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(19) AUSTRALIAN PATENT OFFICE (10) 669300

USE OF RIFAMYCIN DERIVATIVES FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF TOXOPLASMOSIS

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WO 94/25018

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PALO ALTO MEDICAL FOUNDATION

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1. A method of [illegible]

[illegible]

2. A method of [illegible]

[illegible]

(11) AJ-B-69050 94
(19) 669300

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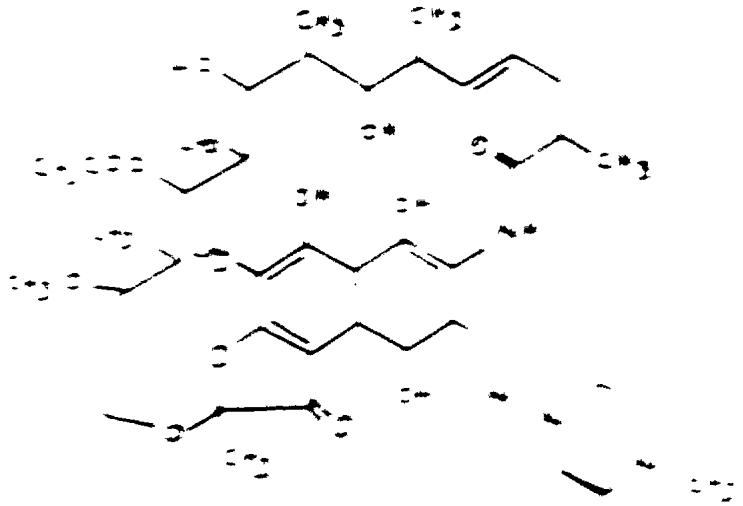


[illegible]

BRIEF DESCRIPTION OF THE DRAWINGS

[illegible]

Figure [illegible] graph [illegible] RIF

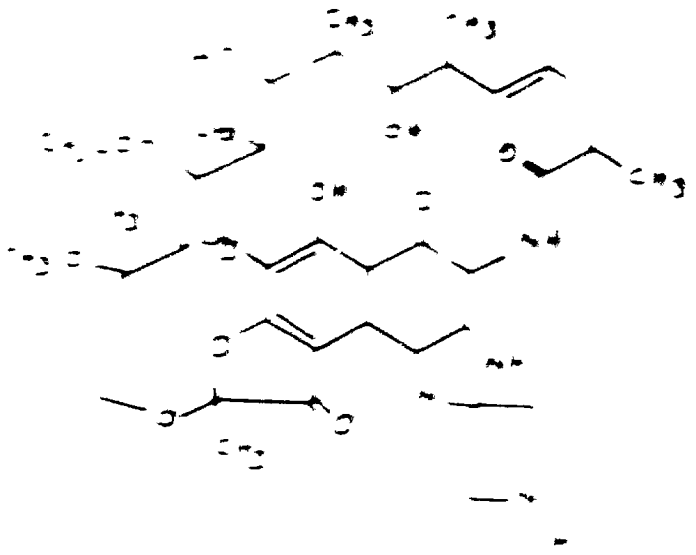
Figure B [illegible] graph [illegible] and treated with [illegible] concentration [illegible] RF

Figure [illegible] graph [illegible]

Figure [illegible] graph [illegible]

Figure [illegible] graph [illegible]

Figure 5 [illegible] graph [illegible] RI [illegible]



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[illegible] ... *T. gondii* ... and *Pneumocystis carinii* ... [illegible] ... organisms of the *Mycobacterium avium* complex ... [illegible] ... frequently encountered ... [illegible]

The invention now being thus described, the following examples are provided for illustration only and are not intended to limit the scope of the invention ... [illegible]

<u>EXAMPLES</u>

<u>EXAMPLE [illegible] Activity ... against *T. gondii* in ... mouse model ... [illegible]</u>

An experiment was carried out to determine the ... [illegible] ... The model used was a mouse model using Swiss Webster female mice weighing approximately 20 grams at the time of the experiment. The ... used in the experiment was obtained commercially ... as a ... [illegible] ... was ... in propylene ... buffered saline, pH 7.2, and then ... [illegible] ... The experiment was carried out in four groups of mice, 3 experimental and one control, with ... mice per group. The mice were infected with *T. gondii* ... the RH strain obtained from the peritoneal fluid of carrier mice ... [illegible] ... The peritoneal fluid was ... [illegible] ... % fetal calf serum and ... [illegible] ... preparation was then filtered through ... [illegible] ... and the sediment was resuspended and ... through a 27 gauge needle. The ... [illegible] ... were counted ... a Neubauer ... hemacytometer ... [illegible] ... Eagle's minimum essential medium (MEM) were made. The ... [illegible] ... RH ... *T. gondii* ... [illegible] ... Mice ... infected with ... RH strain ... [illegible]

[illegible] were [illegible]

[illegible] murine [illegible] different [illegible] administered [illegible] RH [illegible] tachyzoites [illegible] infected [illegible] and apparent [illegible] infected mice treated with these doses did not [illegible] Although pharmacokinetic studies were not conducted in the present [illegible] in normal human volunteers [42] [illegible] from the gastrointestinal tract. The average terminal half-life in humans after oral administration was 36 hours and the bioavailability was 44% [42].

RH tachyzoites and C56 cysts were used in these experiments because [illegible] demonstrated [illegible] different strains of T. gondii [illegible] strain C56 [illegible] and [illegible] the pathogenesis of the infection produced [illegible] tachyzoites or cysts differ [illegible] inoculation of RH tachyzoites results in [illegible] with large numbers of tachyzoites being produced in the peritoneal cavity. One hundred percent mortality usually occurs within 5 to 8 days after infection with an inoculum [illegible] organisms. In contrast, oral inoculation of cysts of the C56 strain results in an infection which progresses slower. Depending on the inoculum size, mortality [illegible] approximately [illegible] days of infection.

<u>EXAMPLE 2 [illegible] in combination with [illegible] against T. gondii in a murine model of acute toxoplasmosis.</u> An experiment was carried [illegible] manner described for Example [illegible] but with the additional administration [illegible] [illegible] were as described in the first example except for the following changes: Seven groups of mice [illegible] mice per group) were infected [illegible] intraperitoneal injection [illegible] tachyzoites of T. gondii and then given oral treatment [illegible] beginning 24 hours after infection and continuing for 10 days. Sulfadiazine sodium salt was obtained from Sigma Chemical [illegible] Chemical [illegible] New York, [illegible] and was administered [illegible] during the entire period of treatment [illegible] in the drinking water [illegible] groups as follows:

Group [illegible]	Rifabutin alone [illegible] mg/kg/day
Group 2	Rifabutin alone [illegible] mg/kg/day
Group 3	Rifabutin alone 200 mg/kg/day

-

[illegible]

[illegible]

[illegible]

[illegible]

Groups 2 and 3 animals were subjected to experimental procedures identical to those described for Example 1 and Figure 1. For procedural details see Example 1. Results are presented in Figure 2. Group 2 animals (the vehicle control group) showed a [illegible] response to the [illegible] challenge. Group 3 animals (the group treated with 100 mg/kg of [illegible]) showed a [illegible] response to the [illegible] challenge. The magnitude of the [illegible] response was significantly [illegible] than that of the vehicle control group. Group 3 animals showed [illegible] for the entire 30-day period. Group 2 animals showed [illegible] for approximately [illegible] days.

These results indicate that [illegible] provided greater protection against [illegible] than did [illegible] alone. There appeared to be a synergistic effect when [illegible] were administered together. Figure 3 shows that [illegible] when treated with [illegible] alone and those receiving [illegible] and [illegible] remained [illegible] for the entire 30-day period. Surprisingly, all of the animals treated with the [illegible] combination survived beyond [illegible] days and four of them survived for the entire 30-day period. Figure 3 also shows that [illegible] at 200 mg/kg/day was highly effective and [illegible] was ineffective. None of the animals survived the entire period when [illegible] alone at this concentration, while [illegible] survived for the entire period.

EXAMPLE 4: [illegible] combination with [illegible]

[illegible] model of acute [illegible]: An experiment was carried out in the manner described for Examples 1 and 2, but with the additional administration of [illegible]. Pentobarbital sodium (Burroughs Wellcome, Research Triangle Park, NC) was dissolved in saline for [illegible] administration. Sprague-Dawley rats were as described in the [illegible] examples. Six animals from the control group and from the [illegible] group as indicated in Figure 5 were treated [illegible] with [illegible] mg/kg/day for [illegible] days. Treatment with [illegible] mg/kg/day was initiated 24 hr after [illegible] of [illegible] with [illegible] mg/kg/day was initiated [illegible]

[illegible]

Group [illegible]

Group 2 Rifabutin [illegible] mg/kg/day

Group 3 Rifabutin [illegible] mg/kg/day

Group 4 Pyrimethamine [illegible] mg/kg/day

Group 5 Rifabutin [illegible] mg/kg/day plus pyrimethamine [illegible] mg/kg/day

Group 6 Rifabutin [illegible] mg/kg/day plus pyrimethamine [illegible] mg/kg/day.

Results of the experiments are set out in Figure 5. The effects of the [illegible] are similar to those seen in the previous examples in which treatment was initiated with the other dosing regimens and protection was observed. The combination of rifabutin and pyrimethamine gave better protection than either drug alone. The higher dose of rifabutin in combination with pyrimethamine gave the best protection.

EXAMPLE 4 This example illustrates the combination of rifabutin and clindamycin against murine toxoplasmosis. Experiments were carried out in the manner described for Examples [illegible] and 3 but with the additional administration of rifabutin and pyrimethamine. Clindamycin hydrochloride salt (C18H33ClN2O5S) purchased from Sigma was dissolved in sterile PBS and administered orally by gavage in a single daily dose of 25 mg/kg. Conditions were as described in the first example except for the following changes. Groups of mice (5 in the control group and 10 in the treatment groups) indicated in Figure 6 were infected by intraperitoneal injection of 2.5 x 10^6 tachyzoites of the RH strain. Treatment with rifabutin (RIF) alone at 50 mg/kg/day or in combination with clindamycin (CLINDA) at 25 mg/kg/day was initiated 24 hours after infection and continued for [illegible] days. Both drugs were administered orally by gavage as a single daily dose either alone or in combination.

Results are shown in Figure 6. The protection afforded by the combination of rifabutin with clindamycin was remarkable. Whereas 10 percent of mice infected with RH tachyzoites survived the infection when treated with a combination of 50 mg/kg rifabutin and 25 mg/kg clindamycin. These doses of the respective drugs had no significant effect on protecting mice against death when administered alone. In a second experiment all infected mice treated with the combination survived.

Clindamycin, the drug most frequently used as an alternative to sulfadiazine when side effects develop during treatment with the pyrimethamine-sulfonamide combination, [illegible]

[illegible]

[illegible] experiments were [illegible] described [illegible] and [illegible] with the additional administration of [illegible] atovaquone [illegible]. Atovaquone (C [illegible]) was prepared in PBS and sonicated [illegible] seconds each. [illegible] solutions [illegible] mg/kg were prepared in 25% [illegible] methylcellulose and sonicated [illegible] seconds [illegible] administration by gavage. Conditions were as described in the first example except [illegible] following change: Four groups of mice (5 in the control group [illegible] in the treatment group as indicated in Figure 7A and 7B) were infected by intraperitoneal injection [illegible] of *T. gondii* RH strain. Treatment with rifabutin (RIF) alone at 100 mg/kg [illegible] or 50 mg/kg/day or in combination with atovaquone (ATO) at 50 mg/kg/day was initiated 24 hours after infection and continued for 10 days. Both drugs were administered orally by gavage as a single daily dose either alone or in combination. Control mice were treated with the drug diluent only.

Results are shown in Figures 7A and 7B. Treatment with combination of 50 or 100 mg/kg of rifabutin with 50 mg/kg of atovaquone resulted in protection of mice against death. One hundred mg/kg of rifabutin plus 50 mg/kg of atovaquone protected at least 60% of the mice against death (Figure 7A). Statistical analysis of the data, however, did not reveal a significant difference between the group of mice treated with rifabutin alone and the group treated with the combination. In contrast, when the dose of rifabutin was reduced to 50 mg/kg and used in combination with a dose of 50 mg/kg/day of atovaquone, prolongation of time to death was obtained and 30% of the mice survived the infection (Figure 7B). The difference between groups of mice treated with rifabutin alone and those treated with the combination was statistically significant (P = [illegible]).

Since atovaquone is active both against *T. gondii* [illegible] and Pneumocystis carinii [33] and rifabutin is active against mycobacteria, being used for treatment and prevention of [illegible] organisms of the Mycobacterium avium complex [34], the results presented herein which demonstrate the effectiveness of the combination of rifabutin/atovaquone against *T. gondii* provide for the use of this combination for treatment and prevention of [illegible] frequently encountered opportunistic agents in AIDS patients.

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[illegible] ... treatment were [illegible] ... mice [illegible] ... adult Swiss Webster female [illegible] ... treatment groups as indicated in Figure 8 were infected [illegible] ... *T. gondii* RH strain. Treatment [illegible] ... THR [illegible] 50 mg/kg [illegible] rifabutin RF [illegible] ... feeding tube was initiated 24 hours after infection and continued [illegible] ... were treated with drug diluent only.

Results are shown in Figure 8. One hundred percent of the mice treated with [illegible] combination survived at 30 days whereas none of the mice treated with [illegible] survived at 30 days.

EXAMPLE 7 [illegible] rifabutin [illegible] *T. gondii* in a murine model of acute toxoplasmosis. Experiments were carried out in the manner described for Examples [illegible] and 3 but with the additional administration of clarithromycin instead of pyrimethamine. Conditions were as described in the first example except for the following changes: Four groups of adult Swiss Webster female mice (n in each group: 5 or [illegible]) in the treatment groups as indicated in Figure 9 were infected intraperitoneally [illegible] 2.5 x 10 tachyzoites of *T. gondii* RH strain. Treatment with 50 mg/kg of rifabutin (RIF) or 50 mg/kg of clarithromycin (CLAR) alone or in combination was administered using a feeding tube, was initiated 24 hours after infection and continued for [illegible] [illegible] mice were treated with drug diluent only.

Results are shown in Figure 9. One hundred percent of the mice treated with [illegible] combination survived at 30 days whereas 20 % of the mice treated with clarithromycin alone survived at 30 days.

EXAMPLE 8 [illegible] activity of rifabutin against *T. gondii* in a murine model of [illegible] encephalitis (TE). [illegible] CBA/Ca (CBA) adult females weighing [illegible] g at the beginning of each experiment were used to determine rifabutin activity in treatment of toxoplasmic encephalitis caused by infection with cysts of the strain ME49 of *T. gondii* [illegible] mice were purchased from [illegible] Laboratories [illegible] and were [illegible] mouse [illegible] and water *ad libitum*. Each CBA mouse was infected [illegible] ME49 strain [illegible] Infected CBA mice were used 8 weeks following infection [illegible] Histological examination of brain of 3 mice euthanized [illegible] time [illegible]

[illegible] administered [illegible] daily [illegible] treated and 5 [illegible] mice were [illegible] treatment and the brains [illegible] collected for determination of numbers [illegible] examination [illegible] are [illegible]

Histological [illegible] brains from [illegible] 30 days after infection were [illegible] in [illegible] treated mice 30 days after infection. H[illegible] mice treated with the [illegible] revealed extensive inflammation [illegible] in the meninges, parenchyma and around small capillaries as well as numerous [illegible] *gondii*. Treatment of infected BALB/c mice with 200 mg/kg/day [illegible] administered [illegible] for 5 days did not significantly reduce the inflammatory response or numbers of *T. gondii* [illegible] in the 5 treated mice as compared with the [illegible]. However, treatment with 200 mg/kg/day [illegible] administered [illegible] reduced [illegible] inflammatory response in each of the treated mice when compared [illegible]. The numbers of *T. gondii* cysts in the brains of the treated mice were not significantly reduced as compared with untreated control.

These results indicate that [illegible] used alone is effective for treatment of the infection in the central nervous system. Since the numbers of *T. gondii* cysts in the brains of treated mice were not significantly reduced at the end of the therapy, these results [illegible] unrecognized anti-inflammatory activity [illegible] against these parasites and [illegible] rapid [illegible] against the [illegible]. However, the present invention is not limited by any particular mechanism of [illegible] action.

EXAMPLE 3 [illegible] activity [illegible] in combination with [illegible] against *T. gondii* in an immunocompromised mammalian host. Treatment of a T. gondii infected immunocompromised mammalian host using [illegible] in combination with [illegible]. Immunocompromised T and B cell deficient SCID [illegible] mice [illegible] were infected [illegible] with [illegible] ME49 strain [illegible] ME49 [illegible] immunocompromised host [illegible] PBS [illegible] doses of the drug combination 50 mg/kg [illegible] with [illegible] mg/kg [illegible]

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The claims defining the invention are as follows:-

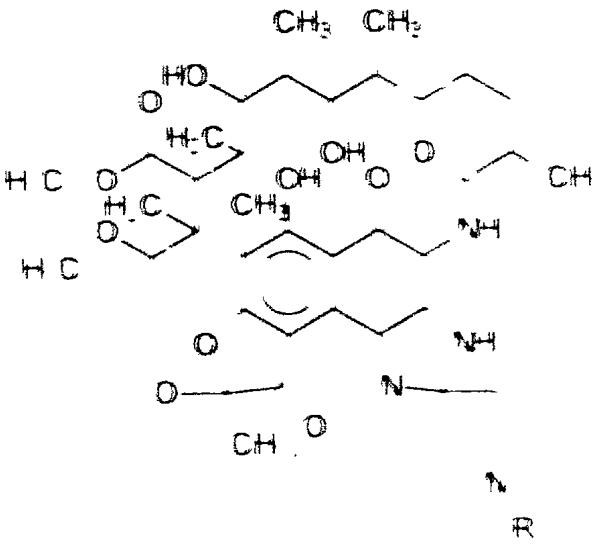
1 A method of reducing the severity of toxoplasmosis resulting from infection of a mammalian host with *Toxoplasma gondii* which comprises

administering to a host in need of said treatment either after infection or before exposure to said infection a therapeutically effective amount of a compound that is a spiropiperidyl derivative of rifamycin S wherein said derivative comprises an imidazole ring that includes carbon atoms 3 and 4 of the rifamycin moiety, the carbon atom at position 2 of said imidazole ring has been replaced by a nitrogen atom and a piperidine ring system thereby forming a spiropiperidyl ring system, said spiropiperidyl ring system optionally comprising a lower hydrocarbon substituent on the nitrogen of said piperidine

2 A method of reducing the severity of a protozoal encephalitis resulting from infection of a mammalian host with *Toxoplasma gondii* which comprises

administering to a host in need of said treatment either after infection or before exposure to said infection a therapeutically effective amount of a compound that is a spiropiperidyl derivative of rifamycin S wherein said derivative comprises an imidazole ring that includes carbon atoms 3 and 4 of the rifamycin moiety, the carbon atom at position 2 of said imidazole ring has been replaced by a nitrogen atom and a piperidine ring system thereby forming a spiropiperidyl ring system, said spiropiperidyl ring system optionally comprising a lower hydrocarbon substituent on the nitrogen of said piperidine

3 The method of claim 1 or claim 2, wherein said compound has the formula



wherein R represents a 3-5 carbon alkyl group

4 The method of claim 1 wherein said compound is rifabutin

5 The method of any one of claims 1 to 4 wherein said compound is administered in combination with a sulfonamide drug

6 The method of any one of claims 1 to 4 wherein said compound is administered in combination with a folate antagonist

The method of any one of claims 1 to 4 wherein said compound is administered in combination with a second drug

6 The method of any one of claims 1 to 4 wherein said compound is administered in combination with a third drug selected from the group consisting of a second drug a third drug and an additional drug

7 The method of any one of claims 1 to 4 wherein said compound is administered in combination with a second drug and a third drug

The method of any one of claims 1 to 4 wherein said compound is administered in combination with an additional drug

9 A pharmaceutical composition for reducing the severity of a psychosis resulting from the infection of a mammalian host with a virus which comprises

a a therapeutically effective amount of a compound that is a spiropiperidine derivative of subformula 5 wherein said derivative comprises an azabicyclic ring that includes carbons at positions 3 and 4 of the subformula ring the carbon at position 2 of said azabicyclic ring is being a ring carbon at position 4 of a piperidine ring system thereby forming a spiropiperidyl ring system said spiropiperidyl ring system optionally comprising a member selected from the group consisting of a second drug a third drug and an additional drug and

b a therapeutically effective amount of a compound selected from the group consisting of a second drug a third drug and an additional drug

10 A method for reducing the severity of a psychosis of a psychosis encephalitis resulting from the infection of a mammalian host with a virus and which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound substantially as hereinbefore described with reference to any one of the Examples

Dated 22 March, 1996
Palo Alto Medical Foundation

Patent Attorneys for the Applicant Nominated Person
SPRUSON & FERGUSON

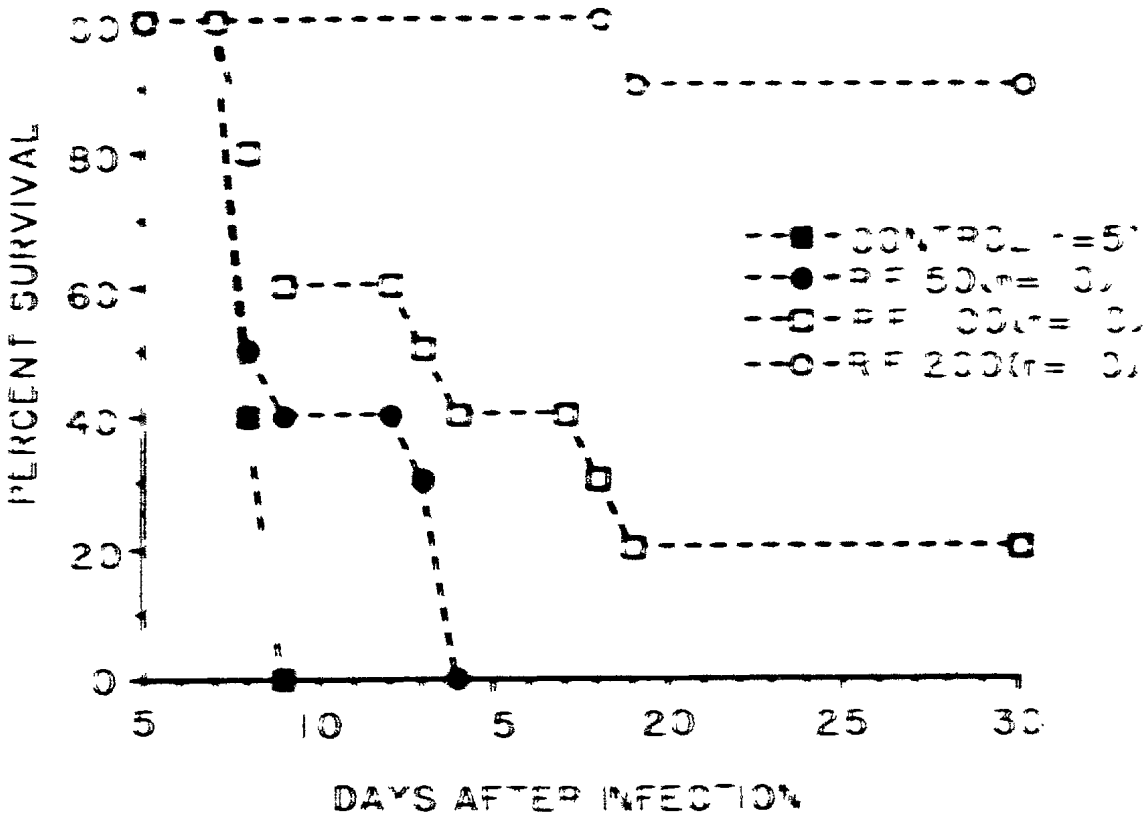

PERCENT SURVIVAL
DAYS AFTER INFECTION

FIG. IA

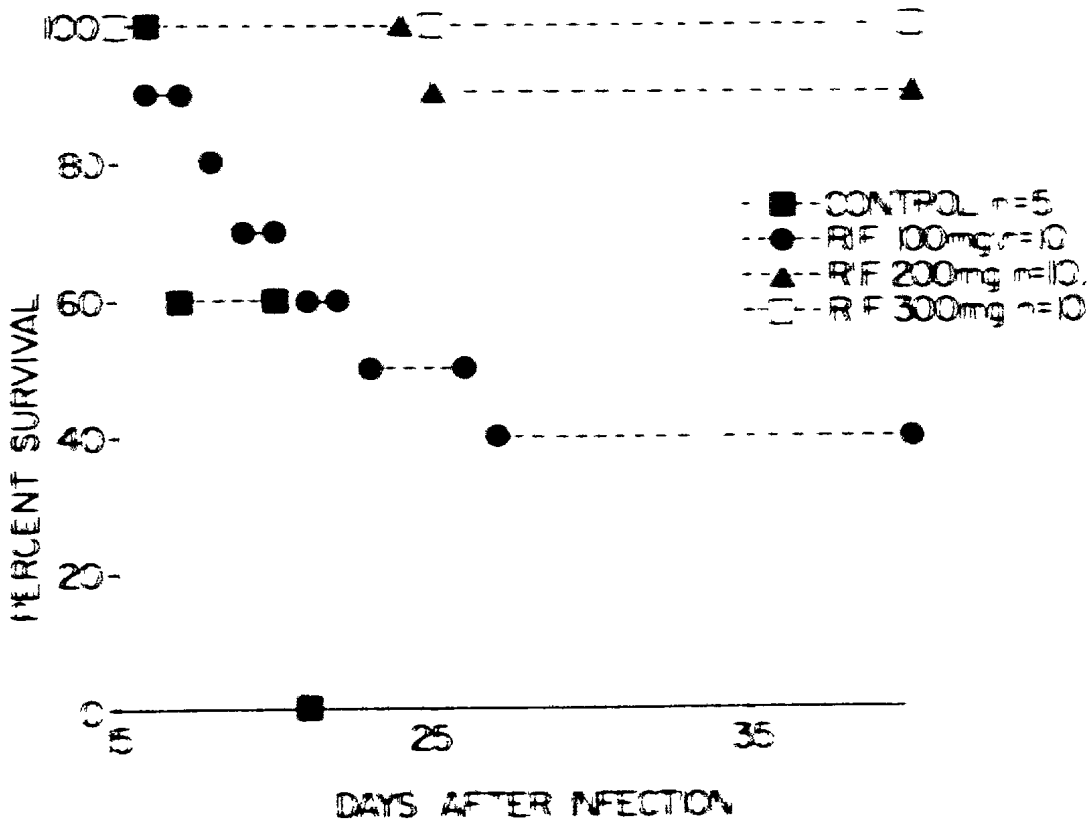

PERCENT SURVIVAL
DAYS AFTER INFECTION

FIG. IB

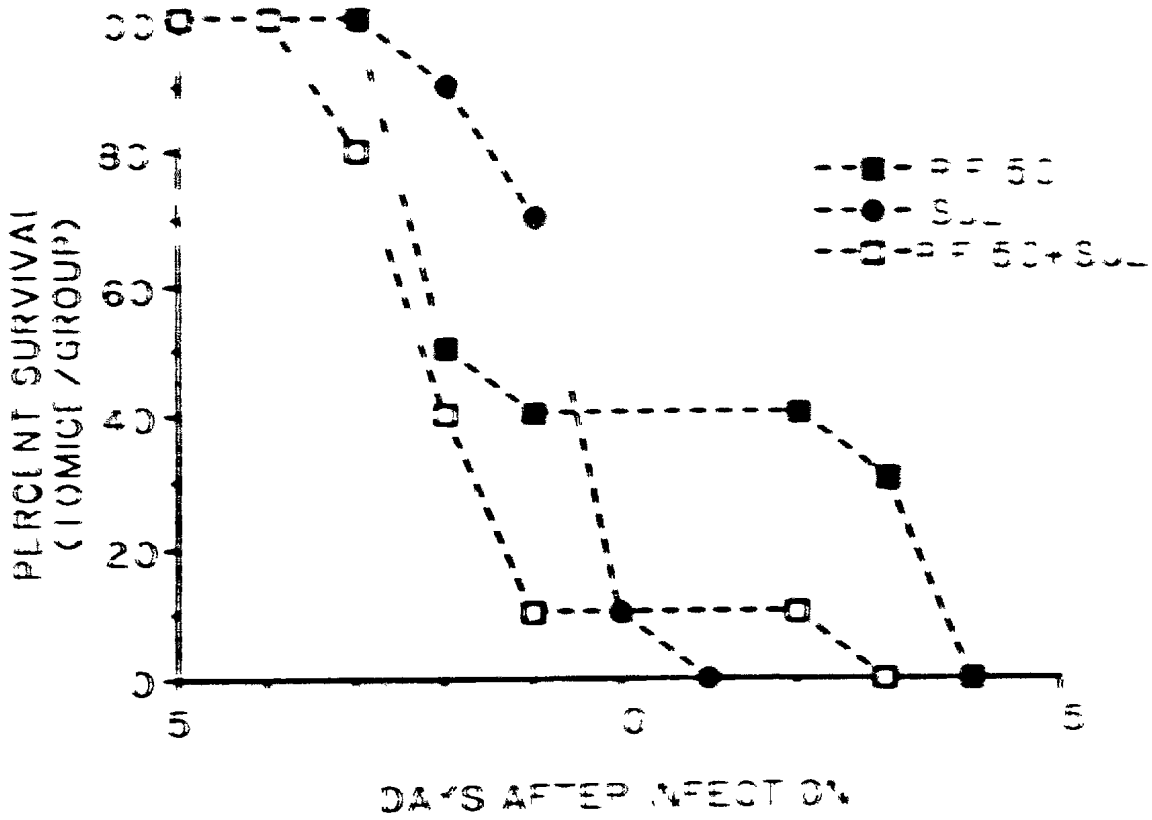

PERCENT SURVIVAL
(10MICE/GROUP)
100
80
60
40
20
0
5
0
5
DAYS AFTER INFECTION

FIG. 2

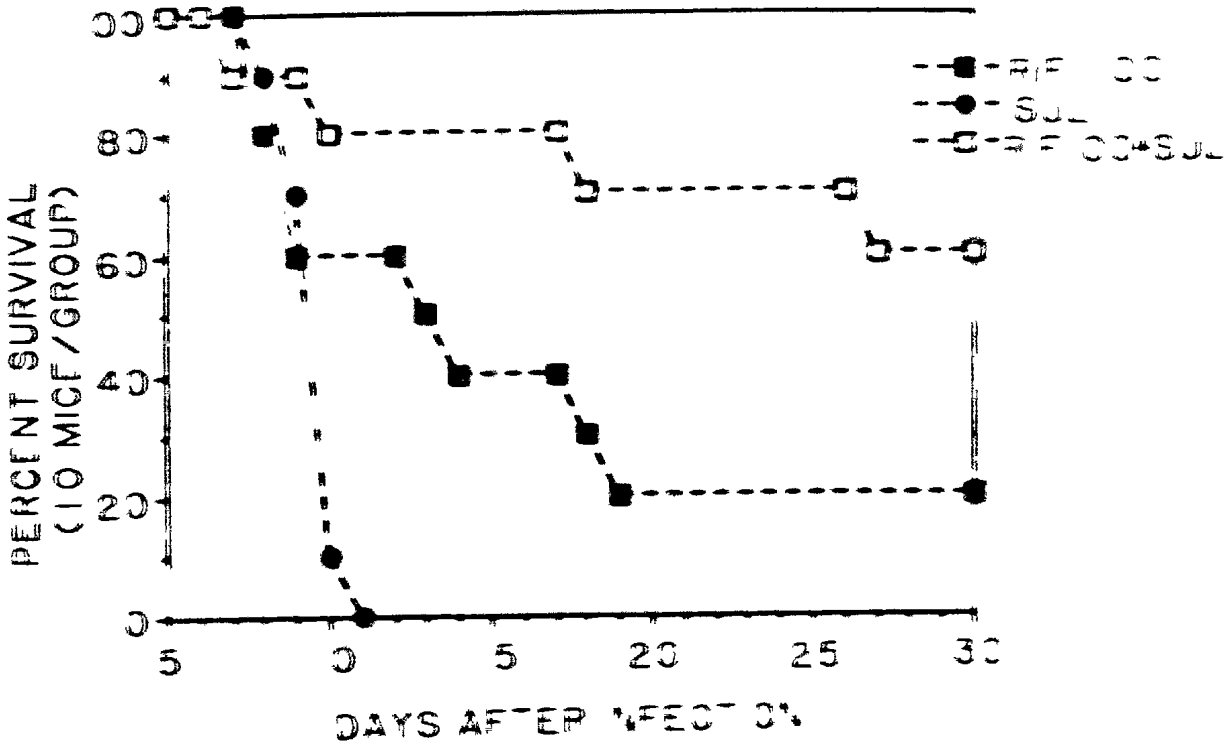

PERCENT SURVIVAL
(10 MICE/GROUP)
100
80
60
40
20
0
5
0
5
20
25
30
DAYS AFTER INFECTION

FIG. 3

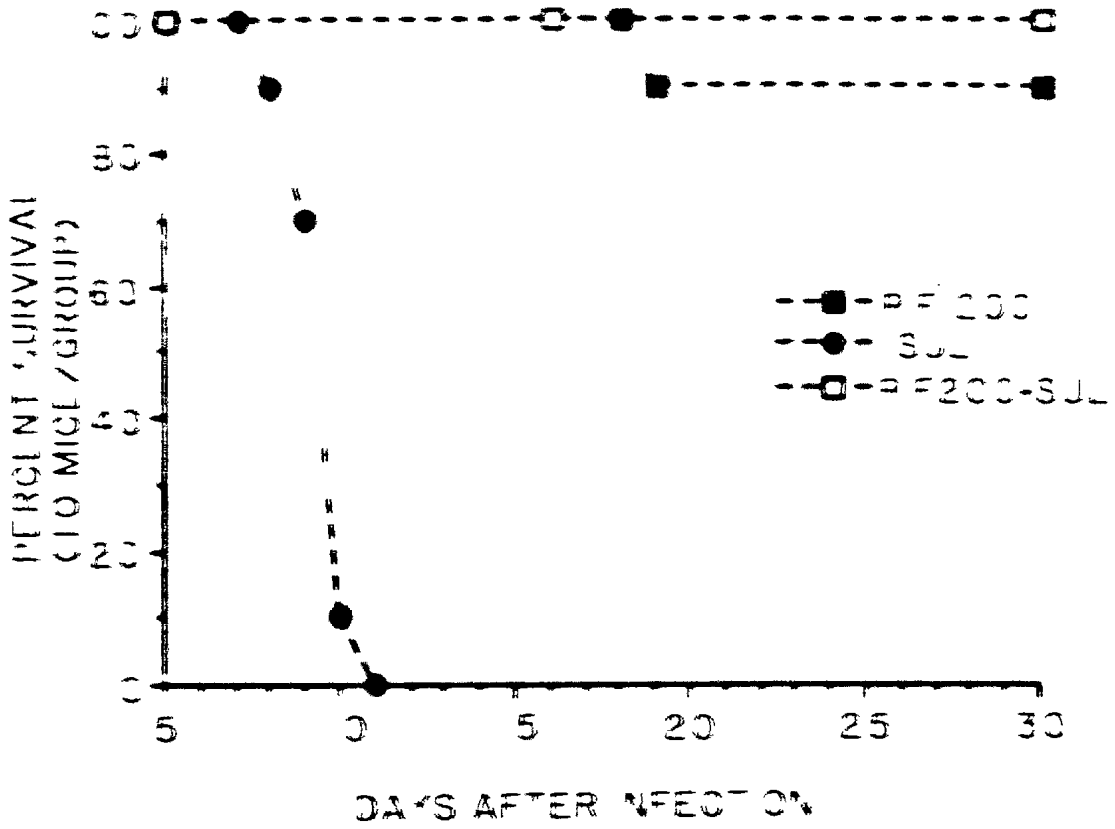

PERCENT SURVIVAL
DAYS AFTER INFECTION

FIG. 4

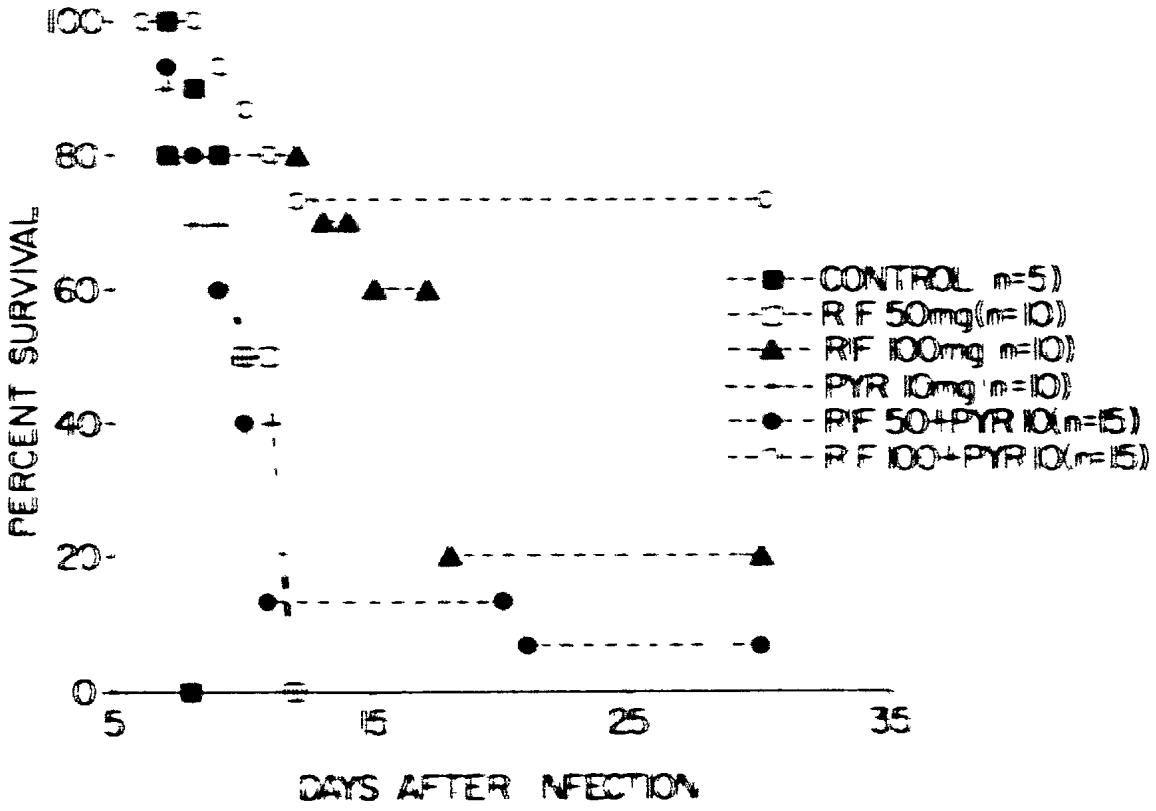

PERCENT SURVIVAL
100
80
60
40
20
0
5
15
25
35
DAYS AFTER INFECTION
CONTROL n=5)
RF 50mg(n=10)
RF 100mg n=10)
PYR 10mg n=10)
RF 50+PYR 10(n=15)
RF 100+PYR 10(n=15)

FIG. 5

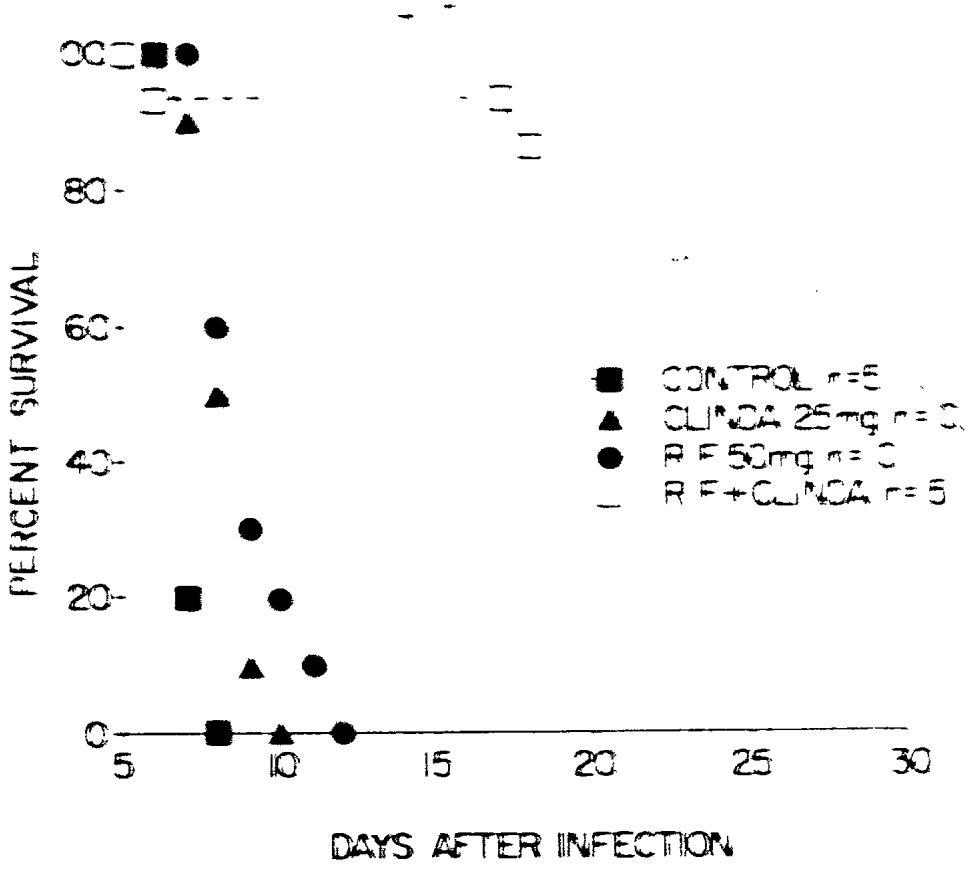

PERCENT SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
30
DAYS AFTER INFECTION
CONTROL n=5
CLINDA 25mg n=5
RIF 50mg n=5
RIF+CLINDA n=5

FIG. 6

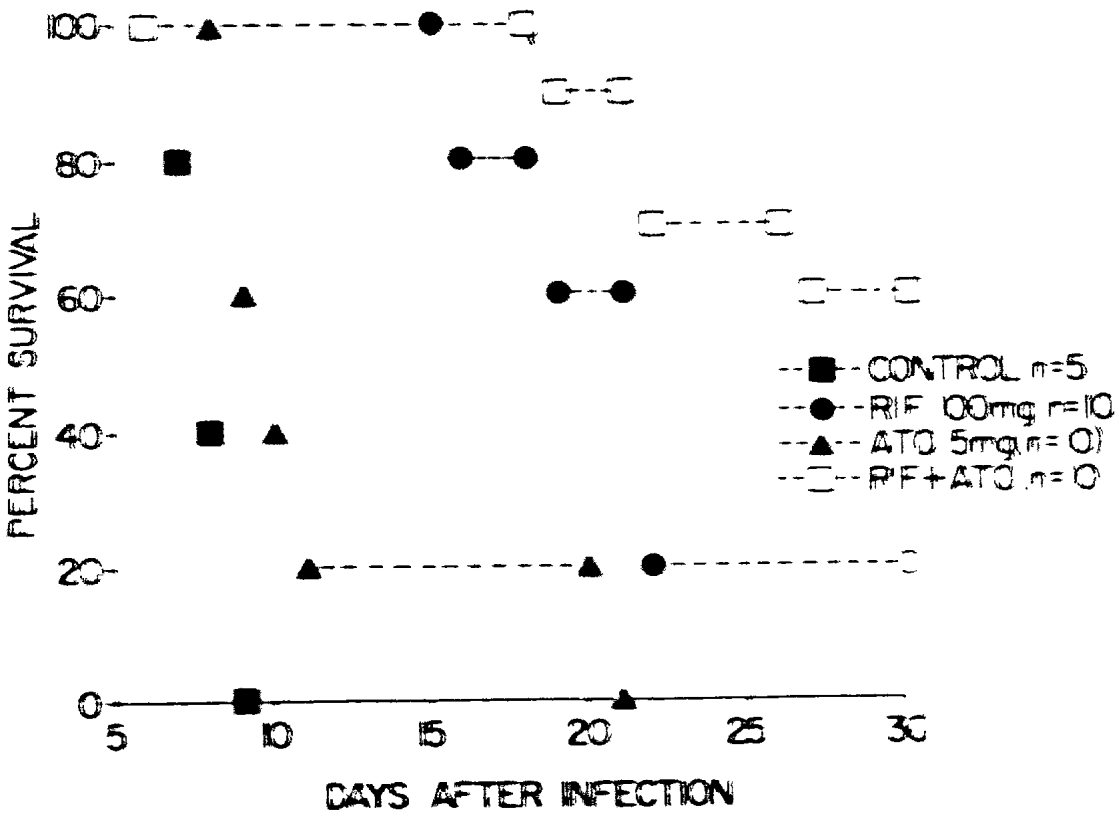

PERCENT SURVIVAL
100
80
60
40
20
0
5
10
15
20
25
30
DAYS AFTER INFECTION
CONTROL n=5
RIF 100mg n=10
ATO 5mg n=10
RIF+ATO n=10

FIG. 7A

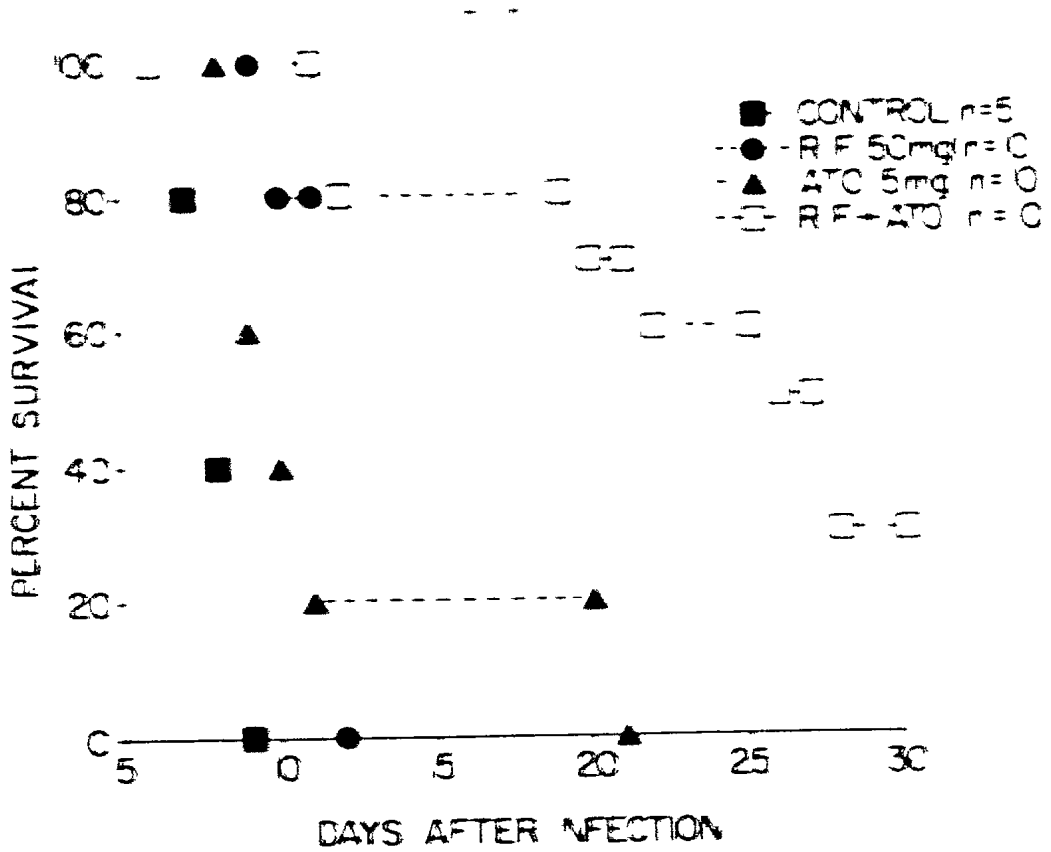

CONTROL n=5
RIF 50mg/kg n=C
AZITHRO 5mg n=0
RIF+AZITHRO n=C
PERCENT SURVIVAL
100
80
60
40
20
0
5
0
5
20
25
30
DAYS AFTER INFECTION

FIG. 7B

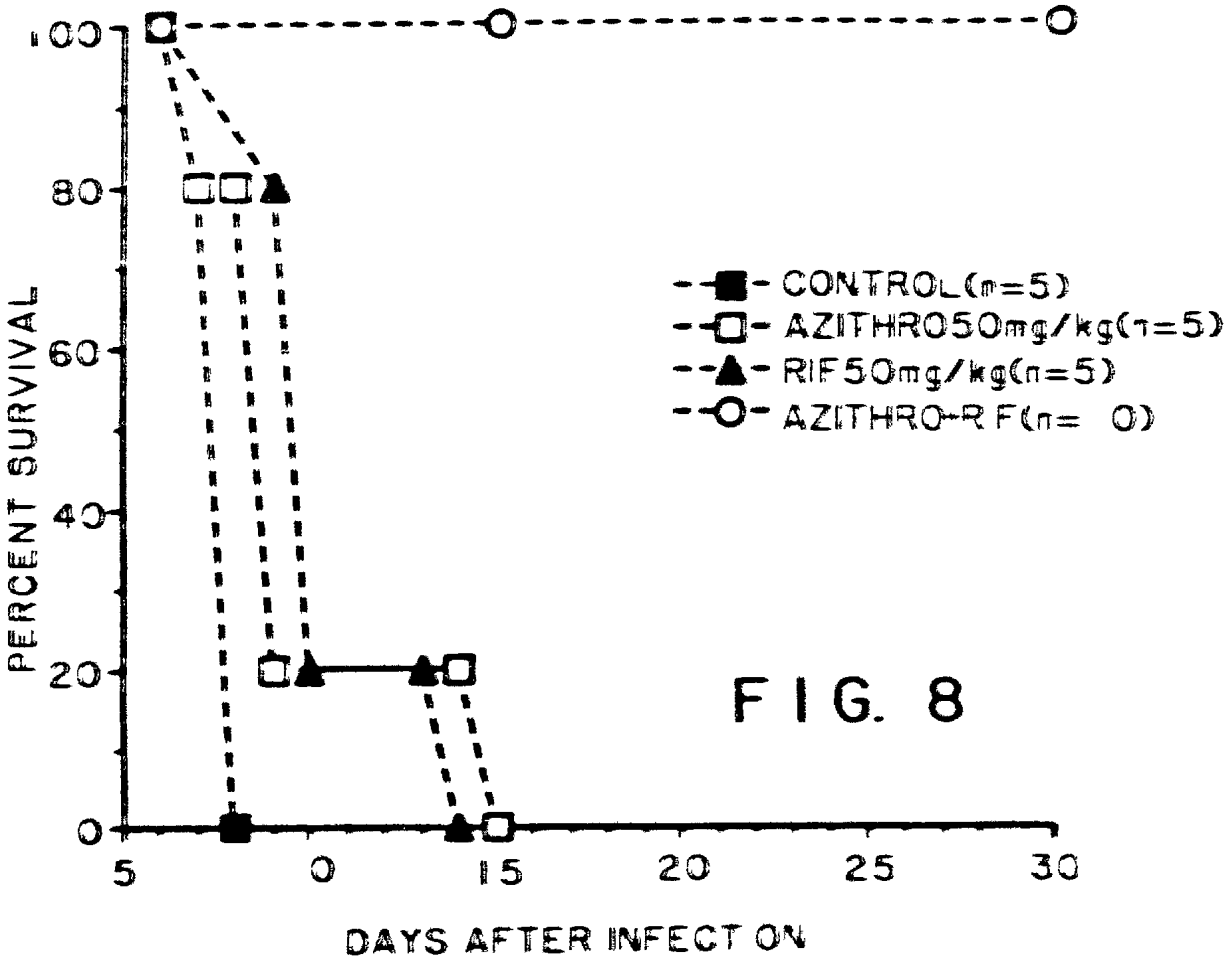

CONTROL(n=5)
AZITHRO 50mg/kg(n=5)
RIF 50mg/kg(n=5)
AZITHRO+RIF(n= 0)
PERCENT SURVIVAL
100
80
60
40
20
0
5
0
15
20
25
30
DAYS AFTER INFECTION

FIG. 8

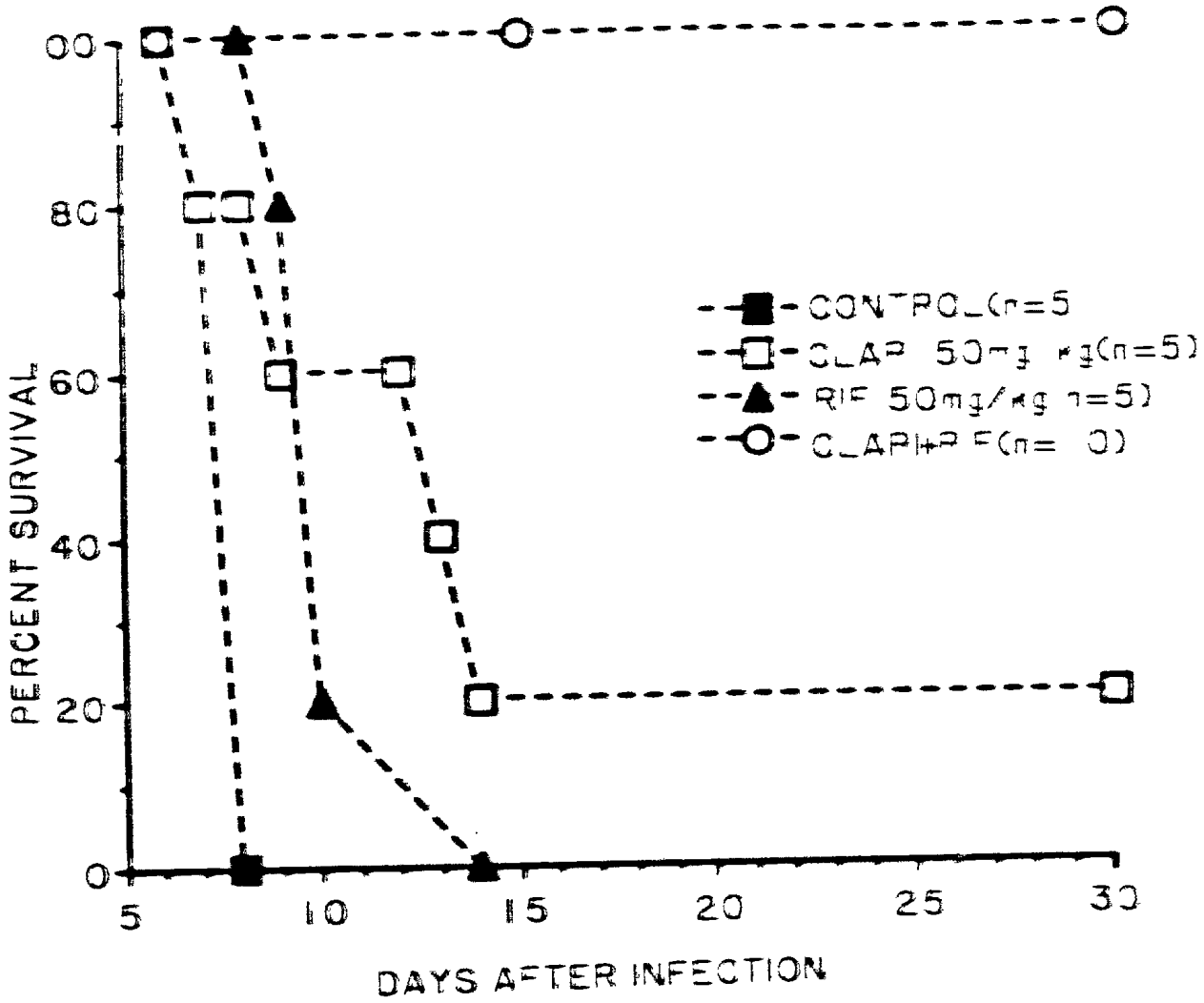

100
80
60
40
20
0
PERCENT SURVIVAL
5
10
15
20
25
30
DAYS AFTER INFECTION
CONTRO_(n=5
C_AP 50mg/kg(n=5)
RIF 50mg/kg n=5)
C_AP+R F(n= 0)

FIG. 9

INTERNATIONAL SEARCH REPORT

IPC 5 A61K31/

Minimum documentation searched
IPC 5 A61K

P,X	ANTIMICROBIAL AGENTS AND CHEMOTHERAPY vol. 38, no. 3, March 1994 pages 570 - 575 FAUSTO G. ARAUJO ET AL. 'Rifabutin is active in murine models of toxoplasmosis' see the whole document	1-12
X	US,A,4 219 478 (LEONARDO MARSILI ET AL.) 26 August 1980 cited in the application see column 2, line 14 - line 65	11

[X] Further documents are listed in the continuation of box.

[X] Patent family members are listed in annex.

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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PCT [illegible]

[illegible]	[illegible]	[illegible]
A	PROC SOC EXP BIOL MED vo 135 1970 pages 167 - 172 JACK S REMINGTON ET AL The effect of rifampin on tuboo asma gonoc c ted in the ace tat on see page 172, eft co umn paragraph 5	[illegible]
A	POSTGRADUATE MEDICINE vo 91, no 8 June 1992 pages 115 - 132 JUDITH FALLOON Current therapy for HI rfection and its rrfect ous comp'rcations see tab'e, 3,4	[illegible]

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[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	A1-A-	448[illegible]
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