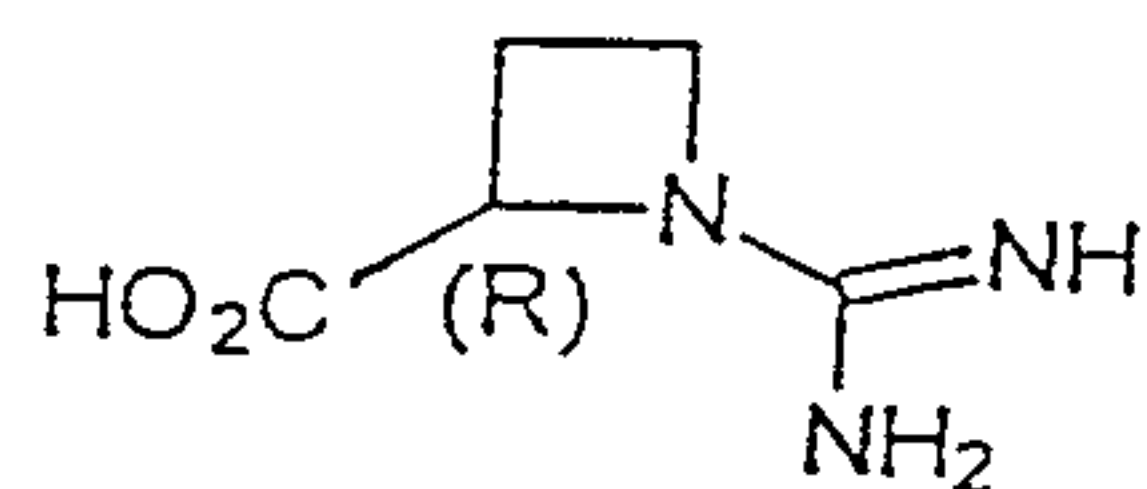
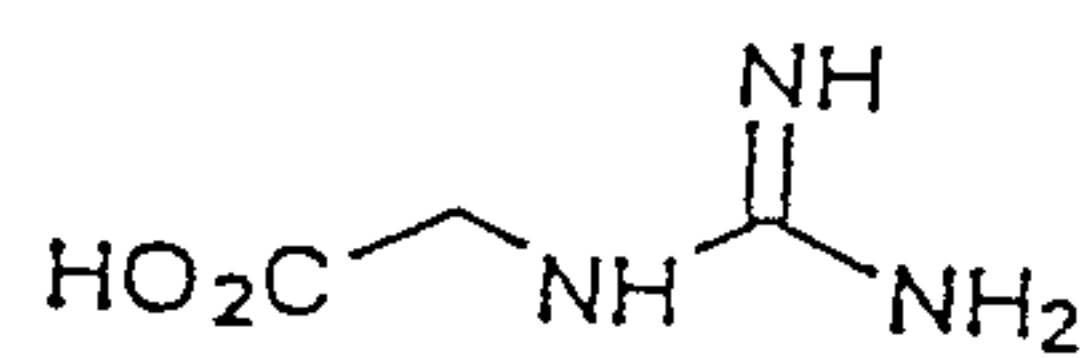
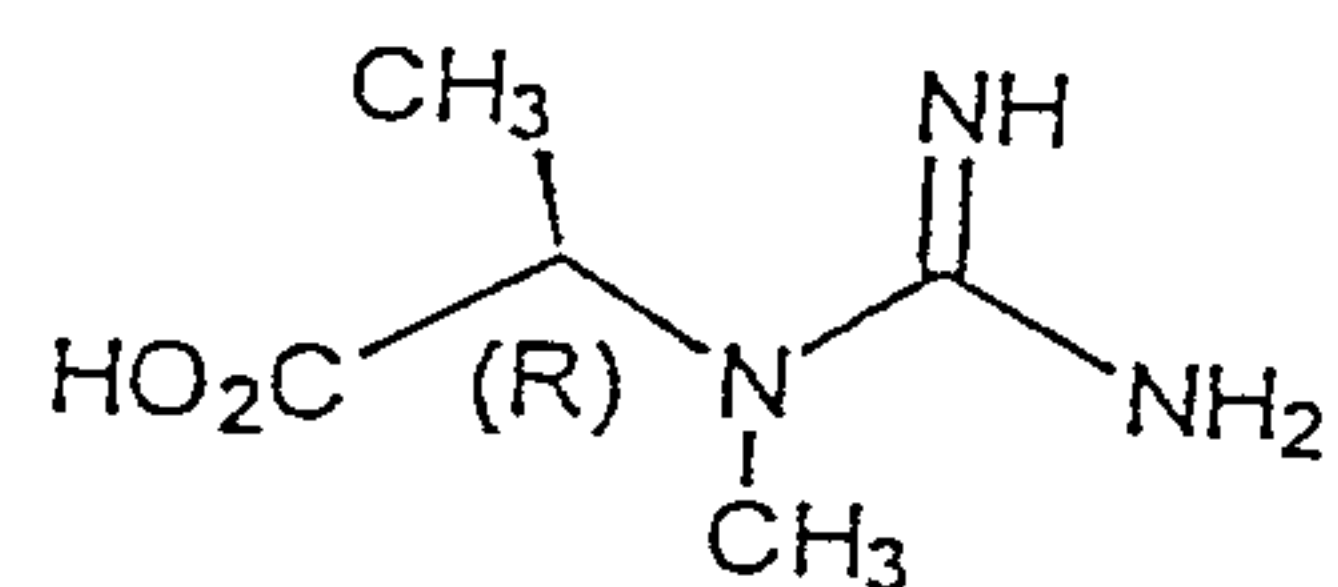
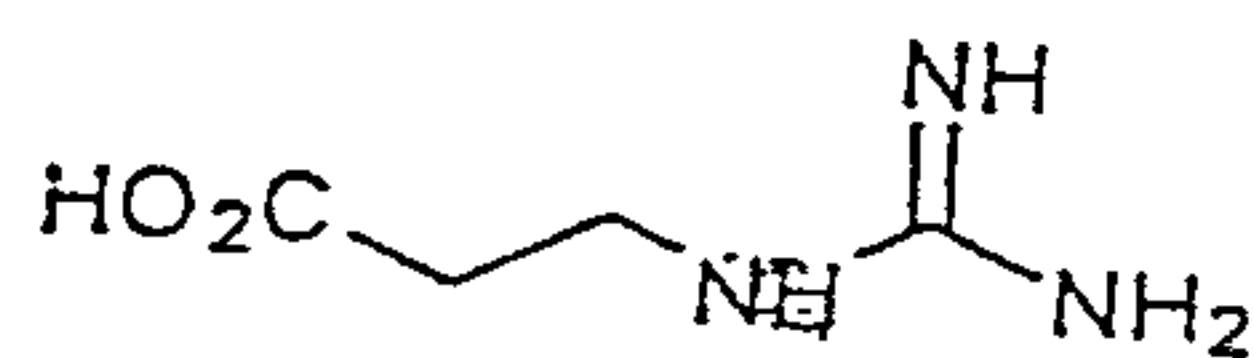
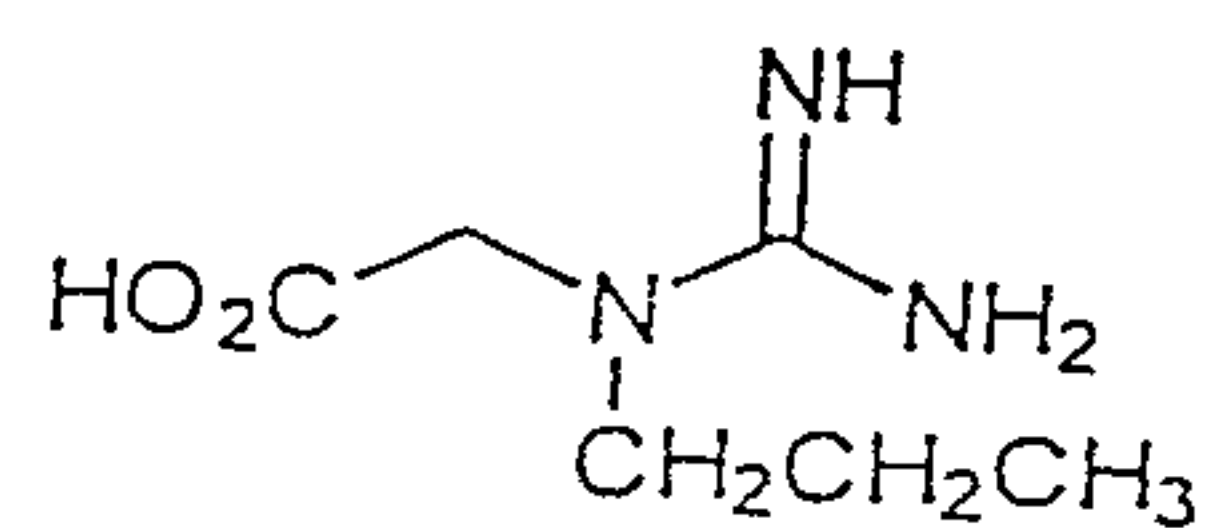
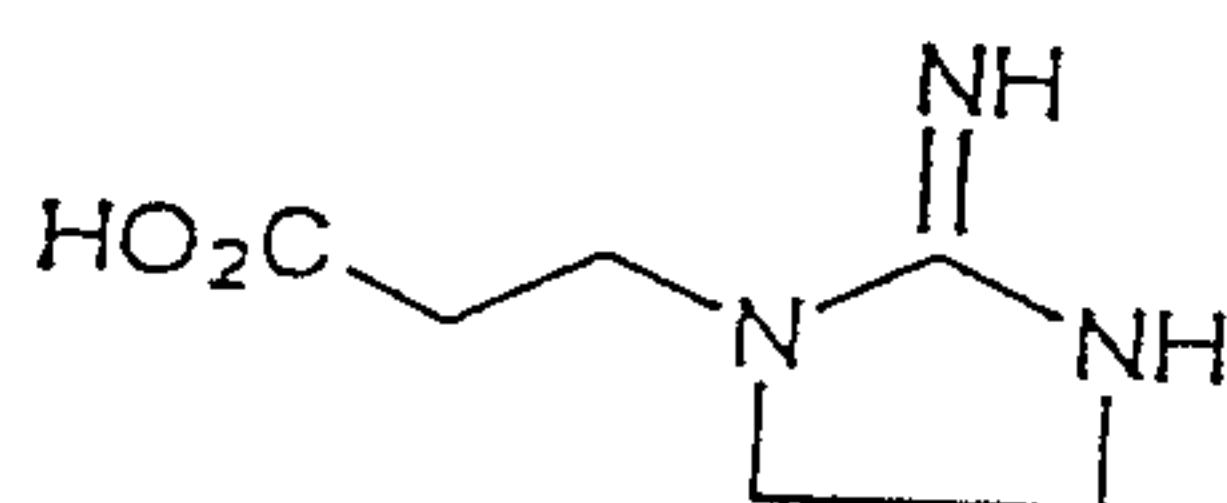
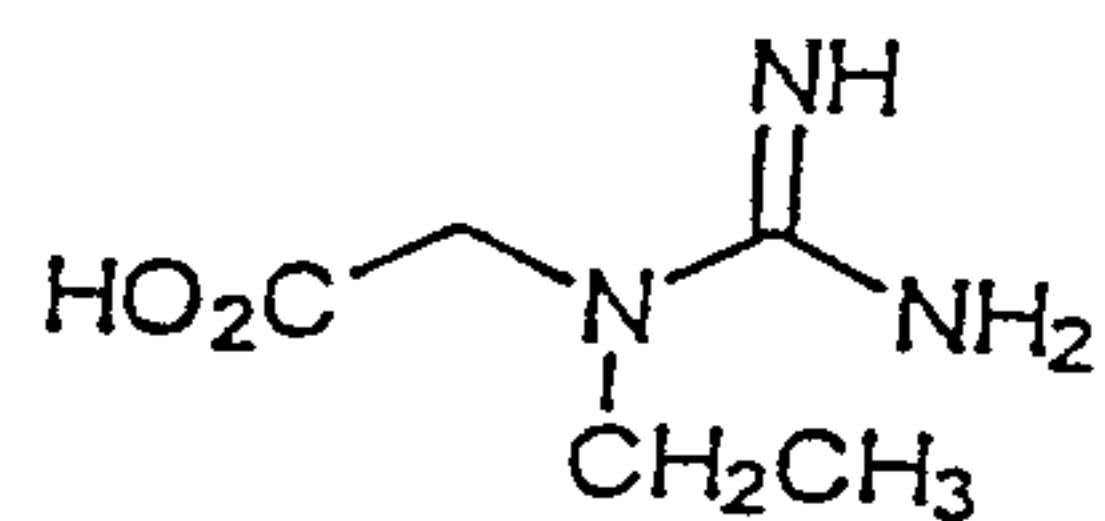
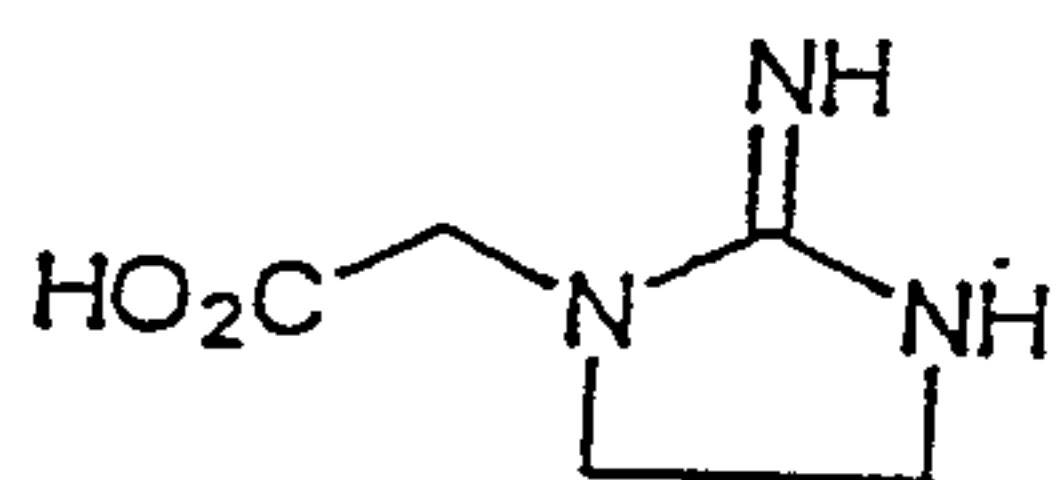
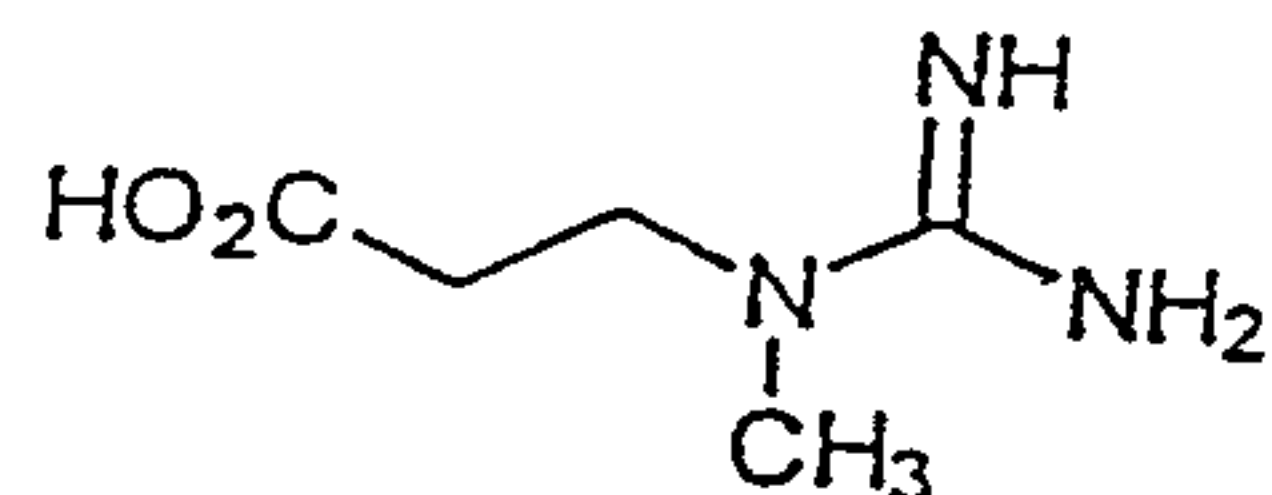
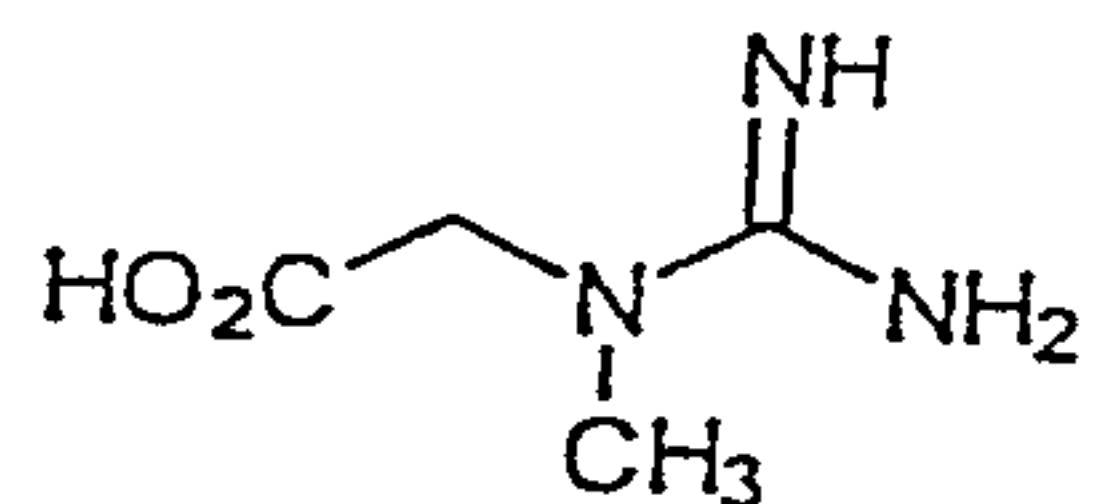
Examples of creatine analogues known or believed to modify the creatine kinase/creatine phosphate system are listed in the following Tables 1 and 2.

WO 97/13507

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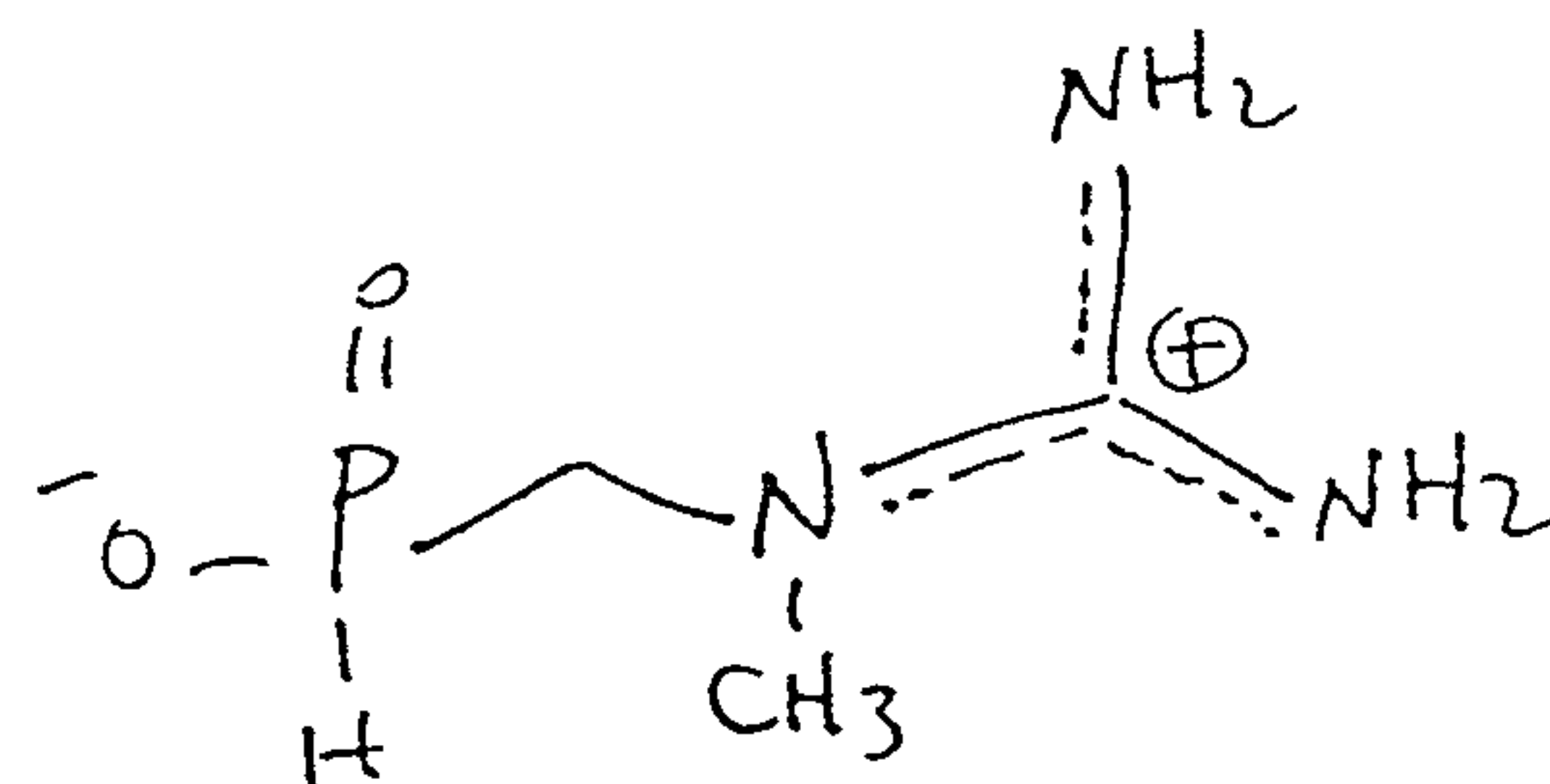
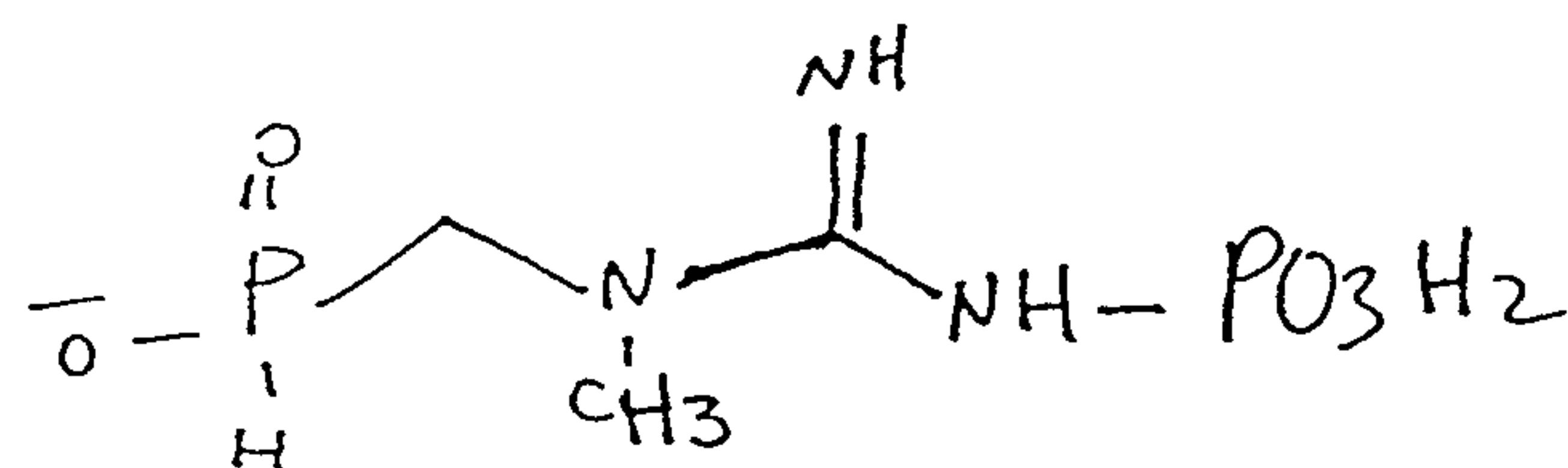
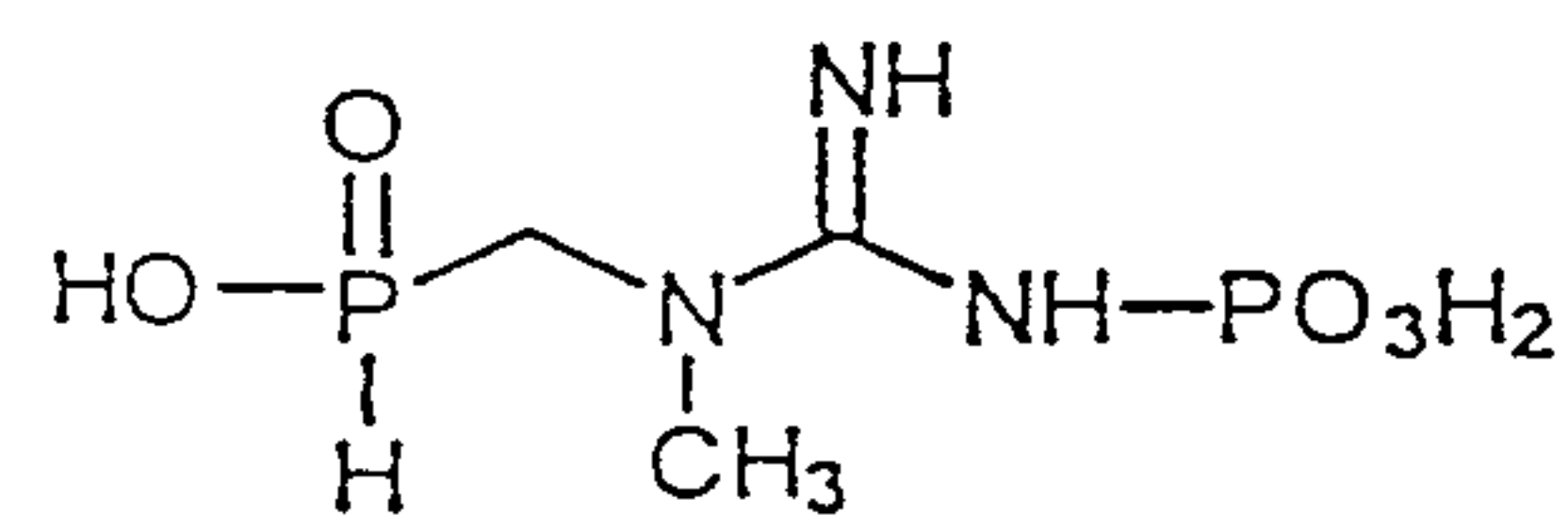
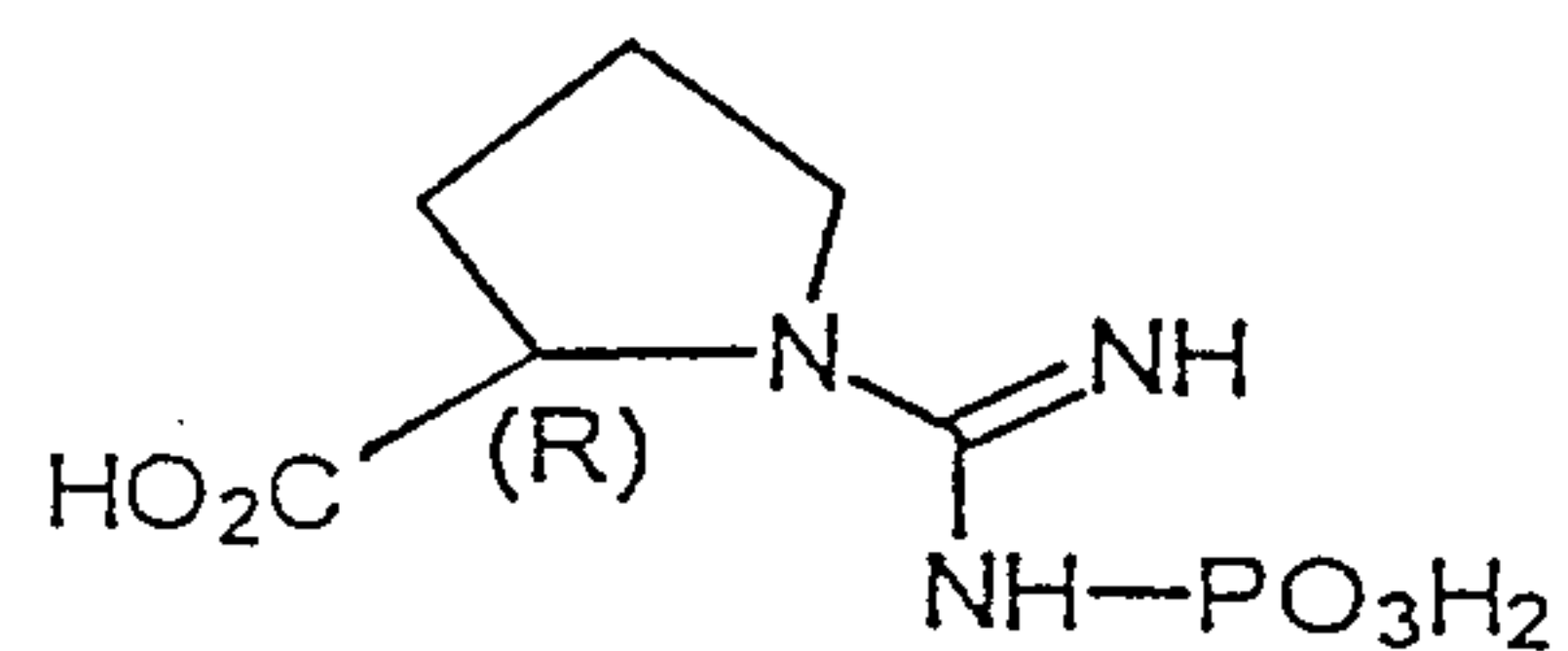
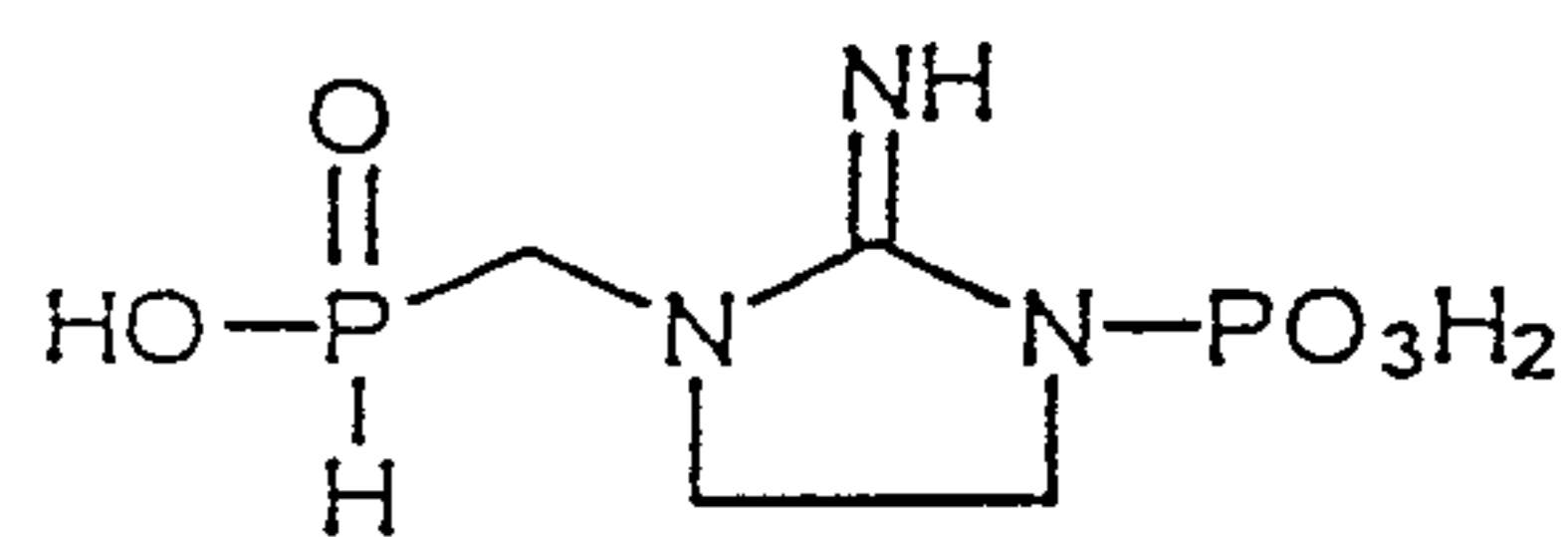
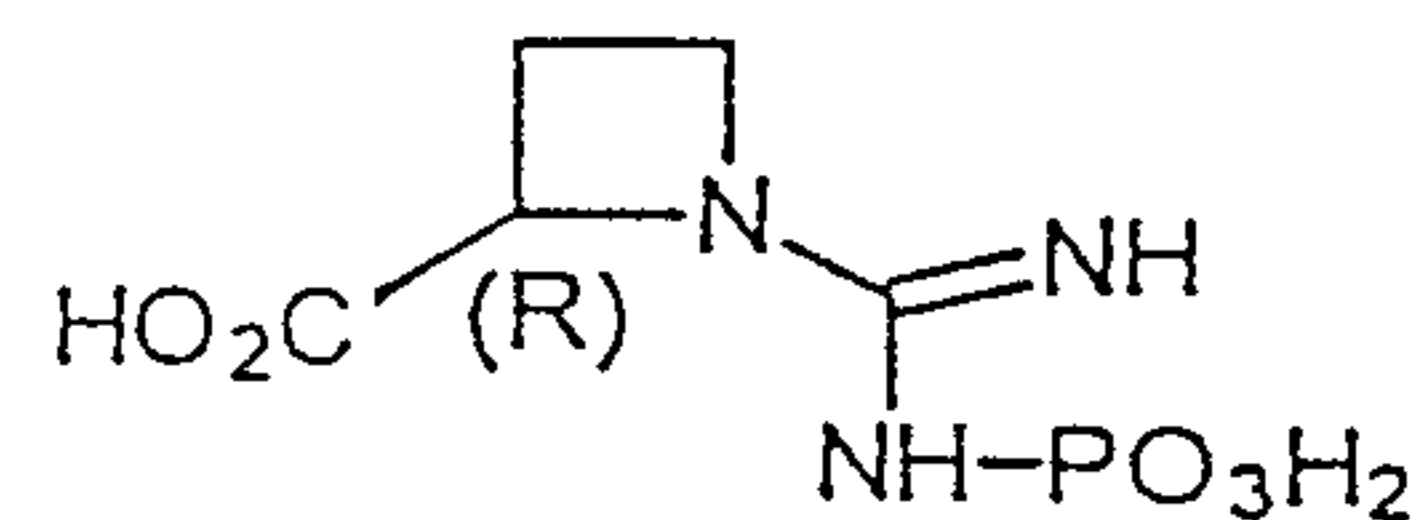
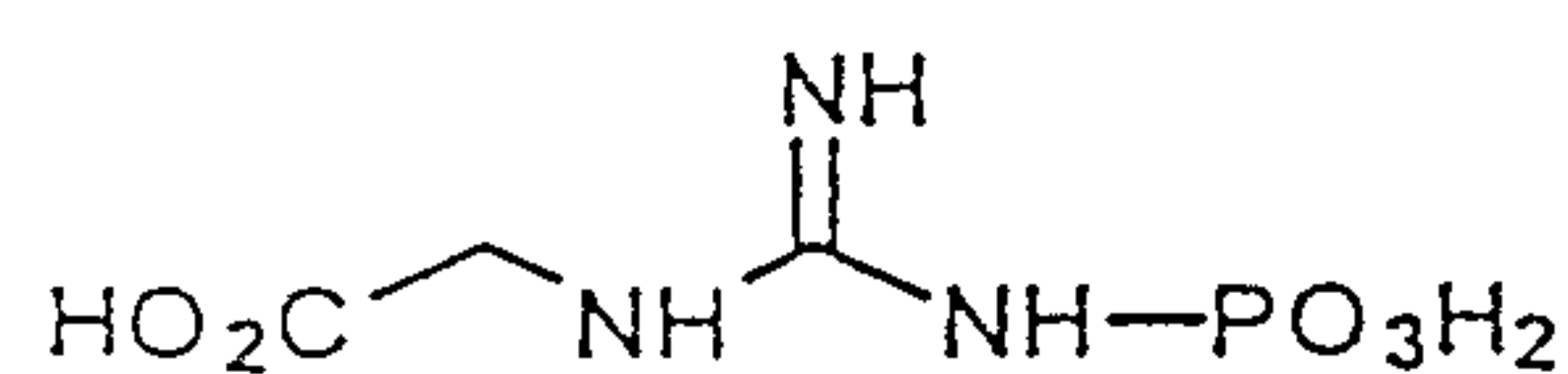
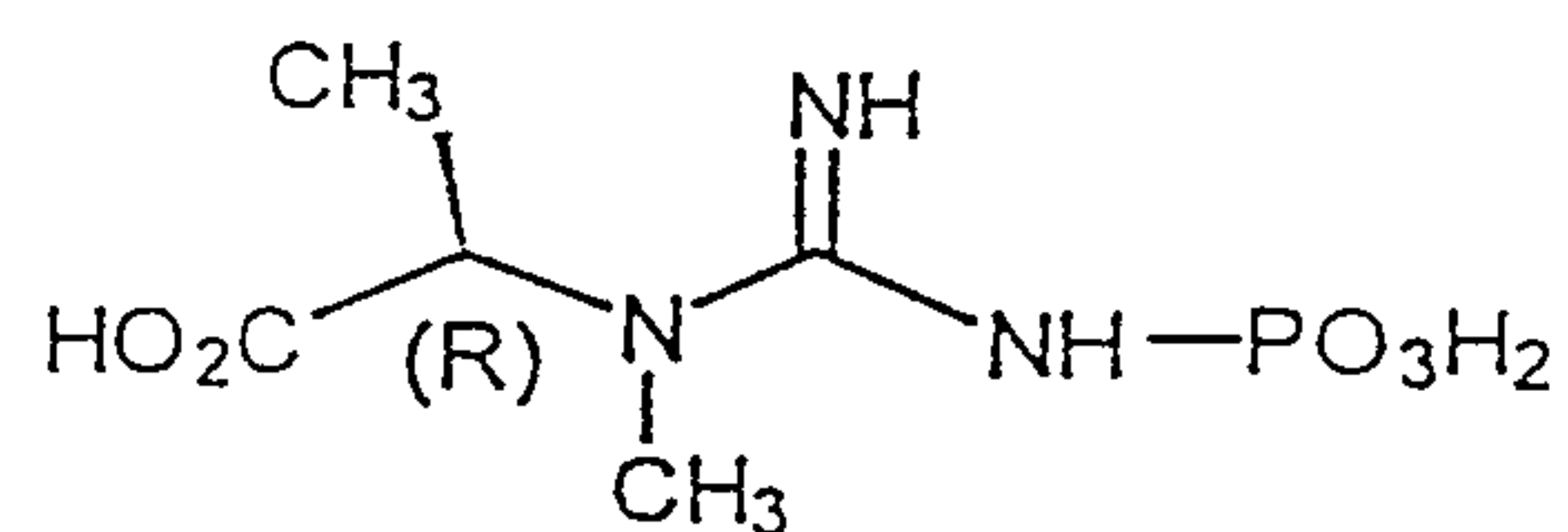
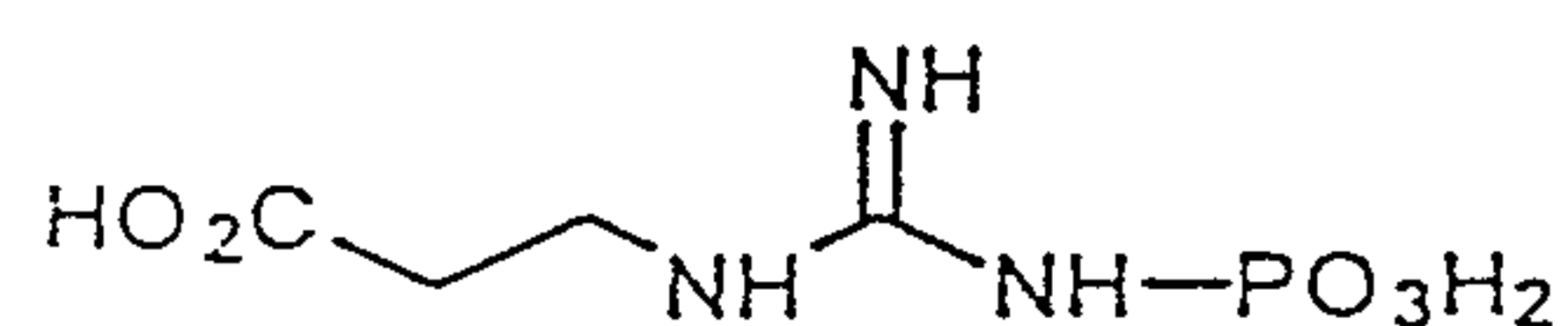
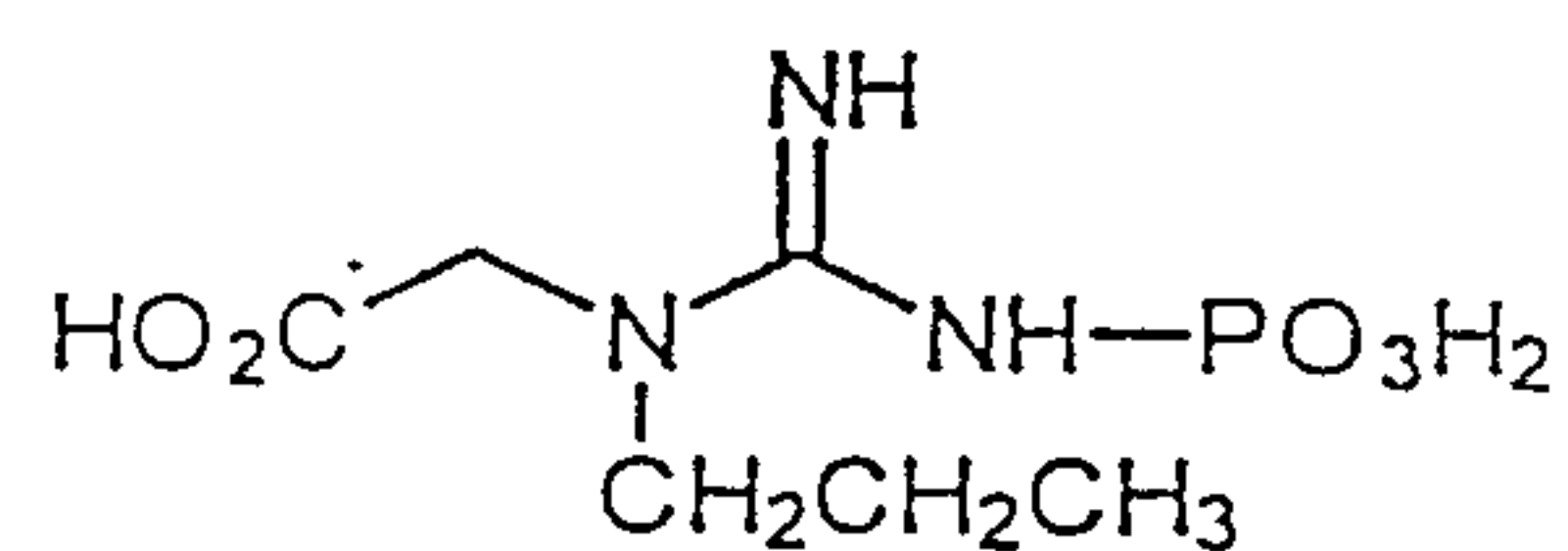
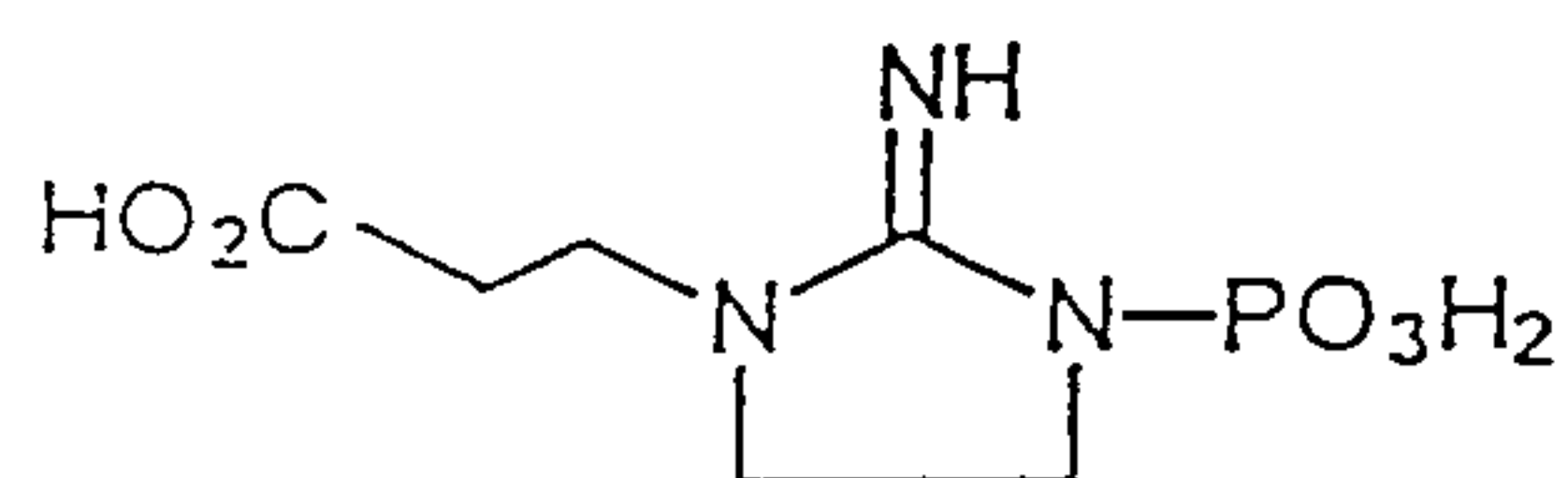
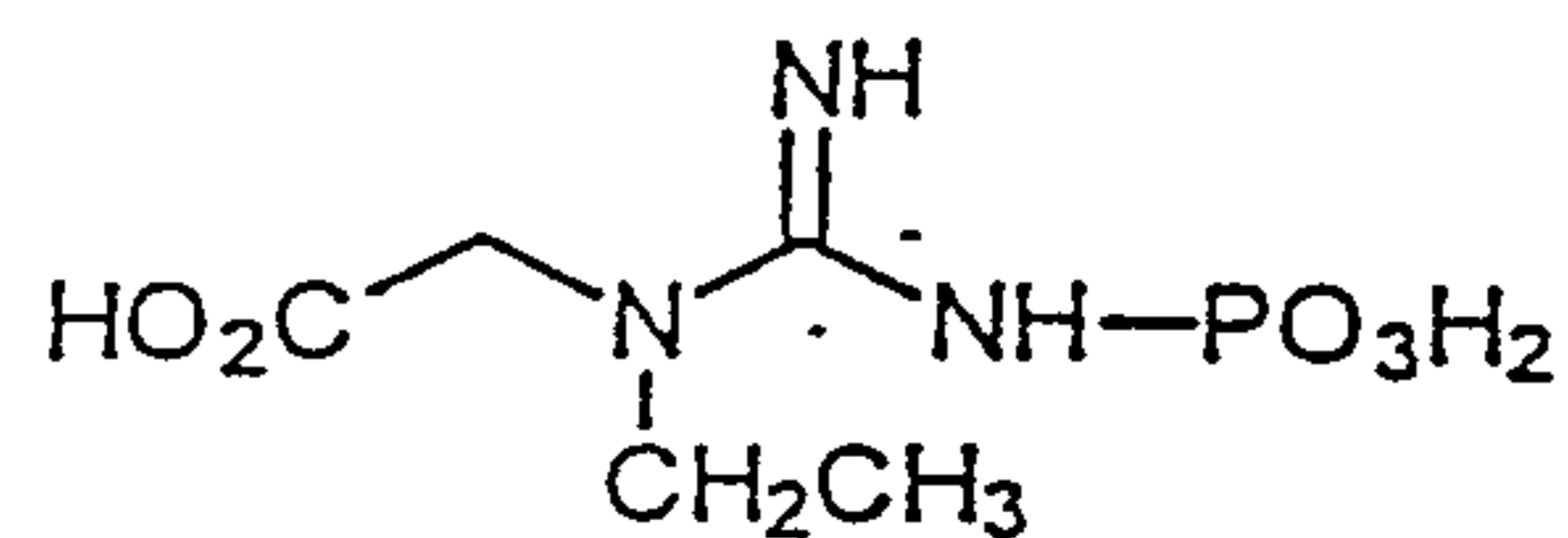
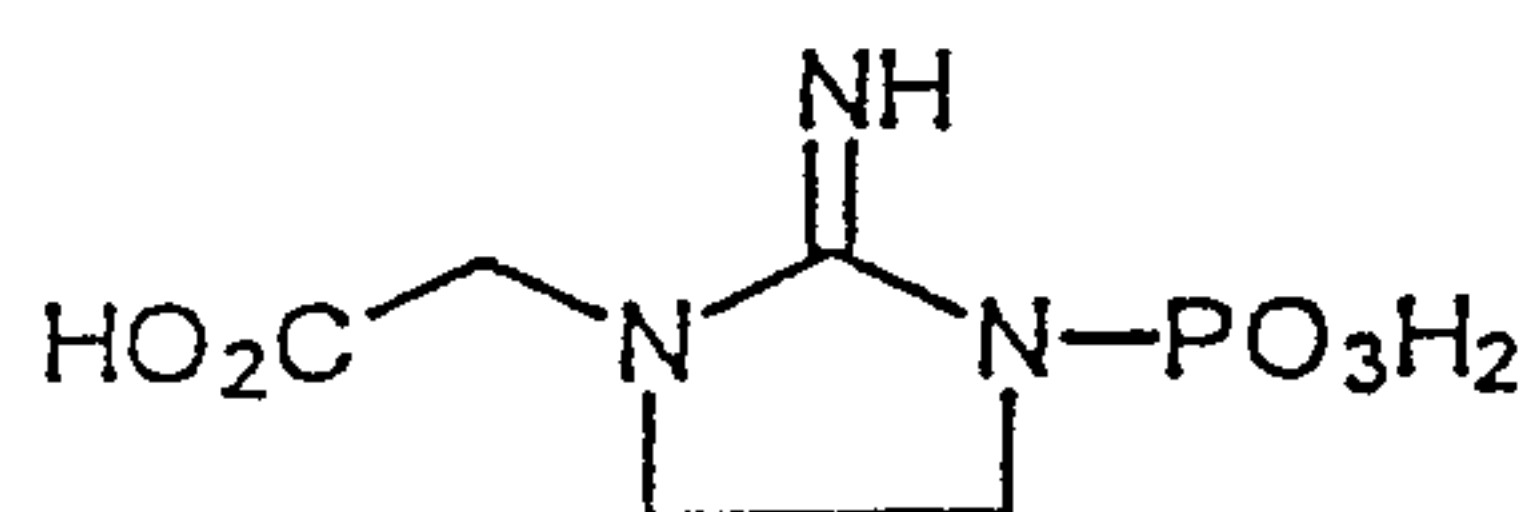
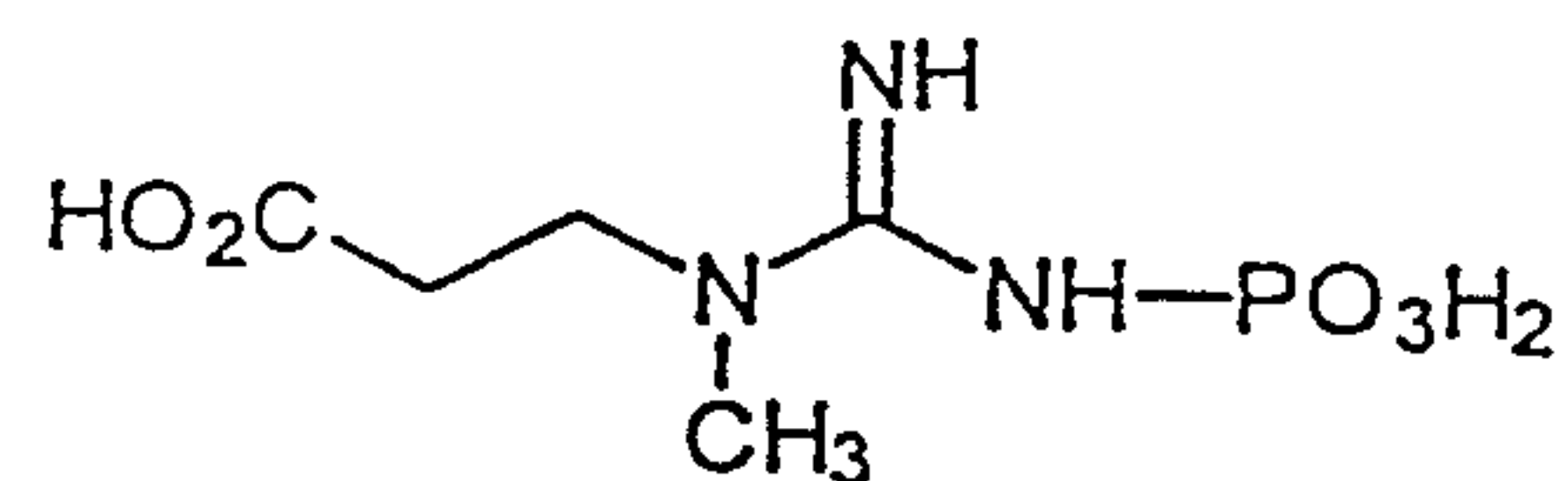
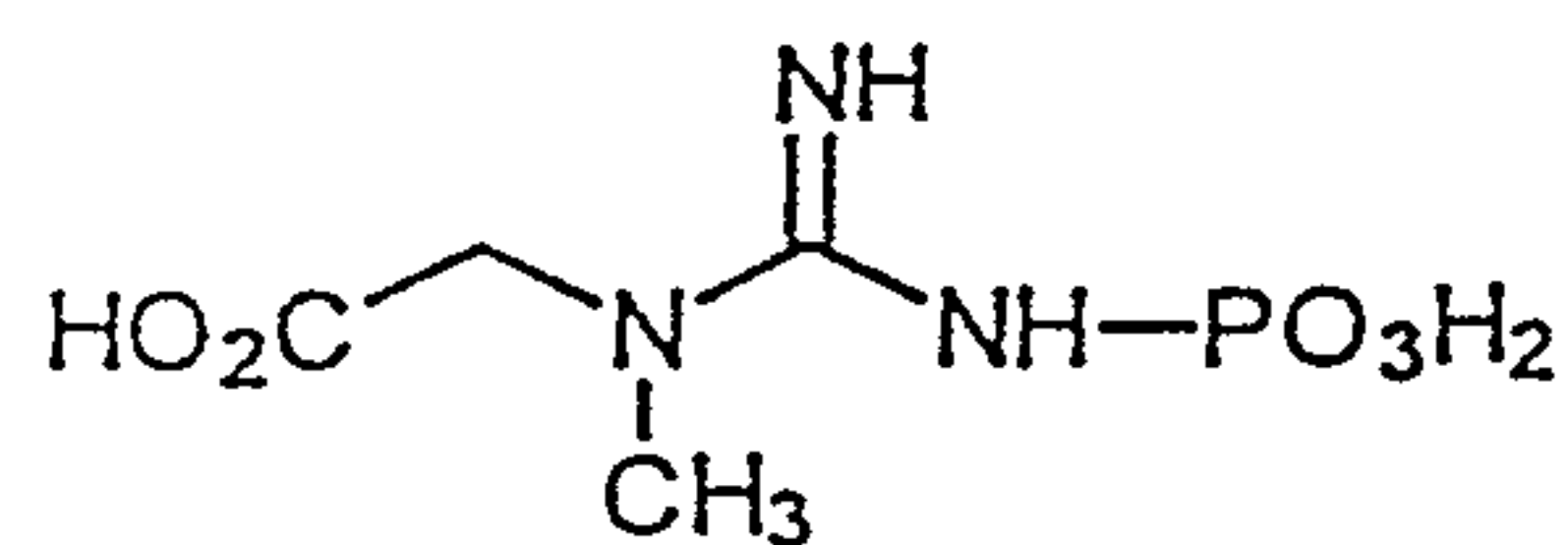
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TABLE 1
CREATINE ANALOGS



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TABLE 2
CREATINE PHOSPHATE ANALOGS



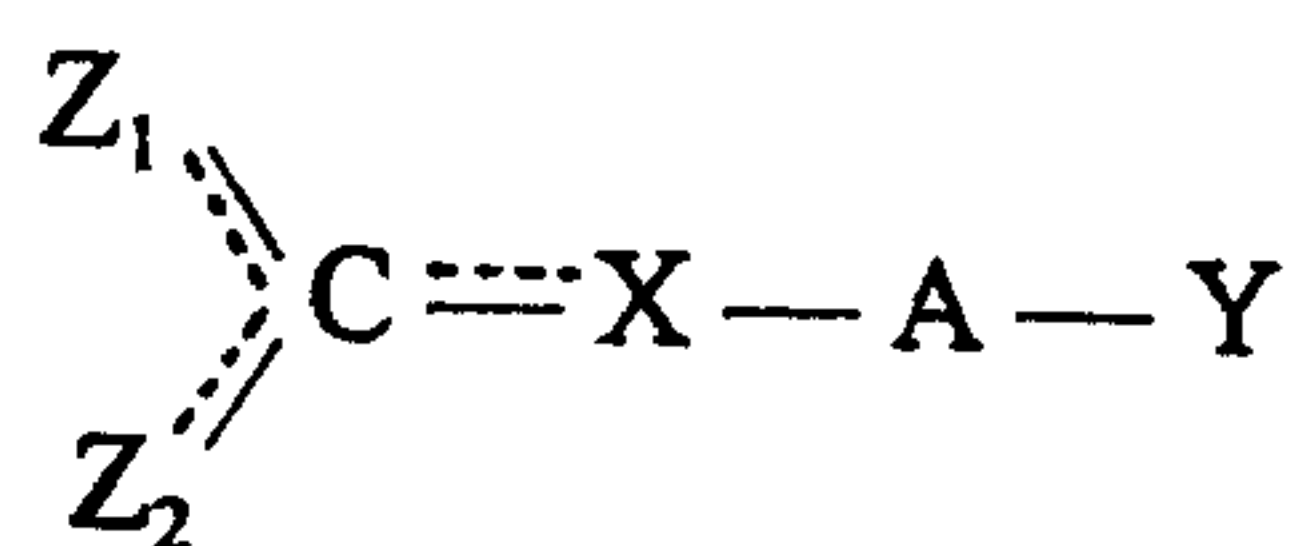
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Most of these compounds have been previously synthesized for other purposes. Rowley et. al., *J. Am. Chem. Soc.*, 93:5542-5551 (1971); Mclaughlin et. al., *J. Biol. Chem.*, 247:4382-4388 (1972); Nguyen, A. C. K., "Synthesis and enzyme studies using creatine analogues", Thesis, Dept of Pharmaceutical Chemistry, Univ. Calif., San Francisco, (1983); Lowe et al., *J. Biol. Chem.*, 225:3944-3951 (1980); Roberts et. al., *J. Biol. Chem.*, 260:13502-13508 (1995); Roberts et. al., *Arch. Biochem. Biophys.*, 220:563-571 (1983), and Griffiths et. al., *J. Biol. Chem.*, 251:2049-2054 (1976). Further to the forementioned references, Kaddurah-Daouk et. al., (WO 92/08456; WO 90/09192; U.S. 5,324,731; U.S. 5,321,030) also provide citations for the synthesis of a plurality of creatine analogues.

It is possible to modify the substances described below to produce analogues which have enhanced characteristics, such as greater specificity for the enzyme, enhanced solubility or stability, enhanced cellular uptake, or better binding activity. Salts of products may be exchanged to other salts using standard protocols.

Bisubstrate analogues of creatine kinase and non hydrolyzable substrate analogues of creatine phosphate (non transferable moieties which mimic the N phosphoryl group of creatine phosphate) can be designed readily and would be examples of creatine kinase modulators. Creatine phosphate compounds can be synthesized chemically or enzymatically. The chemical synthesis is well known. Annesley, T. M., Walker, J. B., *Biochem. Biophys. Res. Commun.*, 74:185-190 (1977); Cramer, F., Scheiffele, E., Vollmar, A., *Chem. Ber.*, 95:1670-1682 (1962).

Creatine compounds which are particularly useful in this invention include those encompassed by the following general formula:



and pharmaceutically acceptable salts thereof, wherein:

- a) Y is selected from the group consisting of: $-\text{CO}_2\text{H}-\text{NHOH}$, $-\text{NO}_2$, $-\text{SO}_3\text{H}$, $-\text{C}(=\text{O})\text{NH}\text{SO}_2\text{J}$ and $-\text{P}(=\text{O})(\text{OH})(\text{OJ})$, wherein J is selected from the group consisting of: hydrogen, C_1 - C_6 straight chain alkyl, C_3 - C_6 branched alkyl, C_2 - C_6 alkenyl, C_3 - C_6 branched alkenyl, and aryl;

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b) A is selected from the group consisting of: C, CH, C₁-C₅alkyl, C₂-C₅alkenyl, C₂-C₅alkynyl, and C₁-C₅alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:

1) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

2) an aryl group selected from the group consisting of: a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy; and

3) -NH-M, wherein M is selected from the group consisting of: hydrogen, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₁-C₄ alkoyl, C₃-C₄ branched alkyl, C₃-C₄ branched alkenyl, and C₄ branched alkoyl;

c) X is selected from the group consisting of NR₁, CHR₁, CR₁, O and S, wherein R₁ is selected from the group consisting of:

1) hydrogen;

2) K where K is selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) a C₅-C₉ α-amino-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon;

5) 2 C₅-C₉ α-amino-ω-aza-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon; and

6) a C₅-C₉ α-amino-ω-thia-ω-methyl-ω-adenosylcarboxylic acid attached via the ω-methyl carbon;

d) Z₁ and Z₂ are chosen independently from the group consisting of: =O, -NHR₂, -CH₂R₂, -NR₂OH; wherein Z₁ and Z₂ may not both be =O and wherein R₂ is selected from the group consisting of:

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1) hydrogen;

2) K, where K is selected from the group consisting of: C₁-C₆ straight alkyl; C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of: -CH₂L and -COCH₂L where L is independently selected from the group consisting of: bromo, chloro, epoxy and acetoxy;

4) 2 C₄-C₈ α-amino-carboxylic acid attached via the ω-carbon;

5) B, wherein B is selected from the group consisting of: -CO₂H-NHOH, -SO₃H, -NO₂, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of: hydrogen, C₁-C₆ straight alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of: C₁-C₂ alkyl, C₂ alkenyl, and C₁-C₂ alkoyl;

6) -D-E, wherein D is selected from the group consisting of: C₁-C₃ straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoyl, aryl and aroyl; and E is selected from the group consisting of: -(PO₃)_nNMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently selected from the group consisting of: C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl, wherein E may be attached to any point to D, and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) -E, wherein E is selected from the group consisting of -(PO₃)_nNMP, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base; -[P(=O)(OCH₃)(O)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; -[P(=O)(OH)(CH₂)]_m-Q, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base; and an aryl group containing 0-3 substituents chosen independently from the group consisting of: Cl, Br, epoxy, acetoxy, -OG, -C(=O)G, and -CO₂G, where G is independently selected from the group consisting of: C₁-C₆ straight

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alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, C₄-C₆ branched alkoyl; and if E is aryl, E may be connected by an amide linkage;

e) if R₁ and at least one R₂ group are present, R₁ may be connected by a single or double bond to an R₂ group to form a cycle of 5 to 7 members;

5 f) if two R₂ groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and

g) if R₁ is present and Z₁ or Z₂ is selected from the group consisting of -NHR₂, -CH₂R₂ and -NR₂OH, then R₁ may be connected by a single or double bond to the carbon or nitrogen of either Z₁ or Z₂ to form a cycle of 4 to 7 members.

10 Currently preferred compounds include cyclocreatine, creatine phosphate and those included in Tables 1 and 2 hereinabove.

The modes of administration for these compounds include, but are not limited to, oral, transdermal, or parenteral (e.g., subcutaneous, intramuscular, intravenous, bolus or continuous infusion). The actual amount of drug needed will depend on factors such as the size, age and
15 severity of disease in the afflicted individual. Creatine has been administered to athletes in the range of 2-8 gms /day to improve muscle function. Creatine phosphate was administered to patients with congestive heart failure also in the range of several gm/day, and was very well tolerated. In experimental animal models of cancer or viral infections, where creatine compounds have been shown to be active, amounts of 1gm/kg/day were administered intravenously or
20 intraperitoneally. For this invention the creatine compound will be administered at dosages and for periods of time effective to reduce, ameliorate or eliminate the symptoms of the disease. Dose regimens may be adjusted for purposes of improving the therapeutic or prophylactic response of the compound. For example, several divided doses may be administered daily, one dose, or cyclic administration of the compounds to achieve the desired therapeutic result.

25 The creatine compounds can be formulated with one or more adjuvants and/or pharmaceutically acceptable carriers according to the selected route of administration. The addition of gelatin, flavoring agents, or coating material can be used for oral applications. For solutions or emulsions in general, carriers may include aqueous or alcoholic/ aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include
30 sodium chloride, potassium chloride among others. In addition, intravenous vehicles can include fluid and nutrient replenishers, electrolyte replenishers among others.

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Preservatives and other additives can also be present. For example, antimicrobial, antioxidant, chelating agents, and inert gases can be added (*see, generally, Remington's Pharmaceutical Sciences, 16th Edition, Mack, (1980)*).

The present invention is demonstrated more fully by the following examples, which are not intended to be limiting in any way:

Example 1

Two creatine compounds, creatine phosphate and cyclocreatine, were injected intravenously into tumor bearing rats, and the level of glucose in the rats was monitored. Beta guanidino propionic acid, also was administered. This compound was previously shown to have no effect on glucose levels in normal animals but was shown to modify glucose levels in NIDDM models. There was no specific reason for using tumor bearing rats, except convenience because the antitumor activity of these compounds also was being studied. The presence of the tumors should not have any effect on the ability of these compounds to regulate glucose levels.

The rats carrying the tumors were described by us previously (*see, Teisher et al., Cancer Chemother. Pharmacol, 35:411-416, 1995*). The schedule and dose selected in these experiments was based on prior experience working with this class of compounds as anticancer or antiviral chemotherapeutic agents. The rat mammary adenocarcinoma 13762 was implanted in the female Fisher 344 rats on day zero. The creatine compounds were administered intravenously on days 4-8 and days 14-18. The amounts used were 1 gm/kg of cyclocreatine, 0.93 gm/kg for beta guanidino propionic acid, and 2.32 gm/kg for creatine phosphate. We were targeting a 1 gm/kg molar equivalent of creatine to achieve mM levels known typically to be needed with creatine analogues to modulate the creatine kinase system intracellularly. Plasma glucose levels were measured at around 11 a.m., by taking a drop of blood from the animals and testing glucose levels using a commercial kit (CHEMSTRIP* bG, Boehringer Mannheim). For animals that were treated with drugs, the treatment was around 9 a.m., and bleeding was also at around 11 a.m.

Figure 1 shows the results of our first experiment graphically. Panel (A): glucose levels in control (unmanipulated animals); Panel (B): glucose levels in cyclocreatine treated animals; Panel (C): glucose levels in beta-guanidino propionic acid treated animals; and Panel (D): glucose levels in creatine phosphate treated animals. The controls showed an average glucose level in rats of 62 mg/dl. The treatment with cyclocreatine showed two drops in glucose levels at the time of drug administration, i.e., between days 4-8 and days 14-18. The drop in glucose level at the second

*Trade-mark

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cycle of drug administration was more dramatic than the first cycle, consistent with what is known about the continuous build up of these compounds in organs high in creatine kinase activity. Minimal changes in glucose levels were seen with beta guanidino propionic acid treatment consistent with previous published data. The compound creatine phosphate induced similar pattern of drops in glucose levels as that seen with cyclocreatine, although cyclocreatine seemed to be more potent.

Example Two

The same above described experiment was repeated. Figure 2 shows the effect of the selected compounds on glucose levels. Panel (A): control (unmanipulated animals); Panel (B): cyclocreatine treated; Panel (C): beta-guanidino propionic acid treated; Panel (D): creatine phosphate treated animals. The same pattern seen in example one is also seen here. Cyclocreatine induced a drop in the level of glucose after each administration. The drop in the second cycle was more dramatic than the first. Beta- guanidino propionic acid had minimal effect, and creatine phosphate seemed to mirror the effect of cyclocreatine.

Example Three

To examine more closely what occurred in the above two experiments, the average readings of glucose levels from experiments one and two were taken in the following time intervals post drug treatment: Days 1-2, Days 4-8, Days 8-12, Days 14-18, Day 15 and Days 19-22. Day 15 demonstrates the largest effect on glucose levels by this class of compounds. Figure 3 outlines these results. Cyclocreatine, Panel (C), shows a drop in glucose level that could be as high as 50% on day 15. Beta-guanidino propionic acid shows minimal effects <15%, and creatine phosphate seems to drop glucose levels by 35% on day 15.

The experiments described above demonstrate that creatine analogues which modulate the creatine kinase system, and that are represented by cyclocreatine and creatine phosphate, can regulate glucose levels. The enzyme system creatine kinase emerges as a novel target for drug design for diseases related to the control of glucose levels.

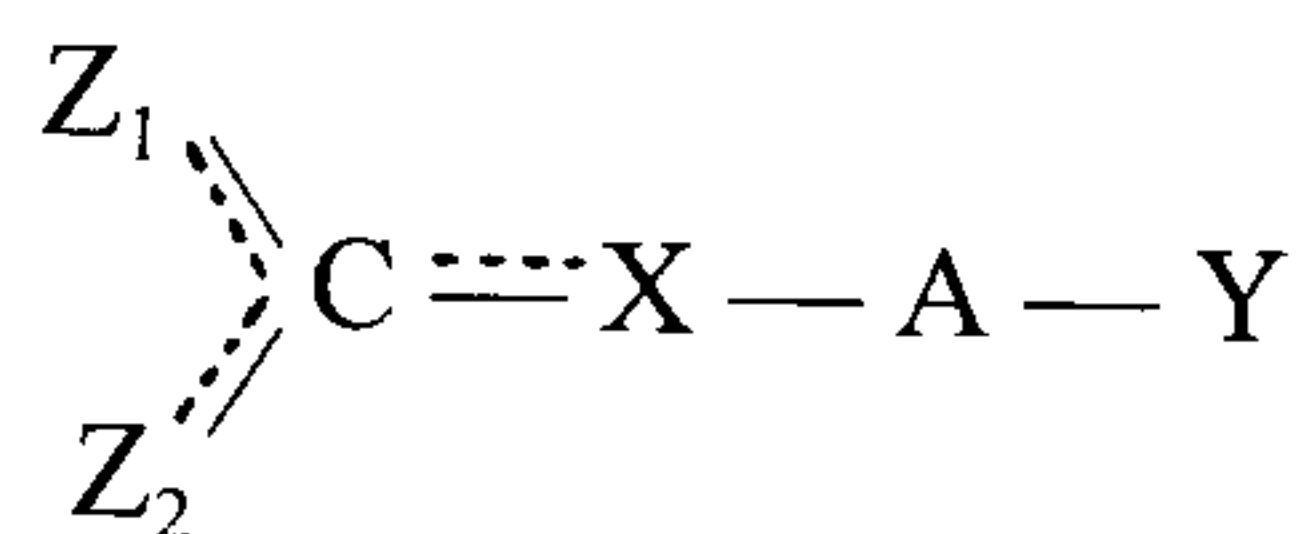
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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. Use of an amount of a creatine compound for the treatment or prevention of a glucose metabolic disorder in a subject afflicted with said disorder or a pharmaceutically acceptable salt of said compound effective to treat, reduce, or prevent said disorder.
2. The use of claim 1 wherein said disorder is hyperglycemia.
3. The use of claim 1 wherein said disorder is insulin dependent diabetes mellitus.
4. The use of claim 1 wherein said disorder is impaired glucose tolerance.
5. The use of claim 1 wherein said disorder is hyperinulinemia.
6. The use of claim 1 wherein said disorder is insulin insensitivity.
7. The use of claim 1 wherein said disorder is diabetes related diseases.
8. The use of claim 1 wherein the subject is a mammal.
9. The use of claim 8 wherein the subject is a human.
10. Use of a therapeutic amount of a creatine analogue for the treatment of a metabolic disorder selected from the group consisting of hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinulinemia, insulin insensitivity, diabetes related diseases, in a subject afflicted with said disorder, the creatine analogue having the general formula



and pharmaceutically acceptable salts thereof, wherein

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- a) Y is selected from the group consisting of $-\text{CO}_2\text{H}-\text{NHOH}$, $-\text{NO}_2$, $-\text{SO}_3\text{H}$, $-\text{C}(=\text{O})\text{NHSO}_2\text{J}$ and $-\text{P}(=\text{O})(\text{OH})(\text{OJ})$, wherein J is selected from the group consisting of hydrogen, C_1 - C_6 straight chain alkyl, C_3 - C_6 branched alkyl, C_2 - C_6 alkenyl, C_3 - C_6 branched alkenyl, and aryl;
- b) A is selected from the group consisting of C, CH, C_1 - C_5 alkyl, C_2 - C_5 alkenyl, C_2 - C_5 alkynyl, and C_1 - C_5 alkoyl chain, each having 0-2 substituents which are selected independently from the group consisting of:
- 1) K, where K is selected from the group consisting of C_1 - C_6 straight alkyl, C_2 - C_6 straight alkenyl, C_1 - C_6 straight alkoyl, C_3 - C_6 branched alkyl, C_3 - C_6 branched alkenyl, and C_4 - C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of bromo, chloro, epoxy and acetoxy;
 - 2) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of $-\text{CH}_2\text{L}$ and $-\text{COCH}_2\text{L}$ where L is independently selected from the group consisting of bromo, chloro, epoxy and acetoxy; and
 - 3) $-\text{NH}-\text{M}$, wherein M is selected from the group consisting of hydrogen, C_1 - C_4 alkyl, C_2 - C_4 alkenyl, C_1 - C_4 alkoyl, C_3 - C_4 branched alkyl, C_3 - C_4 branched alkenyl, and C_4 branched alkoyl;
- c) X is selected from the group consisting of NR_1 , CHR_1 , CR_1 , O and S, wherein R_1 is selected from the group consisting of:
- 1) hydrogen;
 - 2) K, where K is selected from the group consisting of C_1 - C_6 straight alkyl, C_2 - C_6 straight alkenyl, C_1 - C_6 straight alkoyl, C_3 - C_6 branched alkyl, C_3 - C_6 branched alkenyl, and C_4 - C_6 branched alkoyl, K having 0-2 substituents independently selected from the group consisting of bromo, chloro, epoxy and acetoxy;
 - 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of $-\text{CH}_2\text{L}$ and $-\text{COCH}_2\text{L}$ where L is independently selected from the group consisting of bromo, chloro, epoxy and acetoxy;

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- 4) a C₅-C₉ a-amino-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;
 - 5) 2 C₅-C₉ a-amino-w-aza-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon; and
 - 6) A C₅-C₉ a-amino-w-thia-w-methyl-w-adenosylcarboxylic acid attached via the w-methyl carbon;
- d) Z₁ and Z₂ are chosen independently from the group consisting of =O, -NHR₂, -CH₂R₂, -NR₂OH, wherein Z₁ and Z₂ may not both be =O and wherein R₂ is selected from the group consisting of:
- 1) hydrogen;
 - 2) K, where K is selected from the group consisting of C₁-C₆ straight alkyl, C₂-C₆ straight alkenyl, C₁-C₆ straight alkoyl, C₃-C₆ branched alkyl, C₃-C₆ branched alkenyl, and C₄-C₆ branched alkoyl, K having 0-2 substituents independently selected from the group consisting of bromo, chloro, epoxy and acetoxy;
 - 3) an aryl group selected from the group consisting of a 1-2 ring carbocycle and a 1-2 ring heterocycle, wherein the aryl group contains 0-2 substituents independently selected from the group consisting of -CH₂L and -COCH₂L where L is independently selected from the group consisting of bromo, chloro, epoxy and acetoxy;
 - 4) 2 C₄-C₈ a-amino-carboxylic acid attached via the w-carbon;
 - 5) B, wherein B is selected from the group consisting of -CO₂H-NHOH, -SO₃H, -NO₂, OP(=O)(OH)(OJ) and -P(=O)(OH)(OJ), wherein J is selected from the group consisting of hydrogen, C₁-C₆ straight alkyl, C₃-C₆ branched alkyl, C₂-C₆ alkenyl, C₃-C₆ branched alkenyl, and aryl, wherein B is optionally connected to the nitrogen via a linker selected from the group consisting of C₁-C₂ alkyl, C₂ alkenyl, and C₁-C₂ alkoyl;
 - 6) -D-E, wherein D is selected from the group consisting of C₁-C₃ straight alkyl, C₃ branched alkyl, C₂-C₃ straight alkenyl, C₃ branched alkenyl, C₁-C₃ straight alkoyl, aryl and aroyl, and E is selected from the group consisting of - (PO₃)_nNMP, where n is 0-2 and NMP is ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base, -

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$[P(=O)(OCH_3)(O)]_m-Q$, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base, $-[P(=O)(OH)(CH_2)]_m-Q$, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base, and an aryl group containing 0-3 substituents chosen independently from the group consisting of Cl, Br, epoxy, acetoxy, $-OG$, $-C(=O)G$, and $-CO_2G$, where G is independently selected from the group consisting of C_1 - C_6 straight alkyl, C_2 - C_6 straight alkenyl, C_1 - C_6 straight alkoyl, C_3 - C_6 branched alkyl, C_3 - C_6 branched alkenyl, C_4 - C_6 branched alkoyl, wherein E may be attached to any point to D , and if D is alkyl or alkenyl, D may be connected at either or both ends by an amide linkage; and

7) $-E$, wherein E is selected from the group consisting of $-(PO_3)_nNMP$, where n is 0-2 and NMP is a ribonucleotide monophosphate connected via the 5'-phosphate, 3'-phosphate or the aromatic ring of the base, $-[P(=O)(OCH_3)(O)]_m-Q$, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base, $-[P(=O)(OH)(CH_2)]_m-Q$, where m is 0-3 and Q is a ribonucleoside connected via the ribose or the aromatic ring of the base, and an aryl group containing 0-3 substituents chosen independently from the group consisting of Cl, Br, epoxy, acetoxy, $-OG$, $-C(=O)G$, and $=CO_2G$, where G is independently selected from the group consisting of C_1 - C_6 straight alkyl, C_2 - C_6 straight alkenyl, C_1 - C_6 straight alkoyl, C_3 - C_6 branched alkyl, C_3 - C_6 branched alkenyl, C_4 - C_6 branched alkoyl, and if E is aryl, E may be connected by an amide linkage;

- e) if R_1 and at least one R_2 group are present, R_1 may be connected by a single or double bond to an R_2 group to form a cycle of 5 to 7 members;
- f) if two R_2 groups are present, they may be connected by a single or a double bond to form a cycle of 4 to 7 members; and
- g) if R_1 is present and Z_1 and Z_2 is selected from the group consisting of $-NHR_2$, $-CH_2R_2$ and $-NR_2OH$, then R_1 may be connected by a single or double bond to the carbon or nitrogen of either Z_1 or Z_2 to form a cycle of 4 to 7 members.

11. The use of claim 10 wherein the creatine compound is used in combination with insulin

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or a sulphonylurea compound.

12. The use of claim 10 wherein the creatine compound is cyclocreatine.
13. The use of claim 10 wherein the creatine compound is creatine phosphate.
14. Use of an effective therapeutic amount of a substrate of creatine kinase in the treatment of a glucose metabolic disorder selected from the group consisting of hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinulinemia, insulin insensitivity, diabetes related diseases in a subject afflicted with said disorder.
15. Use of an effective therapeutic amount of a creatine phosphate analogue in the treatment of a glucose metabolic disorder selected from the group consisting of hyperglycemia, insulin dependent diabetes mellitus, impaired glucose tolerance, hyperinulinemia, insulin insensitivity, diabetes related diseases in a subject afflicted with said disorder.

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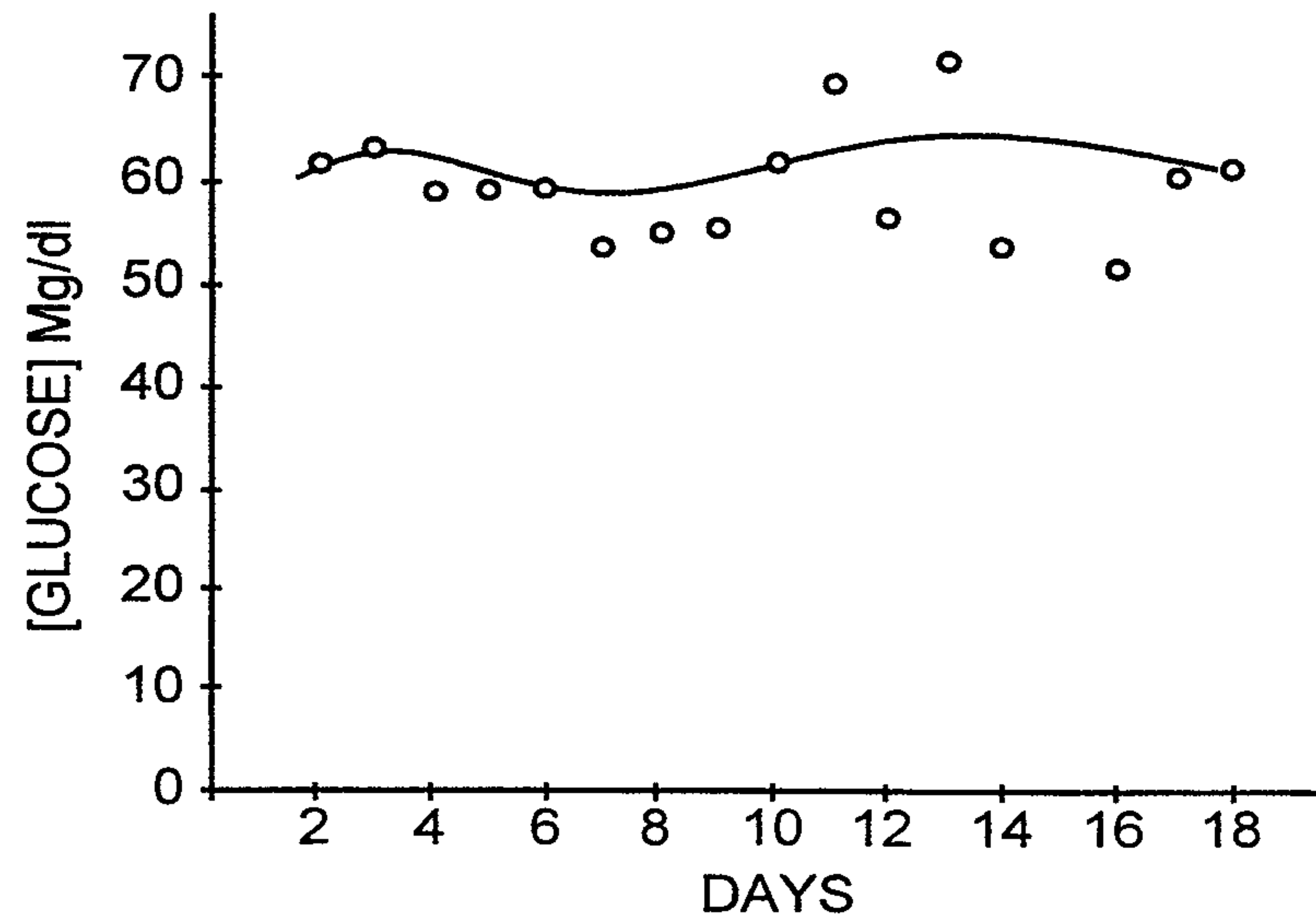


FIG. 1A

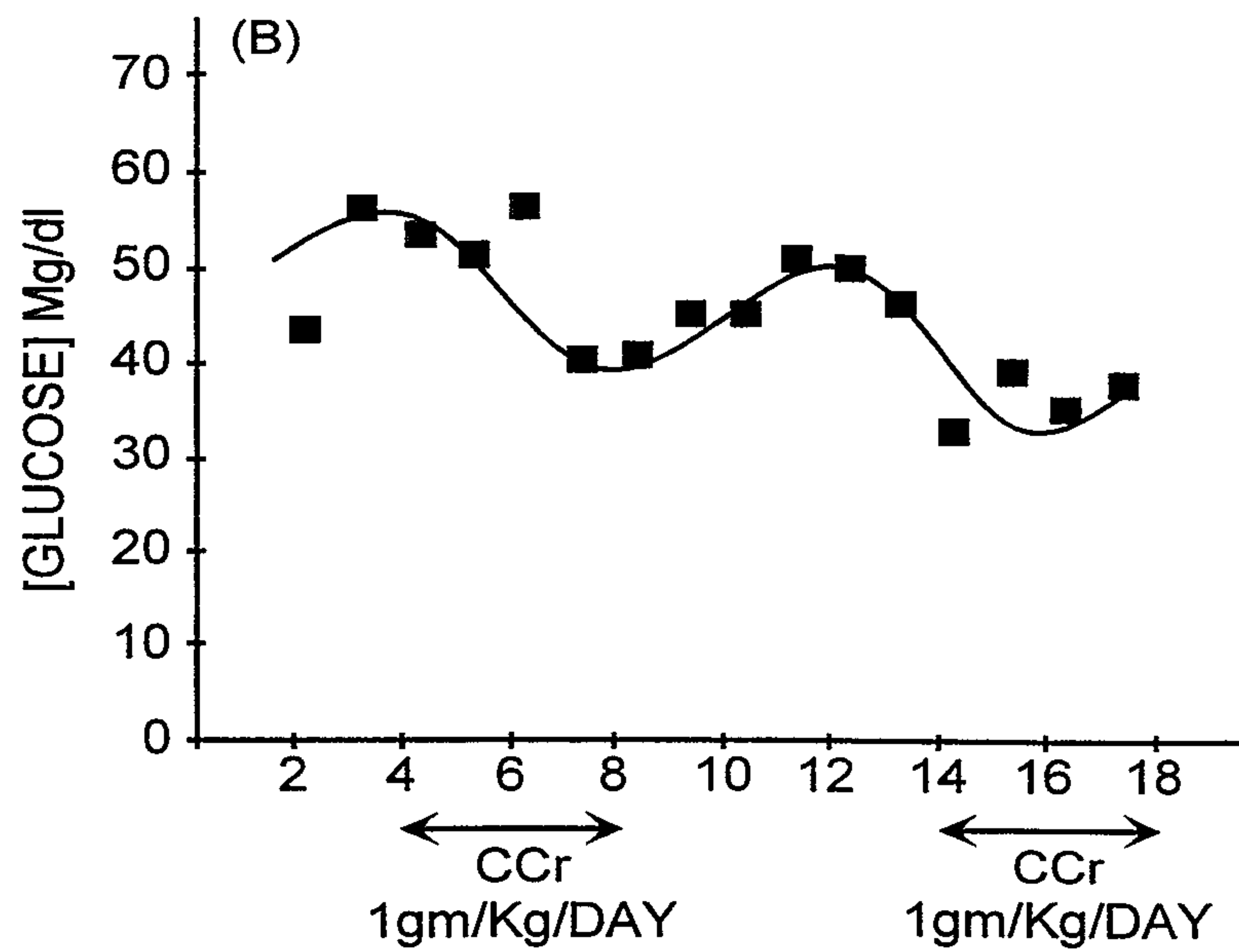


FIG. 1B

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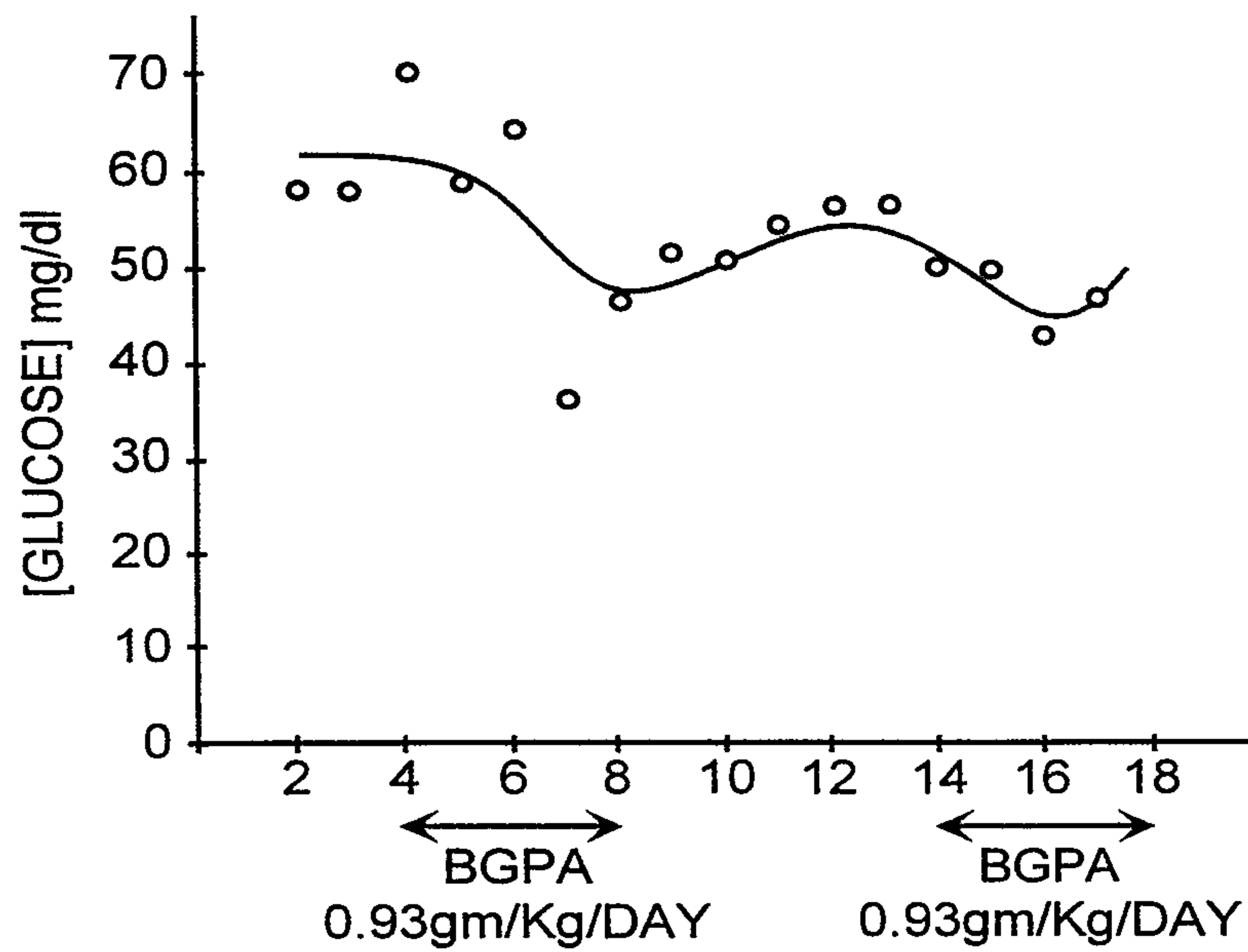


FIG. 1C

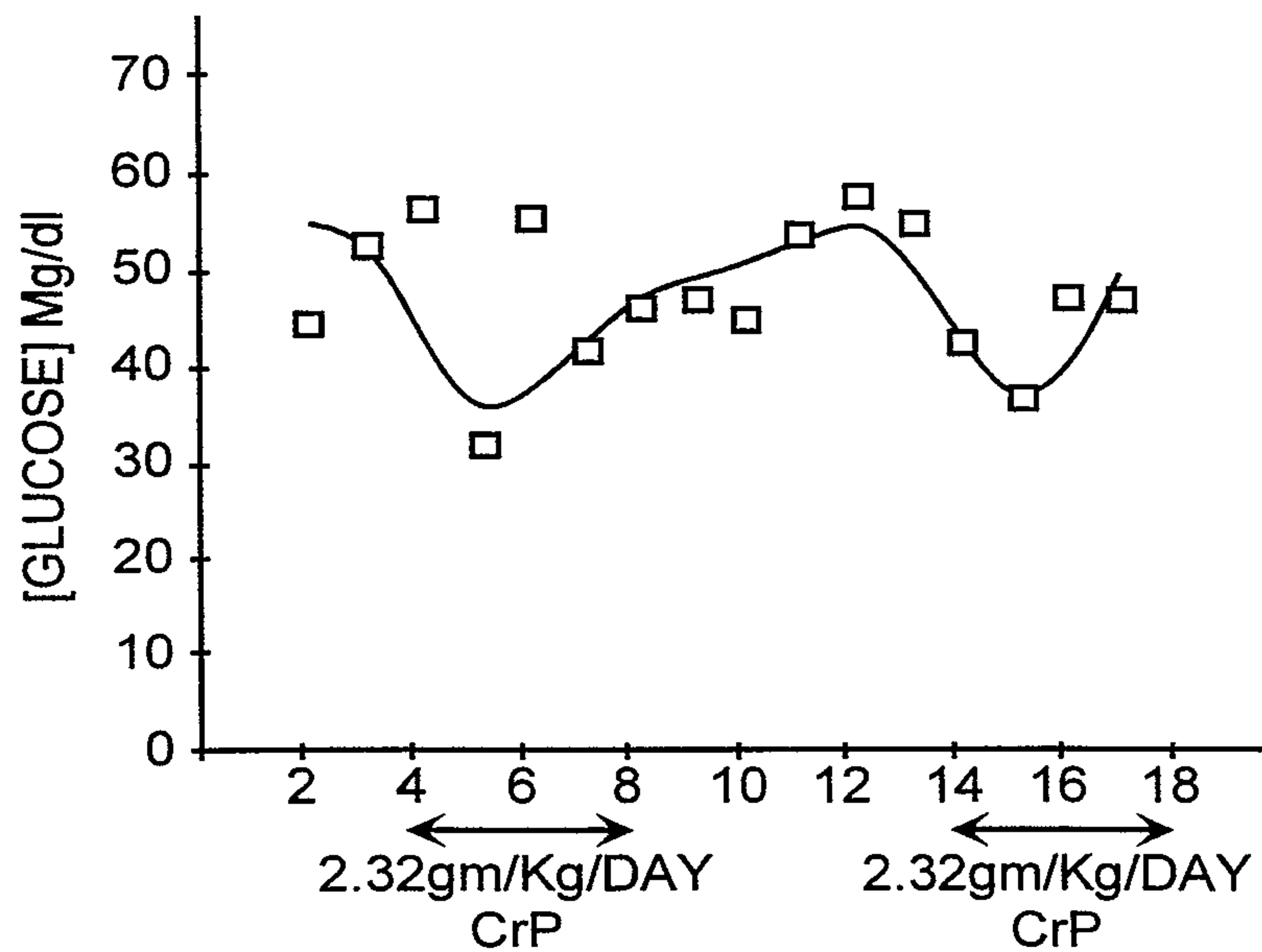


FIG. 1D

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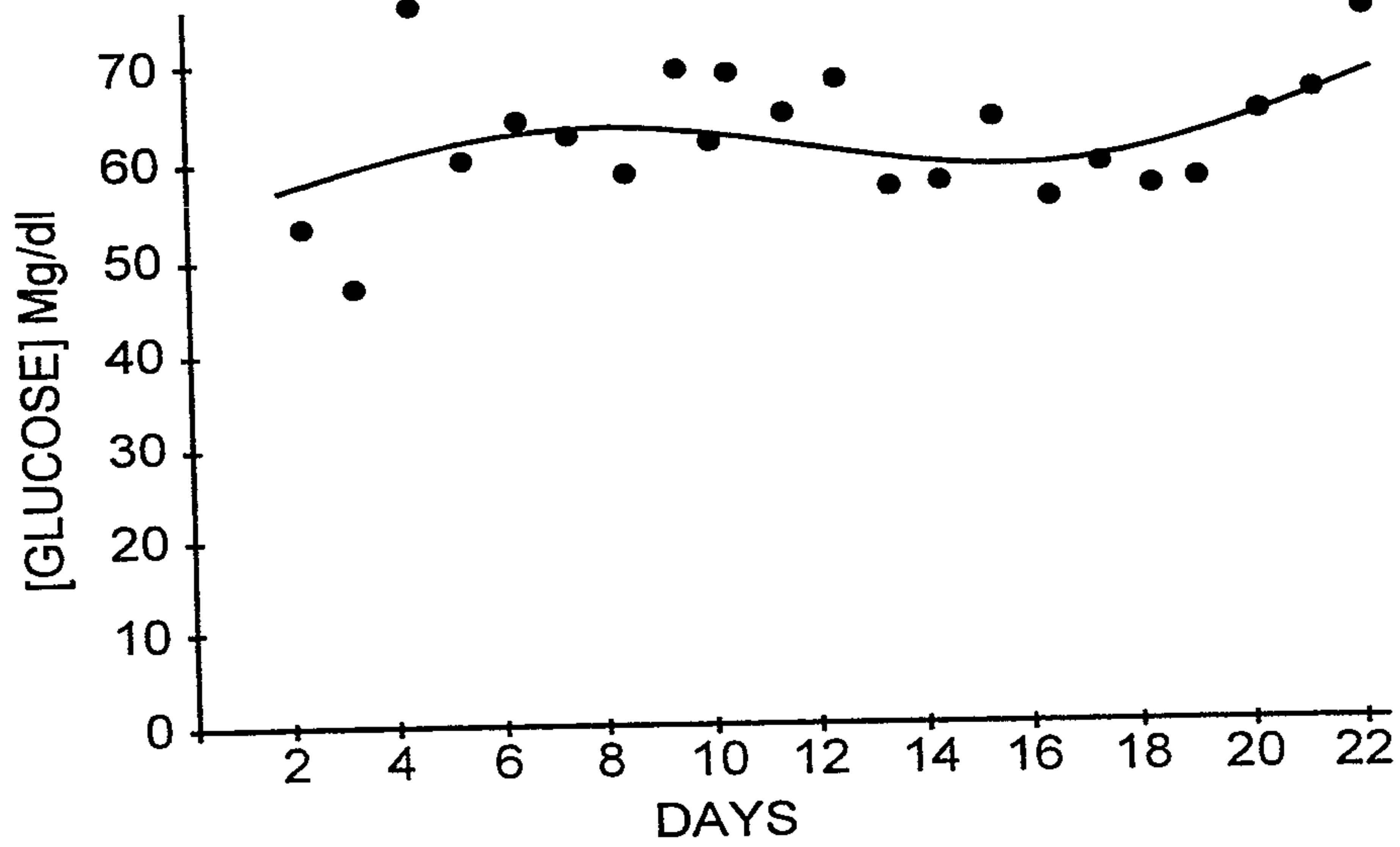


FIG. 2A

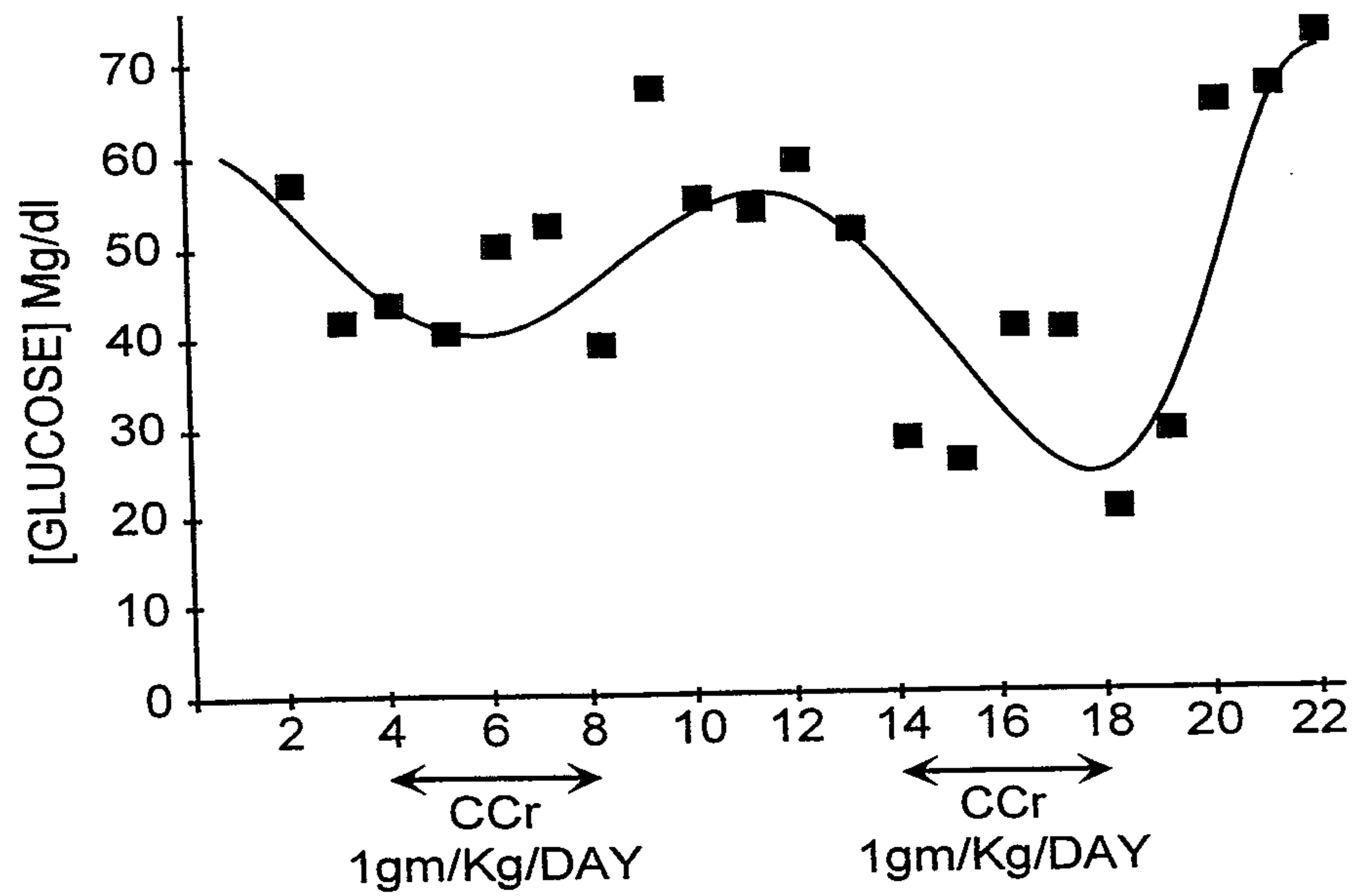


FIG. 2B

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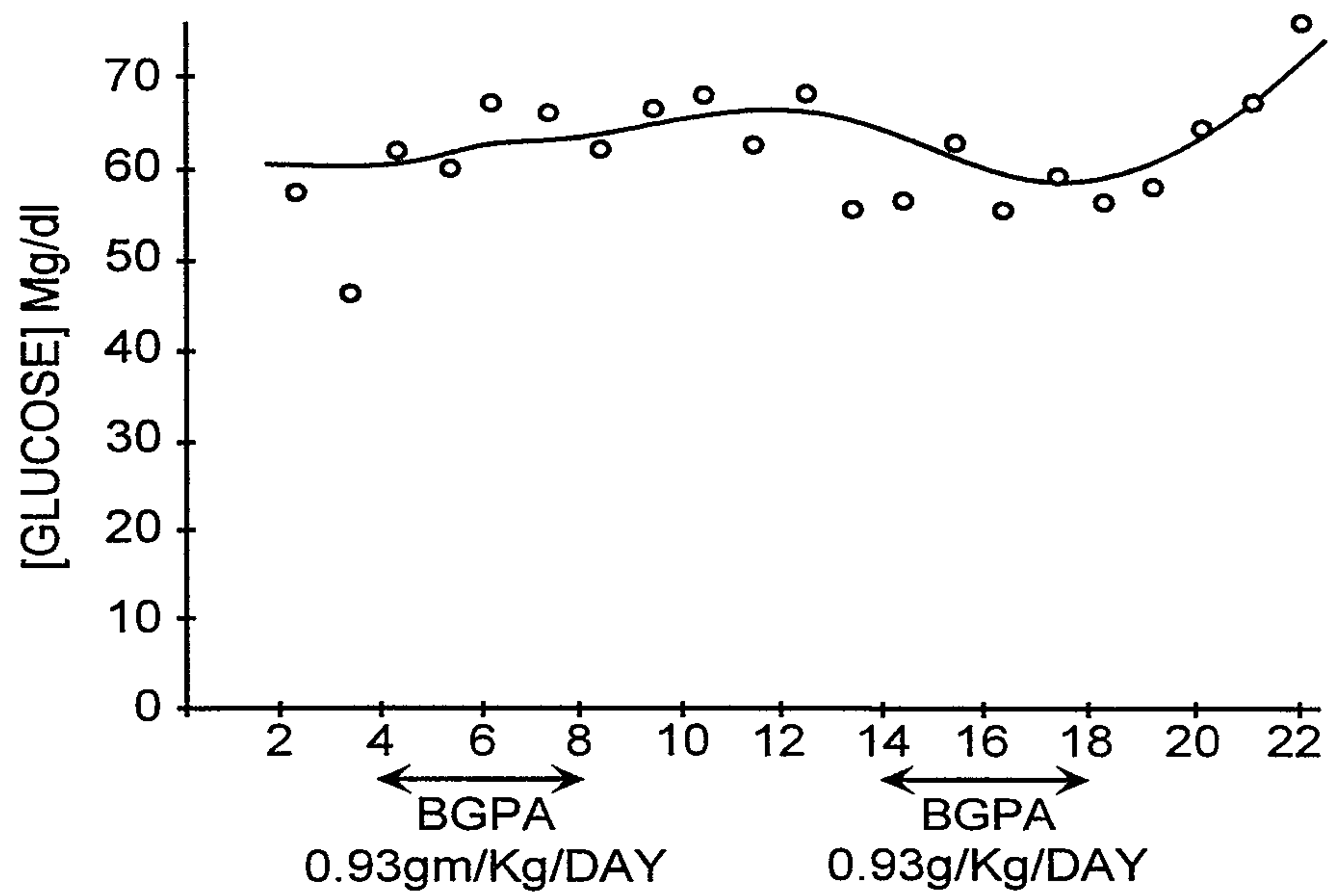


FIG. 2C

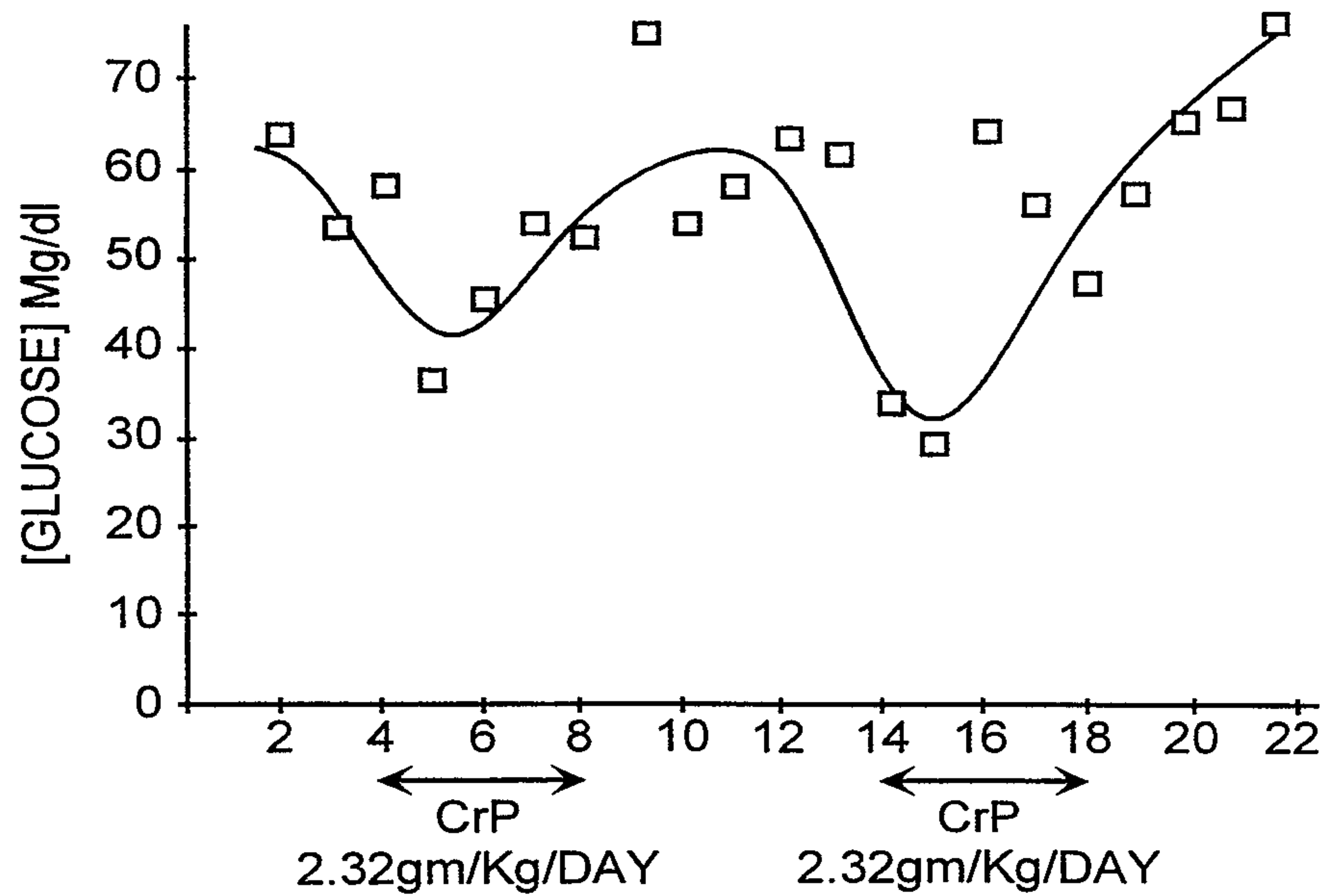


FIG. 2D

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FIG. 3A

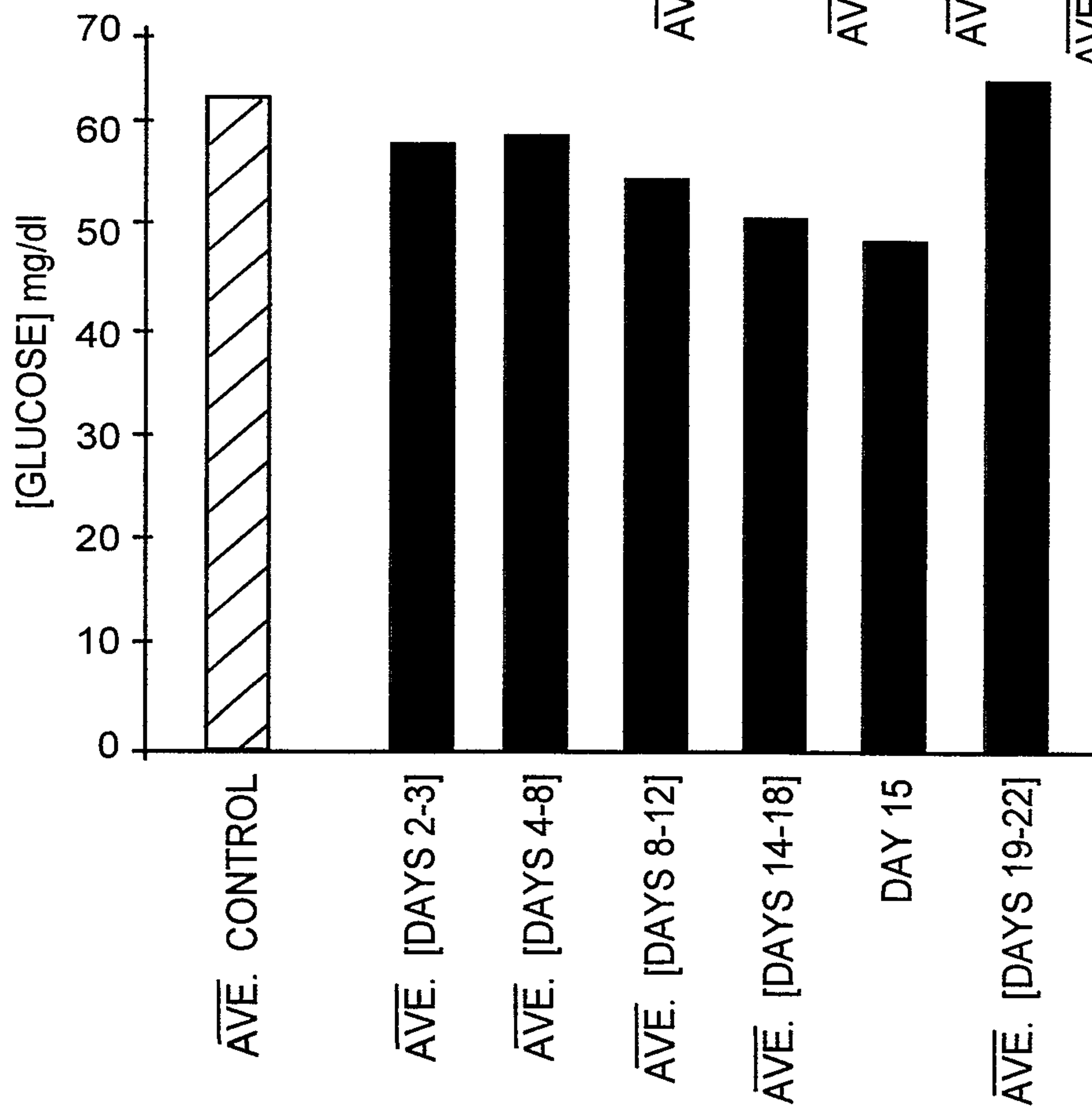
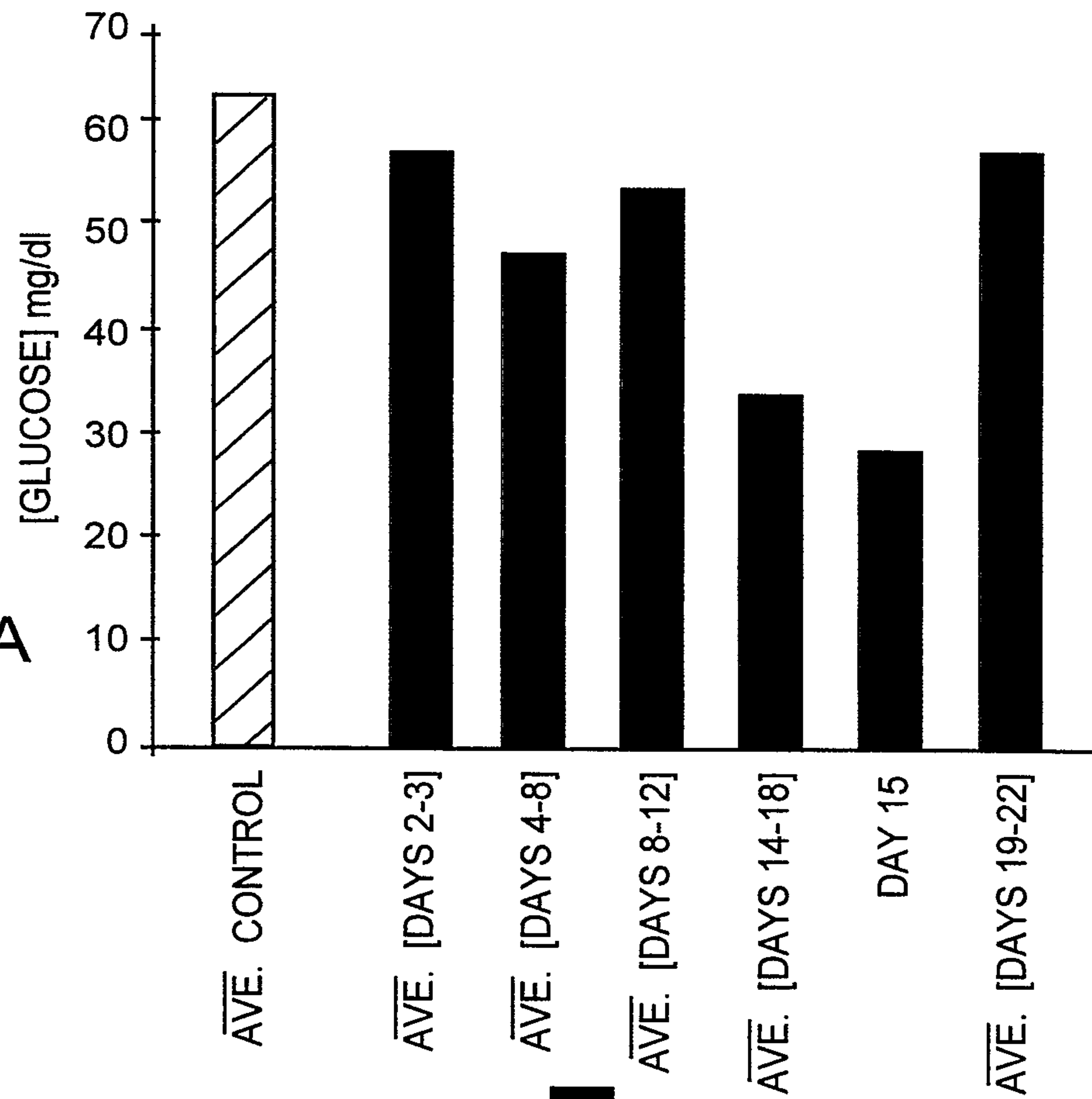


FIG. 3B

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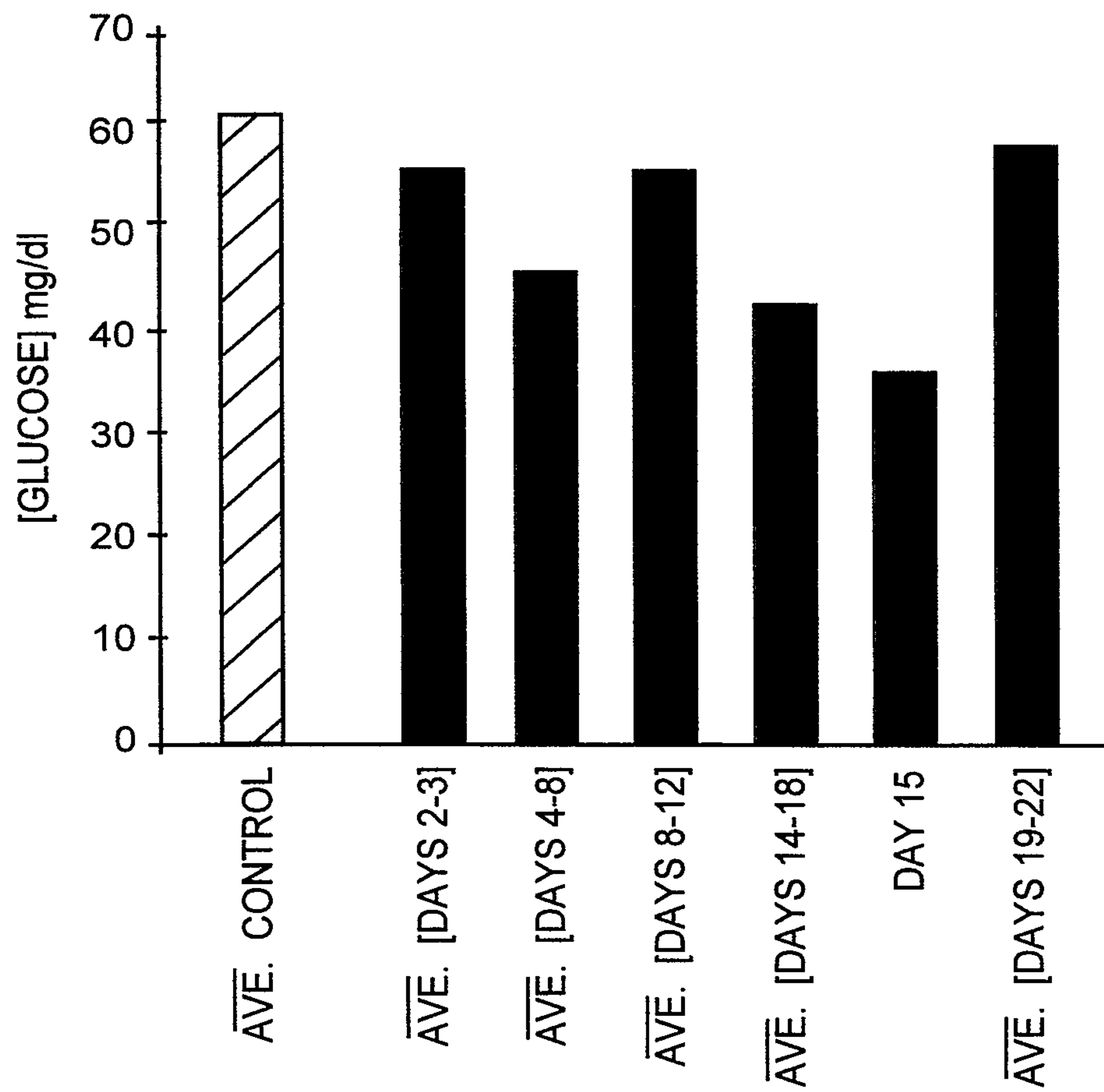


FIG. 3C