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**PHARMACEUTICAL AND/OR DIETETIC COMPOSITIONS WITH ANTIOXIDANT ACTIVITY
CONTAINING CARNOSINE OR DERIVATIVES AND BRANCHED AMINO ACIDS**

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(57) Claim

**1. Pharmaceutical and/or dietetic compositions
containing:**

**a) carnosine and/or derivatives thereof, such as
homocarnosine, anserine, ofidine and/or the
pharmacologically compatible inorganic and organic
salts and/or the acyl derivatives thereof with
pharmacologically compatible organic acids, and
the inorganic salts thereof;**

**b) a branched amino acid selected from leucine,
isoleucine and valine, or salts, and/or acyl
derivatives or esters thereof.**



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(21) International Application Number: PCT/EP96/02079 (22) International Filing Date: 15 May 1996 (15.05.96) (30) Priority Data: MI95A001021 19 May 1995 (19.05.95) IT (71) Applicant (for all designated States except US): FARMILA-FARMACEUTICI MILANO S.R.L. [IT/IT]; Via E. Fermi, 50, I-20019 Settimo Milanese (IT). (72) Inventor; and (75) Inventor/Applicant (for US only): BIANCHI, Roberto [IT/IT]; Piazzale Aquileia, 6, I-20144 Milano (IT). (74) Agent: BIANCHETTI, Giuseppe; Studio Consulenza Brevetuale, Via Rossini, 8, I-20122 Milano (IT).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>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: PHARMACEUTICAL AND/OR DIETETIC COMPOSITIONS WITH ANTIOXIDANT ACTIVITY CONTAINING CARNOSINE OR DERIVATIVES AND BRANCHED AMINO ACIDS (57) Abstract: Pharmaceutical and/or dietetic compositions with antioxidant activity containing carnosine or derivatives thereof and branched amino acids in addition with possible other components selected from carnitine, vitamins, oligoelements, bioflavonoids.		

**PHARMACEUTICAL AND/OR DIETETIC COMPOSITIONS WITH ANTIOXIDANT ACTIVITY
CONTAINING CARNOSINE OR DERIVATIVES AND BRANCHED AMINO ACIDS**

The present invention relates to pharmaceutical and/or dietetic compositions with antioxidant activity, consisting of

- a) carnosine and derivatives thereof, as better
5 specified below, and of
- b) leucine and/or isoleucine and/or valine;
and optionally also of
- c) carnitine, derivatives thereof (as better specified
below) and creatine; and/or
- 10 d) oligoelements; and/or
- e) vitamins; and/or
- f) bioflavonoids.

Technical background

Free radicals and the peroxidative processes caused
15 by them have been known for a long time to be one of the
causes of the structural and functional degradations of
human tissues during aging and of a number of
pathologies related to them: tumours; diabetes;
hypertension; muscular excessive strain; radiation and
20 sunburn damages; intoxications; ischemia;
atherosclerosis; hyperthermia; cerebral traumas;
inflammations; cataract; multiple sclerosis; Down
syndrome, Parkinson's disease, Alzheimer's disease;
dermatitis; muscular dystrophy; obesity; hyperlipidemia;
25 hypercholesterolemia; tabagism; alcoholism, etc. (B.
Cestaro, (1994), in "Per una vita inossidabile",
ETASLIBRI - RCS Medicine, pp. 7-59; G.D. Bompiani, A.
Galluzzo, (1990), in "Radicali Liberi in Fisiologia and

Minerva Medica, pp. 3-280; Supplement to "The American Journal of Clinical Nutrition vol. 53 (n. 1), 1991. pp. 1895-391S; "Radicali Liberi in Medicina", Periodici UTET Scientifici n. 1. dicembre 1993. pp. 1-57; "Lipid Peroxidation: part II Pathological Implications", (1987), Chem and Physics of Lipids, vol. 45 (n. 2-4), pp. 103-353).

Therefore it is important, in order to prevent the aging processes and related pathologies, to keep the concentration of the natural antioxidant molecules, ("free radical scavengers") which are the physiological defences against free radicals, high both in the different tissues of the organism and in the bloodstream, with which a continuous functional interchange occurs; in other words, blood (and the erythrocytes contained therein) can act as a carrier: 1) to provide the necessary antioxydants (both exogenous and deriving from, for example, the oral administration, and taken from endogenous deposits) to a certain tissue (which is at that time subjected to a specific pathological stress condition); 2) to drain the products from the peroxidative processes accumulated in a specific district of the organism.

It is therefore evident that an appropriate chemical-clinical evaluation of the sensitivity of plasma and/or of the erythrocytes (i.e. of the different tissues of the organism) to peroxidation can be an effective prognostic means to evaluate the efficiency of human antioxidant defences and the capability of the body of defending against the damage induced by said dysmetabolic processes, typical of aging and of the

above cited pathologies.

Carnosine (β -alanyl-L-histidine) and some derivatives thereof (homocarnosine, acetylcarnosine, acetylhomocarnosine, etc.) have been known for some time to be among the most important natural antioxidant agents (Boldyrev A., Severin S., (1990), Adv. Enz. Reg., 30, 175-194; Kohen R. et al., (1988), Proc. Natl. Acad. Sci. USA, 85, 3175-79; Yoshikawa T. et al., (1991), Biochim. Biophys. Acta, 1115, 15-22) and the administration of these compounds would allow to cause an effective therapeutical activity in a number of the above mentioned pathologies (Davey C.L., (1960), Arch. Biochem. Biophys., 89, 303-308; Severin S., (1964); Proc. 6th Intern. Biochem. Congress, 45-61; Nagai K. and Suda T., (1988), Meth. Find. Exp. Clin. Pharmacol., 10, 497-507; Boldyrev A., (1990), Int. J. Biochem., 22, 129-132; Kurelle E. et al., (1991), Byul. Exp. Biol. Med., 112, 52-53; Boldyrev A. et al.; (1993), Int. J. Biochem., 25, 1101-1107; Boldyrev A. et al., (1993), Mol. Chem. Neuropathol., 19, 185-192), particularly where the peroxidative damage induced by free radicals is one of the main causes in inducing and/or worsening the pathology. The antiradicalic activity of exogenous carnosine (or of the homologues thereof) is however restricted by the instability shown by said peptide towards the enzyme carnosinase, which is capable of hydrolyzing it into aminoacids components. The carnosinase is present both in the bloodstream and in the various tissue districts.

It has now been surprisingly found that the combination of carnosine with the branched amino acids

leucine, isoleucine and valine induces an effect synergistic with the carnosine antioxidant activity, prolonging it in time, at equal doses. Since a direct antiradicalic effect of said amino acids (even if they are known to promote a therapeutical activity in different tissues, particularly in the skeletal muscle, thanks to their capability of increasing the cell energetic metabolism (R. Bernardi, in "Aminoacidi ed Esercizio", (1992), EDI-ERMES editore, Milan, Italy)), it is believed (without however limiting the scope of the invention) that such a synergistic effect can be ascribed to an inhibitory action on carnosinase, which prolongs the half-life of the circulating dipeptide and increases the bioavailability due to both the reaching of the specific tissue targets and the antioxidant action.

As an alternative, non-limiting interpretation, the increased antiradicalic effectiveness could be related to: 1) the cell energetic metabolic increase by the branched amino acids, which would lead to a reduction in the formation of free radicals, particularly those of the oxygen; 2) the indirect antioxidant action mediated by the cell activation induced by said amino acids, via unknown mechanisms and mediators.

Summary of the invention

The invention provides compositions for pharmaceutical and/or dietetic use, which lead - after in vivo administration by suitable galenical formulations - to an unforeseen synergistic increase in the antioxidant activity of carnosine (and/or of the derivatives thereof).

Said compositions are characterized in that they contain as basic, necessary components, in the suitable ratios:

- 5 a) carnosine and/or derivatives thereof, such as homocarnosine, anserine, ofidine and/or the pharmacologically compatible inorganic and organic salts and/or the acyl derivatives thereof with pharmacologically compatible organic acids, and the inorganic salts thereof;
- 10 b) one or more branched amino acids, in suitable ratios, such as: leucine, isoleucine and valine, both in the free form and in the salified one with inorganic or organic bases or acids, provided that they are pharmacologically compatible, and/or the
- 15 acyl derivatives thereof, salified with inorganic or organic bases, and/or the esters thereof with straight or branched alcohols, optionally salified with inorganic or organic acids.

Moreover, supplementary components of the

20 compositions, depending on the intended final therapeutical characteristics, are :

- c) carnitine and/or water-soluble acyl derivatives thereof (for example acetylcarnitine, propionyl-carnitine, etc.), which are known to have
- 25 antioxidant activity as well as therapeutical activity in a number of the above mentioned pathologies, in particular in those of nervous system (Geremia E. et al., (1988), Med. Sci. Res., vol. 16 (n. 13), 699-700; Calabrese V. et al., in
- 30 "Brain Metabolism and Aging", Meeting in Florence (19/1/1989) pp. 42-44; Ghirardi O. et al., in "VII

General Meeting of Eur. Soc. for Neurochem." Leipzig (23/7/1990); Tesco-Latorraca S. et al., (1992), Dementia, vol. 3 (n. 1) pp. 58-60), and creatinine;

d) oligoelements such as: iron, zinc, manganese, magnesium, copper, cobalt, chrome, molybdenum, vanadium and selenium, in the form of suitable salts of inorganic or organic acids or complexes with pharmacologically compatible amino acids, polypeptides or proteins;

moreover, the following constituents can also be part of the compositions for the alimentary use:

e) vitamins (A, B15, C, D3, E)

f) bioflavonoids (such as those deriving from the citrus: orange, lemon, grapefruit).

The galenical formulations suitable for the oral administration are also part of the invention, provided that they are based on said compositions: swallowable tablets, divisible or not; suitably flavoured chewable tablets; hard- and soft- gelatin capsules; granulates for the extemporary preparation of aqueous solutions, suitably flavoured and added with pharmacologically inert excipients such as various sugars (sachets, solids for use in plunger caps, etc.); suitably flavoured chewing gums; wafer sheets; ready-to-use aqueous solutions, optionally flavoured and added with suitable stabilizers, etc.

The preparation of said galenical formulations can require the simple dry mixing of the components or the previous dry- or humid- granulation, which techniques are known to those skilled in the art.

Said formulations are used successfully in the

prevention and in the treatment of organic aging and/or of the pathologies related thereto and characterized by an increase in the degenerative peroxidation processes induced by free radicals.

5 Detailed disclosure of the invention

The characteristics and the advantages of the compositions according to the present invention will be further illustrated in the following disclosure.

10 Said compositions contain as basic active components:

a) carnosine (β -alanyl-L-histidine) and/or homocarnosine (τ -butyryl-L-histidine) and/or anserine (N1-methyl- β -alanyl-L-histidine) and/or ofidine (N3-methyl- β -alanyl-L-histidine), and/or the pharmacologically compatible inorganic or organic salts thereof and/or the acyl derivatives thereof with pharmacologically compatible acids organic, and the inorganic salts thereof;

15 the content in the constituents a) in the final mixture ranging from 1% to 50% by weight (with the exception of the inert material used for the galenical formulation, also including any sugars added and flavours), preferably from 15% to 25%; and

25 b) one or more branched amino acids, in suitable ratios, such as: leucine, isoleucine and valine, both in the free form and in the salified one with inorganic or organic bases or acids, provided that they are pharmacologically compatible, and/or the acyl derivatives thereof, salified with inorganic or organic bases, and/or the esters thereof with

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straight or branched alcohols, optionally salified with inorganic or organic acids;

the weight ratio of the three amino acids ranging, in the order, from 1:1:1 to 1:0:0, compositions in which leucine is at least 20% of the mixture of the three amino acids being preferred; the content in the constituents b) in the final mixture ranging from 5% to 80% by weight (with the exception of the inert materials used for the galenical formulation, also including any sugars added and flavours), ranging preferably from 50% to 70%.

The compositions according to the invention can moreover contain one or more of the following accessory components:

c) carnitine (3-carboxy-2-hydroxy-N,N,N-trimethyl-1-propanamine inner salt) and/or water-soluble acyl derivatives thereof (for example: acetylcarnitine, propionylcarnitine, etc.) , and creatine;

the content in c) in the final mixture ranging from 0% to 20% by weight (with the exception of the inert materials used for the galenical formulation, also including any sugars added and flavours), preferably from 2.5% to 5%;

d) oligoelements such as: iron, zinc, manganese, magnesium, copper, cobalt, chrome, molybdenum, vanadium and selenium in the form of salts (fumarate, sulfate, oxide, etc.) or complexes with pharmacologically compatible amino acids, polipeptides or proteins;

the amount of c) ranging from 0 mg to 30 mg, depending on the element in the final galenical

formulation, for single administration;

e) vitamins (A, B15, C, D3, E) in the usual amounts made use of in the formulations for the alimentary use;

5 f) bioflavonoids (such as those deriving from citruses: orange, lemon, grapefruit) in the usual amounts made use of in the formulations for the alimentary use.

10 The preparation of the formulations of the present invention in forms for the oral administration, as reported above, needs no specific techniques, since the different powders have a good mixibility and/or are easily water-soluble also in admixture.

15 The compositions of the invention are prepared in the usual oral pharmaceutical forms: swallable tablets, divisible or not; suitably flavoured chewable tablets; hard- and soft- gelatin capsules; granulates for the extemporary preparation of aqueous solutions, suitably flavoured and added with pharmacologically inert
20 excipients such as various sugars (sachets, solids for use in plunger caps, etc.); suitably flavoured chewing gums; wafer sheets; ready-to-use aqueous solutions, optionally flavoured and added with suitable stabilizers, etc.

25 By way of non-limiting example, some formulations according to the invention are reported below.

In the following examples, the compositions are expressed as active ingredient content in grams, independently of the salification, referred to 100 g of
30 final mixture; the components used can be in the form of the free bases or salts, the type of anion being shown

in brackets.

EXAMPLE 1

Example of a formulation of the invention

The composition can be prepared in the form of capsules or soluble, effervescent tablets, sachets etc., as described above, after preparing a humid granulate in which the branched amino acids are dissolved in water and said solution is subsequently sprayed on the homogeneous solid mixture obtained by dry mixing (for example in suitable coating pans) of the other suitably powdered components (for example: in a ball mill). The final mixture is then dried in dry air stream at a temperature below 45°C.

In the case of soluble effervescent formulations, the final product is added, in suitable controlled-humidity environment, with dry tartaric or citric acids, mixed to homogeneity and then the procedure is repeated with sodium bicarbonate. After that, the product is granulated with the usual techniques. The compression of said final mixture gives the effervescent tablets or sachets, whereas, by direct partition of the granulate, before the addition of the effervescent components, the capsules or the tablets are prepared.

Carnosine (base or hydrochloride):15

25 Leucine (base, hydrochloride, sulfate, acetate etc.):15

Isoleucine (base, hydrochloride, sulfate, acetate etc.):15

Valine (base, hydrochloride, sulfate, acetate etc.):15

Carnitine (base, hydrochloride etc.):15

30 Arginine (base, hydrochloride, glutamate etc.):15

Mg (carbonate-hydroxide, basic citrate, lactate,

sulfate):8.5

Zinc (carbonate, lactate, sulfate etc.):0.5

Copper (acetate, basic carbonate, gluconate, sulfate etc.):0.25

5 Iron (gluconate, albuminate, fumarate, proteinate etc.):0.75.

EXAMPLE 2

Carnosine (base or hydrochloride):35

Leucine (base, hydrochloride, sulfate, acetate etc.):50

10 Arginine (base, hydrochloride, glutamate etc.):10

Mg (carbonate-hydroxide, basic citrate, lactate, sulfate):3.5

Zinc (carbonate, lactate, sulfate etc.):0.5

15 Copper (acetate, basic carbonate, gluconate, sulfate etc.):0.25

Iron (gluconate, albuminate, fumarate, proteinate etc.):0.75.

EXAMPLE 3

Carnosine (base or hydrochloride):18

20 Leucine (base, hydrochloride, sulfate, acetate etc.):16

Isoleucine (base, hydrochloride, sulfate, acetate etc.):16

Valine (base, hydrochloride, sulfate, acetate etc.):16

Carnitine (base, hydrochloride etc.):7.5

25 Creatine (base):7.5

Arginine (base, hydrochloride, glutamate etc.):15

Mg (carbonate-hydroxide, basic citrate, lactate, sulfate):2.5

Zinc (carbonate, lactate, sulfate etc.):0.5

30 Copper (acetate, basic carbonate, gluconate, sulfate etc.):0.25

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Iron (gluconate, albuminate, fumarate, proteinate etc.):0.75.

EXAMPLE 4

Carnosine (base or hydrochloride):25

5 Leucine (base, hydrochloride, sulfate, acetate etc.):25

Isoleucine (base, hydrochloride, sulfate, acetate etc.):10

Valine:6

Creatine (base):20

10 Arginine (base, hydrochloride, glutamate etc.):10

Mg (carbonate-hydroxide, basic citrate, lactate, sulfate):2.5

Zinc (carbonate, lactate, sulfate etc.):0.5

15 Copper (acetate, basic carbonate, gluconate, sulfate etc.):0.25

Iron (gluconate, albuminate, fumarate, proteinate etc.):0.75.

EXAMPLE 5

Carnosine (base or hydrochloride):20

20 Leucine (base, hydrochloride, sulfate, acetate etc.):25

Isoleucine (base, hydrochloride, sulfate, acetate etc.):15

Creatine (base):20

Arginine (base, hydrochloride, glutamate etc.):15

25 Mg (carbonate-hydroxide, basic citrate, lactate, sulfate):2.5

Zinc (carbonate, lactate, sulfate etc.):0.5

Copper (acetate, basic carbonate, gluconate, sulfate etc.):0.15

30 Iron (gluconate, albuminate, fumarate, proteinate etc.):0.75

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Manganese (sulfate, gluconate):0.10

Vitamins B15/C/E/D3:150 mg

Lemon and orange bioflavanoids: 200 mg.

Pharmacological and/or dietetic tests

5 In order to study the pharmacological and/or
dietetic characteristics of the compositions according
to the present invention, tests were carried out to
evaluate the following effects in rats subjected to an
10 ipovitaminic, hyperlipidemizing and hypercholesterolemi-
zing diet (which is known to be capable of promoting the
tissue and plasma peroxidation processes);

- 1°) effect of the compositions on the lipoperoxides
gastric content (measured as malonildialdeide
nmoles/ml)
- 15 2°) effect of the compositions on the lipoperoxides
hepatic content (measured as malonyldialdehyde
nmoles/g)
- 3°) effect of the compositions on the lipoperoxides
cerebral content (measured as malonyldialdehyde
20 nmoles/g)
- 4°) effect of the compositions on the lipoperoxides
content in the heart (measured as malonyldialdehyde
nmoles/g).

36 Male rats weighing 180-200 g were used.

25 The animals were subjected for 20 days to a
standard hyperlipidemizing and hypercholesterolemizing
diet consisting of: 20% caseine, a 3.5% mixture of
oligoelements and mineral salts, a 0.1% mixture of
vitamins, 0.2% choline bitartrate, 2% cellulose powder,
30 0.5% chloesterol, 0.25. sodium cholate, 62.44%
saccharose and 10.9% lard. During that time, the animals

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were divided into in 6 groups which received as an "ad libitum" available drink:

- 1° group: control (only water)
- 2° group: Test A (a 0.75% CARNOSINE aqueous solution)
- 3° group: Test B (a 0.25% CARNITINE aqueous solution)
- 4° group: Test C (an aqueous solution of 4% branched amino acids; leucine/isoleucine/valine = 30/0.5/0.5)
- 5° group: Test D (a 0.75% CARNOSINE and 4% branched amino acids aqueous solution as in Test C)
- 6° group: Test E (a 0.75% CARNOSINE aqueous solution plus 0.25% CARNITINE with 4% branched amino acids as in the above Test C).

At end of the test the animals were killed to take both blood samples and the various organs (liver, brain, and heart) on which the lipoperoxides content was evaluated dosing malonyldialdehyde, according to the procedure by Yagi (YAGI K, (1982), in "Lipid peroxides in biology and medicine", Academic Press, New York, pp. 324-40). The results of the lipoperoxides content in plasma are reported in Table I.

15

	malonyldial- dehyde nmoles/ml	percent variation compared with the control

5	<u>Control</u>	4.2 \pm 0.7 --
	<u>Test A</u>	3.6 \pm 0.6 -14.2%
	(Carnosine)	
	<u>Test B</u>	4.2 \pm 0.8 --
10	(Carnitine)	
	<u>Test C</u>	4.3 \pm 0.5 +2.3%
	(Branched A.A.)	
	<u>Test D</u>	3.0 \pm 0.7 -28.5%
	(Carnosine +	
15	Branched A.A.)	
	<u>Test E</u>	2.7 \pm 0.4 -35.7%
	(Carnosine +	
	Carnitine +	
	Branched A.A.)	

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Table I: Changes in lipoperoxides content in the plasma (expressed as malonyldialdehyde nmoles/ml) as the function of the different compositions administered.

25

It is evident that carnosine administration significantly reduces lipoperoxides plasma contents (-14.2%), whereas no effects result from the administration of carnitine alone and an even negative effect to the therapeutical purposes (+2.3%) is observed after administration of the only branched A.A.. A surprisingly favourable, synergistic therapeutical effect, on the other hand, results from the simultaneous administration

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of carnosine + branched A.A. (-28.5%) and of carnosine + branched A.A. + carnitine (-35.7%).

The results referred to the lipoperoxides content in the liver are reported in Table II.

5	-----
	<div>malonyldialdehyde</div> <div>nmoles/g. of fresh</div> <div>tissue</div> <div>percent variation</div> <div>compared with the</div> <div>control</div>

10	<div><u>Control</u> 42 \pm 7 --</div> <div><u>Test A</u> 39 \pm 8 -9.5%</div> <div>(Carnosine)</div> <div><u>Test B</u> 43 \pm 6 +2.3%</div> <div>(Carnitine)</div>
15	<div><u>Test C</u> 44 \pm 7 +4.7%</div> <div>(Branched A.A.)</div> <div><u>Test D</u> 32 \pm 6 -23.8%</div> <div>(Carnosine +</div> <div>Branched A.A.)</div>
20	<div><u>Test E</u> 30 \pm 5 -28.5%</div> <div>(Carnosine +</div> <div>Carnitine +</div> <div>Branched A.A.)</div>

25 Table II: Changes in the lipoperoxides content in the liver (expressed as malonyldialdehyde nmoles/g. of fresh tissue) as a function of the different compositions administered.

30 It is evident that the carnosine administration significantly reduces the hepatic lipoperoxides content (-9.5%). On the contrary, a mildly negative therapeutical effect results from the administration of

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the only carnitine (+2.3%) and of the only branched A.A. (+4.7%). On the other hand, a surprisingly favourable, synergistic therapeutical effect is obtained by the simultaneous administration of carnosine + branched A.A. (-23.8%) and of carnosine + branched A.A. + carnitine (-28.5%).

The results referred to the lipoperoxides content in brain are reported in Table III.

	malonyldialdehyde nmoles/g. of fresh tissue	percent variation compared with the control
Control	102 ± 6	--
15 Test A (Carnosine)	89 ± 7	-12.7%
Test B (Carnitine)	106 ± 5	+3.9%
20 Test C (Branched A.A.)	102 ± 8	--
Test D (Carnosine + Branched A.A.)	69 ± 7	-33.3%
25 Test E (Carnosine + Carnitine + Branched A.A.)	68 ± 4	-32.3%

Table III: Changes of the lipoperoxides content in brain (expressed as malonyldialdehyde nmoles/g. of fresh tissue) as a function of the different compositions administered.

30

From this Table it is evinced that carnosine administration significantly reduces the brain lipoperoxides content (-12.7%), whereas no therapeutically favourable results are observed after the administration of the only carnitine (+3.9%) or of the only branched A.A. (no changes compared with the controls).

On the contrary, a surprisingly favourable therapeutical, synergistic effect results from the simultaneous administration of carnosine + branched A.A. (-32.3%) and of carnosine + carnitine + branched A.A. (33.3%). The results referred to the lipoperoxides content in the heart are reported, finally, in Table IV.

20

other hand, after the simultaneous administration of carnosine + branched A.A. (-35.5%) and of carnosine + carnitine + branched A.A. (-37.7%).

The results from the tests above described show that the compositions of the invention can be used to increase the levels of antioxydants defences and to prevent an increase in the peroxidative processes (in blood and in the different tissues of human body) occurring during aging and in a number of pathologies (see the references above) which are often related to aging. Therefore, said compositions can be used for the preparation of pharmaceutical and/or dietetic forms for the oral administration oral alone [a)+b)+c) or a)+b)] or in combination with other substances such as emulsifiers, other antioxydants, heavy metal chelating agents and pharmacologically (and/or dietotherapeutically) acceptable conventional excipients.

The daily dosage of the compositions [a)+b)+c) or a)+b)] will vary depending on the use which can be both dietotherapeutically prophylactic or therapeutical in the different pathologies, but it will generally range, for prophylactic purposes (dietetic use) from 0.1 to 10 mg/kg/die of carnosine (and derivatives), from 0 to 2.5 mg/kg/die of carnitine (and derivatives) and from 0.1 to 100 mg/kg/die for the branched A.A. (and derivatives) and for the therapeutical purposes (therapeutic use) from 1 to 100 mg/kg/die of carnosine (and derivatives), from 0 to 25 mg/kg/die of carnitine (and derivatives) and from 1 to 500 mg/kg/die for the branched A.A. and derivatives.

CLAIMS

1. Pharmaceutical and/or dietetic compositions containing:
 - 5 a) carnosine and/or derivatives thereof, such as homocarnosine, anserine, ofidine and/or the pharmacologically compatible inorganic and organic salts and/or the acyl derivatives thereof with pharmacologically compatible organic acids, and
10 the inorganic salts thereof;
 - b) a branched amino acid selected from leucine, isoleucine and valine, or salts, and/or acyl derivatives or esters thereof.
2. Compositions according to claim 1 wherein the
15 content in the constituents a) ranges from 15 to 25% by weight.
3. Compositions according to claim 1 or 2 wherein leucine is at least the 20% of the mixture of the three amino acids.
- 20 4. Compositions according to any one of the above claims wherein the content in the constituents b) ranges from 50 to 70% by weight.
5. Compositions according to any one of the above claims containing moreover one or more of the following
25 constituents:
 - c) carnitine, derivatives thereof and creatine;
 - d) oligoelements;
 - e) vitamins;
 - f) bioflavanoids.



AMENDED SHEET

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- c) carnitine, derivatives thereof and creatine;
- d) oligoelements;
- e) vitamins;
- f) bioflavanoids.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/02079

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/05 //(A61K38/05,31:195)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 449 787 (SETRA SRL) 2 October 1991 see claims see page 2, line 1 - line 47 -----	1-5

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- *E* earlier document but published on or after the international filing date
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Date of the actual completion of the international search

8 October 1996

Date of mailing of the international search report

18.10.96

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Information on patent family members

PCT/EP 96/02079

Form PCT/ISA/218 (patent family annex) (July 1992)