PATENT SPECIFICATION

(11) 1 219 785

NO DRAWINGS

1 219 785

(21) Application No. 13452/68 (22) Filed 20 March 1968
(31) Convention Application No. 624 146 (32) Filed 20 March 1967 in
(33) United States of America (US)
(45) Complete Specification published 20 Jan. 1971
(51) International Classification A 61 k 21/00
(52) Index at acceptance
A5B 215 216 21Y 248 24Y 343 34Y 386 38Y

(54) IMPROVEMENTS IN VETERINARY ANTIBIOTIC COMPOSITIONS

(71) We, ELI LILLY AND COMPANY, a Corporation of the State of Indiana, United States of America, of 740 South Alabama Street, City of Indianapolis, State of Indiana, 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 concerns new compositions having enhanced activity against mycoplasma infections in poultry.

The occurrence of chronic respiratory disease (CRD) represents a serious economic problem in the poultry industry. The presence of the disease in a flock is manifested grossly by depressed weight gains as well as by the occurrence of air sac lesions and other histological lesions involving the trachea, lungs, and other tissues. It is now reasonably well established that pleuropneumonia-like organisms (PPLO), specifically, *Mycoplasma gallisepticum*, are implicated as causative agents of CRD in poultry. A limited number of substances have been found to have some activity against mycoplasma infections. Such substances are generally antibiotics, more particularly, such antibiotics are aureomycin, bottromycin, Terramycin (a Registered Trade Mark), erythromycin, spiromycin, lincomycin and tylosin. While such antibiotics have demonstrated activity against the causative organisms producing CRD, their use still achieves only incomplete control of the disease. In the highly competitive poultry industry, any composition or method which would increase the efficacy of therapy would represent a valuable contribution to the art.

It has now been unexpectedly discovered that compositions demonstrating enhanced activity against CRD are obtained by combining an antibiotic effective against PPLO with a polyoxyethylene ether as hereinafter defined.

More particularly the present invention provides a composition having enhanced activity against mycoplasma infections in poultry, which comprises an antibiotic effective against mycoplasma and a polyoxyethylene ether iof the formula $H(OCH_2CH_2)_nOR$ wherein n is a number between 10 and 35 and R is cholesteryl, C_{12}—C_{20} alkyl or C_{12}—C_{20} alkenyl, the weight ratio of antibiotic to polyoxyethylene ether in said composition being between 1:25 and 30:1.

The polyoxyethylene ethers used in this invention are of the general formula $H(OCH_2CH_2)_nOR$ wherein n is a number between 10 and 35 representing the average number of oxyethylene monomer units in the polyoxyethylene ether.

Illustrative of the groups which may be represented by R are cholesteryl, lauryl, myristyl, palmityl, stearyl, cetyl, oleyl and in general the saturated and mono-or polyunsaturated groups having the requisite chain length of 12 to 20 carbon atoms. While the alkyl and alkenyl groups represented by R have been illustrated herein by straight-chain radicals because of the ready availability of the alcohols from which such groups are derived, it will be understood by those skilled in the art that branched-chain radicals having the required carbon content will be equally effective. Illustrative of the antibiotics which can be employed in the practice of the invention are aureomycin, bottromycin, tetramycin, erythromycin, lincomycin and tylosin.

The polkalkylene ethers employed in the compositions of the present invention are all prepared by methods well known in the art. More specifically, they are prepared by reacting an alcohol, ROH, with ethylene oxide in a molar ratio such that the desired number of oxyethylene monomer units will be incorporated into the resulting polyoxyethylene chain. It will be recognized that precise control of such a polymeric reaction is impossible to achieve so that "n" in the general formula must represent an average, rather than an absolute, value for the number of oxyalkylene units incorporated into the polyoxyalkylene chain.

A preferred antibiotic is tylosin and preferred polyoxyethylene ethers are those in

[*Price 5s. 0d. (25p)*]

which n is between 15 and 25 and R is cholesteryl, stearyl, cetyl and oleyl.

The compositions comprising the antibiotic and a polyoxyalkylene ether of the general formula can be employed with equal effectiveness in controlling mycoplasma infections as either solid or liquid formulations. Solid formulations are generally administered to the animals as feed compositions comprising the antibiotic and the polyoxyethylene ether uniformly distributed in a normal poultry ration. Liquid formulations comprise the antibiotic and the polyoxyethylene ether dissolved in the drinking water consumed by the flock. It will be obvious to those skilled in the art that concurrent administration of both solid and liquid formulations can be employed to provide the desired levels of antibiotic and polyoxyethylene ether.

Typically, solid formulations comprise between 50 and 2500 grams of polyoxyethylene ether of the general formula and between 100 and 1500 grams of an antibiotic effective against mycoplasma per ton of feed ration. Preferred formulations comprise between 200 and 500 grams of the polyoxyethylene ether and 600 to 1000 grams of the antibiotic portion of feed. Because of its well-established activity against mycoplasma, a highly preferred antibiotic is tylosin. Various diluents, excipients, supplements, and the like can also be included in the finished feed ration. Preparation of the feed composition is suitably accomplished by employing one or two readily available procedures. Thus for example, a solution comprising the antibiotic and the polyoxyethylene ether can be sprayed onto a feed premix which is then uniformly mixed with the bulk of the feed ration in accordance with procedures recognized in the art. Alternatively, a solution of the polyoxyethylene ether can be sprayed onto the antibiotic and the polyoxyalkylene ether coated antibiotic can then be incorporated by dry-mixing procedures with either a premix or a final feed composition. Either alternative employs as a starting point for the preparation of the feed composition, a concentrate composition comprising the antibiotic and the polyoxyethylene ether, the ratio of antibiotic to polyoxyethylene ether in the concentrate being 1:25 and 30:1 depending upon the relative proportion of the two substances desired in the final feed composition. Typically, such a concentrate composition can be prepared by spraying the antibiotic and, if desired, suitable excipients, diluents, extending agents and the like, with a polyoxyethylene ether in a coating pan or other suitable mixing equipment which provides a rolling bed. Alternatively, with solid polyoxyethylene ethers, the concentrate composition can be prepared by blending the antibiotic, polyoxyethylene ether and, if desired, excipients, diluents, extending agents and the like by suitable dry-mixing procedures.

It will be understood that the term "antibiotic" as employed herein includes the antibiotic as the free base or in the form of any of its nontoxic acid addition salts.

Liquid formulations can readily be prepared at the point of use by dissolving a composition comprising the antibiotic and a polyoxyethylene ether of the general formula in drinking water in a proportion such that the desired concentration is obtained. Suitably, final concentrations of from 0.1 to 5 grams of the polyoxyethylene ether per gallon, preferably from 0.25 gram to 0.5 gram per gallon and 0.25 gram to 5 grams of the antibiotic per gallon of drinking water are employed. The ratio of antibiotic to polyoxyethylene ether in the concentrate compositions employed for the preparation of the liquid formualtions will, therefore, range from 1:20 to 50:1. Other additives such as diluents, excipients, extending agents, and the like can, if desired, be included in the concentrate compositions. Especially desirable additives for such concentrate compositions are substances which permit more rapid solubilization of the polyoxyethylene ether in water in those instances in which the solubility rate of the ether in water is rather slow. Additional compatible chemotherapeutic agents can, if desired, also be included either at the time of preparation of the concentrate compositions or during the preparation of the final liquid formulation.

The compositions of the present invention can be employed chemotherapeutically for the treatment of CRD or prophylactically to prevent the outbreak of the disease. When employed chemotherapeutically, the compositions are usually administered either as solid or liquid formualtions beginning at the time of earliest recognition of the presence of the disease in a flock and continuing for a period of up to five days, preferably from three to five days. Prophylactically, liquid or solid formulations of the compositions of the invention are usually administered during the first three to five days after hatching to minimize the possibility of an outbreak of CRD in the flock. Preferably, a second one- to five-day course of administration should follow after about four weeks.

The compositions of the present invention demonstrate an enhanced efficacy against CRD as compared to tylosin alone. The said enhanced efficacy is clearly evidenced especially by increased weight gains and decreased incidence of air sac lesions.

The practice of the invention is illustrated by the following nonlimiting examples.

EXAMPLE 1

A concentrate composition suitable for the preparation of final formulations having enhanced activity against mycoplasma is prepared as follows:

A mixture of three parts by weight each of gelatin and water is warmed at a temperature of about 50°C. until a homogeneous melt is obtained. To the melt is added with stirring one part by weight of tylosin phosphate and stirring is continued for an additional half hour. The tylosin-gelatin mixture is removed from the mixing equipment, permitted to solidify and coarse ground in a grinder of the sausage-mill type. The ground mixture is vacuum dried at a temperature of about 50°C. for about 48 hours and is then subjected to a fine grinding operation. The gelatinized tylosin phosphate so prepared has a theoretical potency of approximately 250 mg./g. (determined as tylosin). A 10 kg. quantity of gelatinized tylosin phosphate having an assayed potency of 231 mg./g. is placed in a coating pan and while being tumbled in the pan is sprayed with 462 grams of Brij-78 (Brij is a registered Trade Mark) at a pressure of 20 p.s.i. (pounds per square inch) at a rate of about 0.35 gallons per minute. The holding tank, liner and spraying head are heated throughout the operation in order to maintain fluidity of the polyoxyethylene ether. The product so obtained contains approximately 100 grams of tylosin activitiy and 20 grams of Brij-78 per pound of concentrate.

EXAMPLE 2

A solid formualtion of tylosin and a polyoxethylene ether suitable for use as a concentrated premix is prepared as follows:

A mixture of 41.41.kg. of red oil (oleic acid) and 43.91 kg. of Brij-78 (a polyoxyethylene ether of the general formula wherein n is 20 and R is stearyl) is charged into a jacketed solution make-up tank equipped with a propeller-type stirrer. Steam is injected into the tank jacket and when the solids are completely melted, the liquid mixture is stirred for about 10 minutes while the temperature is maintained between about 50°C. and 90°C. The heated mixture is pumped through a spraying system onto a carrier vehicle comprising 1503.7 kg. of extracted soybean feed in an operating vertical mixer and mixing is continued for an additional 15 minutes after all of the liquid mixture has been sprayed onto the carrier vehicle. Mixing is continued for another 30 minutes after 905.61 kg. of gelatinized tylosin (containing 220 kg. of tylosin activity as tylosin phosphate) are added to the mixer. The finished concentrated premix so prepared is transferred by means of a screw conveyor to bagging equipment and packaged in convenient package sizes for subsequent addition to complete feed ration in a proportion such that the final concentration in the feed shall be between about 100 and about 300 grams of Brij-78 and between about 500 and about 1500 grams of tylosin per ton of feed.

EXAMPLE 3

A formulation suitable for the preparation of liquid compositions comprising tylosin and a polyoxyethylene ether is prepared as follows:

Into a suitable container are sieved 350 grams of lactose and 425 grams of powdered sugar. To the mixture are added 25 grams of Brij-78. The resulting mix is placed into an oven and heated until the temperature is about 70—80° throughout. The material is then transferred to a suitable mixer and mixed until uniform and cool. Upon the addition of 200 grams of finely powdered tylosin (activity as tylosin tartrate), the entire lot is again mixed until homogeneous. The resulting homogeneous mixture is sieved through a screen of suitable size into a covered storage container and kept in a cool, dry area prior to filling into individual packages. The product so prepared can be dissolved in the required amounts in drinking water to provide a liquid formualtion having between about 0.1 gram to about 0.6 gram of Brij-78 and between about 0.8 gram to about 4.8 grams of tylosin per gallon.

EXAMPLE 4

Four-week old broiler chickens were divided into 18 groups of 15 birds per group so that each group had the same average weight per bird. The chickens were housed in four-deck batteries in five separate brooder houses. The groups were divided as follows:

A. Four groups, placed in different brooder houses in such a way that one group was at each level in a four-deck battery, were treated for five days with a treated ration containing 1000 g. of tylosin per ton of feed.

B. Four groups, placed as in A, were treated for five days with a treated ration containing 1000 grams of tylosin phosphate and 200 grams of Brij-78 per ton of feed. The feed employed for these groups was prepared by spraying the polyoxyethylene ether onto a tylosin-soybean meal premix and then incorporating the mixture into the finished feed.

C. Four groups, placed as in A, were treated for five days with a feed containing 1000 grams of tylosin phosphate and 200 grams of Brij-78 per ton of feed. The feed employed for these groups was prepared by spraying the polyoxyethylene ether onto the

tylosin phosphate prior to preparation of the premix.

D. Two groups, placed in the lower two decks of a fourth brooder house, served as infected controls and received no treatment subsequent to infection.

E. Two groups, placed in the upper two decks of the brooder house used for D, served as infected controls which received no antibiotic but were given 250 mg. per gallon of drinking water of Brij-78 for three days post-infection.

F. Two groups, placed in two decks in a fifth brooder house, served as non-infected controls.

At the start of the experiment, all of the chickens except those in the non-infected control groups were given 0.2 ml. each of a broth culture of *Mycoplasma gallisepticum* intravenously and into the posterior air sac. Except for the control groups, treated feed was given immediately following infection for a total of five days. After the fifth day all chickens were fed a broiler ration without antibiotic or polyoxyethylene ether.

At the beginning of the trail ten chickens were sacrificed and examined for air sac lesions and blood samples were examined serologically for *M. gallisepticum* antibody. All ten of the chickens were serologically negative and had normal air sacs.

Four weeks after infection, each chicken was weighed, examined at necropsy for air sac lesions, and had a blood sample examined serologically. All positive lesions were cultured for mycoplasma and in those groups having fewer than five chickens with lesions, trachea from five chickens in the group were cultured for the organism. The results obtained are shown in Table I.

13

TABLE I

	Treatment	Replicate	Avg. Weight/Chicken	Air Sac Lesions*	*M. gallisepticum* Antibody Test*	*M. gallisepticum* Culture*
			(gms.)			
A.	Tylosin phosphate, 1000 gms./ton	1	1734	11/15	14/15	6/8
		2	1685	10/15	12/15	8/10
		3	1667	7/15	15/15	8/10
		4	1605	10/15	14/14	9/11
			Avg. 1673	38/60	55/59	31/39
B.	Tylosin phosphate, 1000 gms./ton; Brij-78, 200 gms./ton sprayed on premix	1	1750	3/15	9/15	3/6
		2	1795	1/15	14/15	1/5
		3	1731	7/15	13/15	8/10
		4	1720	8/15	10/14	4/9
			Avg. 1749	19/60	46/59	16/30
C.	Tylosin phosphate, 100 gms./ton Brij-78, 200 gms./ton, sprayed on tylosin phos.	1	1760	4/15	14/15	7/9
		2	1708	1/15	8/15	5/6
		3	1719	3/15	14/15	4/7
		4	1733	4/15	11/15	4/6
			Avg. 1730	12/60	47/60	20/28
D.	Infected control	1	1397	14/14	14/14	6/12
		2	1365	15/15	15/15	8/12
			Avg. 1386	29/29	29/29	14/24
E.	Infected control Brij-78, 250 mg./gal.	1	1435	15/15	15/15	9/10
		2	1439	14/14	14/14	6/14
			Avg. 1437	29/29	29/29	15/24
F.	Normal control	1	1798	0/15	0/15	0/5
		2	1816	0/15	0/15	0/5
			Avg. 1807	0/30	0/30	0/10

*No. positive/no. examined

Statistical evaluation of the data indicates that the group receiving only the polyoxyethylene ether was not benefited and that the two groups receiving tylosin and the polyoxyethylene ether in the feed were benefited to a significantly greater extent than those receiving tylosin alone when evaluated on the basis of weight, frequency of air sac lesions and formation of *M. gallisepticum* antibodies. It will be noted that

in the groups receiving both tylosin and the polyoxyethylene ether, increased weight gains and decreased incidence of air sac lesions is apparent, not only with respect to untreated infected control groups, but also with respect to those groups treated with tylosin alone.

EXAMPLE 5

The enhanced activity of liquid formulations comprising tylosin and a polyoxyethylene ether was demonstrated in an experiment analogous to that described in Example 4. The treatment levels employed and the results are shown in Table II. As in the experiment employing solid formulations, the results clearly show that the combination of tylosin and polyoxyethylene ether produces better weight gains and decreases the incidence of air sac lesions not only with respect to untreated animals but also with respect to those treated with tylosin alone.

TABLE II

	Treatment	Replicate	Avg. Weight/ Chicken	Air Sac Lesions*	*M. gallisepticum* Antibody Test*
			(gms.)		
A.	Tylosin 2.0 gm./gal.	1	1621	9/14	14/14
		2	1577	10/15	15/15
		3	1617	9/15	13/15
		4	1647	9/15	15/15
		Avg.	1615	37/59	57/59
B.	Tylosin 2.0 gm./gal. Brij-78 100 mg./gal.	1	1734	4/14	8/14
		2	1683	10/15	13/15
		3	1663	12/15	14/15
		4	1611	5/15	8/15
		Avg.	1672	31/59	43/59
C.	Tylosin, 2.0 gm./gal. Brij-78 250 mg./gal.	1	1635	4/15	9/15
		2	1648	7/15	8/15
		3	1649	7/15	13/15
		4	1656	7/15	11/15
		Avg.	1647	25/60	41/60
D.	Tylosin, 2.0 gm./gal. Brij-78, 500 mg./gal.	1	1599	4/14	11/14
		2	1709	7/15	9/15
		3	1667	5/14	9/14
		4	1678	2/14	7/14
		Avg.	1663	18/57	36/57
E.	Normal Controls	1	1715	0/15	0/15
		2	1683	0/15	0/15
		Avg.	1700	0/30	0/30
F.	Infected Controls	1	1034	11/12	12/12
		2	1005	12/12	12/12
		Avg.	1020	23/24	24/24

*No positive/no. examined

EXAMPLE 6

The enhancement of the activity of tylosin against mycoplasma infections was observed in the treatment of a natural outbreak of the infection. During the holding period prior to initiation of an induced infection experiment with a large group of chickens, the flock exhibited symptoms of respiratory disease. Blood samples and examination for air sac lesions indicated an early infection with *Mycoplasma gallisepticum*. The chickens were divided into three groups of 75 chickens each and were housed in separate area. One group served as an untreated control, a second group was treated for three days with drinking water containing 2 grams per gallon of tylosin and a third group was treated for three days with drinking water containing 2 grams per gallon of tylosin and 200 mg. per gallon of Solulan C-24, a polyoxyethylene ether of the general formula $H(OCH_2CH_2)_nOR$ wherein n is 24 and R is cholesteryl. Three groups were evaluated after three weeks and the results, shown in Table III, indicate that, especially with regard to weight gains, the group receiving both tylosin and the polyoxyethylene ether was benefited to a considerably greater extent than the group receiving tylosin alone.

TABLE III

	Treatment	Av. Weight/Chicken (gms.)	Air Sac Lesions*
A.	2 gm./gal. tylosin	1655	24/74
B.	2 gm./gal. tylosin 200 mg./gal. Solulan	1731	16/73
C.	Infected Controls	1495	60/74

*No. positive/no. examined.

WHAT WE CLAIM IS:—

1. A composition having enhanced activity against mycoplasma infections in poultry, which comprises an antibiotic effective against mycoplasma and a polyoxyethylene ether of the formula $H(OCH_2CH_2)_nOR$ wherein n representing the average number of polyoxyethylene units is a number between 10 and 35 and R is cholesteryl, C_{12}—C_{20} alkyl or alkenyl, the weight ratio of antibiotic to polyoxyethylene ether in said composition being between 1:25 and 30:1.

2. The composition of claim 1,, wherein the antibiotic is tylosin or a non-toxic acid addition salt thereof.

3. A composition according to claim 1 or 2, wherein the polyoxyethylene ether has the formula $H(OCH_2CH_2)_nOR$ wherein n is 20 and R is stearyl.

4. A composition according to claim 1, 2 or 3, which is a solid feed stuff, wherein the polyoxyethylene ether is present in a concentration of between 50 and 2500 grams per ton of feed and the antibiotic is present in a concentration between 100 and 1500 grams per ton of feed.

5. The composition of claim 4 comprising about 1000 grams per ton of tylosin phosphate and about 200 grams per ton of a polyoxyethylene ether of the formula $H(OCH_2CH_2)_nOR$ wherein n is 20 and R is stearyl.

6. A composition according to claim 1, 2, or 3, in aqueous liquid form wherein the polyoxyethylene ether is present in a concentration of between 0.1 gram and 5 grams per gallon and the tylosin or non-toxic acid addition salt thereof is present in a concentration of between 0.25 gram and 5 grams per gallon.

7. The composition of claim 6 wherein the polyoxyethylene ether is present in a concentration of between 0.25 gram per gallon and the tylosin is present in a concentration of 2 grams per gallon.

8. The composition of claim 6 or 7, wherein the polyoxyethylene ether is present in a concentration of 0.25 gram per gallon.

9. The composition in accordance with claim 1 having enhanced activity against mycoplasma infections in poultry, substantially as herein described, with particular reference to any one of the specific examples.

10. A method for controlling mycoplasma infections in poultry which comprises the oral administration of a prophylactically or chemotherapeutically effective quantity of the composition of any of claims 4 to 8, for a period of 1 to 5 days.

BOULT, WADE & TENNANT.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon), Ltd.—1971.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY,
from which copies may be obtained.

EXAMPLE 6

The enhancement of the activity of tylosin against mycoplasma infections was observed in the treatment of a natural outbreak of the infection. During the holding period prior to initiation of an induced infection experiment with a large group of chickens, the flock exhibited symptoms of respiratory disease. Blood samples and examination for air sac lesions indicated an early infection with *Mycoplasma gallisepticum*. The chickens were divided into three groups of 75 chickens each and were housed in separate area. One group served as an untreated control, a second group was treated for three days with drinking water containing 2 grams per gallon of tylosin and a third group was treated for three days with drinking water containing 2 grams per gallon of tylosin and 200 mg. per gallon of Solulan C-24, a polyoxyethylene ether of the general formula $H(OCH_2CH_2)_nOR$ wherein n is 24 and R is cholesteryl. Three groups were evaluated after three weeks and the results, shown in Table III, indicate that, especially with regard to weight gains, the group receiving both tylosin and the polyoxyethylene ether was benefited to a considerably greater extent than the group receiving tylosin alone.

TABLE III

	Treatment	Av. Weight/Chicken (gms.)	Air Sac Lesions*
A.	2 gm./gal. tylosin	1655	24/74
B.	2 gm./gal. tylosin 200 mg./gal. Solulan	1731	16/73
C.	Infected Controls	1495	60/74

*No. positive/no. examined.

WHAT WE CLAIM IS:—

1. A composition having enhanced activity against mycoplasma infections in poultry, which comprises an antibiotic effective against mycoplasma and a polyoxyethylene ether of the formula $H(OCH_2CH_2)_nOR$ wherein n representing the average number of polyoxyethylene units is a number between 10 and 35 and R is cholesteryl, C_{12}—C_{20} alkyl or alkenyl, the weight ratio of antibiotic to polyoxyethylene ether in said composition being between 1:25 and 30:1.

2. The composition of claim 1,, wherein the antibiotic is tylosin or a non-toxic acid addition salt thereof.

3. A composition according to claim 1 or 2, wherein the polyoxyethylene ether has the formula $H(OCH_2CH_2)_nOR$ wherein n is 20 and R is stearyl.

4. A composition according to claim 1, 2 or 3, which is a solid feed stuff, wherein the polyoxyethylene ether is present in a concentration of between 50 and 2500 grams per ton of feed and the antibiotic is present in a concentration between 100 and 1500 grams per ton of feed.

5. The composition of claim 4 comprising about 1000 grams per ton of tylosin phosphate and about 200 grams per ton of a polyoxyethylene ether of the formula $H(OCH_2CH_2)_nOR$ wherein n is 20 and R is stearyl.

6. A composition according to claim 1, 2, or 3, in aqueous liquid form wherein the polyoxyethylene ether is present in a concentration of between 0.1 gram and 5 grams per gallon and the tylosin or non-toxic acid addition salt thereof is present in a concentration of between 0.25 gram and 5 grams per gallon.

7. The composition of claim 6 wherein the polyoxyethylene ether is present in a concentration of between 0.25 gram per gallon and the tylosin is present in a concentration of 2 grams per gallon.

8. The composition of claim 6 or 7, wherein the polyoxyethylene ether is present in a concentration of 0.25 gram per gallon.

9. The composition in accordance with claim 1 having enhanced activity against mycoplasma infections in poultry, substantially as herein described, with particular reference to any one of the specific examples.

10. A method for controlling mycoplasma infections in poultry which comprises the oral administration of a prophylactically or chemotherapeutically effective quantity of the composition of any of claims 4 to 8, for a period of 1 to 5 days.

BOULT, WADE & TENNANT.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon), Ltd.—1971.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY,
from which copies may be obtained.