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(54) Title: SYNERGISTIC ANTIBIOTIC COMPOSITIONS CONTAINING A PERPHYRIN AND AN ANTIBIOTIC

(57) Abstract

Porphyrins are used as antimicrobial agents in synergistic antibiotic composition, comprising as an antibiotic active ingredient a mixture of one or more porphyrins and at least one antibiotic compound. An antimicrobial composition, comprises as an active ingredient a porphyrin or a mixture of one or more porphyrins, alone or in admixture with conventional antibiotic materials and/or pharmaceutically acceptable or beneficial agents or additives, in a water-based cream composition. Also described is a sterilizing liquid composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, in an aqueous solution, together with a surface-active agent.

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SYNERGISTIC ANTIBIOTIC COMPOSITIONS CONTAINING A PERPHYRIN AND AN ANTIBIOTIC

Field of The Invention

The present invention relates to antimicrobial and to pharmaceutical preparations. More particularly, the invention relates to the use of porphyrins to increase antibiotic efficacy, to antimicrobial liquid compositions useful for hygienic and medical uses, and to antimicrobial cream compositions.

BACKGROUND OF THE INVENTION

It is accepted in modern medicine that the ever increasing need for large amounts of antibiotic compounds is undesirable, since it adversely affects the patients and gives rise to antibiotic-resistant strains, which in turn need new and more potent antibiotics. It is therefore a substantial goal of today's research to be able to increase the efficacy of antibiotic compounds, without administering them in large dosages.

Furthermore, antibiotic materials are widely used in today's medicine, both for the treatment and for the prevention of infections. Antibiotics are administered mostly systemically, orally or intravenously, but for a number of uses, such as for superficial cuts and skin wounds, antibiotic ointments are also available.

However, in a number of cases antibiotic ointments are ineffective or cannot be used. One such case is the treatment of severe injuries, which are prone to become seriously infected by gram-positive bacteria.

Additionally, sterilization of surfaces is also an important task, particularly for medical applications. One of the problems encountered is that of sterilizing the hands of surgeons and of medical staff involved in surgery. This problem is particularly severe, because gram-positive bacterial nest in the various irregularities of the skin, whence they are very difficultly removed.

The Prior Art

Porphyrins have been known for some time to be efficient in the treatment of infections by photodynamic therapy (PDT) [Malik et al., in "Photodynamic Therapy, Basic Principles and Clinical Applications", B. W. Henderson and T.J. Dougherty, Eds., Rosewell Park center Institute, Buffalo, New York]. The antibacterial properties of hemin have also been described [Ladan et al., *FEMS Microbiology Letters,* **112**(1993), 173-178].

However, nowhere in the art an attempt has been made to exploit porphyrins to increase the efficacy of antibiotics, and to reduce the dosages of antibiotic materials needed to combat infections.

Another problem which has remained so far unsolved, is that of infection resulting from exposed bones, either from injuries or from surgery. It is not uncommon to discover that severe infection results after an exposed bone injury, requiring heavy systemic antibiotic treatment, which is not always capable of overcoming the problem. In chest surgery, the problem is complicated by the advent of staphylococcus infection, which may lead to the death of an otherwise successfully treated patient. To overcome this problem, the chest bones are today covered with honey. However, this treatment is

only partially effective because, although it has some preventive effect, honey does not possess any antibiotic activity.

The problem of sterilization of surfaces is dealt with in the art by providing soaps of various types, which are effective to remove superficial bacteria. However, such soaps are practically ineffective against deep-nested bacteria which are not easily removed by a simple washing operation.

Another problem which has remained so far unsolved, is that of sterilizing exposed bones, either from injuries or from surgery, to avoid infection. It is not uncommon to discover that severe infection results after an exposed bone injury requiring heavy systemic antibiotic treatment, which is not always capable of overcoming the problem.

Porphyrins are known to be effective antimicrobial agents. However, the art has so far not addressed the use of porphyrins and components of sterilizing solutions for sterilizing hands or other surfaces which come into contact with exposed wounds.

Summary of the Invention

It is an object of the present invention to provide a synergistic method and compositions for increasing the efficacy of antibiotic compounds.

It is another object of the invention to provide means to reduce the amount of antibiotic material needed to be administered to a patient in need thereof.

It is still another object of the invention to provide synergistic compositions useful for the treatment of infections caused by gram-positive bacteria.

It is a further object of the invention to provide synergistic compositions useful for the treatment of infections caused by gram-negative bacteria.

It has further been found, and this is another object of the present invention, that it is possible to provide antibiotic creams which can be used effectively to treat and to prevent gram-positive infection in severe wounds.

It is another object of the invention to provide a cream which can be used to treat burns and to prevent infections resulting from skin burns.

It is a further object of the invention to provide a method an compositions for sterilizing exposed bones in the course of surgery or the treatment of exposed bone injuries.

It is yet another object of the present invention to provide solutions which can be used effectively to sterilize hands and other surfaces which may house bacteria in recesses from which a mechanical action is ineffective to remove them.

It is still another object of the invention to provide a solution which can be used to sterilize bones which have become exposed in the course of surgery, or as the result of an injury.

Other objects of the invention will become apparent as the description proceeds.

In one aspect, therefore, the invention is directed to a synergistic antibiotic composition, comprising as an antibiotic active ingredient a mixture of one or more porphyrins and at least one antibiotic compound. In another aspect, the invention is directed to a method of treating bacterial infections using reduced amounts of antibiotic materials.

In a further aspect, the invention is directed to an antimicrobial composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, alone or in admixture with conventional antibiotic materials and/or pharmaceutically acceptable or beneficial agents or additives, in a water-based cream composition, as well as to the use of porphyrins for the treatment and the prevention of bacterial infections, and to the method of treatment which utilizes such compositions. Preferred porphyrins include deuteroporphyrin and hemin.

The invention has as another objective a sterilizing liquid composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, in an aqueous solution, together with a surface-active agent. The combined action of the porphyrin and the surface-active agent is effective to reduce the concentration of bacteria on a surface, even when deeply nested, and to maintain it at a low value for a long period of time.

Brief Description of the Drawings

In the drawings:

Fig. 1 shows the effect of the combined treatment of tetracycline and hemin on the growth of *S. aureus*;

Fig. 2 illustrates the effect of the combined treatment of methicillin and hemin on cell growth of *S. aureus*;

Fig. 3 illustrates the effect of methicillin, ampicillin, hemin and their combinations, on the viability of *S. aureus*;

Fig. 4 shows the effect of the combined treatment of tetracycline and photoactivated deuteroporphyrin on cell growth of *S. aureus*;

Fig. 5 shows the effect of the combined treatment of tetracycline and photoactivated deuteroporphyrin on the viability of *S. aureus*;

Fig. 6 shows the synergistic effect of a combination of an irradiated composition comprising deuteroporphyrin and polymyxin B nonapeptide, with tetracycline, on the gram negative bacteria *E. coli*;

Fig. 7 illustrates the effect of deuteroporphyrin, alone and with hemin, in a specific base cream (Merck);

Fig. 8 illustrates the effect of tetracycline, and of deuteroporphyrin, alone and with hemin, in another specific base cream (Johnson's);

Fig. 9 illustrates the effect of tetracycline, and of deuteroporphyrin, alone and with hemin, in yet another specific base cream (Carbopol);

Fig. 10 illustrates the effect of tetracycline and of hemin, alone and in combination, in the Merck base cream; and

Fig. 11 illustrates the activity of different sterilizing liquid compositions on *S. aureus*.

Detailed Description of The Invention

The synergistic antibiotic composition of the invention comprises as an antibiotic active ingredient a mixture of one or more porphyrins and at least one antibiotic compound.

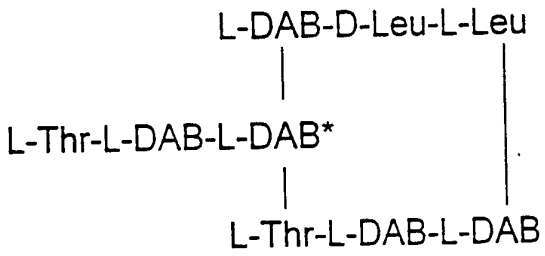
The invention can be conveniently exploited with a variety of antibiotics. Examples of suitable antibiotic materials are those which owe their activity to their ability to inhibit the synthesis of bacterial cell walls by blocking the terminal cross-linking of linear glycopeptides into the complex peptidoglycan, and which activate autolytic enzymes in the cell walls. Examples of such antibiotic materials are the penicillins and the cephalosporins. Other antibiotic materials which can be used according to the invention are those which are active because they inhibit protein synthesis. Illustrative and non-limitative examples of such materials are, e.g., the tetracyclines, which act by inhibiting the binding of aminoacyl-tRNA to the 30S unit of bacterial ribisomes, chloroamphenicol, which blocks the attachment of amino acids to the nascent peptide chain on the 50S unit of ribosomes by interfering with the action of peptidyl transferase, and the macrolide antibiotics, which attach to a receptor on the 50S subunit of the bacterial ribosome.

As can be seen, the antibiotic materials useful in conjunction with the invention are many and of many different types. Accordingly, while the following description will be limited to specific materials, for the sake of brevity, it is not to be construed as intending to limit the invention to any particular antibiotic material.

Illustrative, but non-limitative, examples of specific antibiotic materials which can be conveniently used in the context of the invention are penicillin (G and V), ampicillin, methicillin, oxacillin, amoxicillin, cephalexin, cephradine, tetracycline, erythromycin and chloroamphenicol, and mixtures of two or more of such compounds with or without additional antibiotic and/or antibiotic synergistic materials.

According to a preferred embodiment of the invention, the porphyrin is selected from the group consisting essentially of deuteroporphyrin, hemin, hematoporphyrin, protoporphyrin, and meta-, para- or orto-hydroxy phenylporphyrin.

The composition of the invention can further comprise a peptide of the formula:



(I)

wherein DAB* is a 2,4-diaminobutyric acid residue, and DAB is a 2,4-diaminobutyric acid residue containing a free 4-amino group. This compound is a known material, which is useful to disorganize the bacterial membrane of gram-negative bacteria. A specific peptide is polymyxin B nonapeptide. Therefore, it can be conveniently used when the invention is exploited to combat infections generated by gram-negative bacteria. However, alternative additives can of course be used for this purpose, and the invention is not limited to the use of any particular additive. It should be understood that the compositions of the invention can be used together with any useful and pharmaceutically-acceptable additive, carrier and adjuvant, which are not listed nor discussed in detail herein, for the sake of brevity, since they are readily apparent to the skilled person.

Thus, in one aspect, the invention is directed to the use of a composition comprising one or more porphyrins and one or more antibiotic materials, for the manufacture of a pharmaceutical preparation for the treatment of infections caused by gram-positive bacteria.

In another aspect, the invention is directed to the use of a composition comprising one or more porphyrins and one or more antibiotic materials, for the manufacture of a pharmaceutical preparation for the treatment of infections caused by gram-negative bacteria.

The synergistic composition of the invention is useful, *inter alia*, in a method of treating an infection in an animal, including humans, which infection is susceptible of treatment by a given antibiotic material or mixture of antibiotic materials, comprising administering to the animal in need thereof an amount lower than the effective amount of the said antibiotic material or mixture of

antibiotic materials, together with one or more porphyrins. The compositions of the invention can be used effectively to treat infections which, for practical purposes, cannot be treated by the antibiotic material or by the combination of antibiotic materials contained therein alone, because the dosage required to affect the bacteria exceeds acceptable boundaries. Thus, the synergistic compositions of the invention not only substantially reduce the amount of antibiotic material needed for a given treatment, which is an extremely important result by itself, but also permits to use antibiotic materials which are essentially ineffective as such, for the treatment of infections so far untreatable or only difficulty treatable by the same antibiotics.

Illustrative examples of bacteria which can be treated according to the invention are Gram-Positive bacteria, Anaerobic Gram-Negative bacteria, *Streptococci sp., Diplococci sp., Staphylococci sp., P. aeruginosa, E. coli, Klebsiella sp.,* and *Proteus vulgaris.*

As stated, in another aspect the invention is directed to an antimicrobial composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, alone or in admixture with conventional antibiotic materials and/or pharmaceutically acceptable or beneficial agents or additives, in a water-based cream composition.

According to a preferred embodiment of the invention, the porphyrin is deuteroporphyrin or hemin. One preferred composition of the invention comprises a mixture of deuteroporphyrin and hemin. Another preferred embodiment of the invention comprises a mixture of hemin and tetracycline.

Without wishing to be bound by any specific theory, the inventors believe that the high efficiency of the cream compositions of the invention derives from the fact that the cream is water based. Creams which are based on organic solvents, such as oils, tend to dissolve the porphyrins which thus loose their efficacy.

By "cream" is meant to indicate all water-based compositions which are creamy in consistence, and which can be easily applied in thin layer to a surface. Water-based compositions may, of course, contain small amounts of materials which may be considered water-unmiscible solvents, such as for the purpose of creating emulsions, or as jellyfying agents, or for any other purpose, as long as such materials do not substantially dissolve the porphyrin therein.

An illustrative and non-limitative antimicrobial cream composition according to the invention may comprise, along with other components, the following major components: paraffin, propylenglycol, polysorbat 40, cetylstearyl alcohol, Vaseline and water.

Another illustrative and non-limitative antimicrobial cream composition according to the invention may comprise, along with other components, the following major components: synthetic beeswax, glyceryl stearate, stearic acid, propylene glycol, alkylparaben(s), preservatives, and water.

The invention is also directed to the use of a cream composition according to the invention, for the treatment of infections caused by gram-positive bacteria. Such infections may comprise, inter alia, infections caused by flesh wounds, surgical cuts, or exposed bone injuries.

The cream composition according to the invention can be conveniently employed for the sterilization of bones. A particularly important use, which addresses a need so far unsolved, is the sterilization of chest bones exposed during chest surgery, to prevent the occurrence of post-surgical infection.

In all the uses described above, when a photoporphyrin is employed, the treatment may further be rendered more effective by irradiating the treated area with light. Such irradiation procedure is within the scope of the routineer, and is therefore not described herein in detail, for the sake of brevity.

The invention therefore provides a method of treating or preventing the occurrence of an infection caused by Gram-positive bacteria, comprising applying to the area to be treated a cream composition as described above. Of course, the amount of active material used in a given composition depends on the type or porphyrin, or porphyrin combination or antibiotic used, as well as on additional additives that may be employed for a specific purpose. The skilled person will easily determine the amount of each active material which it is desirable to employ in a given composition, by simple experiments of the kind described in the examples given below. As an indicative value, however, an average composition may comprise each of the porphyrin(s) and of the antibiotic material(s) in an amount comprised between 5 and 100 μg/ml.

The synergistic antibiotic compositions described above, which comprise, as an antibiotic active ingredient, a mixture of one or more porphyrins and at least one antibiotic compound, can of course be employed also in conjunction

with the antibiotic creams of the invention. However, the creams of the invention are not limited to those which comprise synergistic compositions, and are intended to encompass all the antibiotic combinations described or referred to herein, which can be incorporated in water-based creams, whether they exhibit synergistically improved activity or not. Of course, all the antibiotic materials described above, as well as many others, can be used in conjunction with the creams of the invention.

The cream compositions of the invention can of course be used together with any useful and pharmaceutically-acceptable additive, carrier and adjuvant, which are not listed nor discussed in detail herein, for the sake of brevity, since they are readily apparent to the skilled person.

As stated, the invention has as a further objective a sterilizing liquid composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, in an aqueous solution, together with a surface-active agent.

It has been found that in order to obtain a quick and prolonged sterilizing effect, and to reduce the amount of bacteria present on a surface, it is necessary to utilize both a porphyrin and a surface-active agent. The combined action of these two components is effective quickly to reduce the concentration of bacteria on a surface, even when deeply nested, and to maintain it at a low value for a long period of time.

By "sterilizing composition" is meant to indicate a composition that, when applied to a surface infected with bacteria, is capable of reducing the concentration of the bacteria to a non-dangerous level, preferably to a non-

detectable level. However, in some instances a reduction of several orders of magnitude will be considered a disinfection, when appropriate according to the case, even if the bacteria are still detectable on the sterilized surface.

While a variety of porphyrins can be used in the solutions of the invention, according to a preferred embodiment of the invention the porphyrin is deuteroporphyrin or hemin, or a mixture thereof. These porphyrins are preferred because they are widely used and mostly do not present regulatory problems, but as stated, other porphyrins are also useful. Other porphyrins include, e.g,, hematoporphyrin, protoporphyrin, and meta-, para- or ortho-hydroxy phenylporphyrin.

The surface-active agent appears to be instrumental in allowing a better contact between the bacteria and the porphyrin, although the actual mechanism through which it acts has not been fully elucidated. The art is crowded with many surface-active agents, some of which may be unsuitable for the purposes of the invention because the may be hazardous to health. According to a preferred embodiment of the invention, therefore, the surface-active agent is a detergent, preferably a detergent which is in use and is recognized as being non-hazardous to health. Illustrative and non-limitative examples of suitable detergents which are commercially available include Triton X-100 (which is iso-octylphenoxypolyethoxyethanol, containing approximately 5 moles/lit of ethylene oxide, manufactured by BDH Chemicals Ltd., England), sodium dodecyl sulphate and sodium lauryl sulphate, or a mixture of two or more of such agents.

The concentration of the porphyrin in the sterilizing solution may vary, according to the porphyrin or mixture of porphyrins employed, the surface-

active agent used, and the purpose for which it is desired to employ it. The skilled person will be able to tailor specific sterilizing solutions for specific uses, which is within the scope of the routineer. By way of example, however, in sterilizing compositions of the invention the porphyrin may be present in a concentration of 1 to 100 μg/ml of sterilizing solution.

Likewise, the concentration of the surface-active agent may vary over a wide range, depending on the surface-active agent employed, the porphyrin used and the purpose for which the solution is intended. Illustrative concentrations of the surface-active agent in the solution are 0.5 - 5 wt%.

The invention also encompasses the use of a liquid composition according to the invention, for the sterilization of surfaces. The surfaces to be sterilized can be of many types, e.g. the human hand or a bone. A particularly important case of bone sterilization is when the bones are chest bones exposed during chest surgery.

While in most cases this may be unnecessary, it is possible to enhance the sterilizing activity of the solution of the invention, or to speed-up the sterilization process, by irradiating the area to be sterilized with light. Such a use is of course also within the scope of the invention.

The invention therefore provides a method of preventing the occurrence of an infection caused by Gram-positive bacteria, comprising applying to the area to be treated a liquid sterilizing composition as described above.

Of course, the synergistic antibiotic compositions described above, which comprise, as an antibiotic active ingredient, a mixture of one or more

porphyrins and at least one antibiotic compound, can be employed also in conjunction with the sterilizing compositions of the invention. However, conventional antibiotics are not required in the liquid compositions of the invention. Nevertheless, the addition of amounts of conventional antibiotics to the sterilizing liquid compositions does not exceed the scope of the invention.

The compositions of the invention can of course be used together with any useful and pharmaceutically-acceptable additive, carrier and adjuvant, which are not listed nor discussed in detail herein, for the sake of brevity, since they are readily apparent to the skilled person. Furthermore, when the human hand is to be sterilized, conventional dermatologically-acceptable additives, e.g., perfumes, can of course be employed.

All the above and other characteristics and advantages of the invention will be better understood through the following illustrative and non-limitative examples.

Experimental Procedures

Bacterial Strains

This experiments detailed below were carried out using both Gram-positive and Gram-negative bacteria. Illustrative Gram-positive bacteria are the coagulase and DNAse positive Staphylococcus aureus which belong to phage type 0[88/89/95] and resistant to the majority of the common antibiotics such as Methicillin, Ampicillin, Chloramphenicol, Tetracycline, Ofloxacin, Imipinen, Ciprofloxacin, Gentamicin, and Erythromycin. Illustrative Gram-

negative bacteria are *Escherichia coli* of serotype 0111/B4, resistant to Ampicillin, Chloramphenicol, Tetracycline and Sulfamethoxazol trimethoprin. All of these strains were recovered from clinical material submitted to the Bacteriological Laboratory of the Meir Hospital, Kfar-Saba, Israel.

Bacterial Growth

Overnight cultures of *S. aureus* grown in Nutrient Agar (Difco, U.S.A.) were transferred into Nutrient Broth (Difco, U.S.A.) at a pH of 6.5, to a final volume of 25 ml, at an initial density of 0.1 at 660 nm and allowed to grow at 37°C with aeration. Porphyrins (10μg/ml) were added at the beginning of the log phase. When photoactive (excluding hemin) porphyrins were used, the cultures were then irradiated, using two tungsten lamps which were placed 30 cm above both sides of the flasks. Bacterial growth was determined by the increase of the optical density as a function of time at 660 nm with a Novaspec Biochrom LKB spectrophotometer. Viable bacteria were monitored by counting the number of colony-forming units on nutrient agar plates.

Antibiotics

The indicated antibiotics were added, as described specifically in each example, at the indicated concentrations, to the cultures at the time of porphyrin addition, not prior to that, and the experiment was continued as described above.

Cream Preparations

The relevant base cream were mixed with pre-dissolved deuteroporphyrin or hemin, from a stock solution. The stock solution was prepared by dissolving

5 mg of porphyrin in 2 drops of 0.1 N NaOH, and then diluting in phosphate buffer to pH 6.8, to a concentration of 1 mg/ml. The diluted solution was mixed with a base cream, to a final concentration of porphyrin of 100 µg/ml and, when used, 100 µg/ml concentration of antibiotic was added.

Preparation of Sterilizing Solutions

The sterilizing solutions were prepared by adding pre-dissolved deuteroporphyrin or hemin, from a stock solution. The stock solution was prepared by dissolving 5 mg of porphyrin in 2 drops of 0.1 N NaOH, and then diluting in phosphate buffer to pH 6.8, to a concentration of 1 mg/ml. The diluted solution was mixed with phosphate buffer saline (PBS) 0.15 M, pH 7, to a final concentration of porphyrin of 10 µg/ml.

The procedures detailed above were used throughout the following examples, unless otherwise specified, and therefore are not reproduced again in each example, for the sake of brevity. Throughout the following examples, concentrations given in µg/ml refer to ml of nutrient broth, or culture broth, or buffer, as appropriate.

Example 1

The effect of the combined treatment of tetracycline and hemin was tested on the cell growth of *S. aureus*. The following compositions were tested:

a. 10 µg/ml of hemin;

b. 30 µg/ml of tetracycline ;

c. 10 µg/ml of hemin + 10 µg/ml of tetracycline ;

d. control cells.

The optical density of the nutrient broth containing the calls was measured at 660 nm, as detailed above, as a function of time. The results are shown in Fig. 1, from which it can be seen that a combination of 10 μg/ml hemin + 10 μg/ml tetracycline was the most effective, and was substantially more effective than 30 μg/ml tetracycline alone.

Example 2

The effect of the combined treatment of methicillin and hemin was tested on the cell growth of *S. aureus*. The following compositions were tested:

a. 10 μg/ml of hemin;

b. 10 μg/ml of ampicillin;

c. 5 μg/ml of methicillin;

d. 10 μg/ml of hemin + 10 μg/ml of ampicillin;

e. 10 μg/ml of hemin + 5 μg/ml of methicillin;

The optical density of the nutrient broth containing the calls was measured at 660 nm, as detailed above, as a function of time, and the results are plotted in Fig. 2. From the results of this experiment it is apparent that the control cells (♦ line), to which no active material was added, behaved very similarly to those to which there were added 5 μg/ml methicillin (O line), or 10 μg/ml ampicillin (o line). The optical density remained low were 10 μg/ml of hemin (◊ line) were added, but increased rapidly after about 5 hours. In contrast, the optical density of composition "d" (n line) and of composition 'e" (X line), remained substantially lower than that of the corresponding compositions

without hemin (compositions "b" and "c"). It should be noted that hemin alone inhibits the growth of *S. aureus* for only 5 hours, while the combined effect of hemin and methycillin (in amounts in which methicillin alone does not show any effect) inhibit the growth for 7-8 hours, which represents a striking synergistic effect.

Example 3

The effect of the synergistic combinations of the invention was tested using *S. aureus*, and methicillin, ampicillin and hemin as the active materials. Viable counts were taken at the beginning of the experiment, and after one and two hours. The results are shown in Fig. 3.

The tested compositions are identified as follows:

control - no active materials added;

AMP - ampicillin, 10 μg/ml;

MET - methicillin, 10 μg/ml;

HM - hemin, 10 μg/ml;

As it can be seen from the figure, there is virtually no difference in the viable count after two hours, between the control, AMP and MET samples. The HM sample shows an improved antibiotic effect, and the HM+AMP and HM+MET show a further improvement of one order of magnitude, over the HM.

Example 4

Penicillase activity in *S. aureus* was measured by the phenol red test. In the test there were used 10^4 cells induced by methicillin, 50 μg/ml penicillin (in the mother liquor), and 0.5% phenol red. The experiment was carried out according to the method described by Escamilla J., [Susceptibility of Haemophilus influenzae to ampicillin as determined by use of a modified, one-minute beta-lactamase test", *Antimicrob. Agents Chemoter.*, 9, 196-198 (1976)].

The results are shown in Table I below. "++" indicates that color change from red to yellow takes place in less than 1 minute, and "-" indicates that no color change from red to yellow took place in the course of the experiment.

Table I

Penicillinase activity in *S. aureus* treated bacteria

Penicillinase activity	Treatment		
Time (min.) after treatment	Penicillin 10μg/ml	Hemin 10μg/ml	Penicillin + Hemin 10μg/ml + 10μg/ml
30	++	-	-
60	++	-	-

Example 5

The combined effect of tetracycline and photoactivated deuteroporphyrin was tested on cell growth of *S. aureus*. The photoactivation was effected as detailed above under "Experimental Procedures". Optical density of the various samples was measured at 660 nm, as a function of time, and the results are plotted in Fig. 4. The compositions used are identified as follows:

C -	control cells;
Dp 0.1+L -	deuteroporpohyrin, 0.1 μg/ml, irradiated with light at 5 J/cm^2;
Tc 30 μg/ml -	tetracycline, 30 μg/ml;
Tc+Dp0.1+L -	tetracycline 2 μg/ml + deuteroporpohyrin, 0.1 μg/ml, irradiated with light at 5 J/cm^2;
Tc 2 μg/ml -	tetracycline, 2 μg/ml.

As can be seen with the figure, the best results were obtained using tetracycline 2 μg/ml + deuteroporpohyrin, 0.1 μg/ml, irradiated with light at 5 J/cm^2, and they were substantially better than those obtained with 30 μg/ml of tetracycline.

Example 6

The effect of the synergistic combination of the tetracycline and photoactivated deuteroporphyrin was tested using *S. aureus*. Viable counts were taken at the beginning of the experiment, and after one and two hours. The results are shown in Fig. 5. The tested compositions are identified as in Example 5.

Example 7

The effect of the synergistic combination of tetracycline with a composition comprising photoactivated deuteroporphyrin together with the nonapeptide if Formula (I), was tested on the Gram negative bacteria *E. coli*. The *e. coli* cells were grown to the log phase under the conditions shown in Fig. 6. Fig. 6A shows the optical density that was measured to determine total biomass, and Fig. 6B represents the same results, given in terms of colony forming units. The following materials were used:

Tc = 10μg/ml of tetracycline;

DP = 5 μg/ml of deuteroporphyrin;

NP = 650 μg/ml of polymyxin B nonapeptide.

In all experiments the cells were irradiated with 10 J/cm^2 of light.

As can be clearly seen from Fig. 6, the *E. coli* cells were essentially insensitive to the polymyxin B nonapeptide alone, to its combination with tetracycline, and to tetracycline alone. The cells treated with these compositions behaved essentially as the untreated control cells (Cont). The combination of the invention, on the other hand, comprising polymyxin B nonapeptide, deuteroporphyrin and tetracycline shows a surprising activity against *E. coli*.

Example 8

The experiments described above were repeated with a number of porphyrins and antibiotics, and an illustrative spectrum of activity on different bacteria is illustrated in Table II below. Results comparable to those detailed in Examples 1-7 where obtained with the bacteria, porphyrins and antibiotics listed in Table II, and these experiments are therefore not described herein in detail, for the sake of brevity.

Table II:

Spectrum of porphyrins and antibiotics activity on bacteria.

IIA - Porphyrins

deuteroporphyrin
hematoporphyrin
protoporphyrin
hemin
m. p. or *o.* hydroxy phenyl porphyrins

IIB - Antibiotics

ampicillin
methicillin
tetracycline
erythromycin
chloramphenicol
polymixin B nanopeptide

Table II (Continued)

IIC - Bacteria

General to Gram (+)
Streptococci sp.
Diplococci sp.
Staphylococci sp.
P. aeruginosa
E. coli
Klebsiella sp.
Proteus vulgaris
Anaerobic Gram (-)

Example 9

The following base cream (DECODERM, manufactured by Merck & CO., U.S.A.) was used (contents in mg/1 gr base cream):

2 mg sorbic acid

1 mg silicum dioxide

20 mg medium chain triglyceride

30 mg glycerol monostearate

30 mg paraffin

50 mg propylenglycol

80 mg polysorbat 40

90 mg cetylstearyl alcohol

320 mg vaseline

double distilled water, q.s.

The following compositions were tested:

control (base cream only);

deuteroporphyrin, 100 μg/ml - in the dark (Dp);

deuteroporphyrin, 100 μg/ml - with a light irradiation of 10 J/cm^2;

deuteroporphyrin, 100 μg/ml + hemin, 100 μg/ml - in the dark.

Staphylococcus aureus bacteria grown in nutrient broth media to their log phase, were spinned and bacterial concentrate containing 10^8 - 10^9 bacteria were added to the various cream compositions. Creams tested in the dark were kept at 37°C, while irradiated creams were illuminated as described above. At the end of the incubation intervals, 100 μg samples of the cream were diluted in PBS and plated on nutrient agar plates to determine the colony forming units / ml sample. Incubation intervals of 30 minutes and 180 minutes were employed.

The results are shown in Fig. 7. It is clearly seen that deuteroporphyrin in the dark is entirely ineffective, and the CFU found are of the same order as the control cream, irradiation of the deuteroporphyrin was only partially effective, while creams containing a combination of deuteroporphyrin and hemin were highly effective even in the dark.

Example 10

Example 9 was repeated, with the following changes:

The base cream was a commercially available cream, the so-called Johnson's baby moisturizer cream (manufactured by Johnson & Johnson Ltd., U.K.), containing:

dimethicone;
isopropyl palmitate;
polysorbate 61;
sorbitan stearate;
myristyl myristate;
cetyl alcohol;
stearyl alcohol;
synthetic beeswax;
glyceryl stearate;
stearic acid;
oleic acid;
propylene glycol;
carbomer 941;
methylparaben;
propylparaben;
butylparaben;
BHT;
stearoxytrimethylsilane;
stearyl alcohol;
benzyl alcohol;
sodium hydroxide;
essence;

water.

Instead of an irradiated deuteroporphyrin composition, a 100 μg/ml tetracycline composition was tested.

The results are shown in Fig. 8. As before, deuteroporphyrin in the dark is entirely ineffective. The tetracycline-containing cream was also ineffective. Creams containing a combination of deuteroporphyrin and hemin were highly, as was the case with the previous base cream.

Example 11

Example 10 was repeated, but instead of the Johnson & Johnson base cream, Carbopol 940 was used. Carbopol 940 is a commercially available acrylic copolymer (produced by B. F. Goodrich Company, U.S.A.).

The results are shown in Fig. 9, from which it can be seen that the behavior is essentially identical to that obtained in Example 10.

Example 12

Using the base cream of Example 9, the following compositions were tested:

hemin, 100 μg/ml;

tetracycline (Tc), 100 μg/ml;

hemin, 100 μg/ml + tetracycline, 100 μg/ml.

The results are shown in Fig. 10. The results show that hemin alone in the base cream was effective, but the combination of hemin and tetracycline was substantially more effective.

Examples 13 - 16

Examples 9 - 12 were repeated, but instead of adding the bacterial concentrate directly to the cream, it was smeared on a bovine bone, and left to incubate at 37°C on the bone before a thin layer of cream was applied thereto. At the end of the incubation periods, the bone was washed with equal volumes of PBS, and the washings were plated on nutrient agar plates to determine the CFU.

Example 17

Example 16 was repeated, using *S. epidermidis* instead of *S. aureus*, and allowing incubation times of up to 6 hours. The results obtained were comparable to those obtained with *S. aureus*.

When an incubation time of up to 6 hours was allowed, complete disinfection of the bone was observed, using compositions containing deuteroporphyrin and hemin (100 µg/ml of each) and hemin with tetracycline (also 100 µg/ml of each). This was checked by immersing the bone in PBS buffer for 30 minutes, and using the PBS buffer in a growth experiment on agar plates. No CFU were observed in these experiments.

Example 18

The creams of Examples 9 - 12 were tested in mice, to treat surgical cuts infected with *S. aureus* concentrate. The cuts were of identical lengths, and the bacterial concentrate was taken from the same stock solution. The cuts were tested periodically for bacterial presence. The results were essentially the same as those obtained in Examples 9 - 12.

Example 19

The following sterilizing compositions were tested, the liquid base solution being in all cases a PBS buffer 0.15 M at pH 7:

Con -	Control: PBS without any addition;
Dp-	Deuteroporphyrin, 10 µg/ml;
Hm-	Hemin, 10 µg/ml;
Dp+Det	Deuteroporphyrin, 10 µg/ml, with 1% of the relevant detergent;
Hm+Det	Hemin, 10 µg/ml, with 1% of the relevant detergent;

Three detergents were tested, all of which gave comparable results: Triton X-100, sodium dodecyl sulphate, and sodium lauryl sulphate.

Staphylococcus aureus bacteria grown in nutrient broth media to their log phase, were spinned and bacterial concentrate containing 10^8 - 10^9 bacteria were added to the various sterilizing compositions. The solutions were kept at 37°C. At the end of the incubation intervals, 10 ml samples of the solution

were plated on nutrient agar plates to determine the colony forming units / ml sample. Incubation intervals of 1, 2 and 3 hours were employed.

The results are shown in Fig. 11. The results obtained with the combination of porphyrin and detergent were excellent, and were better by one order of magnitude than those obtained with the porphyrins alone, and eight orders of magnitude better than the control.

Example 20

Example 19 was repeated, with the exception that the bacterial concentrate was smeared on a human hand, which was allowed to incubate for 10 minutes and was then washed with one of the solutions of Example 19. The washing liquid was then plated on an agar plate and tested for bacterial growth. The control solution showed substantial bacterial concentration, the deuteroporphyrin and the hemin solution showed traces of bacteria, which the bacteria were non-detectable in the washings containing both a porphyrin and a detergent.

Example 21

Example 20 was repeated, but this time the hands which had previously been washed with one of the solutions were immersed in PBS for 2 minutes, and the PBS was then analyzed for bacterial growth. The results obtained were consistent with those of Example 20.

Example 22

Example 21 was repeated, but instead of smearing the bacterial concentrate onto a human hand, it was smeared on a bovine bone, and left to incubate at 37°C on the bone before the bone was washed with one of the solutions of Example 19. At the end of the incubation periods, the bone was washed with equal volumes of PBS, and the washings were plated on nutrient agar plates to determine the CFU.

Example 23

Example 22 was repeated, using *S. epidermidis* instead of *S. aureus*. The results obtained were comparable to those obtained with *S. aureus*.

Example 24

The solutions of Example 19 were tested in mice, to treat surgical cuts infected with *S. aureus* concentrate. The cuts were of identical lengths, and the bacterial concentrate was taken from the same stock solution. The cuts were tested periodically for bacterial presence. The results were essentially the same as those obtained in Example 19 - 23.

All the above examples have been given for the purpose of illustration, and are not intended to limit the invention in any way. The invention extends to the synergistic activity obtainable using porphyrins together with antibiotic materials, in general, and is not intended to be limited to any specific porphyrin/antibiotic combination, or to any particular bacteria. As will be apparent to the skilled person, many different cream and liquid compositions

can be used, and many different combinations of porphyrins and other antibiotic materials, synergists and additives can be employed, to combat a variety of infections, all without exceeding the scope of the invention.

CLAIMS

1. A synergistic antibiotic composition, comprising as an antibiotic active ingredient a mixture of one or more porphyrins and at least one antibiotic compound.

2. A composition according to claim 1, wherein the antibiotic compound is a compound which inhibits protein synthesis.

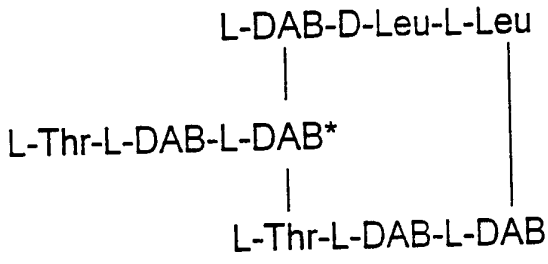
3. A composition according to claim 1, wherein the antibiotic compound is a compound which inhibits the synthesis of bacterial cell walls.

4. A composition according to claim 1, wherein the antibiotic compound is selected from among the group consisting essentially of penicillins, cephalosporins, tetracyclines, chloroamphenicol and macrolide antibiotics.

5. A composition according to claim 4, wherein the antibiotic is selected from the group consisting essentially of ampicillin, methicillin, tetracycline, erythromycin and chloroamphenicol, and mixtures of two or more of such compounds with or without additional antibiotic and/or antibiotic synergistic materials.

6. A composition according to any one of claims 1 to 5, wherein the porphyrin is selected from the group consisting essentially of deuteroporphyrin, hemin, hematoporphyrin, protoporphyrin, and meta-, para- or orto-hydroxy phenylporphyrin.

7. A composition according to any one of claims 1 to 6, further comprising a peptide of the formula:



(I)

wherein DAB* is a 2,4-diaminobutyric acid residue, and DAB is a 2,4-diaminobutyric acid residue containing a free 4-amino group.

8. A composition according to claim 7, wherein the peptide of Formula (I) is polymyxin B nonapeptide.

9. Use of a composition according to any one of claims 1 to 6, for the manufacturing of a pharmaceutical preparation for the treatment of infections caused by gram-positive bacteria.

10. Use of a composition according to claim 7 or 8, for the manufacturing of a pharmaceutical preparation for the treatment of infections caused by gram-negative bacteria.

11. A method of treating an infection in an animal, including humans, which infection is susceptible of treatment by a given antibiotic material or mixture of antibiotic materials, comprising administering to the animal in need thereof an amount lower than the effective amount of the said antibiotic

material or mixture of antibiotic materials, together with one or more porphyrin.

12. A method according to claim 11, wherein the antibiotic compound is a compound which inhibits protein synthesis.

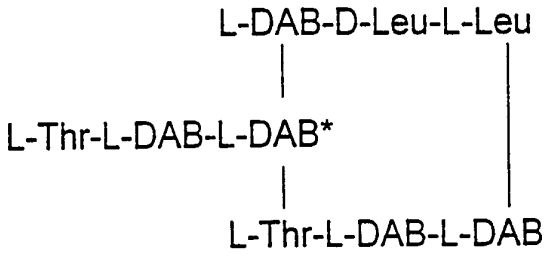
13. A method according to claim 10, wherein the antibiotic compound is a compound which inhibits the synthesis of bacterial cell walls.

14. A method according to claim 11, wherein the antibiotic compound is selected from among the group consisting essentially of penicillins, cephalosporins, tetracyclines, chloroamphenicol and macrolide antibiotics.

15. A method according to claim 14, wherein the antibiotic is selected from the group consisting essentially of ampicillin, methicillin, tetracycline, erythromycin and chloroamphenicol, and mixtures of two or more of such compounds with or without additional antibiotic and/or antibiotic synergistic materials.

16. A method according to any one of claims 11 to 15, wherein the porphyrin is selected from the group consisting essentially of deuteroporphyrin, hemin, hematoporphyrin, protoporphyrin, and meta-, para- or orto-hydroxy phenylporphyrin.

17. A method according to any one of claims 11 to 16, further comprising administering to the animal in need thereof, together with the mixture of porphyrin(s) and antibiotic material(s), also a peptide of the formula:



(I)

wherein DAB* is a 2,4-diaminobutyric acid residue, and DAB is a 2,4-diaminobutyric acid residue containing a free 4-amino group.

18. A method according to claim 17, wherein the peptide of Formula (I) is polymyxin B nonapeptide.

19. A method according to any one of claims 11 to 16, for the treatment of infections caused by gram-positive bacteria.

20. A method according to claim 18 or 19, for the treatment of infections caused by gram-negative bacteria.

21. A method according to claim 11, wherein the infection is caused by bacteria selected from among the group consisting essentially of Gram-Positive bacteria, Anaerobic Gram-Negative bacteria, *Streptococci sp., Diplococci sp., Staphylococci sp., P. aeruginosa, E. coli, Klebsiella sp.,* and *Proteus vulgaris.*

22. An antimicrobial composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, alone or in admixture

with conventional antibiotic materials and/or pharmaceutically acceptable or beneficial agents or additives, in a water-based cream composition.

23. A composition according to claim 22, wherein the porphyrin is deuteroporphyrin or hemin.

24. A composition according to claim 22 or 23, comprising a mixture of deuteroporphyrin and hemin.

25. A composition according to claim 22 or 23, comprising a mixture of hemin and tetracycline.

26. An antimicrobial cream composition according to claim 22, wherein the base cream comprises: paraffin, propylenglycol, polysorbat 40, cetylstearyl alcohol, vaseline and water.

27. An antimicrobial cream composition according to claim 22, wherein the base cream comprises: synthetic beeswax, glyceryl stearate, stearic acid, propylene glycol, alkylparaben(s), preservatives, and water.

28. Use of a cream composition according to any one of claims 22 to 27, for the treatment of infections caused by gram-positive bacteria.

29. Use of a cream composition according to any one of claims 22 to 27, for the treatment of flesh wounds.

30. Use according to claim 29, wherein the wound is a surgical cut.

31. Use of a cream composition according to any one of claims 22 to 27, for the sterilization of bones.

32. Use according to claim 31, wherein the bones are chest bones exposed during chest surgery.

33. Use according to any one of claims 29 to 32, further comprising irradiating the treated area with light.

34. A method of treating or preventing the occurrence of an infection caused by Gram-positive bacteria, comprising applying to the area to be treated a cream composition according to any one of claims 22 to 27.

35. A method according to claim 34, wherein each of the porphyrin(s) and of the antibiotic material(s) is present in an amount comprised between 5 and 100 μg/ml.

36. A cream composition for the treatment or the prevention of bacterial infections, essentially as described.

37. Use of a porphyrin for the manufacture of an antibacterial cream composition, essentially as described.

38. A sterilizing liquid composition, comprising as an active ingredient a porphyrin or a mixture of one or more porphyrins, in an aqueous solution, together with a surface-active agent.

39. A composition according to claim 38, wherein the porphyrin is deuteroporphyrin or hemin, or a mixture thereof.

40. A composition according to claim 38 or 39, wherein the surface-active agent is a detergent.

41. A composition according to claim 40, wherein the surface-active agent is selected from among Triton X-100, sodium dodecyl sulphate and sodium lauryl sulphate, or a mixture of two or more of such agents.

42. A composition according to any one of claims 38 to 41, wherein the porphyrin is present in a concentration of 1 to 100 µg/ml of sterilizing solution.

43. A composition according to any one of claims 38 to 42, wherein the surface-active agent is present in an amount of 0.5 - 5 wt%.

44. Use of a liquid composition according to any one of claims 38 to 43, for the sterilization of surfaces

45. Use according to claim 44, wherein the surface to be sterilized is the human hand.

46. Use according to claim 44, wherein the surface to be sterilized is a bone.

47. Use according to claim 46, wherein the bones are chest bones exposed during chest surgery.

48. Use according to any one of claims 44 to 47, further comprising irradiating the area to be sterilized with light.

49. Use according to any one of claims 44 to 48, for sterilizing areas infected with Gram-positive bacteria.

50. A sterilizing liquid composition, substantially as described.

51. Use of a sterilizing liquid composition, substantially as described.

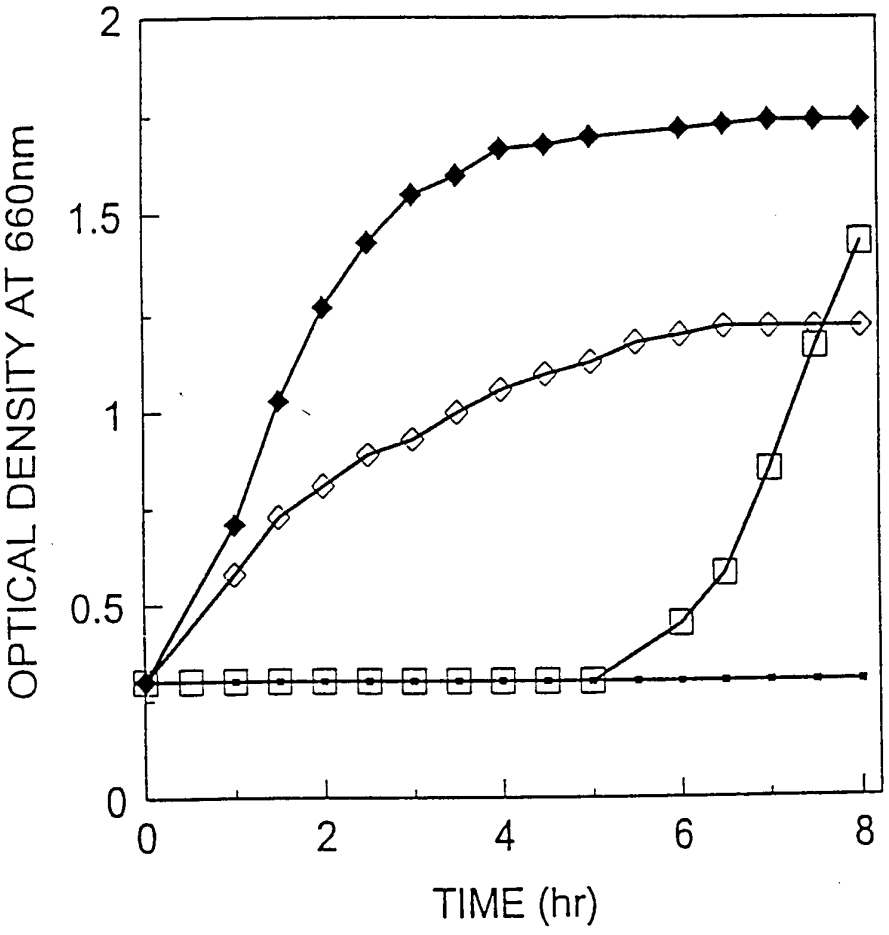


Fig. 1

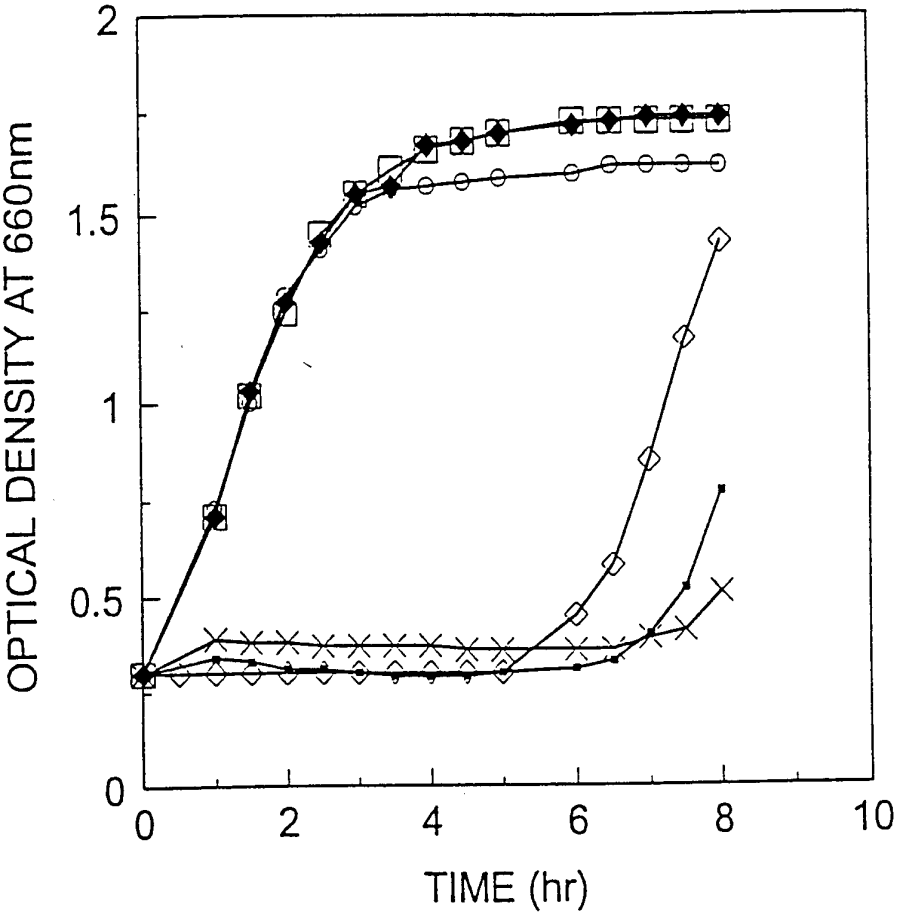


Fig. 2

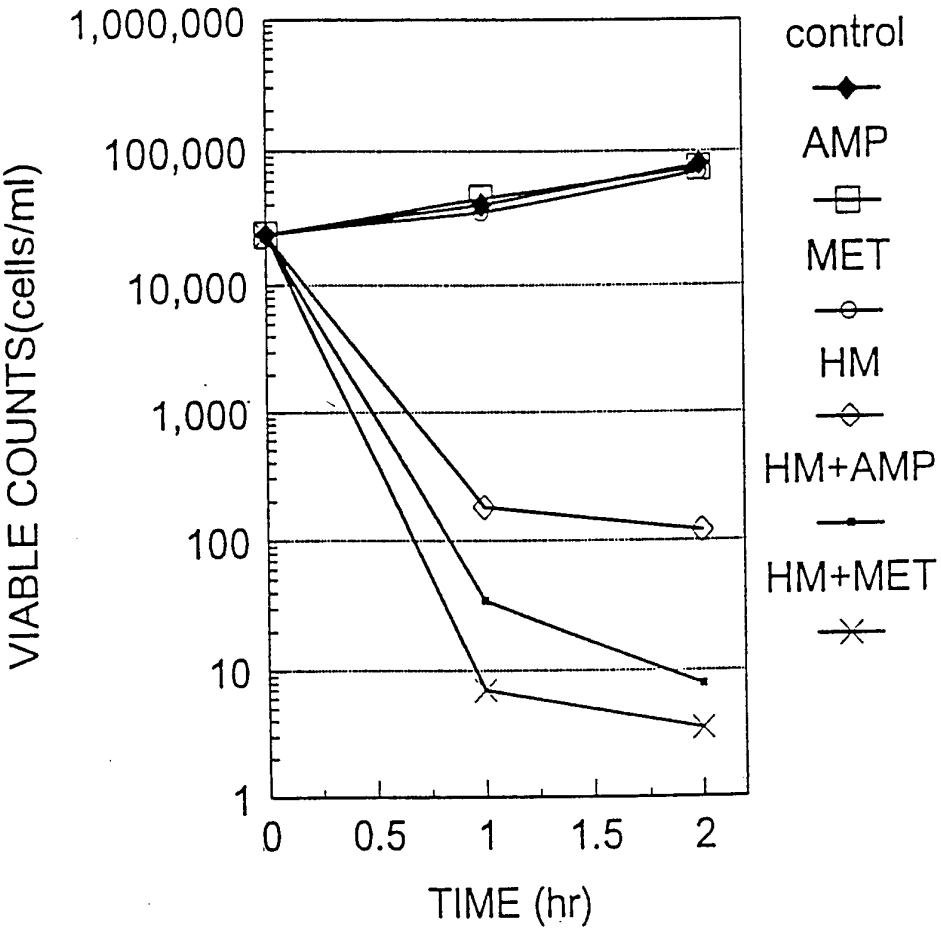

1,000,000
100,000
10,000
1,000
100
10
1
VIABLE COUNTS(cells/ml)
0
0.5
1
1.5
2
TIME (hr)
control
AMP
MET
HM
HM+AMP
HM+MET

Fig. 3

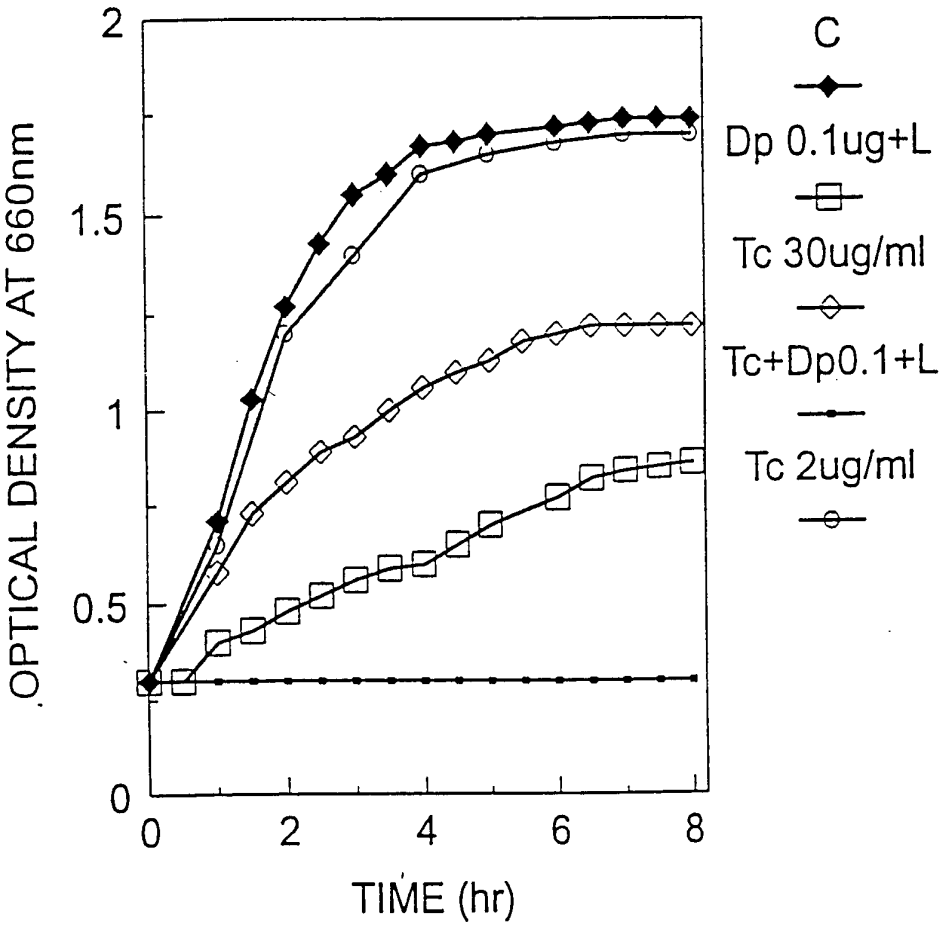

OPTICAL DENSITY AT 660nm
2
1.5
1
0.5
0
0
2
4
6
8
TIME (hr)
C
Dp 0.1ug+L
Tc 30ug/ml
Tc+Dp0.1+L
Tc 2ug/ml

Fig. 4

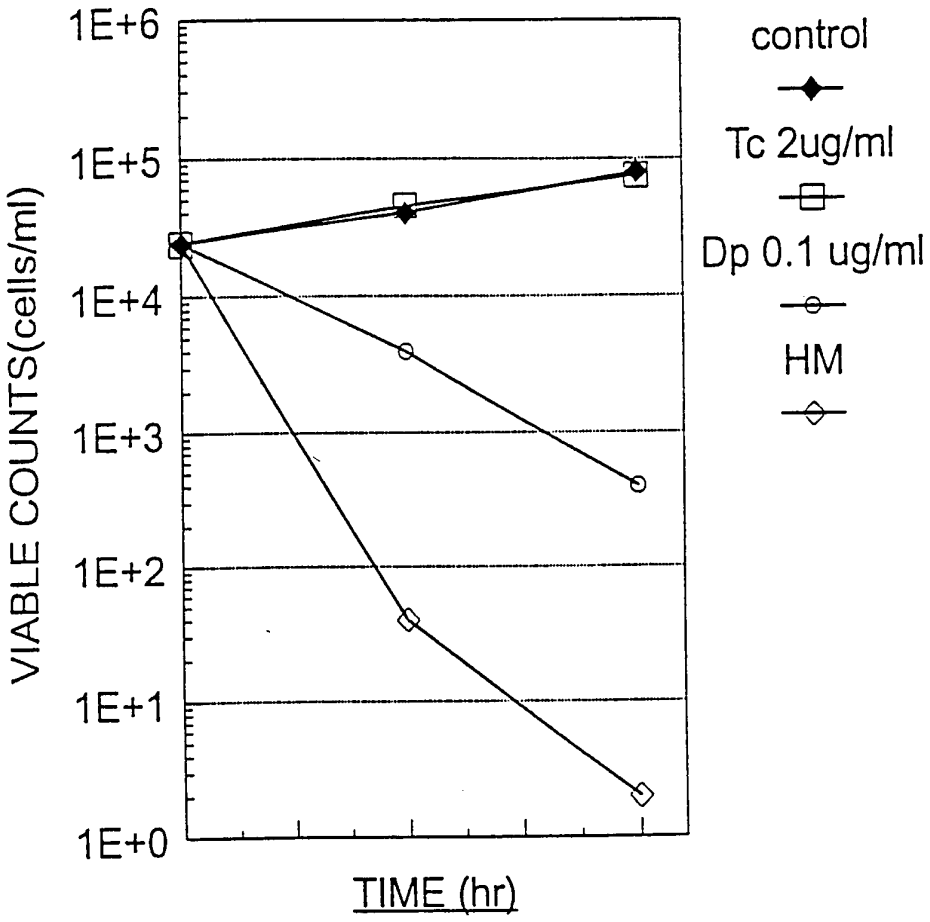

1E+6
1E+5
1E+4
1E+3
1E+2
1E+1
1E+0
VIABLE COUNTS(cells/ml)
TIME (hr)
control
Tc 2ug/ml
Dp 0.1 ug/ml
HM

Fig. 5

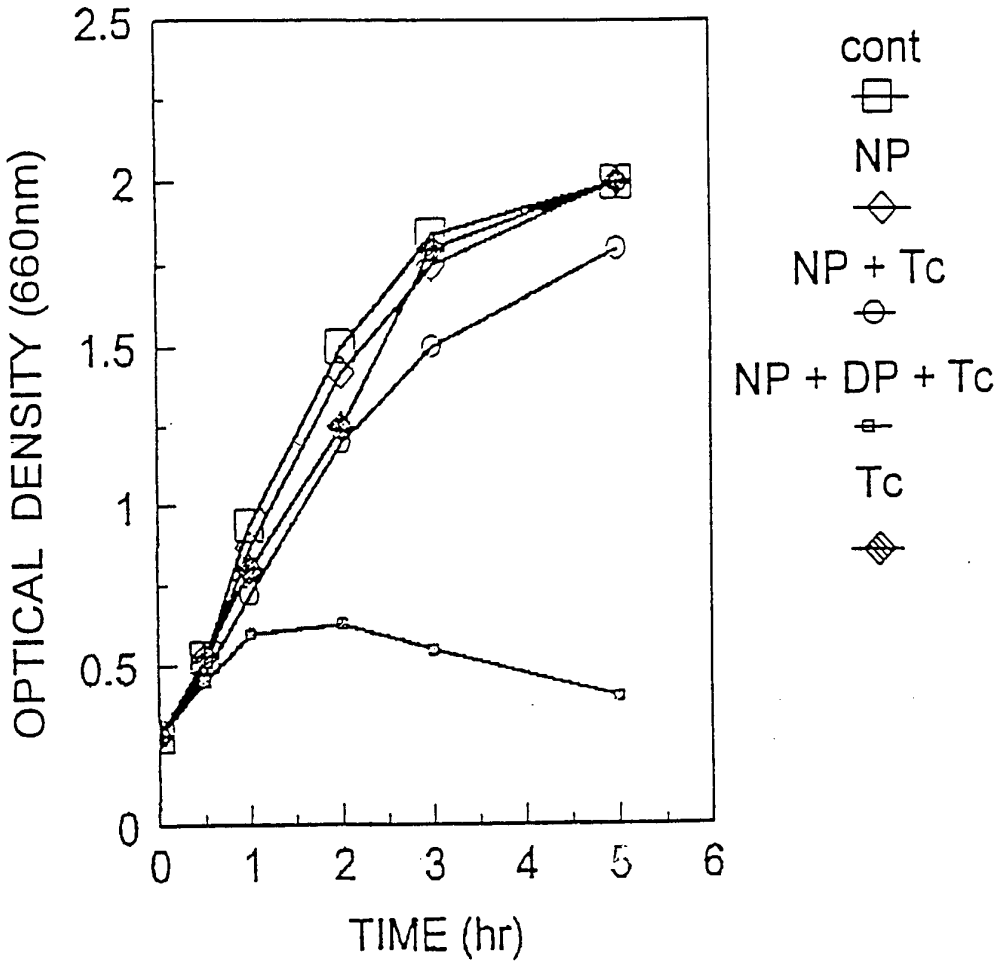

OPTICAL DENSITY (660nm)
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6
TIME (hr)
cont
NP
NP + Tc
NP + DP + Tc
Tc

Fig. 6A

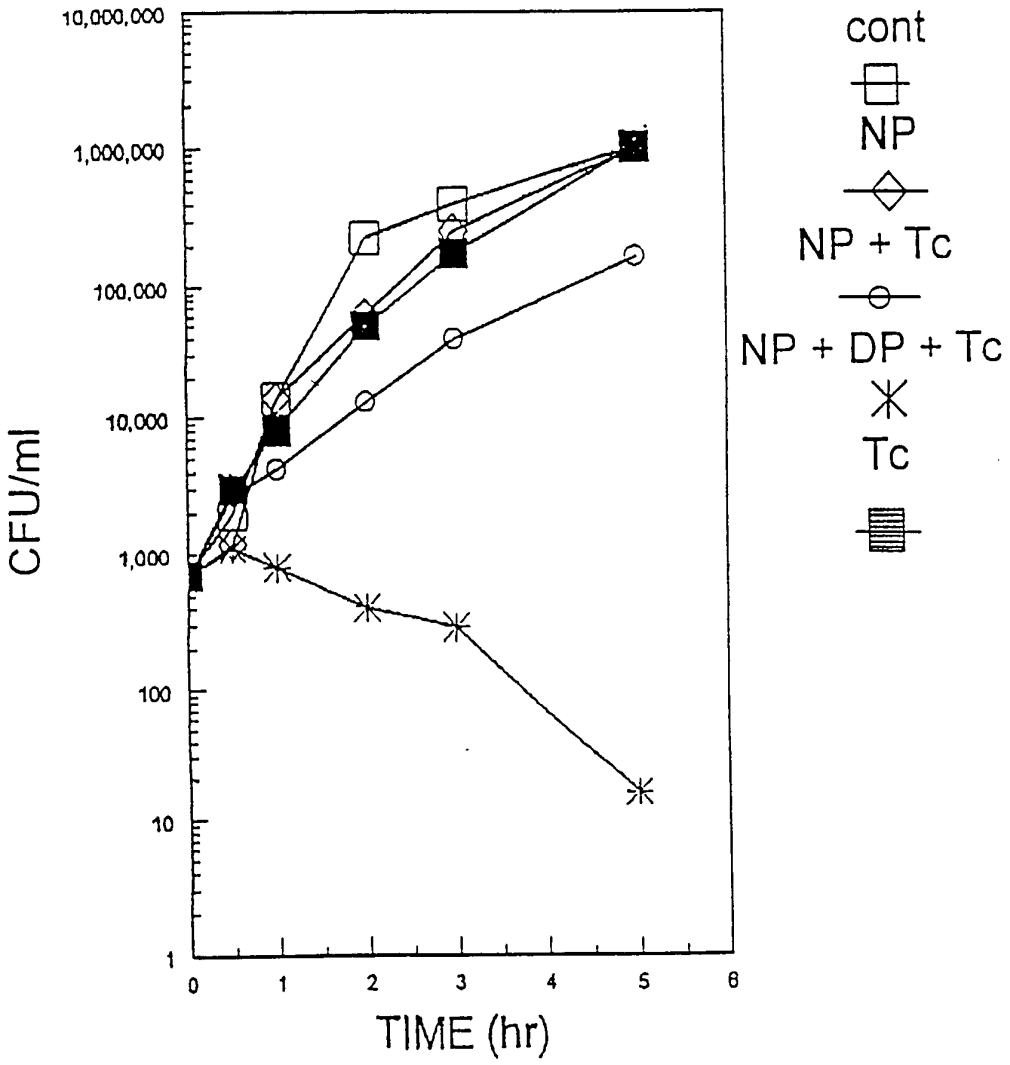

10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
CFU/ml
0
1
2
3
4
5
6
TIME (hr)
cont
NP
NP + Tc
NP + DP + Tc
Tc

Fig. 6B

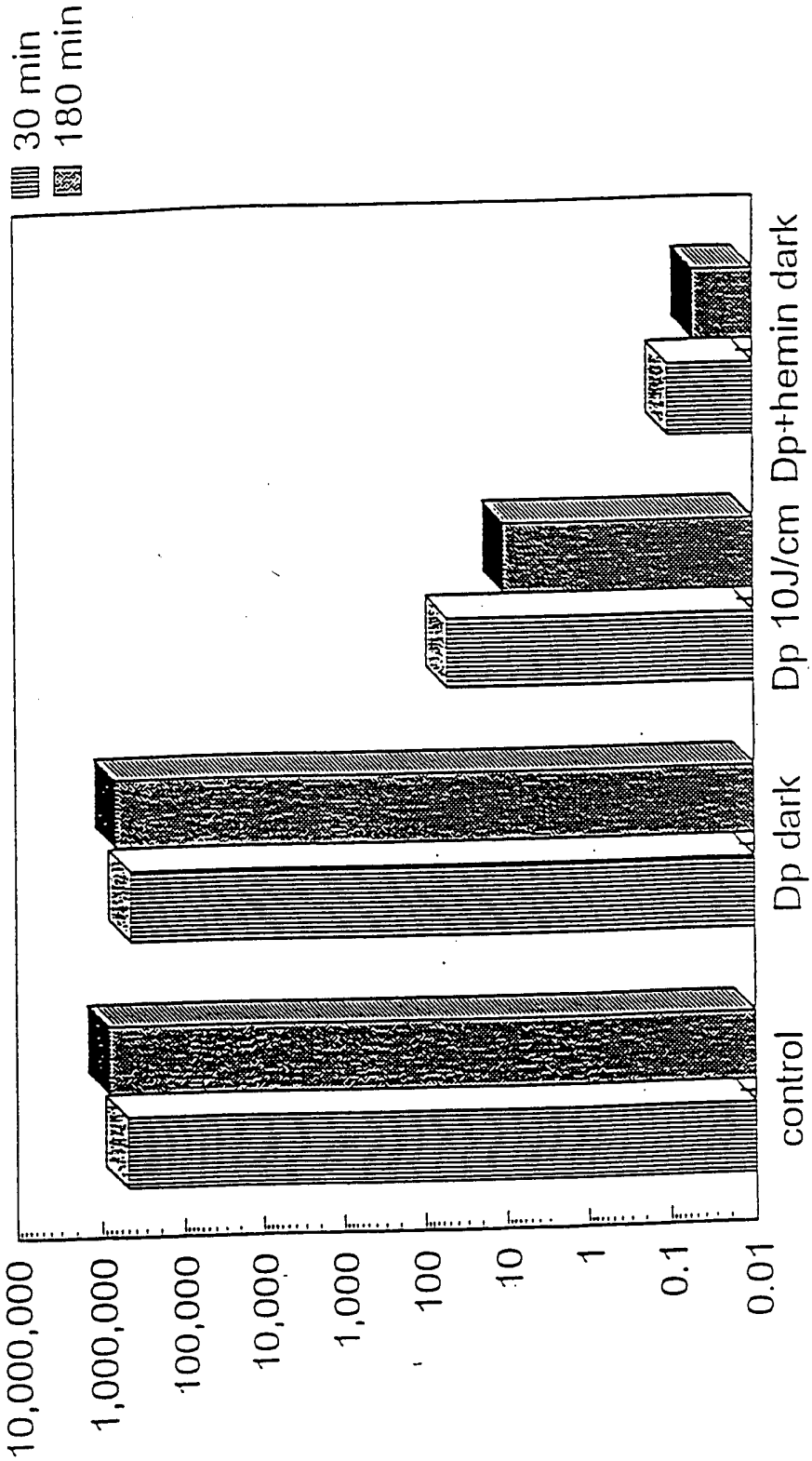

30 min
180 min
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
0.1
0.01
CFU X10E3/ml
control
Dp dark
Dp 10J/cm
Dp+hemin dark

Fig. 7

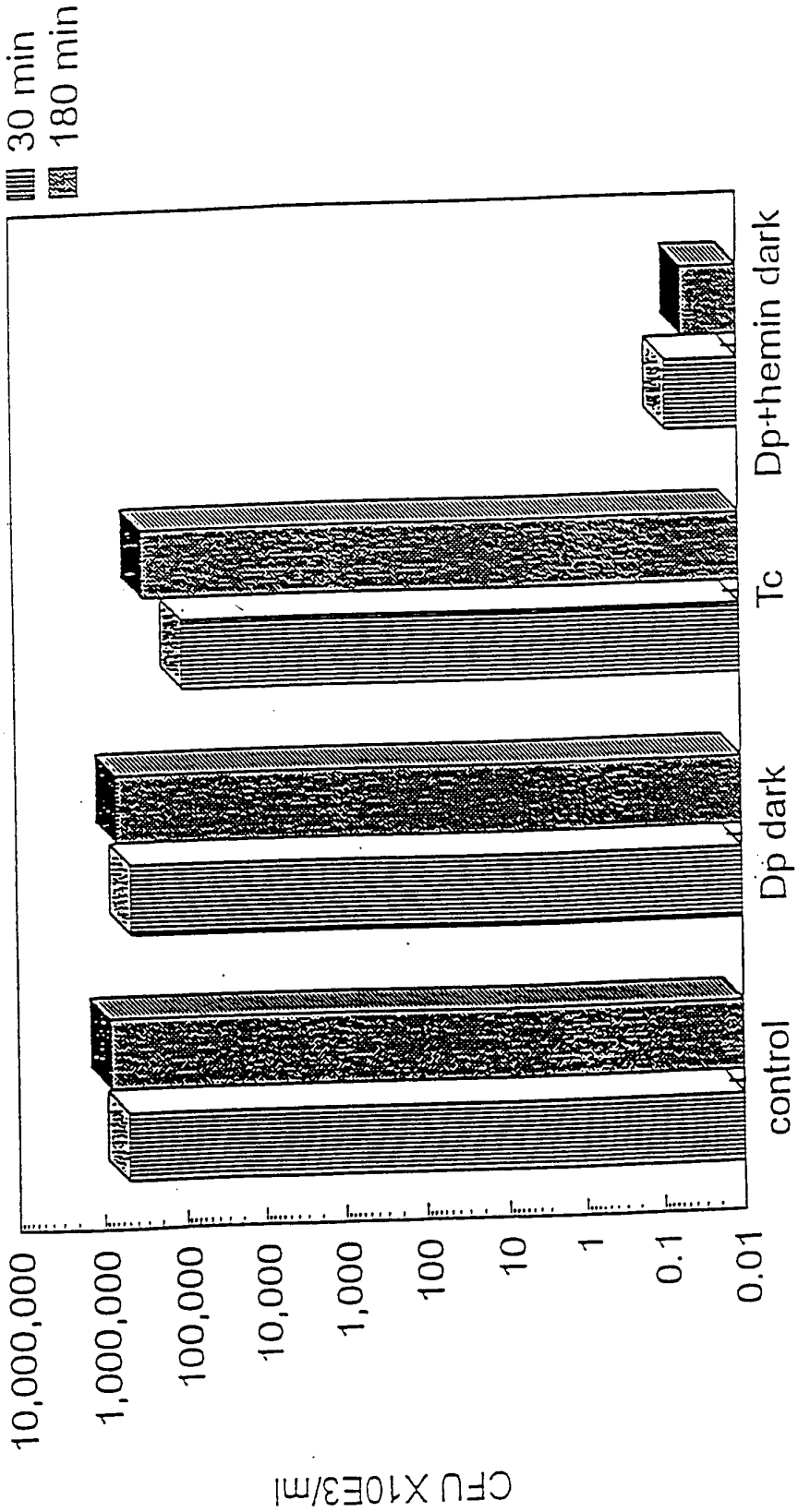

30 min
180 min
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
0.1
0.01
CFU X10E3/ml
control
Dp dark
Tc
Dp+hemin dark

Fig. 8

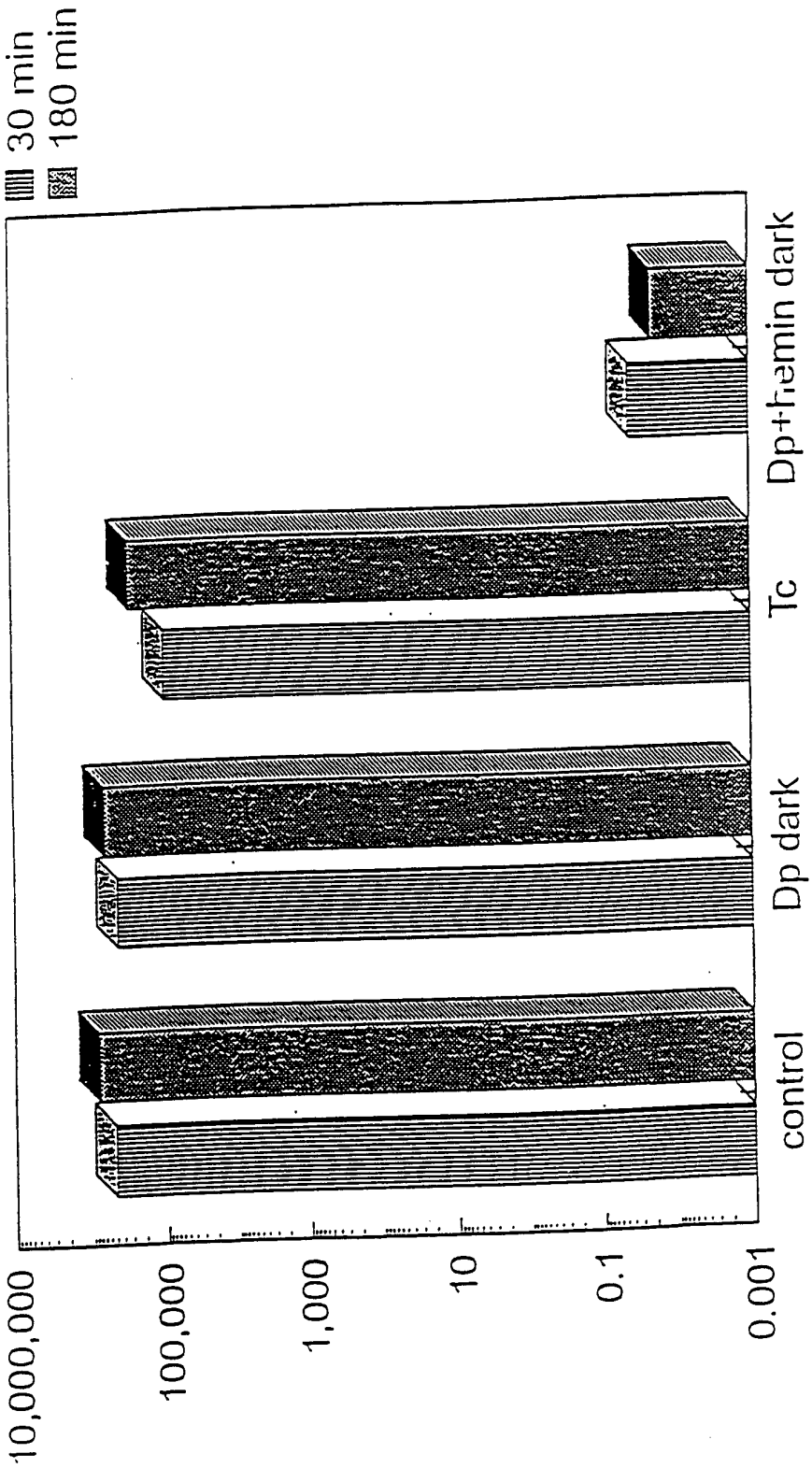

30 min
180 min
10,000,000
100,000
1,000
10
0.1
0.001
CFU X10E3/ml
control
Dp dark
Tc
Dp+hemin dark

Fig. 9

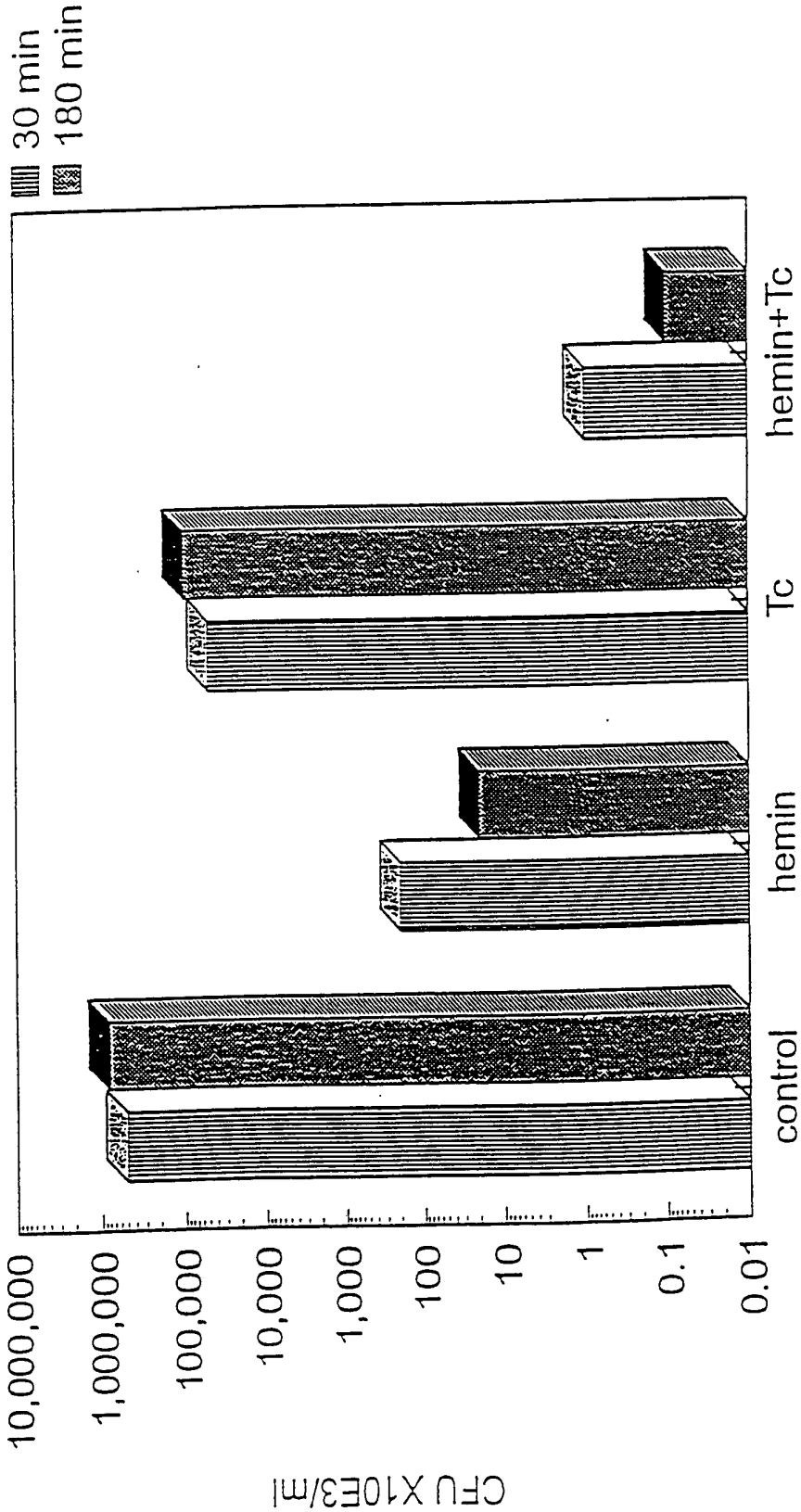

30 min
180 min
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
0.1
0.01
CFU X10E3/ml
control
hemin
Tc
hemin+Tc

Fig. 10

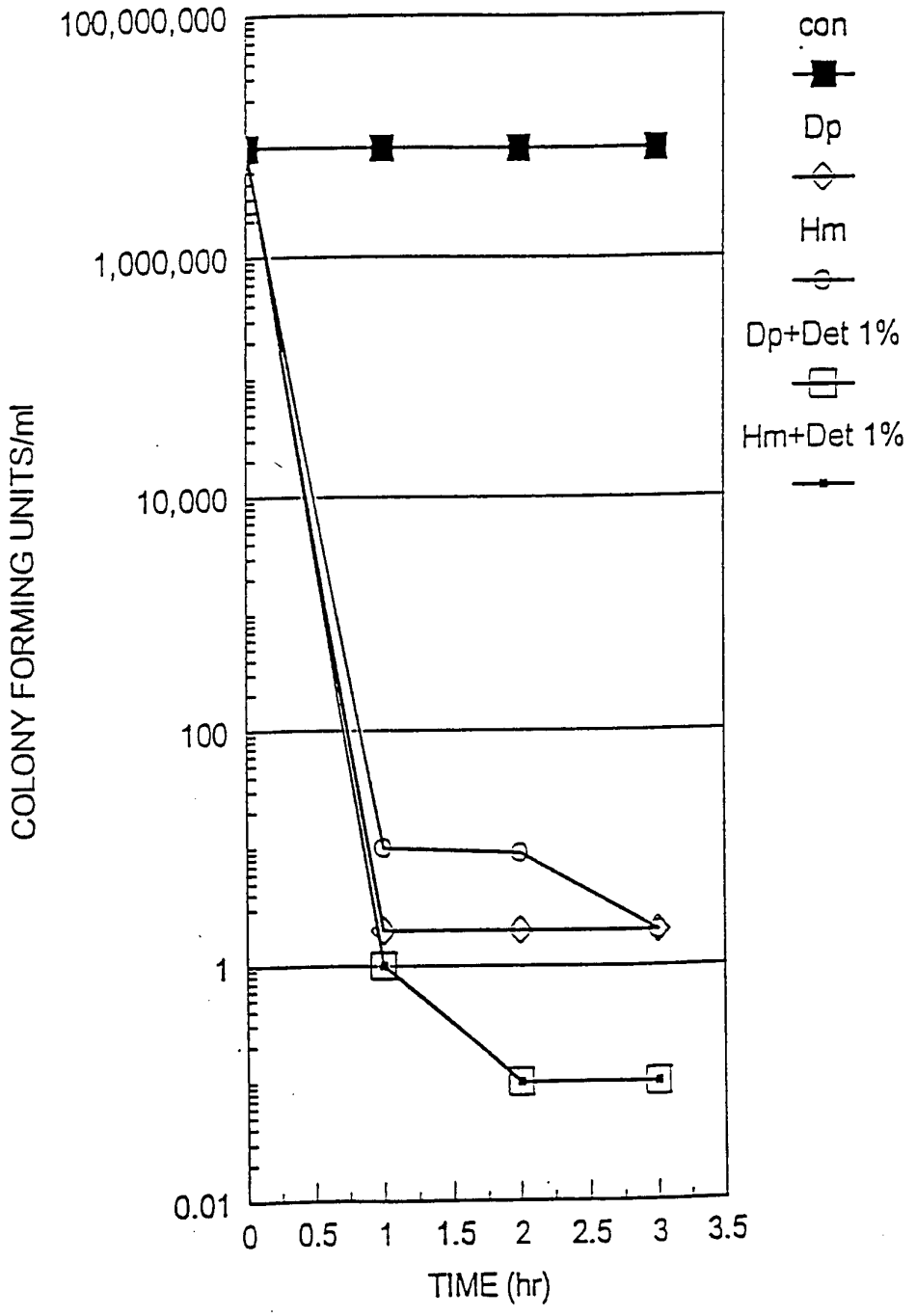

100,000,000
1,000,000
10,000
100
1
0.01
COLONY FORMING UNITS/ml
0
0.5
1
1.5
2
2.5
3
3.5
TIME (hr)
con
Dp
Hm
Dp+Det 1%
Hm+Det 1%

Fig. 11