

PATENT SPECIFICATION

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DRAWINGS ATTACHED.

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COMPLETE SPECIFICATION.

Improvements in or relating to Antibiotics and the Manufacture Thereof.

We, THE UPJOHN COMPANY, a Corporation organized and existing under the laws of the State of Delaware, United States of America, of 301 Herrietta Street, Kalamazoo, State of Michigan, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to an antibiotic in its crude and pure form and to a process for the production thereof. More particularly, this invention relates to a new antibiotic lincomycin formerly known as lincolnensin, to a process for the production thereof, and therapeutic compositions containing the same.

The antibiotic lincomycin is a biosynthetic product obtained as an elaboration product of a lincomycin-producing actinomycete. It is a basic compound which has the property of adversely affecting the growth of various micro-organisms, particularly Gram positive bacteria, and can be used either as the free base or as an acid addition salt, alone or in admixture with other antibacterial agents, to prevent the growth of or to reduce the number of micro-organisms present in various environments. For example, it is useful in wash solutions for sanitation purposes, as in the washing of hands and the cleaning

of equipment, floors, or furnishings/ of contaminated rooms or laboratories; it is also useful as an industrial preservative, for example, as a bacteriostatic rinse for laundered clothes and for impregnating paper and fabrics; and it is useful for suppressing the growth of sensitive organisms in plate assays, and other biological media. It can also be used as a feed supplement to promote the growth of mammals and birds, either alone or in admixture with other antibiotics.

The actinomycete used according to this invention for the production of lincomycin has been designated as *Streptomyces lincolnensis* var. *lincolnensis*. One of its strain characteristics is the production of the antibiotic lincomycin. A subculture of this variety can be obtained from the permanent collection of the Northern Utilization and Research Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. Its accession number in this repository is NRRL 2936. The NCIB accession number is 9413.

Streptomyces lincolnensis var. *lincolnensis* has a compact cream pink aerial growth, yellow-tan vegetative growth and is melanin positive. Spore chains are flexuous. Spores are smooth. Its growth characteristics on standard biological media and its carbon assimilation are given in the following tables.

[Price 4s. 6d.]



TABLE I.
Appearance on "Ektachrome"^{*}
(Registered Trade Mark)

Agar Medium	Surface	Reverse
1. Bennett's	Cream pink	Brown
2. Czapek's sucrose	Cream pink	Yellow tan
3. Maltose tryptone	Cream pink	Brown
4. Peptone iron	Trace cream pink	Brown
5. 0.1% Tyrosine	Trace cream pink	Trace brown
6. Casein starch	Cream pink	Yellow tan

* Dietz, A., Ektachrome transparencies as aids in actinomycete classification, *Annals of the N. Y. Academy of Science* 60: 152-154, 1954.

TABLE II.

*Assimilation of Carbon Compounds in Synthetic Medium**

D-xylose	+	Cellobiose	+	Salicin	(+)
D-arabinose	(+)	Raffinose	+	Na formate	(-)
Rhamnose	+	Dextrin	+	Na oxalate	(-)
D-fructose	+	Inulin	+	Na tartrate	(-)
D-galactose	+	Soluble starch	+	Na salicylate	-
D-glucose	+	Glycerol	+	Na acetate	(+)
D-mannose	+	Dulcitol	(-)	Na citrate	(+)
Maltose	+	D-mannitol	+	Na citrate	(+)
Sucrose	+	D-sorbitol	(-)		
Lactose	+	Inositol	+	Control	(-)

+ good growth, positive assimilation.

(+) slight growth, positive assimilation.

(-) slight growth, no assimilation.

- no growth.

* Pridham, T. G., and Gottlieb, D., "Assimilation of Carbon Compounds in Synthetic Medium", *J. Bact.* 56: 107-114, 1948.

TABLE III.

Cultural Characteristics.

Medium	Aerial	Vegetative	Other
35 Plain gelatin	None	Colorless dropping to base	Brown pigment. Complete liquefaction.
40 Nutrient gelatin	None	Colorless dropping to base	Brown pigment. Complete liquefaction.
Nutrient nitrate broth	White on surface ring	Colorless	Yellow tan pigment. No reduction.
45 Synthetic nitrate broth	White on surface pellicle	Colorless slightly flocculent throughout and at base	Trace yellow pigment. No reduction.
Litmus milk	Pink-white on surface ring	Blue	Peptonization. pH 7.5.

TABLE III—cont.
Cultural Characteristics.

	Medium	Aerial	Vegetative	Other
	Peptone-iron agar	Pink-white	Brown	H ₂ S darkening.
	Calcium malate agar	~~Cream-white~~	Cream	Malate solubilized. — pink
	Skin milk agar	Pink-white	Yellow	Yellow tan pigment. Casein hydrolyzed.
	Glucose asparagine agar	~~Cream-white~~	Yellow tan	Trace yellow pigment. — pink
10	Casein starch agar	Cream	Yellow	Starch hydrolyzed.
	Nutrient starch agar	Cream	Yellow	Starch hydrolyzed.
	Tyrosine agar	Cream	Brown	Brown tan pigment. Tyrosine solubilized.
15	Xanthine agar	Cream	Yellow	Yellow pigment. Xanthine solubilized.
	Maltose tryptone agar	Olive green	Brown	Tan pigment.
20	Bennett's agar	Cream	Brown	Tan pigment. Good growth and sporulation at 18, 24, 28°; fair at 37°; slight vegetative at 55°.
25	Czapek's sucrose agar	Cream	Olive tan	Yellow pigment. Good growth and sporulation at 18, 24, 28°; fair at 37°; slight vegetative at 55°.

The antibiotic of the invention is produced when the elaborating organism is grown in an aqueous nutrient medium under submerged aerobic conditions, and in a nutrient medium containing a carbon source, for example, an assimilable carbohydrate, and a nitrogen source, for example, an assimilable nitrogen compound or proteinaceous material. It is to be understood also that for the preparation of limited amounts surface cultures in bottles can be employed. Preferred carbon sources include glucose, brown sugar, sucrose, glycerol, starch, corn starch, lactose, dextrin or molasses. Preferred nitrogen sources include corn steep liquor, yeast, autolyzed Brewer's yeast with milk solids, soybean meal, cottonseed meal, corn meal, milk solids, pancreatic digest of casein, distiller's solubles, animal peptone liquors, meat and bone scraps. Mixtures of these carbon and nitrogen sources can be used advantageously. Trace metals, for example, zinc, magnesium, manganese, cobalt or iron, need not be added to the fermentation media

since tap water and unpurified ingredients are used as media components.

Production of the compound of the invention can be effected at any temperature conducive to satisfactory growth of the micro-organism, for example, between 18° and 40° C. and preferably between 26° and 30° C. Ordinarily, optimum production of the antibiotic is obtained in from about 2 to 10 days. The medium normally stays fairly close to neutral, or on the alkaline side, during the fermentation. The final pH is dependent, in part, on the initial pH of the culture medium which is advantageously adjusted to pH 6—8 prior to sterilization, and the buffers present, if any.

When growth is carried out in large vessels and tanks, it is preferable to use the vegetative form of the micro-organism for inoculation to avoid a pronounced lag in the production of the antibiotic and the attendant inefficient utilization of the equipment. Accordingly, it is desirable to produce a vegetative inoculum in a nutrient broth culture by

example

inoculating the broth culture with an aliquot from a soil or slant culture. When a young, active vegetative inoculum has thus been secured, it is transferred aseptically to large vessels or tanks. The medium in which the vegetative inoculum is produced can be the same as, or different from, that utilized for the production of the antibiotic as long as it is such that a good growth of the micro-organism is obtained.

The antibiotic of the invention is a nitrogenous base having the empirical formula $C_{18}H_{34}N_2O_6S$. It is monobasic having a pK_a^1 of 7.6 and under ordinary conditions is more stable in the protonated, that is, salt form. It is soluble in lower-alkanols, e.g., methanol, ethanol, isopropanol or the butanols; lower-alkyl esters of lower-alkanoic acids, e.g., ethyl acetate, *n*-butyl acetate or amyl acetate; lower-alkanones, e.g., acetone, methyl ethyl ketone or isopropyl *n*-butyl ketone; and chlorinated lower-alkanes, e.g., methylene chloride, chloroform or ethylene dichloride wherein the term lower denotes 1 to 8 carbon atoms inclusive. It has some solubility in water but can be extracted from aqueous solutions with water-immiscible solvents, e.g., *n*-butanol, *n*-butyl acetate, methyl ethyl ketone or methylene chloride.

An antibiotic lincomycin according to the present invention assays at least 20 mcg. per mg. of the pure antibiotic or an acid addition salt thereof.

In accordance with a preferred procedure for the recovery of the antibiotic of the invention, the whole beer is adjusted, if necessary or desirable, to a near neutral pH or below, suitably between pH 2 and pH 8 and filtered. A filter aid, for example, diatomite can be used. The pH of the filtered beer is then adjusted to place the compound in the non-protonated form. This is accomplished by neutralizing the solution with suitable base, for example, sodium hydroxide or sodium carbonate, to a pH greater than pH 7.5, advantageously to a pH from 9 to 11. The resulting solution is then extracted with a water-immiscible solvent for the antibiotic lincomycin and the antibiotic recovered from the solvent extract. If desired, the solvent phase can be acidified and the antibiotic recovered in the protonated form. This can be accomplished by precipitating the antibiotic as an insoluble salt or by extracting the solvent extract with an aqueous solution of an acid which forms a water soluble salt, for example, hydrochloric acid, sulfuric acid, phosphoric acid, and acetic acid. Advantageously, the latter is accomplished by adjusting the pH to less than 7.5, preferably from pH 2 to pH 6. The salt is then recovered by evaporation and/or crystallization.

If desired, the above extraction procedure can be repeated as necessary to effect desired

purification before the salt is recovered. Also a change of water-immiscible solvent can be utilized to effect further purification. For example, methylene chloride can be utilized to wash out impurities from the aqueous solutions of the salt form or to extract the free base from aqueous solutions of the non-protonated compound.

The antibiotic of the invention can also be recovered from the filtered beer by adsorption on cation exchange resins. Both the carboxylic and sulphonic acid types can be used. Suitable carboxylic acid resins include the polyacrylic acid resins obtained by the copolymerization of acrylic acid and divinylbenzene by the procedure given on page 87 of Kunin, *Ion Exchange Resins*, 2nd Ed. (1958), John Wiley and Sons, Inc. Carboxylic acid cation exchange resins of this type are marketed under the trade names Amberlite IRC-50 (Registered Trade Mark) and Zeo-karb (Registered Trade Mark) 226. Suitable sulphonic acid resins include nuclear sulphonated polystyrene resins cross-linked with divinylbenzene obtained by the procedure given in page 84 of Kunin, *supra*. Sulphonated cation exchange resins of this type are marketed under the trade names Dowex-50 (Registered Trade Mark), Amberlite IR-120 (Registered Trade Mark), Nalcite HCR, Chempro C-20, Permutit Q (Registered Trade Mark) and Zeocarb 225.

The protonated antibiotic is eluted from the resin with water at an acid pH, advantageously at a pH lower than the pK_a^1 of the cation exchange resin used. Satisfactory results are obtained with a pH of 1 to 6. The excess acid in the eluate is neutralized to pH 6 to 7 with NaOH or a strongly basic anion exchange resin so as to remove excess acid over that necessary to protonate the basic groups. Suitable anion exchange resins for this purpose are obtained by chloromethylating by the procedure given on pages 88 and 97 of Kunin, *supra*, polystyrene cross-linked, if desired, with divinylbenzene prepared by the procedure given on page 84 of Kunin, *supra*, and quaternizing with trimethylamine, or dimethylalkanolamine by the procedure given on page 97 of Kunin, *supra*. Anion exchange resins of this type are marketed under the trade names Dowex 2, Dowex 20, Amberlite IRA-400, Duolite A-102 and Permutit S-1, all of which are Registered Trade Marks.

The antibiotic of the invention can also be recovered from harvest beers and other aqueous solutions by adsorption on a surface active absorbent, for example, decolorizing carbon or decolorizing resins, and eluting the adsorbed material with a solvent for the antibiotic. Any of the solvents mentioned above can be used. A suitable decolorizing resin is Permutit DR (U.S. Patent 2,702,263).

The antibiotic of the invention can be



lincomycin



purified by successive transfers from protonated to non-protonated forms and vice versa, especially with intervening other types of treatments, as for example, solvent extractions and washings, recrystallization, and fractional liquid-liquid extractions. It can also be purified by conversion of the protonated or non-protonated compounds to less soluble in water forms, for example by reaction with helianthic acid, Reinecke's acid, azobenzene sulphonic acid or picric acid. The salts thus obtained can be used for the same purpose as the free base, or they can be converted back to the free base and then converted to other salts such as the hydrochloride, phosphate, and sulphate.

Fractional liquid-liquid extraction is accomplished in partition chromatographic columns or in countercurrent distribution apparatus, using such solvent systems as cyclohexane-methyl ethyl ketone-pH 10 buffer (7:3:2, by volume) and 2-butanol-water (1:1, by volume).

Recrystallization is accomplished by dissolving the crystalline salt in water, adding a water-miscible solvent, e.g., acetone, methanol, ethanol, or isopropanol, and cooling to induce or complete crystallization. The crystals are filtered and washed with water-solvent solution and, if desired, by an anhydrous solvent, and then vacuum dried.

The salts can be converted to the free base by neutralizing with an alkali or by contacting with an anionic resin, advantageously to pH 9 to 11. Specific acid salts can then be made by neutralizing the free base with the appropriate acid to below about pH 7.5, and advantageously to pH 2 to pH 6. Suitable acids for this purpose include hydrochloric, sulphuric, phosphoric, acetic, succinic, lactic, maleic and fumaric, methanesulphonic, benzenesulphonic, helianthic, Reinecke's, azobenzenesulphonic and picric acid.

The antibiotic of the invention is active against bacteria, for example, *Streptococcus lactis*, which cause the souring of milk, and can be used to prevent or delay souring of dairy products, for example, milk and cheese. Concentrations as low as 1.6 mcg./ml. can be used. The antibiotic has little oral toxicity. Its LD₅₀, in rats, the dose lethal to 50% of the animals, exceeds 3000 gm./kg. The antibiotic can also be used to inhibit Gram-positive sporeformer spreaders on agar plates when isolating molds, yeasts, Streptomycetes, and Gram-negative organisms. It can be used, for example, in the isolation of microorganisms from soil samples as well as in the isolation of Gram-negative organisms, for example, *Pseudomonas*, *Proteus*, and *Escherichia coli*, from mixed infections in the presence of Staphylococci and/or Streptococci.

The following Examples 1 to 7 are illustrative of the process for the preparation of

the characterised products of the present invention. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1.

A. A soil slant of *Streptomyces lincolnensis* var. *lincolnensis*, NRRL 2936 was used to inoculate a series of 500-ml. Erlenmeyer flasks each containing 100 ml. of seed medium consisting of the following ingredients:—

Yeastolac*	...	...	10 grams	
Glucose monohydrate	...	...	10 grams	
N-Z-Amine B*	...	...	5 grams	
Tap water qs	...	...	1 liter	80

*Yeastolac is a protein hydrolysate of yeast cells and N-Z-Amine B is Sheffield's enzymatic digest of casein.

The seed medium presterilization pH was 7.3. The seed was grown for two days at 28° C. on a Gump rotary shaker operating at 250 r.p.m.

B. A 5% inoculum of the seed described above (5 ml.) was added to each of thirty 500-ml. Erlenmeyer flasks each containing 100 ml. of the following fermentation medium:—

Glucose monohydrate	...	...	20 grams	
Molasses	...	...	20 grams	
Corn Steep Liquor	...	...	20 grams	95
Wilson's Peptone				
Liquor 159*	...	...	10 grams	
Calcium Carbonate	...	...	4 grams	
Tap water qs	...	...	1 liter	

*Wilson's Peptone liquor 159 is a preparation of enzymatically hydrolyzed proteins from animal origin.

The pH of the medium after sterilization was 7.0. The shake flasks were harvested after four days of fermentation at 28° C. on a Gump rotary shaker at 250 r.p.m. The harvested beer was at pH 8.6.

C. The whole beer (2430 ml.) was filtered at harvest pH (pH 8.6) using a filter aid as required. The cake was washed with $\frac{1}{3}$ volume (490 ml.) of water. The wash was added to the filtered beer and the cake discarded. The clear beer was adjusted to pH 10 with 50% aqueous NaOH solution and extracted twice with $\frac{1}{3}$ volume of 1-butanol. The extracts were combined and the spent beer discarded. To the butanol extract was added $\frac{1}{2}$ volume of water. The water-butanol mixture was adjusted to pH 2.0 under constant agitation with concentrated sulphuric acid (95%). The mixture was allowed to equilibrate and the phases were separated. The extract phase was discarded. The

aqueous phase was adjusted to pH 10 with 50% aqueous NaOH solution and extracted twice with $1/3$ volume of 1-butanol. The combined 1-butanol extracts were washed with $1/10$ volume of water. The washed 1-butanol extracts were concentrated *in vacuo* to a dried residue (RES-30) having the characteristic papergram pattern shown in Figure 1 of the accompanying drawings.

The solvent systems were as follows:—

- I 1-butanol-water (86:16 v/v) 16 hrs.
 II 1-butanol-water (84:16 v/v) plus 0.25% *p*-toluene-sulphonic acid w/v 16 hrs.

- III 1-butanol-acetic acid-water (2:1:1 v/v) 16 hrs.
 IV 1-butanol-water (86:16 v/v) plus 2% piperidine (v/v) 16 hrs.
 V 1-butanol-water (4:96 v/v) 5 hrs.
 VI 1-butanol-water (4:96 v/v) plus 0.25% *p*-toluene-sulphonic acid (w/v) 5 hrs

The long tailing in solvent system V is characteristic of pure antibiotic lincomycin, but sometimes does not occur with beers or impure preparations.

The material balance is given in the following table:—

Fraction	Volume (ml.)	Assay* (Biounits/ml.)	Total Biounits
Whole beer	2430	28.5	61,255
Clear beer + wash	2020	20	40,400
1st butanol extract	1380	40	55,200
Spent beer	1890	0.6	1,134
Aqueous extract	770	50	38,500
Spent butanol	1360	1.3	1,768
2nd butanol extract	635	62	39,370
Spent aqueous	640	1.2	768
Dried Prep (RES-30)	268.5 mg.	140 biounits/mg.	37,590

*Assayed against *Sarcina lutea* on agar buffered to pH 6—8 with pH 7.0 Phosphate buffer (0.1 M). A unit volume (0.08 ml.) of solution containing the material to be assayed is placed on a 12.7 mm. assay disc which is then placed on an agar plate seeded with the assay micro-organism. A biounit is that amount of material per ml. which, when contained in one ml. of solution, gives a zone of inhibition of 20 mm. when a unit volume of solution is assayed as above under standard microbiological conditions.

This preparation gave almost complete protection at 5 mg./kg. (subcutaneously) against *Streptococcus hemolyticus* infected mice. This was the lowest level tested. In mice infected with *Staphylococcus aureus*, the CD50 was 23.8 (17.0—30.6) mg./kg. (subcutaneously).

This preparation had the following *In vitro* spectrum.

TEST ORGANISMS	MIC (γ /ml.)*
<i>Diplococcus pneumoniae</i>	<1
<i>Escherichia coli</i>	1000
<i>Klebsiella pneumoniae</i>	500
<i>Pasteurella multocida</i>	8
<i>Proteus vulgaris</i>	1000
<i>Pseudomonas aeruginosa</i>	>1000
<i>Salmonella paratyphi</i>	>1000
<i>Salmonella pullorum</i>	250
<i>Salmonella typhi</i>	500
<i>Staphylococcus aureus</i>	<1
<i>Staphylococcus albus</i>	<1
<i>Streptococcus fecalis</i>	<1
<i>Streptococcus hemolyticus</i>	<1
<i>Streptococcus viridans</i>	<1
<i>B. Subtilis</i>	64

*Minimum inhibitory concentration at 2-fold dilutions in brain-heart broth.

EXAMPLE 2.

A. FERMENTATION.

Two 250-liter fermentations were conducted in the following manner:—

a. Seed inoculum.

A 40 liter fermentor containing 20 liters of the following sterile medium (pH 7.2)

Glucose monohydrate ...	10 g./liter
Wilson's peptone liquor No. 159 ...	10 g./liter
Corn steep liquor ...	10 g./liter
Pharmamedia* ...	2 g./liter
Lard oil ...	2 ml./liter
Tap water ...	Balance

*Pharmamedia is an industrial grade of cottonseed flour produced by Traders Oil Mill Co., Fort Worth, Texas,

was inoculated with 100 ml. of a preseed inoculum, prepared according to Example 1, Part A, and grown at 28° C. for two days while agitating with a stirrer at 400 r.p.m. and aerating at the rate of ten standard liters of air per minute.

b. Fermentation.

Each of two 380-liter fermentors contain-

ing 250 liters of the following sterile medium (pH 6.45)

Starch*	...	20 g./liter
Black strap molasses	...	20 g./liter
Corn steep liquor	...	20 g./liter
Wilson's peptone liquor		
No. 159	...	10 g./liter
Calcium carbonate	...	4 g./liter
Lard oil	...	5 g./liter
Tap water	...	Balance

*Glucose monohydrate can be used to replace all or part of the starch

was inoculated with 12 liters of the seed inoculum and fermented at 28° C. for 5 days while agitating at 280 r.p.m. and aerating at the rate of 100 standard liters of air per minute. In one fermentor 700 ml. of sterile lard oil was added during the fermentation to control foaming and in the second, 900 ml. of sterile lard oil was added. At the end of the fermentation (114 hrs.), 250 liters of beer was harvested from first fermentor (pH 7.9; assay, 24 biounits/ml.) and 210 liters from the second fermentor (pH 7.9; assay, 43 biounits/ml.).

B. EXTRACTION.

The whole beer from the first fermentor was adjusted from a harvest pH 7.9 to pH 6.7 with 70 ml. of concentrated sulphuric acid and filtered using 4% filter aid. The filter cake was washed with $\frac{1}{10}$ volume of water, based on the whole beer, and added to the clear beer. The clear beer (250 liters; assay, 20 biounits/ml.) was adjusted to pH 10 with 300 ml. of 50% sodium hydroxide solution, and extracted two times with $\frac{1}{3}$ volume of 1-butanol. The combined 1-butanol extracts (160 liters; assay 28 biounits/ml.) were mixed with $\frac{1}{2}$ volume of water (80 liters) and adjusted to pH 2 with 50 ml. of concentrated sulphuric acid. The aqueous extract (110 liters; assay, 36 biounits/ml.) was separated off, adjusted to pH 10.1 with 120 ml. of 50% aqueous sodium hydroxide solution and extracted two times with $\frac{1}{3}$ volume of 1-butanol. The combined butanol extracts (80 liters; assay, 48 biounits/ml.) was washed with $\frac{1}{10}$ volume of water. The washed butanol extract was freeze-dried to give about 21 g. of dried preparation (DEG) 54-11), assaying 145 biounits/mg.

The procedure above was repeated on the whole beer of the second fermentation to give 105 g. of dried preparation (HRV 134-11) assaying 125 biounits/mg.

C. PURIFICATION.

Preparation DEG 54-11 and 24.31 g. of preparation HRV 134-11 were combined (combined weight 45.31 g.—21 g. of DEG

54-11 and 24.31 g. of HRV 134-11/ assay, 177 biounits/mg.) and dissolved in 252 ml. of water. The solution was adjusted to pH 2.0 with concentrated sulphuric acid and a brown precipitate was removed by filtration. The filtrate was extracted once with 250 ml. of methylene chloride to remove impurities. The aqueous layer was adjusted to pH 5.0 with 50% aqueous sodium hydroxide and extracted once with 250 ml. of methylene chloride. The methylene chloride extracts removed 3.6 grams of impurities at a potency of 12 biounits/mg.

The aqueous layer was adjusted to pH 10.2 with 50% aqueous sodium hydroxide and extracted 5 times with 250 ml. portions of methylene chloride. One hundred ml. of water was added to the combined extracts and the methylene chloride removed *in vacuo*. The resulting aqueous solution was freeze dried to give 32 grams of dry preparation (MEB-2) assaying 232 biounits/mg.

D. CRYSTALLIZATION.

Further purification of preparation MEB-2 was accomplished by use of a partition column. The column was prepared and developed as follows: A solvent system consisting of cyclohexane, methyl ethyl ketone, and pH 10 buffer (70:30:20 by volume) was thoroughly mixed and equilibrated. (The pH 10.0 buffer was made by adding sufficient NaHCO_3 to a 0.2 M Na_2CO_3 solution to adjust the pH to pH 10.0). One hundred grams of diatomite was slurried in upper phase of the system described above; forty ml. of lower phase was added and the whole slurry homogenized. This solvent-diatomite mixture was poured into a glass column (1½" inside diameter) and packed to a constant height using 2 p.s.i. gauge of air pressure. The feed for the column, 2.5 g. of preparation MEB-2, was dissolved in 2 ml. of lower phase and then homogenized with upper phase and placed onto the top of the column bed. The column was developed with upper phase at a rate of approximately 2 ml./minute. Two hundred 20-ml. fractions were collected. Fractions 1 through 85 were inactive; fractions 90 through 200 all possessed about the same activity.

Fractions 100 through 200 were pooled and concentrated *in vacuo* (less than 50° C.) to dryness. The residue was dissolved in 20 ml. of water and the pH was adjusted to pH 2.0 using concentrated hydrochloric acid. The aqueous solution was extracted 2 times with 10 ml. of 1-butanol and the aqueous layer was distilled *in vacuo* at less than 50° C. to 10 ml. to remove dissolved 1-butanol. Acetone (50—60 ml.) was added slowly to the aqueous concentrate and crystallization started. The aqueous acetone solution stood for 30 minutes at room temperature. The crystals were filtered off and



vacuum dried to a constant weight. There was thus obtained 388 mg. of lincomycin hydrochloride crystals (MEB 12) assaying 138 biounits/mg., having a melting point of

- 5 145—147° C., an optical rotation $[\alpha]_D^{25}$ = +133 (C,=1% in water), no UV absorption maxima (220 to 400 millimicrons), and the following characteristic IR absorption.
- | Group | Bands cm^{-1} |
|----------|---|
| 10 OH/NH | 3500(shoulder), 3400, 3340, 3240, 3150, 3060. |
| | 6 μ region 1690, 1675, 1600, 1590. |
| 15 | Other bands: 1315, 1305, 1276, 1265, 1233, 1155, 1140, 1115, 1100, 1093, 1078, 1042, 990, 985, 970, 875, 793. |

EXAMPLE 3.

- A solution of 5 grams of the amorphous material MEB-2 in 50 ml. of water was adjusted with hydrochloric acid to pH 2.0 (1 ml. of concentrated HCl) and extracted twice with 25 ml. portions of 1-butanol. The aqueous phase was then concentrated *in vacuo* at less than 50° C. to a volume of 25 ml. Four hundred ml. of acetone was added and the mixture was cooled to -5° F. The resulting crystals were removed by filtration, washed with acetone, and dried *in vacuo* to a constant weight yielding 3.46 g. of lincomycin hydrochloride crystals (MEB-13) assaying 129 biounits/mg., having a melting point of 138—145° C., an optical rotation $[\alpha]_D^{25}$ = +144 (C,=1% in water), and no UV absorption, maxima.

EXAMPLE 4.

- A solution of 23 grams of amorphous material MEB-2 in 100 ml. of water was adjusted with 4.1 ml. of concentrated hydrochloric acid to pH 1.8—2.0 and the solution was extracted with two 100 ml. portions of 1-butanol. The aqueous layer was then concentrated *in vacuo* at less than 50° C. to a volume of 50 ml. Seven hundred and fifty ml. of acetone was added and the mixture was cooled to -5° F. The crystals were removed by filtration, washed with acetone, and vacuum dried to a constant weight yielding 14.0 g. of lincomycin hydrochloride crystals (MEB-14) assaying 120 biounits/mg., having the melting point of 145—146° C., an optical rotation $[\alpha]_D^{25}$ = +139 (C,=1% in water), and no UV absorption, maxima.

EXAMPLE 5.

- Following the procedure of Example 2, parts A, B, and C, 12.7 g. of preparation

MEB-147 assaying 270 biounits/mg. was obtained. After an unsuccessful effort to purify this material in a Craig countercurrent distribution apparatus charged with equal volumes of the equilibrated phases of a butyl acetate:water solvent system, the Craig fractions were pooled and concentrated to 10 ml. of aqueous solution (pH 9.1; assay, 16 biounits/mg.; solids, 82.57 mg./ml.; total solids, 8.26 g.). The pH was adjusted to pH 2.3 with 1.9 ml. of concentrated hydrochloric acid. The aqueous solution was extracted twice with 100 ml. portions of MEK (methyl ethyl ketone). The MEK extracts removed 471 mg. of impurities assaying 41 biounits/mg. The aqueous layer was stripped of the MEK by concentrating *in vacuo*. To the aqueous concentrate (40 ml.) was added 600 ml. of acetone. The solution was chilled to -5° F. and the crystals which separated were recovered by filtering, washed with acetone, and vacuum dried to constant weight. There was thus obtained 7.4 g. of lincomycin hydrochloride crystals (MEB 19-1) having a melting point of 139—142° C., an optical rotation $[\alpha]_D^{25}$ of +138 (C,=1% in water), and no UV absorption, maxima.

EXAMPLE 6.

Preparations MEB 13, MEB 14, and MEB 19-1 were pooled (total of 24.86 g.) and recrystallized from aqueous acetone by dissolving in 125 ml. of water and adding 1000 ml. acetone to effect crystallization. There was thus obtained 19.2 g. of lincomycin hydrochloride crystals (MEB 20) having an optical rotation $[\alpha]_D^{25}$ = +122 (C,=1% in water), an equivalent weight of 455, and the following elemental analysis.

Analysis

Calculated for $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S}\cdot\text{HCl} (\frac{1}{2} \text{H}_2\text{O})$
C, 47.83; H, 8.03; N, 6.20;
O, 23.01; S, 7.09; Cl, 7.84.

Found: C, 47.95; H, 7.81; N, 6.07;
O, 22.93 (by difference);
S, 7.21; Cl, 9.97; ash, 0.06.

Preparation MEB 20 has the following *in vivo* effectiveness in mice.

Organism	CD50 (mg./kg.)	CD50 (mg./kg.)	
	Subcutaneously	Oral	
<i>Streptococcus hemolyticus</i>	0.81	3.8	
<i>Diplococcus pneumoniae</i>	20	145	110
<i>Staphylococcus aureus</i>	7.1	16	

The CD50 is the dose effective to give 50% survival. The preparation has the following *in vitro* spectrum.





TEST ORGANISMS	MIC (γ/ml.)*	
	BHI	PYE
<i>Diplococcus pneumoniae</i>	0.4	NG
<i>Escherichia coli</i>	>100	>100
<i>Klebsiella pneumoniae</i>	>100	50
<i>Pasteurella multocida</i>	0.4	0.1
<i>Proteus vulgaris</i>	>100	>100
<i>Pseudomonas aeruginosa</i>	>100	>100
<i>Salmonella paratyphi</i>	>100	>100
<i>Salmonella pullorum</i>	100	>100
<i>Salmonella typhi</i>	>100	>100
<i>Staphylococcus aureus</i>	0.2	0.8
<i>Staphylococcus albus</i>	0.1	0.4
<i>Streptococcus fecalis</i>	0.8	0.8
<i>Streptococcus hemolyticus</i>	0.4	0.4
<i>Streptococcus lactis</i>	0.4	—
<i>Clostridium perfringens</i>	0.4	—
<i>Clostridium tetani</i>	1.5	—
<i>Bacillus subtilis</i>	25	50

*Minimum inhibitory concentration by two fold dilutions in brain-heart infusion broth (BHI) and peptone-yeast extract broth (PYE). End point at 20 hrs., 37° C. NG=no growth of the control.

Two grams of this preparation (MEB-20) was dissolved in 100 ml. of water and 100 ml. of 2-butanol. The starting material was placed in tubes 0—19 of a Craig counter-current distribution apparatus and distributed through 500 transfers. The distribution was analyzed by running solid determinations, and an excellent agreement with the theoretical curve was obtained. Tubes from 100—140 were pooled.

The pool from tubes 100—140 was concentrated to a volume of 10 ml., filtered through diatomite, and concentrated further to 5 ml. Fifty ml. of reagent grade acetone was added whereupon crystallization began. The mixture was chilled to -5° F. and the crystals were collected, washed with acetone, and vacuum dried to a constant weight. The

yield was 1.42 grams of lincomycin hydrochloride crystals (MEB 30) having a melting point of 135—140° C., an optical rotation $[\alpha]_D^{25} = +137^\circ$ (C=1% in water), no UV absorption, an equivalent weight of 454.7 pKa¹ of 7.60, a characteristic papergram pattern as shown in Figure 2 of the accompanying drawings, a characteristic IR absorption at the following frequencies expressed in reciprocal centimeters and as shown in Figure 3 of the accompanying drawings, where the top curve represents the absorption curve for the mineral oil mill and the lower and more variable curve represents the antibiotic suspended in the mineral oil mill.

3340 (S)	1600 (M)	1185 (W)	990 (M)
3240 (S)	1590 (M)	1155 (M)	985 (M)
3150 (S)	1450 (S) (oil)	1140 (M)	970 (W)
3080 (S)	1375 (M) (oil)	1115 (M)	924 (W)
2930 (S) (oil)	1315 (M)	1100 (M)	906 (W)
2850 (S) (oil)	1305 (M)	1093 (S)	875 (M)
2160 (W)	1276 (M)	1078 (S)	855 (W)
1690 (S)	1265 (M)	1042 (S)	827 (W)
1675 (S)	1233 (W)	1006 (W)	793 (W)
			717 (W) (oil)
			690 (M)

and the following elemental analysis:—

Analysis

Calculated for $C_{18}H_{34}N_2O_6S \cdot HCl (\frac{1}{2} H_2O)$
 C, 47.83; H, 8.03; N, 6.20;
 O, 23.01; S, 7.09; Cl, 7.84.
 Found: C, 47.92; H, 7.96; N, 6.08;
 O, 23.22; S, 7.34; Cl, 7.94.

EXAMPLE 7.

A. The procedure of Example 2, part A was repeated and the two fermentations were combined to give 490 liters of whole beer assaying 11.2 biounits/ml. This beer was extracted by the procedure of Example 2, part B to give 62.3 g. of a solid preparation (EAK 137-11) assaying 70 biounits/mg. The

fermentation of Example 2, part A was scaled up to a 2000 gallon fermentor to give 4900 liters of whole beer assaying 22 biounits/ml. which on extraction by the same procedure, except that the final butanol extract was not evaporated to dryness, gave 40 liters of aqueous solution (WTP 123-11) assaying 1000 biounits/ml.

B. Preparation EAK-137-11 was added to aqueous solution WTP 123-11 and the resulting 40 liters of solution was concentrated to 4 liters. This aqueous concentrate was adjusted from pH 8.0 to pH 2.0 with 40 ml. of concentrated sulphuric acid, and the resulting brown insoluble material was filtered off. The clear filtrate (pH 2.0) was extracted with 5.0 liters of methylene chloride. The aqueous layer was adjusted to pH 5.0 with 13 ml. of 50% aqueous sodium hydroxide and extracted again with 5 liters of methylene chloride. The aqueous layer was readjusted to pH 10.0 with 13 ml. of 50% aqueous sodium hydroxide and extracted 5 times with about 5 liters of methylene chloride for each extraction. The last five methylene chloride extracts were pooled and concentrated *in vacuo* to a volume of 700 ml., at which time 1.1 liters of deionized water was added. Concentration was continued until approximately 1500 ml. of aqueous solution remained. The aqueous concentrate was adjusted to pH 2.2 with 28.5 ml. of concentrated hydrochloric acid and extracted twice with 1 liter of 1-butanol. The aqueous layer was concentrated to a volume of 1.1 liters and 15 liters of acetone was added to promote crystallization. The aqueous acetone solution was held overnight at 0° C. The crystals were filtered off, washed with a small volume of acetone and dried *in vacuo* at room temperature, yielding (108 grams) of lincomycin hydrochloride (WTP 123-18) assaying on repeated assays an average of 215 biounits/mg. (equivalent to 920 mcg. free base/mg.), having an equivalent weight of 451.3, an optical rotation $[\alpha]_D^{25} = +137$ (C=1% in water), no UV absorption maxima, and the following elemental analysis:—

50 Analysis

Calculated for $C_{18}H_{34}N_2O_6S \cdot HCl$ ($\frac{1}{2} H_2O$)
 C, 47.83; H, 8.03; N, 6.20;
 O, 23.01; S, 7.09; Cl, 7.84.
 Found: C, 47.61; H, 8.29; N, 6.40;
 O, 22.57 (diff.); S, 7.06;
 Cl, 8.02.

The therapeutic compositions of the present invention are useful in the treatment of humans and animals for various pathological conditions. The compositions provide a means for administering the therapeutic ingredient by the oral and parenteral routes for

systemic treatment as well as topical and localized treatment. The compositions provide a method of therapy for tonsillitis, pneumonia, otitis, conjunctivitis, boils, carbuncles and other infectious conditions of humans due to the presence of bacteria. In animals the compositions can be used prophylactically. For example, rats can be protected from *Streptococcus viridans* during shipment. Animals raised for meat can be given prophylactic treatment for increased weight gains. Further, chickens infected with PPLO (pleuropneumonia-like organisms) can be treated. 65 75

The compositions of the present invention comprise the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof as the principal active ingredient in admixture with a pharmaceutical carrier or diluent. 80

The compositions of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the antibiotic lincomycin or its pharmacologically acceptable acid addition salts. 85 90

For oral administration either solid or fluid unit dosage forms can be prepared. For preparing solid compositions such as tablets, the principal active ingredient is mixed with conventional ingredients such as talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulphate, starch, lactose, acacia or methylcellulose, similar pharmaceutical diluents or carriers. The tablets can be liminated or otherwise compounded to provided a dosage form affording the advantage of prolonged or delayed action or predetermined successive action of the enclosed medication. For example, the tablet can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids or mixtures of polymeric acids with such materials as shellac, cetyl alcohol, cellulose acetate phthalate or styrene maleic acid copolymer. Alternatively, the two component system can be utilized for preparing tablets containing two or more incompatible active ingredients. Wafers are prepared in the same manner as tablets, differing only in shape and the inclusion of sucrose or other sweetener and flavor. In their simplest em- 95 100 105 110 115 120 125



bodiment, capsules, like tablets, are prepared by mixing the antibiotic with an inert pharmaceutical diluent and filling the mixture into a hard gelatin capsule of appropriate size. In another embodiment, capsules are prepared by filling hard gelatin capsules with polymeric acid coated beads containing the antibiotic. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the antibiotic with an acceptable vegetable oil, light liquid petrolatum or other inert oil.

Fluid unit dosage forms for oral administration such as syrups, elixirs, and suspensions can be prepared. The water-soluble antibiotics can be dissolved in an aqueous vehicle together with sugar, aromatic flavoring agents and preservatives to form a syrup. An elixir is prepared by using an aqueous-alcoholic (ethanol) vehicle with suitable sweeteners such as sugar, saccharin, and cyclamate together with an aromatic flavoring agent. Suspensions can be prepared of the insoluble antibiotics with a syrup vehicle with the aid of a suspending agent such as acacia, tragacanth or methylcellulose.

Topical ointments can be prepared by dispersing the antibiotic in a suitable ointment base such as petrolatum, lanolin, polyethylene glycols or mixtures thereof. Advantageously, the antibiotic is finely divided by means of a colloid mill utilizing light liquid petrolatum as a levigating agent prior to dispersing in the ointment base. Topical creams and lotions are prepared by dispersing the antibiotic in the oil phase prior to the emulsification of the oil phase in water.

For parenteral administration, fluid unit dosage forms are prepared utilizing the antibiotic and a sterile vehicle, water being preferred. The antibiotic, depending on the form and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the water-soluble antibiotic can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection is supplied to reconstitute the powder prior to use. Parenteral suspensions are prepared in substantially the same manner except that the antibiotic is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The antibiotic can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the com-

position to facilitate uniform distribution of the antibiotic.

The term unit dosage form as used in the Specification and claims refers to physically discrete units suitable as unitary dosages for human subjects and animals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the novel unit dosage forms of this invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for therapeutic use in humans and animals, as disclosed in detail in this Specification. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, pills, troches, suppositories, powder packets, granules, wafers, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and other forms as herein described.

In addition to the administration of the antibiotic lincomycin as the principal active ingredient of compositions for the treatment of the conditions described herein, the said lincomycin can be included with other types of compounds to obtain advantageous combinations of properties. Such combinations include the antibiotic lincomycin with antibiotics such as actinospectacin, chloramphenicol, tetracyclines (e.g., tetracycline, oxytetracycline and chlortetracycline), penicillin, erythromycin, novobiocin, kanamycin, streptomycin, neomycin, polymyxin, bacitracin, filipin, fumagillin and endomycin to broaden the bacterial spectrum of the composition and for synergistic action against particular bacteria; steroid having anti-inflammatory activity such as hydrocortisone, prednisolone, 6 α -methylprednisolone, or 6 α -fluoroprednisolone; analgesics such as aspirin, sodium salicylate, (acetylsalicylic acid)-anhydride, N-acetyl-p-aminophenol and salicylamide; antihistamines, such as chlorpheniramine maleate, diphenhydramine, promethazine, or pyrazinazine sulphate drugs such as sulphadiazine, sulphamethazine, sulphamerazine, sulphacetamide, sulphadimethyloxazole or sulphamethizole, antifungal agents, such as undecylenic acid, sodium propionate, salicylanilide, sodium caprylate, and hexetidine; and the vitamins.

The dosage of lincomycin for treatment depends on route of administration; the age, weight, and condition of the patient; and the particular disease to be treated. A dosage schedule of from 50 to 500 mg., 1 to 4 times daily (every six hours), embraces the effective range for the treatment of most



aromatic

lc
70



80

lc
85

90

95



100

105

110

115

120

125

chlorpheniramine

conditions for which the compositions are effective. For children the dosage is calculated on the basis of 6 to 8 mg./kg. by weight to be administered every six hours.

- 5 The lincomycin is compounded with a suitable pharmaceutical carrier in unit dosage form for convenient and effective administration. In the preferred embodiments of this invention, the dosage units contain
10 lincomycin in: 50, 100, 200 and 500 mg. amounts for systemic treatment; in 0.25, 0.5, 1, 2 and 5% amounts for topical or localized treatment; and 0.5 to 25% w/v for parenteral treatment. The dosage of compositions containing lincomycin and one or more
15 other active ingredients is to be determined with reference to the usual dosage of each such ingredient.

EXAMPLE 8.
Capsules.

- 20 One thousand two-piece hard gelatin capsules for oral use, each containing 200 mg. of lincomycin hydrochloride are prepared from the following types and amounts of materials:—

Lincomycin hydrochloride	...	200 gm.
Corn starch	...	150 gm.
Talc	...	75 gm.
Magnesium stearate	...	25 gm.

- 30 The materials are thoroughly mixed and then encapsulated in the usual manner.

- The foregoing capsules are useful for the systemic treatment of infection in adult humans by the oral administration of 1 capsule every 4 hours.

- 35 Using the procedure above, capsules are similarly prepared containing lincomycin hydrochloride in 50, 100, and 500 mg. amounts by substituting 50, 100 and 500 mg. of lincomycin hydrochloride for the 200 gm. used above.

EXAMPLE 9.
Capsules.

- 45 One thousand two-piece hard gelatin capsules for oral use, each containing 200 mg. of lincomycin hydrochloride and 250 mg. of tetracycline hydrochloride, are prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride	...	200 gm.
Tetracycline hydrochloride	...	250 gm.
Talc	...	75 gm.
Magnesium stearate	...	25 gm.

- 55 The ingredients are thoroughly mixed and then encapsulated in the usual manner.

- The foregoing capsules are useful for the systemic treatment of infection in adult humans by the oral administration of 1 capsule every 6 hours.

- 60 Using the procedure above, capsules are

similarly prepared containing lincomycin and each of the following antibiotics in place of tetracycline by substituting 250 gm. of such other antibiotic for tetracycline: chloramphenicol, oxytetracycline, chlortetracycline, fumagillin, erythromycin, streptomycin dihydrostreptomycin and novobiocin. Where a penicillin, such as potassium penicillin G is to be used in place of tetracycline, 250,000 units per capsule is employed.

Such combination products are useful for the systemic treatment of mixed infections in adult humans by the oral administration of 1 capsule every 6 hours.

EXAMPLE 10.

Tablets.

One thousand tablets for oral use, each containing 500 mg. of lincomycin hydrochloride are prepared from the following types and amounts of materials:—

Lincomycin hydrochloride	...	500 gm.
Lactose	...	125 gm.
Corn starch	...	65 gm.
Magnesium stearate	...	25 gm.
Light liquid petrolatum	...	3 gm.

The ingredients are thoroughly mixed and slugged. The slugs are broken down by forcing through a number sixteen screen. The resulting granules are then compressed into tablets, each tablet containing 500 mg. of lincomycin hydrochloride.

The foregoing tablets are useful for systemic treatment of infections in adult humans by oral administration of 1 tablet every 4 hours.

Using the above procedure, except for reducing the amount of lincomycin hydrochloride to 200 gm., tablets containing 200 mg. of lincomycin hydrochloride are prepared.

EXAMPLE 11.

Tablets.

One thousand oral tablets, each containing 200 mg. of lincomycin hydrochloride and a total of 250 mg. (83.3 mg. each) of sulphadiazine, sulphamerazine, and sulphamethazine, are prepared from the following types and amounts of materials:—

Lincomycin hydrochloride	...	200 gm.
Sulphadiazine	...	83.3 gm.
Sulphamerazine	...	83.3 gm.
Sulphamethazine	...	83.3 gm.
Lactose	...	50 gm.
Corn starch	...	50 gm.
Calcium stearate	...	25 gm.
Light liquid petrolatum	...	5 gm.

The ingredients are thoroughly mixed and slugged. The slugs are broken down by forcing through a number sixteen screen [an American standard screen used in this type

operation]. The resulting granules are then compressed into tablets, each containing 200 mg. of lincomycin hydrochloride and a total of 250 mg. (83.3 mg. each) of sulphadiazine, sulphamerazine, and sulphamethazine.

The foregoing tablets are useful for systemic treatment of infections by the oral administration of 4 tablets first and then 1 very six hours.

- 16 For the treatment of urinary infections, the triple sulfa drugs in the above formulation is advantageously replaced by 250 gm. of sulphamethylthiadiazole or 250 gm. of sulphacetamide.

15 **EXAMPLE 12.**
Granules.

- 20 2367 gm. of a granulation suitable for reconstitution with water prior to use is prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride ...	150 gm.
Tetracycline hydrochloride ...	150 gm.
Lecithin	5 gm.
Sucrose, powdered	2,000 gm.
25 Flavor	60 gm.
Sodium metabisulphite ...	2 gm.

- 30 The tetracycline is finely divided and coated with the lecithin. The coated tetracycline, lincomycin, sugar, flavor, and sodium metabisulphite are mixed together until thoroughly blended. The powder mixture is wetted with water and forced through a screen to form granules. The granules are dried and 23.67 gm. filled into 60 cc. bottles.
- 35 Prior to use sufficient water is added to the granules to make 60 cc. of composition.

- 40 The foregoing composition is useful for systemic treatment of infection, particularly in children at a dose of one teaspoonful 4 times daily.

EXAMPLE 13.
Oral Syrup.

- 45 One thousand cc. of an aqueous suspension for oral use, containing in each 5 cc. dose, one-half gram of total sulfas drugs and 200 mg. of lincomycin hydrochloride, is prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride ...	40 gm.
50 Sulphadiazine	33.3 gm.
Sulphamerazine	33.3 gm.
Sulphamethazine	33.3 gm.
Citric acid	2 gm.
Benzoic acid	1 gm.
55 Sucrose	700 gm.
Tragacanth	5 gm.
Lemon oil	2 cc.
Deionized water q.s. ...	1,000 cc.

The citric acid, benzoic acid, sucrose, tra-

gacanth, and lemon oil are dispersed in sufficient water to make 850 cc. of solution. The lincomycin sulphate and finely powdered sulpha drugs are stirred into the syrup until uniformly distributed. Sufficient water is added to make 1,000 cc.

The composition so prepared is useful in the systemic treatment of pneumonia in adult humans at a dose of 1 teaspoonful 4 times a day.

EXAMPLE 14.
Parenteral Solution.

A sterile aqueous solution for intramuscular use, containing in 1 cc. 200 mg. of lincomycin hydrochloride is prepared from the following types and amounts of materials:—

Lincomycin hydrochloride ...	200 gm.
Lidocaine hydrochloride ...	4 gm.
Methylparaben	2.5 gm.
Propylparaben	0.17 gm.
Water for injection q.s. ...	1,000 cc.

The ingredients are dissolved in the water and the solution sterilized by filtration. The sterile solution is filled into vials and the vials sealed.

EXAMPLE 15.
Parenteral Solution.

A sterile aqueous solution for intramuscular use, containing in 1 cc. 50 mg. of lincomycin hydrochloride, is prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride ...	50 gm.
Lactose	50 gm.
Water for injection q.s. ...	1,000 cc.

The lincomycin salt and lactose are dissolved in the water and the solution sterilized by filtration. The sterile solution, in the amount of 2 cc., is aseptically filled into sterile vials and frozen. The water is removed under high vacuum and the vials containing the lyophilized powder are sealed. Just prior to use, sufficient sterile water for injection to make 2 cc. of solution is added to the vial.

EXAMPLE 16.
Topical Ointment.

One thousand gm. of 0.25% ointment is prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride ...	2.5 gm.
Zinc oxide	50 gm.
Calamine	50 gm.
Liquid petrolatum (heavy) ...	250 gm.
Wool fat	200 gm.
White petrolatum q.s. ...	1,000 gm.

The white petrolatum and wool fat are

melted, and 100 gm. of liquid petrolatum added thereto. The lincomycin, zinc oxide and calamine are added to the remaining liquid petrolatum and the mixture milled until the powders are finely divided and uniformly dispersed. The powder mixture is stirred into the white petrolatum mixture and stirring continued until the ointment congeals.

The foregoing ointment is usefully applied topically to the skin of mammals for the treatment of infection.

The foregoing composition can be prepared by omitting the zinc oxide and calamine.

Following the procedure above, ointments are similarly prepared containing lincomycin in 0.5, 1, 2 and 5% amounts by substituting 5, 10, 20 and 50 gm. of lincomycin for the 2.5 gm. used above.

EXAMPLE 17.

Cream.

One thousand gm. of a vaginal cream are prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride	...	50 gm.
Tegacid Regular ¹	...	150 gm.
Spermaceti	...	100 gm.
Propylene glycol	...	50 gm.
Polysorbate 80	...	5 gm.
Methylparaben	...	1 gm.
Deionized water q.s.	...	1,000 gm.

¹ Self-emulsifying glyceryl monostearate from Goldschmidt Chemical Corporation, New York, N.Y.

The Tegacid and spermaceti are melted together at a temperature of 70–80° C. The methylparaben is dissolved in about 50 gm. of water and the propylene glycol, Polysorbate 80, and lincomycin are added in turn, maintaining a temperature of 75–80° C. The methylparaben mixture is added slowly to the Tegacid and spermaceti melt, with constant stirring. The addition is continued for at least 30 minutes with continued stirring until the temperature has dropped to 40–45° C. The pH of the final cream is adjusted to 3.5 by incorporating 2.5 gm. of citric acid and 0.2 gm. of dibasic sodium phosphate dissolved in about 50 gm. of water. Finally, sufficient water is added to bring the final weight to 1,000 gm. and the preparation stirred to maintain homogeneity until cooled and congealed.

The foregoing composition is useful for the treatment of vaginal infections in humans.

EXAMPLE 18.

Ointment, Ophthalmic.

One thousand gm. of an ophthalmic ointment containing 0.5% lincomycin hydro-

chloride are prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride	...	5 gm.
Bacitracin	...	12.2 gm.
Polymyxin B sulphate (10,000 units/mg.)	...	1 gm.
Light liquid petrolatum	...	250 gm.
Wool fat	...	200 gm.
White petrolatum q.s.	...	1,000 gm.

The antibiotics are finely divided by means of an air micronizer and added to the light liquid petrolatum. The mixture is passed through a colloid mill to uniformly distribute the antibiotics. The wool fat and white petrolatum are melted together, strained, and the temperature adjusted to 45–50° C. The liquid petrolatum slurry is added and the ointment stirred until congealed. Suitably the ointment is packaged in one dram ophthalmic tubes.

The foregoing ointment is usefully applied to the eye for treatment of localized infection in humans and other animals.

Advantageously the foregoing composition can contain 5 gm. (0.5%) of methylprednisolone for the treatment of inflammation, and, alternatively, the bacitracin and polymyxin B sulfate can be omitted.

EXAMPLE 19.

Eye-ear Drops.

One thousand cc. of a sterile aqueous solution for eye or ear use containing 10 mg. of lincomycin (free base) and 10 mg. of prednisolone in each cc. is prepared from the following types and amounts of ingredients:—

Lincomycin	...	10 gm.
Prednisolone succinate sodium	...	10 gm.
Sodium citrate	...	4.5 gm.
Polyethylene glycol 4,000	...	120 gm.
Myristyl-γ-picolinium chloride	...	0.2 gm.
Polyvinylpyrrolidone	...	1 gm.
Deionized water q.s. ad	...	1,000 cc.

The ingredients are dissolved in the water and the resulting solution is sterilized by filtration. The solution is aseptically filled into sterile dropper containers.

The composition so prepared is useful in the topical treatment of inflammation and infection of the eye and ear as well as other sensitive tissues of the animal body.

EXAMPLE 20.

Troches.

Ten thousand troches are prepared from the following types and amounts of ingredients:—

Lincomycin hydrochloride	...	100 gm.
Neomycin sulphate	...	50 gm.



	Polymyxin B sulphate (10,000 units/mg.)	...	...	1 gm.
	Ethyl aminobenzoate	...	...	50 gm.
	Calcium stearate	...	...	150 gm.
5	Powdered sucrose q.s.	...	...	5,000 gm.

The powdered materials are mixed thoroughly and then compressed into half gram roches following the usual techniques for the preparation of compressed tablets.

- 10 The troches are held in the mouth and allowed to dissolve slowly to provide treatment for the mouth and throat of humans.

EXAMPLE 21.
Chewing Gum.

- 15 Pieces of uncoated chicle chewing gum of tablet-like shape weighing about one gram each are used as starters for the coating process. A coating of sucrose is first applied, using a syrup (80% sucrose solution) and the usual coating pan. A finely powdered highly hydrogenated castor oil, such as Thixcin, is dusted on the sucrose-covered starters. The second coating step is the application of a dispersion of lincomycin hydrochloride and benzocaine (10:1) in absolute ethanol. Successive applications are made until 50 mg. of lincomycin hydrochloride and 5 mg. of benzocaine have been deposited on each piece. Thereafter, a second dusting of the hydrogenated castor oil is applied. A final finishing coating is applied of sucrose solution containing flavor and color. Advantageously a polishing coat of wax is added.
- 30 The foregoing chewing gum composition is chewed in the mouth to provide the slow release of lincomycin for the topical treatment of infections of the mouth and throat.

EXAMPLE 22.
Suppository, Rectal.

- 40 One thousand suppositories, each weighing 2.5 gm. and containing 100 mg. of lincomycin hydrochloride are prepared from the following types and amounts of ingredients:—

	Lincomycin hydrochloride	...	100 gm.
	Polymyxin B sulphate (10,000 units/mg.)	...	1.25 gm.
	6 α -methylprednisolone	...	1 gm.
50	Ethyl aminobenzoate	...	75 gm.
	Zinc oxide	...	62.5 gm.
	Propylene glycol	...	162.5 gm.
	Polyethylene glycol 4,000 q.s.	...	2,500 gm.

- 55 The lincomycin hydrochloride, polymyxin B sulphate, 6 α -methylprednisolone, ethyl aminobenzoate, and zinc oxide are added to the propylene glycol and the mixture milled until the powders are finely divided and uniformly dispersed. The polyethylene glycol 4,000 is melted and the propylene glycol dis-

persion added slowly with stirring. The suspension is poured into unchilled molds at 40° C. The composition is allowed to cool and solidify and then removed from the mold and each suppository foil wrapped.

The foregoing suppositories are inserted rectally for local treatment of inflammation and infection.

Alternatively, the foregoing composition can be prepared omitting the steroid.

EXAMPLE 23.
Mastitis Ointment.

One thousand gm. of an ointment for the treatment of mastitis in dairy cattle is prepared from the following types and amounts of ingredients:—

	Lincomycin hydrochloride	...	50 gm.
	Prednisolone acetate	...	0.5 gm.
	Light liquid petrolatum	...	300 gm.
	Chlorobutanol, anhydrous	...	5 gm.
	Polysorbate 80	...	5 gm.
	2% Aluminum monostearate-peanut oil gel	...	400 gm.
	White petrolatum q.s.	...	1,000 gm.

The lincomycin hydrochloride and prednisolone acetate are milled with the light liquid petrolatum until finely divided and uniformly dispersed. The chlorobutanol, polysorbate 80, peanut oil gel and white petrolatum are heated to 120° F. to form a melt and the liquid petrolatum dispersion stirred in. With continued stirring the dispersion is allowed to cool (and congeal) to room temperature and is filled into disposable mastitis syringes in 10 gm. doses.

EXAMPLE 24.
Animal Feed.

One thousand gm. of a feed mix is prepared from the following types and amounts of ingredients:—

	Lincomycin hydrochloride	...	10 gm.
	Soybean meal	...	400 gm.
	Fish meal	...	400 gm.
	Wheat germ oil	...	50 gm.
	Sorghum molasses	...	140 gm.

The ingredients are mixed together and pressed into pellets.

The composition can be fed to laboratory animals, i.e., rats, mice, guinea pigs, and rabbits for prophylaxis during shipping.

For larger animals the composition can be added to the animal's regular feed in an amount calculated to give the desired dose of lincomycin.

EXAMPLE 25.

Following the procedure of each of the preceding Examples 8 through 24, each member selected from the group consisting



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prophylaxis

of lincomycin sulphate, lincomycin nitrate, lincomycin phosphate, lincomycin citrate, lincomycin lactate, lincomycin acetate, lincomycin tartrate, and lincomycin succinate, is substituted in an equivalent amount for the lincomycin hydrochloride shown in the example to provide similar therapeutic properties.

EXAMPLE 26.

Following the procedure of each of the preceding Examples 8 to 11, sodium novobiocin, calcium novobiocin, chlorotetracycline hydrochloride, oxytetracycline hydrochloride, tetracycline, tetracycline hydrochloride, or tetracycline phosphate complex is added in 50, 100 and 250 gm. amounts to provide a mixture having a wider spectrum of therapeutic effectiveness in the treatment of infectious diseases resulting from mixed organisms susceptible to antibiotic lincomycin as indicated in the present Specification and the above indicated antibiotics as already well known to the medical art.

WHAT WE CLAIM IS:—

1. The antibiotic lincomycin which when isolated in the essentially pure free base form is characterised by:—

(a) Being monobasic and having a pK_a of 7.6;

(b) Having no UV absorption maxima from 220 to 400 millimicrons;

(c) A formula of $C_{18}H_{34}N_2O_6S$;

(d) Soluble in lower-alkanols, lower alkyl esters of lower-alkanoic acids, lower-alkanones and chlorinated lower alkanes as hereinbefore defined; and, in the form of its crystalline hydrochloride by

(e) Characteristic infrared absorption in mineral oil mull at the following frequencies expressed in reciprocal centimeters;

3,340, 3,240, 3,150, 3,080, 2,930, 2,850, 2,160, 1,690, 1,675, 1,600, 1,590, 1,450, 1,375, 1,315, 1,305, 1,276, 1,265, 1,233, 1,185, 1,155, 1,140, 1,115, 1,100, 1,093, 1,078, 1,042, 1,006, 990, 985, 970, 924, 906, 875, 855, 827, 793, 717, 690;

(f) Having a melting point of 135—140° C., an optical-rotation $[\alpha]_D^{25} = +137^\circ$ (C. = 1% in water) and an equivalent weight of 454.7 and an acid addition salt thereof.

2. A crude antibiotic lincomycin assaying at least 20 mcg. per mg. of the antibiotic or an acid addition salt thereof as defined in Claim 1.

3. The hydrochloride of the antibiotic lincomycin as defined in Claim 1.

4. A process for the production of the antibiotic lincomycin which comprises cultivating *Streptomyces lincolnensis* var. *lin-*

colnensis in an aqueous nutrient medium containing a source of carbon and nitrogen under aerobic conditions until substantial activity is imparted to said medium by production of lincomycin.

5. A process as claimed in Claim 4 wherein the nutrient medium contains source of assimilable carbohydrate and a assimilable nitrogen compound or proteinaceous material and the lincomycin so produced is isolated therefrom.

6. A process as claimed in Claim 4 or 5 in which the cultivation is effected at a temperature of 18° C. to 40° C. for a period between 2 to 10 days.

7. A process as claimed in Claim 4 or 5 wherein the culture filtrate is adjusted to a pH 7.5 or greater and then extracted with a water-immiscible solvent for the antibiotic lincomycin and the antibiotic lincomycin is recovered from the solvent extract.

8. A therapeutic composition comprising the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof as an essential active ingredient in admixture with a pharmaceutical carrier.

9. A therapeutic composition as claimed in Claim 8 and in unit dosage form comprising from 50 to 500 mg. of the active ingredient.

10. A therapeutic composition as claimed in Claim 8 comprising from 0.25% to 25% of the active ingredient.

11. A sterile therapeutic composition for parenteral administration comprising from 0.5% to 25% w/v of the antibiotic lincomycin or its pharmacologically acceptable acid addition salts thereof as an essential active ingredient in admixture with a sterile vehicle.

12. A process for the treatment of animals and birds to promote the growth thereof which comprises feeding to said animals and birds the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof.

13. A process as claimed in Claim 12 which comprises administering in dosage unit form from 50 to 500 mg. of the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof in admixture with a pharmaceutical carrier.

14. A process for the preparation of the antibiotic lincomycin and acid addition salts thereof as claimed in any of Claims 1 to 3 substantially as herein described with reference to any one of Examples 1 to 7.

15. The antibiotic lincomycin and the acid addition salts thereof as claimed in any of Claims 1 to 3 when prepared by a process as claimed in any of Claims 4 to 7, or 14.

16. A therapeutic composition comprising as the active ingredient the antibiotic

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antibiotic

of lincomycin sulphate, lincomycin nitrate, lincomycin phosphate, lincomycin citrate, lincomycin lactate, lincomycin acetate, lincomycin tartrate, and lincomycin succinate, is substituted in an equivalent amount for the lincomycin hydrochloride shown in the example to provide similar therapeutic properties.

EXAMPLE 26.

Following the procedure of each of the preceding Examples 8 to 11, sodium novobiocin, calcium novobiocin, chlorotetracycline hydrochloride, oxytetracycline hydrochloride, tetracycline, tetracycline hydrochloride, or tetracycline phosphate complex is added in 50, 100 and 250 gm. amounts to provide a mixture having a wider spectrum of therapeutic effectiveness in the treatment of infectious diseases resulting from mixed organisms susceptible to antibiotic lincomycin as indicated in the present Specification and the above indicated antibiotics as already well known to the medical art.

WHAT WE CLAIM IS:—

1. The antibiotic lincomycin which when isolated in the essentially pure free base form is characterised by:—
 - (a) Being monobasic and having a pK_a of 7.6;
 - (b) Having no UV absorption maxima from 220 to 400 millimicrons;
 - (c) A formula of $C_{18}H_{34}N_2O_6S$;
 - (d) Soluble in lower-alkanols, lower alkyl esters of lower-alkanoic acids, lower-alkanones and chlorinated lower alkanes as hereinbefore defined; and, in the form of its crystalline hydrochloride by
 - (e) Characteristic infrared absorption in mineral oil mull at the following frequencies expressed in reciprocal centimeters;

3,340, 3,240, 3,150, 3,080, 2,930, 2,850, 2,160, 1,690, 1,675, 1,600, 1,590, 1,450, 1,375, 1,315, 1,305, 1,276, 1,265, 1,233, 1,185, 1,155, 1,140, 1,115, 1,100, 1,093, 1,078, 1,042, 1,006, 990, 985, 970, 924, 906, 875, 855, 827, 793, 717, 690;
 - (f) Having a melting point of 135–140° C., an optical-rotation $[\alpha]_D^{25} = +137^\circ$ (C. = 1% in water) and an equivalent weight of 454.7 and an acid addition salt thereof.
2. A crude antibiotic lincomycin assaying at least 20 mcg. per mg. of the antibiotic or an acid addition salt thereof as defined in Claim 1.
3. The hydrochloride of the antibiotic lincomycin as defined in Claim 1.
4. A process for the production of the antibiotic lincomycin which comprises cultivating *Streptomyces lincolnensis* var. *lin-*

colnensis in an aqueous nutrient medium containing a source of carbon and nitrogen under aerobic conditions until substantial activity is imparted to said medium by production of lincomycin.

5. A process as claimed in Claim 4 wherein the nutrient medium contains source of assimilable carbohydrate and assimilable nitrogen compound or proteinaceous material and the lincomycin so produced is isolated therefrom.

6. A process as claimed in Claim 4 or 5 in which the cultivation is effected at a temperature of 18° C. to 40° C. for a period between 2 to 10 days.

7. A process as claimed in Claim 4 or 5 wherein the culture filtrate is adjusted to a pH 7.5 or greater and then extracted with a water-immiscible solvent for the antibiotic lincomycin and the antibiotic lincomycin is recovered from the solvent extract.

8. A therapeutic composition comprising the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof as an essential active ingredient in admixture with a pharmaceutical carrier.

9. A therapeutic composition as claimed in Claim 8 and in unit dosage form comprising from 50 to 500 mg. of the active ingredient.

10. A therapeutic composition as claimed in Claim 8 comprising from 0.25% to 25% of the active ingredient.

11. A sterile therapeutic composition for parenteral administration comprising from 0.5% to 25% w/v of the antibiotic lincomycin or its pharmacologically acceptable acid addition salts thereof as an essential active ingredient in admixture with a sterile vehicle.

12. A process for the treatment of animals and birds to promote the growth thereof which comprises feeding to said animals and birds the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof.

13. A process as claimed in Claim 12 which comprises administering in dosage unit form from 50 to 500 mg. of the antibiotic lincomycin or a pharmacologically acceptable acid addition salt thereof in admixture with a pharmaceutical carrier.

14. A process for the preparation of the antibiotic lincomycin and acid addition salts thereof as claimed in any of Claims 1 to 3 substantially as herein described with reference to any one of Examples 1 to 7.

15. The antibiotic lincomycin and the acid addition salts thereof as claimed in any of Claims 1 to 3 when prepared by a process as claimed in any of Claims 4 to 7, or 14.

16. A therapeutic composition comprising as the active ingredient the antibiotic



lincomycin or an acid addition salt thereof
as claimed in any of Claims 1 to 3 substan-
tially as herein described with reference to
any one of Examples 8 to 26.

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