Pharmaceutically therapeutic use of glutathione derivatives

All higher organised multicellular living systems, including man, cover the energy requirement of their cells and tissue according to the principle of biological oxidation.

Carbohydrates, amino acids, fatty acids and nucleotides emit on dehydrating (dehydrating = oxidation) in various metabolic paths a part of the chemical energy stored in these molecules and are finally passed to the common final disintegration in the citric acid cycle.

In the metabolic cycle of healthy cells or tissue, which represents the common final stage for monosaccharides, fatty acids and amino acids, the C-6 compounds (citric acid) entering into the cycle are broken down during a circulation in the cycle to C-4 compounds (oxalic acetic acid) by two decarboxylation reactions; the carbon chain is therefore shortened by two carbon atoms.

Moreover, in the citric acid cycle 8 H-atoms are oxidized via the respiration chain with an energy gain into four molecules or water.

The hydrogen occurring on the various disintegration paths on dehydration is transported by hydrogen-transferring coenzymes NAD and FAD to the inner membrane of the mitochondria, the cell organelles, which are considered to be power units of a cell. The hydrogen, with structurally linked enzymes of the respiration chain, the cytochromes, forms an electron transport chain which - as also the entire metabolism - can only function when in the final member of the chain the hydrogen can react in a redox reaction with oxygen to water. The normal potential E_o of this redox reaction lies with a pH value of 7 at + 0.810 volt, so that a suction is exerted on the electron transport chain.

Oxygen is therefore the terminal hydrogen- or electron acceptor in the metabolic process and through its presence it maintains the electron flow in the methabolism of a cell.

The respiration chain in the mitochondria therefore performs a type of biochemical oxyhydrogen gas reaction, in which the respiration substrates are dehydrogenated. Through the gradual reaction of the hydrogen or of the electrons via a cascade of intermediate carriers, the high reaction enthalpy emitted on water formation from hydrogen and oxygen is gradually released and a considerable component is stored in the form of chemical energy as adenosine triphosphate (ATP) for energy-consuming cell reactions.

Every healthy cell having mitochondria covers its energy requirement in this way. Depending on the metabolic tasks, the number of mitochondria varies between 10 and 6 x 10 of a cell; the oxygen requirement thereof is also correspondingly different.

Oxygen must be available at every second in the life of an oxidizing living system to a sufficient extent in the mitochondria of all cells. Necessary for this is a constant undisturbed flow of oxygen from the respiration air with a sufficient oxygen partial pressure via the respiratory pathways, the transition of the oxygen at the lung/blood barrier of the alveoli of the lungs, the diffusion through the erythrocyte membrane, the formation of haemoglobin and the releasing of haemoglobin in the blood capillaries of the tissue, the oxygen diffusion through the intermediate cell space, the interstitial space, through the cell membrane and the mitochondria membrane up to the respiration chain described above. A further, but important path of the oxygen.

This oxidation, which occurs continuously in an indisturbed manner in all cells is the important precondition for

maintaining health in the organism. In an approximate comparison with electrical or electronic components and their circuits, one can speak in terms of a performance characteristic of a healthy cell. On this, an optimum working point is present, which can only move slightly on the performance characteristic. If the working point is unduly far removed from its optimum, this is an indication of a considerable disturbance in the metabolism of the cell. Such a disturbance is caused for example by the phenomenon of peroxidation, which is also designated as oxidative stress. The oxidative stress can either arise through an oversupply of activated oxygen stages (oxygen radicals) and/or through a reduction of those molecules which are normally able to collect this radical energy and are designated as scavenger molecules. If the cells are not able, themselves, to eliminate the disturbance, then a destruction of biomolecules and cell structures is brought about, such as lipid peroxidation of the cell membranes, also various cell function disturbances, cancer-like degeneration of a cell and finally cell destruction.

In living systems, from the outset, nature had to protect its molecular and cell structures from the effect of destructive oxygen radicals, which arise through exogenous noxious substances and also continuously in the cell metabolism, and developed protective enzymes and protective molecules as scavengers, which are able to catch these radicals reliably and quickly.

If it is not possible for the individual disturbed cells to catch the energy-rich radicals, then as a consequence diseases can occur, such as cancer of every genesis, malignant diseases of the blood cells, hepatopathies, fatty liver, fat cirrhosis, liver cirrhoses of every genesis, disturbances of the immunological defence functions in the field of natural killer cells, complex disturbances of lymphokine biosynthesis in T-helper cells, cardiomyopathies

of every genesis, neurological diseases of inflammatory, allergic or degenerative genesis, blood cell diseases, damage to the lens of the eye, proliferation disturbances and differentiation disturbances of epithelial, endothelial and mucous membrane tissue and many more. The type of disease which will occur depends substantially on which cells or cell tissue is most severely affected.

The result of this is that the oxidations which are necessarily required in living systems, must not be allowed to lead to superoxidations.

From a chemical point of view, oxidation means:

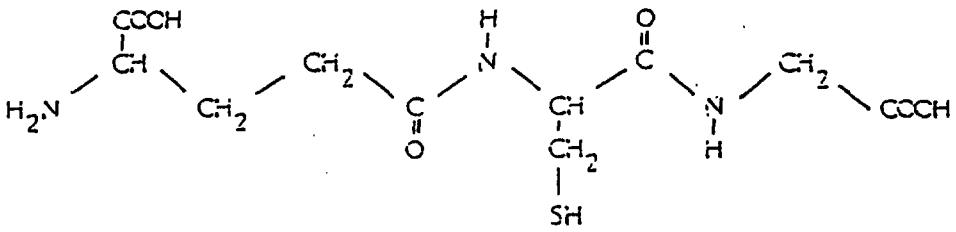
1. Issue of electrons.
2. Issue of hydrogen.
3. Admission of oxygen.

Reduction means accordingly:

1. Admission of electrons.
2. Admission of hydrogen.
3. Issue of oxygen.

Considered thermodynamically, all reductions are endothermic processes, and all oxidations, on the other hand, are exothermic. This means that the oxidized stage is "poorer in energy" and the reduced stage is "richer in energy". The red-stage can always issue "energy" = electrons and the ox-stage is reduced by "energy admission", by the admission of electrons.

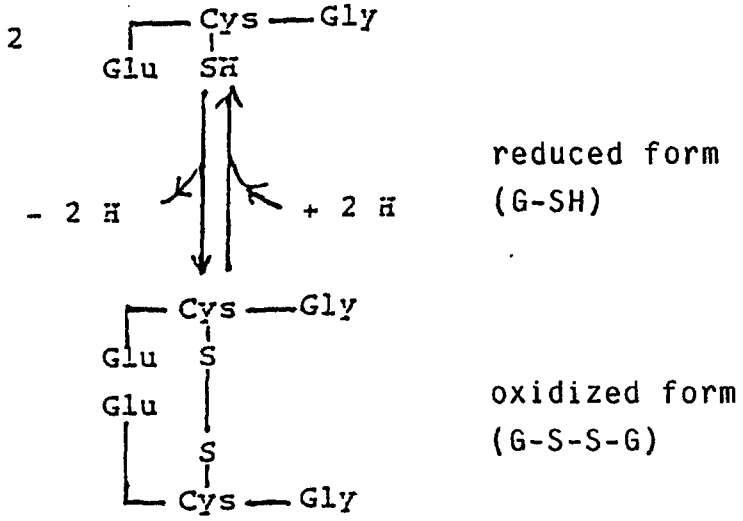
The basis for all reductive potency in the blood and in most cells of man and mammals is the tripeptide glutathione in its reduced form (G-SH), consisting of the three amino acids glutamic acid, cysteine and glycine with the following structural formula:



Glutathione (gamma-glutamyl-cysteinyl-glycine = G-SH)

The functionality of living systems, which is based on the principle of biological oxidation, is only possible through the existence of a reductive protection by this important molecule: G-SH.

Through dehydration or through oxidation, two molecules of reduced glutathione (G-SH) can pass over with disulphide formation into a molecule of oxidized glutathione (G-S-S-G).



The redox system 2 G-SH / G-S-S-G.

In numerous investigations, it was able to be established that cells which comply with their specific function without any problem have a concentration ratio of reduced glutathione (G-SH) to oxidized glutathione (G-S-S-G) of approximately 400 to 1. This means that the intact viable cell has a high reductive potential in the form of the content of reduced glutathione. The concentration ratio of G-SH to G-S-S-G in the range of 400 to 1 can be evaluated as

evidence of optimum function and a reliable maintainting of structure.

To maintain the functionality of several, perhaps all enzymes of the cell metabolism, to prevent oxidative changes to their catalytic and allosteric centres, to maintain an optimum conformation, the reductive potential of the reduced glutathione, i.e. its optimum, high intracellular concentration, is of the utmost importance.

All the conventional therapies existing at present support either the phenomenon of oxidation, i.e. the oxidative side of living systems, or intervene in the "oxidative power" of the metabolism through medicaments which as xenobiotics require oxidation for their breakdown and for metabolite formation.

Of course, the reduced glutathione itself and also particular derivatives of gluthatione have also already been investigated as to their suitability as therapeutically effective substances. The GB-A-144 024 proposes a cancer-inhibiting additive for food or fodder, which consists of a range of substances designated as being essential, one of which can be glutathione. The journal Cancer Res., 15.01.1987, Vol. 47(2), pages 477-485 contains an investigation as to how treatment with reduced glutathione influences the development of tumours. In the journal Biochemical and Biophysical Research Communications, Vol. 35, No. 5, 1969, R.Vince et al.: "Glyoxalase inhibitors as potential anticancer agents", pages 593-598, a series of in vitro experiments with S-aklylglutathione derivatives is described for inhibiting the enzyme glyoxalase I. This is an enzyme which connects methylglyoxal to S-lactoylglutathione. In the in vitro experiments, inhibiting of the enzyme occurs at various intensities. However, no information is given as to what pharmaceutical relevance this inhibition could have.

In the journal Archives of Biochemistry and Biophysics, Vol. 239, No. 2, June 1985, Academic Press,Inc.(US), it is illustrated that glutathione is essential for the cell metabolism and protects the cell from free radicals, oxidative forces and intoxication. It has also already been recognised that glutathione itself has a poor bio-availability, i.e. does not easily arrive into the cells. This is compared with two derivatives of glutathione, namely glutathione monomethyl ester and glutathione monoethyl ester, which have a better bio-availability and disintegrate in the cell to glutathione, inter alia, so that a dose of these derivatives leads to an increased glutathione level in the cells. These are derivatives in which the glycine carboxyl group of the glutathione is esterified.
The invention is based on the problem of making available glutathione derivatives which, used as medicaments, are suitable to strengthen the redox potential of the cells which are disturbed in the metabolism, or even to monopolise them, and in so doing to have a good bio-availability.

To solve this problem, two glutathione-thiol derivatives are made available in accordance with the invention, which are characterised in Claim 1, namely the mono-acetyl-glutathionyl-(thio)-ester and the mono-phosphoric acid-glutathionyl-(thio)-ester of glutathione. With particular advantage, these derivatives are used as medicaments in combination with further substances in accordance with Claims 2 to 4.

The first-mentioned S-acetyl-glutathione is previously known - without an indication of a pharmaceutical effectiveness - from Chemical Abstracts, Vol. 81,1974, Abstract No. 116020 H.

Through the central molecular-biological significance of the

reduced gluathione in a physiologically suitable, intracellular concentration, the following physiological, biochemical and vital cell functions are all able to be explained as being solely dependent on the intracellular glutathione status (G-SH > mixed disulphides > G-S-S-G).

* Redox potential
* Optimum working capacity of all enzyme reactions (active centre and also allosteric centres of an enzyme molecule).
* Maintaining the membrane integrity of all biological membranes (cell- and cell organelle membranes).
* This is also particularly important for the function optimisation of structurally linked enzymes, for membrane carrier mechanisms, for the function and allosteric specificity of all cell receptors.
* Cofactor and reduction potential of many enzymes, especially those which are included in decontamination.
* For the regulation and regulation normalisation of the complex space/time pattern of cell growth- and cell differentiation processes.
* To prevent, weaken and terminate radical reactions and radical chain reactions in living systems.

Thus the physiological intracellular concentration of reduced glutathione is a primary precondition for all basic cell functions.

And therefore the normalisation of the intracellular G-SH concentration is of causal-therapeutic significance for the therapy and for the prophylaxis of many diseases, of most diseases, also of differing etiology and by phenomenologically differing pathobiochemical harmful agents.

A reliable basic therapy for the normalisation of a cell metabolism which has been damaged by the most varied of

harmful agents becomes possible for the first time through the glutathione derivatives which have good bio-availability and are effective, proposed by the invention :

mono-acetyl-(thio)-ester of gamma-glutamyl-cysteinyl-glycine,

mono-phosphoric acid -(thio)-ester of gamma-glutamyl-cysteinyl-glycine.

Essential advantages in the pharmaceutically therapeutic application of the derivatives, newly described here, of the reduced glutathione (G-SH) in diseases of man and animal are as follows:

A) The good permeation capability of these derivatives through biological membranes, wherebyan effective intracellular therapy only becomes possible with glutathione with all its positive physiological consequences.

B) Protection of the SH-group of glutathione which is so important for the therapeutic effects on the way through biological compartments up to the desired effective site.

C) No inhibition of the enzymes included in the endogenous glutathione biosynthesis (gamma-glutamylcysteine-synthetase, the regulation enzyme by glutathione in the sense of a negative feed-back; glutathione-synthetase).

Thereby the possibility exists of a glutathione- (G-SH) substitution for the intracellular space, without an endogenous glutathione biosynthesis, still possible depending on the degree of cell damage, being inhibited or blocked in the manner of a competitive and/or allosteric inhibition.

D) The pharmaceutical/therapeutic application of

glutathione derivatives also does not lead to any inhibition of the endogenous glutathione biosynthesis in healthy cells.

In order that a therapeutically usable glutathione therapy is possible in therapeutically effective and justifiable intracellular concentrations via various ways of application (oral-enteral, nasal, buccal, sublingual, per inhalationem, vaginal, rectal, intracutaneous, subcutaneous, intramuscular, intravenous, per infusionem, intraarterial), the molecule must be able to come into effect intracellularly with an intact SH-group and must be able to pass easily through membranes.

This essential requirement for the relatively broad capability of therapeutic use of glutathione (G-SH) becomes possible for the first time through the derivatives proposed in accordance with the invention.

Owing to the basic effect of G-SH in suitable intracellular concentrations for the normalisation of basal mechanisms of cell metabolism, the described derivatives can be used alone and/or in combination with themselves or with preparations in accordance with Claims 2- 4:

In all inflammatory (also allergic and autoaggressive), chemical-toxic, physical-toxic (also radiation damage by electromagnetic waves and/or corpuscular radiation), infectious-toxic damage to cells, tissues and organs;
and also in pathological inclusions, deposits on and into tissue; in atrophies, hypertrophies, dys- to anaplasiae, i.e. in benign tumours and in malignant neoplasiae with and without metastasis.
In denaturation processes on and with biological molecules, in normotrophic and bradytrophic tissues.
In immune weakness of every genesis.
For cancer prophylaxis and for adjuvant cancer treatment within other cancer therapies and those with

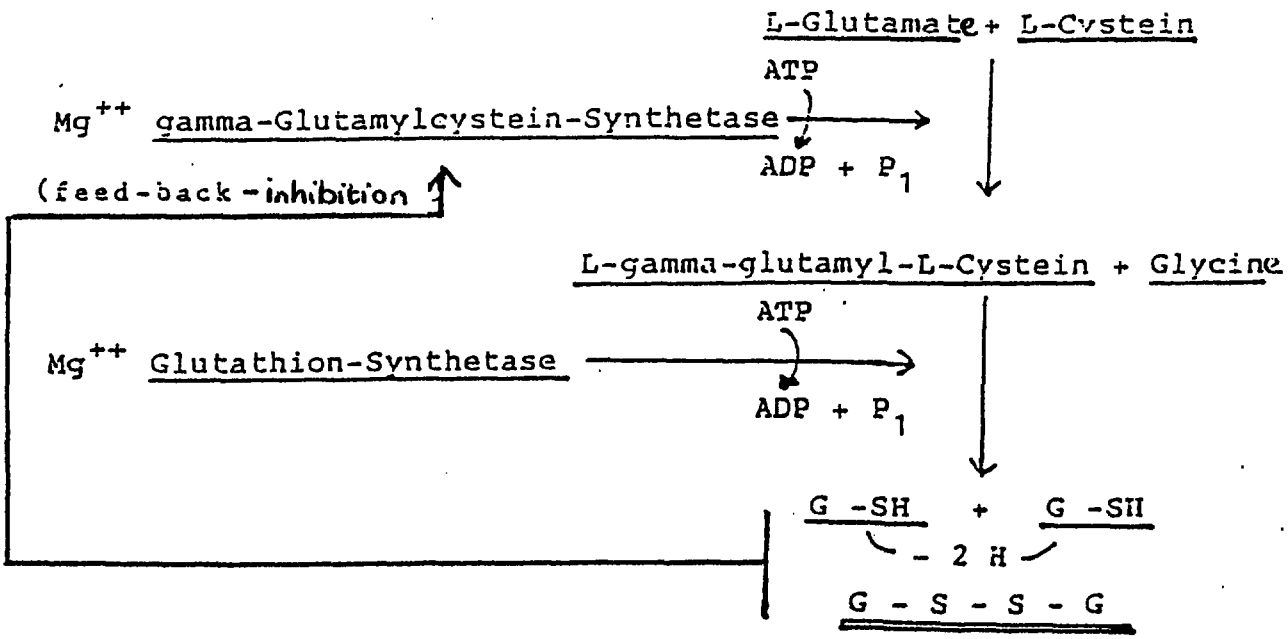
chemotherapeutics or with a radiation treatment.

In addition the increase in oxygen radicals caused by lack of reduced glutathione can be optimally intercepted, which radicals destroy the molecule- and cell structure and therefore can bring about malignant degeneration or the death of the cell.

The prevention of a "peroxidation" by a physiological adjustment of the redox potential of cells and tissues using the proposed thiol derivatives of reduced glutathione as a therapeuticum is a fundamentally new departure for the treatment of many diseases, including the prophylaxis and treatment of malignant tumours.

The glutathione-reductase, a flavoprotein, which fulfils the function of the electron transferrer, sets in all human cells a concentration ratio of (G-SH) to (G-S-S-G) of aproximately 400 : 1. This value reflects the vitality or the physiological function capability of a cell.

The biosynthesis of glutathione from the three amino acids glutamic acid, cystein and glycine takes place intracellularly, independent of DNA, with the inclusion of two enzymes, gamma-glutamyl-cysteine-synthetase and glutathione-synthetase, using 2 mol adenosine-triphosphate (ATP).



Diagrammatic cycle of intracellular glutathione biosynthesis

The relatively unusual peptide bond via the gamma-carboxyl group of glutamic acid prevents an uncontrolled breakdown of the glutathione molecule by peptidases.

The groups of the reduced glutathione (G-SH) are very reactive radical scavengers, by issuing their hydrogen to carbon, oxygen and nitrogen radicals. In addition to the function of the G-SH / G-S-S-G system as a fundamental redox system of living systems, the previously mentioned hydrogen transfer aspect of the reactive SH-group has a particularly important significance for the comprehensive biological role of G-SH.

Possible single electron transfer processes are illustrated below:

A. Hydrogen removal — thiyl radical

$$RSH + R^{\bullet\prime} \longrightarrow RS^{\bullet} + R'H$$

$$(RS^{\bullet} + RS^{\bullet} \longrightarrow RSSR)$$

B. Photo-Ionisation

$$RS^{-} \longrightarrow RS + e^{-}$$

C. Single electron transfer to a metal ion

$$RS^{-} + M^{2+} \longrightarrow RS\cdot + M^{+}$$

D Single electron reduction of disulphides

$$RSSR + e_{aq}^{-} \longrightarrow RS\bar{S}R$$
$$(R\bar{S}SR \longrightarrow RS^{-} + RS\cdot)$$

Also two-electron transfers are possible, thus, for example, when two G-SH transfer into one G-S-S-G.

In the various human tissues, the biological half-life period of glutathione is different. In the erythrocyte approximately 100 hours, in the brain approximately 70 hours, in the lens of the eye approximately 30 hours and in the liver only approximately 4 hours.

In addition to the single- and two-electron transitions, the formation of mixed disulphides and the thio-disulphide exchange reaction are also possible in glutathione.

Glutathione is a component of many important enzymes such as glutathione peroxidase or glutathione reductase. The glutathione peroxidase protects intracellularly for example from many hydroperoxides which have occurred in various ways, through its ability to reduce these to water in close cooperation with catalases.

The selenium-dependent glutathione peroxidase splits H_2O_2 and also organic peroxides (ROOH). A second selenium-dependent glutathione peroxidase splits exclusively

organic peroxides.

The glutathione reductase provides intracellularly for the physiological concentration ratio previously already mentioned of G-SH to G-S-S-G. The intracellular G-SH concentrations lie at 1 - 50 x 10^{-4} M; the intracellular G-S-S-G concentrations lie only at 6 - 200 x 10^{-6} M.

Approximately 30% of the G-SH are present in the form of mixed disulphides (prot-S-S-G).

The tasks of glutathione in the intermediary metabolism of humans are in particular:

1.) Redox system
2.) Thiol disulphide exchange reaction with enzymes and structure proteins.
3.) Decontamination of alkylating medicaments and chemicals.
4.) Amino acid transport through cell membranes.
5.) Chelate formation and -bonding.
6.) Protection from "free" radicals; energy absorption of "free" radicals.
7.) Reaction partners of defined enzyme reactions:
glutathione-peroxidase,
glutathione-reductase;
glutathione-S-transferases, such as
glutathione-S-aryltransferases,
glutathione-S-alkyltransferases,
glutathione-S-epoxide transferases and also
glutathione-dihydroascorbate-reductase inter alia.

Taking into account the functions listed above, the following effects of the reduced glutathione are characteristic:

1.) Improvement to all specific cell functions through

normalisation of the redox potential and optimization of the protection from the destruction of important biomolecules and cell structures through peroxidation, and also protection from lipid peroxidation inter alia.

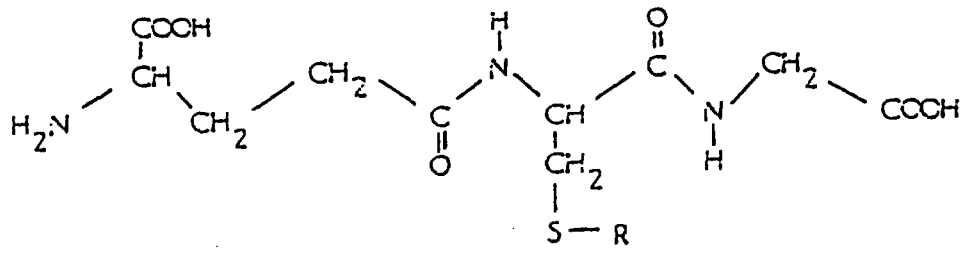
2.) Optimization of the function of many cell enzymes through the setting of an optimum redox potential for the cell, partially via the formation of mixed disulphides.

3.) Normalisation of the mitosis power of a cell via the setting of the redox potential, thereby protective with respect to tumours and effective therapeutically with respect to tumours.

4.) Carrying out enzyme reactions, because these can only occur when sufficient glutathione is available. Glutathione is a component of many defined enzymes such as glutathione-peroxidase, glutathione-S-transferases, glutathione-dihydroascorbate-reductase.

5.) Normalisation of all physiological cell functions, which are possible through the action of glutathione.

The positive effects of the reduced glutathione in the cell are brought about in a particularly marked and reliable manner through doses of the monoacetyl derivative of reduced glutathione and the monophosphoric acid ester of reduced glutathione:



with R = $\overset{O}{\overset{\|}{C}} - CH_3$: mono-acety;-glutathionyl-(thio)-ester

or R = PO_3H_2: mono-phosphoric acid-glutathionyl-(thio)-ester

Excellent compatibility and increased efficacy are shown by combination preparations of the derivatives in connection with substances which contain semiconductor elements such as silicon, selenium or germanium in an organic bond. The same applies to combination preparations with vitamins such as vitamin A, vitamin E or the provitamin beta-carotene and also those with amino acids such as L-cysteine or L-methionine. Several of the said substances can also be contained in such combination preparations. Such preparations are suitable for the treatment of numerous diseases in a broad scope of indications.

Fields of indication

The treatment of cancerous diseases of every genesis, also for malignant diseases of the blood cells and in their initial stages.

Substitution and metabolism regulation in carrying out other cancer therapies, chemotherapies, radiotherapies and/or naturopathic therapies.

Prophylaxis and treatment of metastases within the framework of malignant tumorous diseases.

Hepatopathies, in particular acute and chronic diseases in hepatitis, such as chemo-toxic and infectious-toxic hepatitis, hepatitis caused by virus, rikettsia, bacteria or protozoa and also chronic-aggressive hepatitis, fatty liver, fat cirrhosis and liver cirrhoses of every genesis.

Disturbances of the immunological defence function in the field of natural killer cells, monocytes, macrophages, granulocytes, T- and B-lymphocytes, plasma cells, and also disturbances of the complementary factors and antibody synthesis.

Complex disturbances of the lymphokine biosynthesis in T-helper cells, macrophages and other cells.

Treatment of cardiomyopathy of every genesis, also in combination with other therapies, all forms of coronary disease, angina pectoris, myocardial infarction prophylaxis and also emergency therapy of cardiac infarction in combination with other emergency medicaments.

Acquired and also congenital forms of skeletal muscular disturbances.

Neurological diseases of inflammatory, allergic or degenerative genesis.

All forms of blood cell diseases, anaemia, leucopenia, lymphopenia and thromobocytopenia.

Prophylaxis of damage to the lens of the eye, toxic disturbances to the retina and to the vitreous humour, and also cataract prophylaxis.

All forms of peroxidation or of oxidative stress, for example within the framework of the application of oxygen therapies or therapies with activated oxygen stages (oxygen radicals) and also for protection in the application of hyperbaric oxygen therapy, oxygen multiple-step therapy, in ozone therapies and HCT-therapies.

Intoxications, which lead to biomolecule- and tissue damage in the human organism via radical chain reactions.

Accompanying therapy in radiation treatments, cytostatic treatments and also to diminish or prevent sickness, nausea inter alia.

After narcoses, in particular after full narcoses in patients with damaged heart or liver.

Intoxications with xenobiotics, in particular with toxic trace elements and with heavy metals.

Proliferation disturbances and differentiation disturbances of epithelial, endothelial and mucous membrane tissue.

Treatment of pathophysiological and differently occurring arteriosclerosis.

Base treatment and adjuvant treatment of allergies.

Treatment of impotentia coeundi and impotentia generandi, and also fertility disturbances and potency disturbances of every genesis. Also in premature ageing, the wear of all tissues due to age, and for prophylaxis in activities which lead to premature ageing or to wear of the organs.

Forms of application

The proposed thiol derivatives of reduced glutathione can be applied for therapy in the following forms:

1.) intravenous, also as infusion,
2.) intramuscular,
3.) intracutaneous and subcutaneous,
4.) oral
5.) nasal, buccal and sublingual,
6.) per inhalationem,
7.) vaginal and
8.) rectal.

The intravenous injection or infusion is preferably recommandable in serious and acutely threatening diseases, in which infusion in particular is to be preferred in higher

dosages over a lengthy period of time.

As a basis for the preparation of the infusion solution, the following are suitable in each case in pure form or as a mixture: physiological saline solution (0.9% NaCl in water), Ringer's solution or a monosaccharide solution (5% glucose, 5% laevulose), in which the pH value of the solution lies in the range of between 6.8 to 7.4.

Storage should be undertaken protected from light, so that dark glass is recommended for ampoules.

Whereas in the intavenous application to the organism, a maximum quantity of glutathione can be supplied in reduced form directly into the intravasal space, in other forms of application, a partial oxidation of the substance on the resorption path is to be taken into account.

Nevertheless, the subcutaneous, intramuscular, intraarterial and also oral form of administration is advantageous in long-term treatment. Oral administration is possible in the form of tablets, granulate, capsules, powder or drops. It goes without saying that on the oral-enteral intake path, a reduction can take place in the effect through oxidative reactions, as is also the case in inhalation.

In so far as the medical preconditions exist, intratumoral and also intraperitoneal application is possible.

Likewise, the application in the form of suppositories is possible, i.e. the rectal or vaginal administration, with the restriction of a slightly poorer systematic, but relatively good local bioavailability.

In particular cases of wounds which are healing poorly, such as burns, frostbite or gangrene ulcers, the application in the form of ointments, spray or as liquid comes into

consideration and leads to good therapeutic results.

The different dosaging and galenics for the respective type of administration corresponds to the different bioavailability for each application, biological compatibility, therapeutic reliability and harmlessness at the correct dose.

Dosage

The therapeutic dosage which has proved to be non-toxic in animal experiments on small animals such as grey mice, Wistar rats and rabbits, lies for humans in a range of 2 - 5 mg/kg body weight for a single dose with slow intravenous injection, in which the injection volume amounts to 5 ml with a content of reduced glutathione of 150 mg.

As intravenous infusion, in a carrier volume of 250 ml, daily doses of up to 20 mg/kg body weight and in cases of a special nature even higher doses can be administered. With a dosage of 20 mg/kg body weight, one should not fall short of a minimum infusion time of 30 minutes.

Injection- and infusion solutions which contain reduced glutathione are not to be mixed with other medicaments. Infusion solutions are always to be produced fresh before application, and they are intended for immediate use.

As a daily dose, basically the dose range of 2 to 20 mg/kg body weight is recommended, in which the precise dose is dependent on the individual conditions within the framework of the disease. Only in cancerous diseases with metastases with a total mass of the tumour of more than 100 g can daily doses higher than 20 mg/kg body weight be used.

The duration of the therapy depends on the basic disease which is to be treated. With a duration of therapy of more

than 4 weeks, the trace element serum level should be monitored. For quantitative detection, it is recommended to determine the serum zinc content as screening test parameter.

The potassium and magnesium contents can serve as therapeutic guide parameters of the serum electrolytes.

Galenics

The injectable solutions of thiol derivatives of reduced glutathione are to be present as solutions, free of heavy metals, in sterile and pyrogen-free water with a conductivity value of less than 5 µS, in which storage in dark glass ampoules is recommended.

Infusion solutions are always to be prepared fresh by injecting the contents of the ampoules into the carrier infusion solutions.

In all other forms of preparation, care is to be taken in the selection of adjuvant- and filler substances, that the latter can not undergo any undesired reaction with the free SH-group of the reduced glutathione, so that the effective substance can be prepared, stored and administered unchanged in the respective form of preparation.

Results of experiments

In vitro experiments

1.1) In vitro experiment on human erythrocytes to improve and normalize the redox behaviour:

Of 20 patients with various diseases, in each case 10 ml human venous blood was removed and its ability to coagulate was counteracted by the addition of heparin. As a parameter

for the optimum erythrocyte function, before and after incubation with 0.5 mg reduced glutathione at 37° C during a period of 30 minutes, the content of intraerythrocytary reduced glutathione was determined.

n = 20 (indication of the mean value and the standard deviation)

Start value: 1.50 ± 0.47 mU

Value after incubation: 1.99 ± 0.15 mU

1.2) In vitro experiment on cell systems of human erythrocytes:

Under the same conditions as in the experiment described above, the enzymes glucose-6-phosphate-dehydrogenase and glutathione-reductase are examined in vitro from erythrocytes of 22 patients.

n = 22 (indication of the mean value and the standard deviation)

Start values:
glucose-6-phosphate-dehydrogenase 148 ± 22 mU/ 10^9 erys.
glutathione-reductase 9.5 ± 0.8 U/ 10^{11} erys.

Values after incubation:
glucose-6-phosphate-dehydrogenase 179 ± 16 mU/ 10^9 erys.
glutathione-reductase 11.3 ± 1.2 U/ 10^{11} erys.

In vivo experiments

2.1) Animal experiments on the rabbit to improve and/or normalise the redox status of the blood:

in 5 different rabbits, in 30 experiments, the following

parameters were measured in venous blood in accordance with the method and VINCENT apparatus:

ohmic resistance: r
redox potential E_H or electron potential: rH_2
pH value: pH.

The values were measured in the venous blood of the ear after a defined oxygen deficiency stress.

n = 30 (indication of the mean value and the standard deviation)

r	= 199 $\pm$ 12 Ohm/cm^3	values after stress
pH	= 7.38 $\pm$ 0.21	without dose of
rH	=24.9 $\pm$ 2.3	reduced glutathione

Thereafter, reduced glutathione was administered to the animals intravenously in a dose of 10 mg/kg body weight.

5 - 10 minutes after the application of reduced glutathione, the following values were determined:

r	= 202 $\pm$ 14 Ohm/cm^3	values after dose of
pH	= 7.15 $\pm$ 0.16	reduced glutathione
rH	= 21.8 $\pm$ 0.95	and stress

Marginal conditions: Temperature 20 $^\circ$C, air pressure 767 mm Hg.

2.2) Auto-experiment on human to improve and/or to normalise the redox status of the blood:

In human venous blood, after a defined oxygen deficiency stress, the same parameters were determined as in the experiment set out above. In total, 50 experiments were carried out on various days.

n = 50 (indication of the mean value and the standard deviation)

r = 197 ± 14.5 Ohm/cm	values after stress
pH = 7.41 ± 0.18	without dose of
rH = 26.3 ± 2.1	reduced glutathione

Thereafter, reduced glutathione was slowly administered intravenously in a dose of 600 mg.

10 minutes after the application of reduced glutathione, the following values were determined:

r = 204 ± 12.3 Ohm/cm^3	values after dose of
pH = 7.23 ± 0.11	reduced glutathione
rH_2 = 23.1 ± 1.4	and load

Marginal conditions: Temperature 20 °C, air pressure 758 - 770 mm Hg.

2.3) Experiment on 8 healthy grey mice after transplantations of a malignant melanoma from man

In Fig. 1, the growth of the tumour is illustrated as a function of time.

After super-inoculation of in each case 10^6 cells of a malignant melanoma from man on n = 8 healthy grey mice, four animals not treated with reduced glutathione died (control group) in a period of 8 to 14 days. Four animals, which were treated three times weekly with in each case 250mg reduced glutathione (test group) showed a distinctly slower tumour growth and survived up to the seventh week.

2.4) Experiment on 40 healthy grey mice after transplantation of a malignant melanoma from man

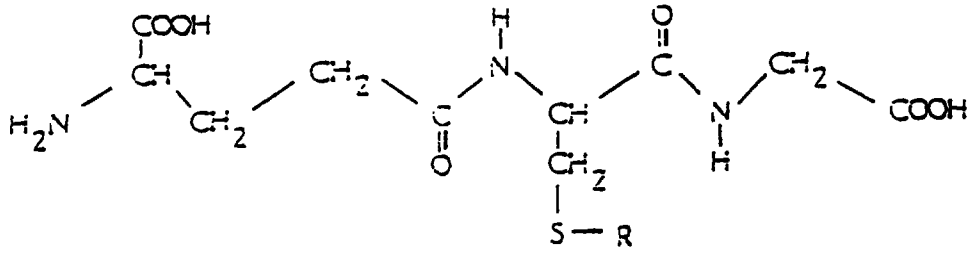
40 healthy grey mice were super-inoculated with in each case 10^6 malignant melanoma cells. 10 untreated control animals died in the period of 8 - 12 days.

30 animals were treated from the start of the series of experiments three times weekly with in each case 300 mg reduced glutathione. Of these animals, three died within 15 days, a further five within 20 days and a further seven within 30 days.

15 animals, i.e. 50 % of the animals treated with reduced glutathione survived the period of observation, lasting 30 days, after inoculation with regressive tumour cells.

CLAIMS

1. A glutathione-thiol derivative of the formula



with $R = \overset{O}{\overset{\|}{C}} - CH_3$

or $R = PO_3H_2$

for use as a medicament.

2. A glutathione-thiol derivative according to claim 1 in combination with at least one vitamin.

3. A glutathione-thiol derivative according to claim 1 or 2 in combination with at least one substance which contains an inorganically or organically bound semiconductor element.

4. A glutathione-thiol derivative according to claim 1, 2 or 3 in combination with at least one amino acid.