SUSCEPTIBILITY OF MICRO-ORGANISMS TO CHLORAMPHENICOL (CHLOROMYCETIN) [1, 2]

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The antibiotic, chloramphenicol, produced by *Streptomyces venezuelae,* was originally reported by Ehrlich *et al.* (1, 2) and independently described by Carter, Gottlieb *et al.* (3, 4). Since these original reports the active principle has been chemically isolated and characterized (5, 6), synthesized (7) and is at present commercially available for clinical use.

Originally (1) activity was described against several Gram-negative bacteria, in particular *Shigella sonnei,* and against *Rickettsia prowazekii* in chick embryos. Smadel *et al.* (8–11) have notably extended this spectrum to include other rickettsial agents as well as two viruses, lymphogranuloma venereum and psittacosis. Smith *et al.* (12) reported that chloramphenicol was active, under the conditions tested, against Gram-negative bacteria, *R. prowazekii* and *Borellia recurrentis;* moderately active against Gram-positive bacteria and *Mycobacterium tuberculosis;* and inactive in the concentrations used against yeasts, filamentous fungi, protozoa and certain viruses. Youmans *et al.* (13) tested strains of virulent human-type *M. tuberculosis in vitro* and concluded that chloramphenicol was only moderately active when compared with streptomycin or para-amino salicylic acid.

Clinically, confirmation of laboratory activity has been obtained for certain of the rickettsial diseases (14–18), typhoid fever (19), brucellosis (20) and in urinary infections caused by certain Gram-negative bacteria (21). With the drug now available in quantity for clinical trial, it seems opportune at this time to collect the known information with respect to the microbiological spectrum of this new chemotherapeutic agent.

METHODS

Viruses and Rickettsiae

Borreliota: Vaccinia virus (New York Public Health strain from Dr. S. K. Muckenfus, New York City Dept. of Public Health, N. Y., egg-adapted) was tested as a representative of this group of viruses. Two types of tests in chick embryos were used. The first consisted of mixing the chloramphenicol with the virus and inoculating 0.1 ml. of the mixture onto the chorioallantoic membrane of 10-day chick embryos. After four days incubation at 35° C. the membranes were examined and compared with controls for reduction in pock formation. The second method consisted of inoculating groups of treated, 10-day chick embryos *via* the yolk sac with a lethal dose of virus and repeating treatment on the following day by the same route. The eggs were candled twice daily and delay in death time and seven-day survivors recorded.

Erro: St. Louis encephalitis virus (egg-adapted from Dr. Carl Duffy, Wayne University, Detroit, Michigan, and a mouse-adapted laboratory strain) was tested to represent the group of virus encephalitides. Tests were conducted in embryonated eggs and mice. For the egg test, nine-day embryos were infected *via* the allantoic sac and treated 30 minutes before infection and on the 1st day after infection. The eggs were candled twice daily and time of death and seven-day survivors noted. In the mouse test, groups of 10 to 12 Gm. white Swiss mice were infected intracerebrally and treated intraperitoneally before infection and twice daily thereafter for 10 doses. Mean death time was compared with that of a control group.

Formido: The National Institutes of Health mouse-adapted strain of rabies virus was used, as well as a variant line of this strain adapted to the intramuscular route of infection. For the former, mice were injected intracerebrally with a lethal dose and daily treatments given intraperitoneally for four days. A similar procedure was used with the intramuscular strain except for the different route of virus administration. Time of paralysis and death of the treated mice was noted for comparison with control groups.

Legio: The anti-poliomyelitis virus tests were conducted by Drs. J. L. Melnick and F. R. Corria (22) of the Yale University School of Medicine, New Haven, Conn. The Y-S.K. and Lansing strains (mouse-adapted) were used as well as Theiler's intestinal virus of mice.

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[2] Parke, Davis & Company trade-name.

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Rabula: The egg-adapted mumps virus tested was obtained from Dr. J. E. Smadel of the Army Medical School, Washington, D. C. The tests were conducted in seven-day embryonated eggs infected *via* the allantoic sac. Treatments were given 30 minutes before infection and on the second day. Allantoic fluid was harvested from each egg after seven-days incubation and tested for virus multiplication by the addition of washed chicken red blood cells. In addition a hemagglutination titration was performed on an aliquot pool of allantoic fluid from the groups of eggs. An untreated control group was included in the test for comparison of the number of positive eggs and level of group hemagglutinin titer.

Tarpeia: Tests against three strains of type A, seven strains of type A′ and two strains of type B influenza virus have been conducted in 11-day chick embryos. The virus was inoculated into the allantoic sac and treatments given by the same route 30 minutes before infection and 60 minutes later. After 40 hours incubation at 35° C. the presence of infection in each egg and the hemagglutination titer of an aliquot pool of allantoic fluid were determined for each group as described above for mumps virus. Considerable work has been done using the mouse-adapted PR8 strain of type A in mice. In general, groups of 12 to 14 Gm. white Swiss mice were infected intranasally and treated by various routes and at different times with chloramphenicol. In the test reported here treatment was given intraperitoneally for seven days starting at the time of infection.

One inconclusive test has been made with the Green strain of distemper virus (commercial distemperoid, ferret-adapted) in ferrets. Two ferrets were infected intranasally and one was treated intraperitoneally at the time of infection and daily thereafter for three days. Severity of illness and time of development of fever were noted. The animals were sacrificed at the height of the disease; hence duration of illness could not be determined.

Tortor: Newcastle disease virus (California 14 from Dr. L. T. Giltner, Bureau of Animal Industry, Washington, D. C.) was tested in 11-day embryonated eggs and white Leghorn pullets. The test in eggs was conducted in the same manner as the influenza virus tests described above except that infection was determined by death or survival of the embryo. Comparisons of the average time of death of treated and untreated groups were made. In chickens the virus was administered intramuscularly and treatments were given approximately every 12 hours for four doses starting at the time of infection. Time of the appearance of symptoms and number of survivors compared with a control group were noted.

The chick bronchitis virus (obtained from Dr. C. H. Cunningham, Michigan State College, East Lansing, Michigan) was tested in chick embryos as described above for Newcastle disease.

Laryngo-tracheitis virus (commercial vaccine strain) was tested on the membrane of chick embryos using the technique described for vaccinia virus.

Rickettsia: Considerable work has been done with epidemic typhus (*R. prowazekii,* Breinl strain) in chick embryos. In the work reported here, groups of six-day chick embryos were infected *via* the yolk sac and treated once on the third day after infection by the same route. Average time of death and number of survivors were compared with an untreated control group.

The results with murine typhus (*R. typhi*), scrub typhus (*R. tsutsugamushi*) and rickettsialpox (*R. akari*) were taken from the work of Smadel *et al.* (8–11). Using the method described above, their findings for murine typhus in eggs have been confirmed in this laboratory.

Dermacentroxenus, Coxiella and Miyagawanella: The reports of Smadel *et al.* (8–11) are included in the table for Rocky Mountain spotted fever (*D. rickettsi*), Q fever (*C. burnetii*), lymphogranuloma venereum (*M. lymphogranulomatis*) and psittacosis (*M. psittacii*).

Bacteria

The bacterial species reported here were tested for their susceptibility to chloramphenicol by inoculating a specified dilution or quantity of liquid culture into media containing varying concentrations of the drug, the end point being taken as the lowest concentration which caused complete inhibition of growth for 18 hours. Except as indicated the original transplants for preparation of suspensions were made from cultures dried from the frozen state.

The aerobes were tested by adding 1.0 ml. of a standardized dilution of a tryptose broth culture to 1.0 ml. quantities of tryptose broth containing varying concentration of chloramphenicol. The final concentrations used varied from 0.1 to 200 μg/ml. The tubes were incubated for 18 hours and the results recorded as "growth" or "no growth."

It was necessary to utilize special media for some of the more fastidious organisms. However, the same technique was used, replacing the tryptose broth with the special medium required. *Brucella abortus* was grown under 10 per cent CO_2 for 48 hours (No. 1335).

The anaerobes were tested by adding varying concentrations (ranging from 0.1 to 500 μg/ml.) of chloramphenicol to melted agar medium cooled to 40° C. To each tube, 0.1 ml. of a 24-hour liquid culture of the organism to be tested was added. The tubes were then shaken thoroughly and allowed to gel. Growth was recorded as positive or negative after 24 hours incubation.

Beef infusion agar (2 per cent) containing 2 per cent glucose (pH 8.2) was used for *Clostridium perfringens, novyi* and *septicum. Cl. feseri* was tested in 2 per cent liver infusion agar (pH 8.2) and the anaerobic agar recommended by Records and Vawter (23) was utilized for *Cl. hemolyticum. Cl. tetani* was grown in Taylor medium containing 0.5 per cent agar and incubated in an atmosphere of hydrogen and carbon dioxide.

Fungi

In determining the susceptibility of fungi to chloramphenicol, a broth dilution method was employed. Serial dilutions of the drug in broth were dispensed in 1 ml. amounts in Wassermann tubes and to each tube was added

1 ml. of inoculum in broth. The tubes were incubated at 30° C. for one to seven days, depending upon the species, and then observed for inhibition of growth. The minimum concentration of the drug causing complete inhibition of growth was taken as the end point.

Modified Sabouraud's broth (dextrose 2 per cent, neo peptone 1 per cent) was used in preparing the dilutions of the drug and the fungus suspensions. In the case of the actinomycetes, Brain-Heart Infusion Broth (Difco) was employed.

Three types of inocula were used. *1*) Spore suspension: In heavily sporulating species suspensions were prepared from either agar slant or agar plate cultures of the fungus. In the case of agar slants, broth was added to the tube, the fungus stirred up with an inoculating needle and the tube then shaken. When using agar plate cultures the mycelium was scraped from the surface of the plate, placed in broth and shaken. The suspensions were then filtered through cotton and the spores counted on the hemocytometer. *2*) Spores and mycelial fragments: Species having limited sporulation were removed from the surface of the culture medium, emulsified with broth either in a Waring Blender, by shaking with glass beads or by grinding in a mortar. The suspension was filtered through a loose cotton mat and in some instances the number of fragments and spores per ml. was determined by means of the hemocytometer. *3*) Mycelial suspension: The mycelium of non-sporulating species was

TABLE I

Viruses and rickettsiae

Organism	Host	Route of infection*	Treatment (mg.)		Total dose	Result
			Schedule	Route		
					mg.	
Vaccinia	Emb.(10)†	C.A.M.	V+Cm. mixed	Memb.	0.05	Negative
Vaccinia	Emb.(10)	Y.S.	0.5×2	Y.S.	1.0	Negative
Variola (22)	Emb.	—	—	—	—	Negative
St. Louis Enceph.	Emb.(9)	Al.S.	0.5×2	Al.S.	1.0	Negative
St. Louis Enceph.	Mice	I.C.	0.3 q.d.×10	I.P.	3.0	Negative
Japanese Enceph.	Mice	—	—	—	—	Negative
Rabies	Mice	I.C.	0.5+0.2 q.d.×4	I.P.	1.3	Negative
Rabies	Mice	I.M.	0.25+1 q.d.×2	I.P.	2.25	Negative
Polio. (Lansing) (22)	Mice	—	—	—	—	Negative
Polio. (Y-SK) (22)	Mice	—	—	—	—	Negative
Theiler's Intestinal (22)	Mice	—	—	—	—	Negative
Mumps	Emb.(7)	Al.S.	0.5×2	Al.S.	1.0	Negative
Influenza						
A (PR8)	Mice	I.N.	2+1 q.d.×6	I.P.	8.0	Delayed death
A (3 strains)	Emb.(11)	Al.S.	0.25×2	Al.S.	0.5	Negative
A′ (7 strains)	Emb.(11)	Al.S.	0.25×2	Al.S.	0.5	Negative
B (2 strains)	Emb.(11)	Al.S.	0.25×2	Al.S.	0.5	Negative
Distemper	Ferrets	I.P.	2+1 q.d.×3	I.P.	6.0	Negative
Newcastle Disease	Emb.(11)	Al.S.	0.3×2	Al.S.	0.6	Negative
Newcastle Disease	Chickens	I.M.	16.5 q.d.×12h×4	I.M.	66.0	6/8 Survivors
Chick Bronchitis	Emb.(11)	Al.S.	0.5×2	Al.S.	1.0	Negative
Laryngo-tracheitis	Emb.(10)	C.A.M.	V+Cm. mixed	Memb.	0.05	Negative
Lymphogranuloma (L.A.) (8–11)	Emb.(7)	Y.S.	Pre-inf.	Y.S.	0.06	Significant
Psittacosis (6-BC) (8–11)	Emb.(7)	Y.S.	Pre-inf.	Y.S.	0.06	Significant
Psittacosis (6-BC) (8–11)	Mice	I.P.	0.75 q.d.	I.P.	—	Significant
Psittacosis (P-4) (8–11)	Emb.(7)	Y.S.	Pre-inf.	Y.S.	0.06	Significant
Epidemic Typhus	Emb.(6)	Y.S.	0.5×1 (3rd)	Y.S.	0.05	Significant
(Breinl) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.06	Significant
Murine Typhus						
(Wilmington) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.06	Significant
(Wilmington) (8–11)	Mice	I.P.	5.0 q.d.×13	Oral	65.0	Significant
Scrub Typhus						
(Gilliam) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.13	Significant
(Seerangayee) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.13	Significant
(Seerangayee) (8–11)	Mice	I.P.	2.5 q.d.×20	Oral	55	Significant
(Karp) (8–11)	Mice	I.P.	2.5 q.d.×12	I.P.	30	Significant
Rickettsialpox (MK) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.13	Significant
Rickettsialpox (MK) (8–11)	Mice	I.P.	0.75×10	Oral	7.5	Significant
R. M. spot. fever						
(Bitterroot) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.06	Significant
(Bitterroot) (8–11)	G. pigs	I.P.	50 q.d.	Oral	—	Doubtful
Q Fever (Henzerling) (8–11)	Emb.(6)	Y.S.	Pre-inf.	Y.S.	0.5	Significant

* C.A.M. = chorioallantoic membrane; Y.S. = yolk sac; Al.S. = allantoic sac; I.C. = intracerebral; I.M. = intramuscular; I.N. = intranasal; I.P. = intraperitoneal.

† Emb.(10) = chick embryos and (age of incubation).

TABLE II

Bacteria

Organism	P-D Culture Bureau No.	Source	Inoculum	Inhibiting conc.
				μg./ml.
Aerobacter aerogenes	0126	P. D. Stock	1–20,000,000	0.5
Aerobacter aerogenes	0126	Frequent transfer	1–10,000,000	2.5
Alcaligenes faecalis	01602	Pus	1–10,000,000	1
Alcaligenes metalcaligenes	—	Urine (B)	1–10,000,000	200
Alcaligenes metalcaligenes	—	Urine (B)	1–10,000,000	>200
Alcaligenes metalcaligenes	—	Urine (B)	1–10,000,000	>200
Bacillus anthracis	01172	B. A. I.	1:20	5
Bacillus anthracis	01176	B. A. I.	1:20	5
Bacillus anthracis	03191	College Station, Tex.	1:10	0.75
Bacillus anthracis	04809	A. T. C. C.	1:20	5
Bacillus cereus var. *mycoides*	04545	Frequent transfer	1–10,000,000	2.5
Bacillus subtilis	04771	Frequent transfer	1–10,000,000	2.5
Brucella abortus	1335	Dr. Huddleson, M. S. C.	1:20	10
Brucella abortus	02522	Aborted bovine fetus N. I. H.	1:20	2.5
Brucella bronchisepticus	03710	Trachea of dog	1:20	10
Brucella bronchisepticus	03854	Lung	1:20	10
Brucella melitensis	Gol.	Camp Detrick	1:20	5
Brucella melitensis	04091	N. I. H.	1:20	5
Brucella suis	1772	Dr. Huddleson, M. S. C.	1:20	5
Clostridium feseri	01409	K. S. A. C.	0.1 cc. undiluted	>500
Clostridium feseri	01416	K. S. A. C.	0.1 cc. undiluted	>500
Clostridium hemolyticum	04779	Univ. Nev.	0.1 cc. undiluted	>500
Clostridium hemolyticum	04780	Univ. Nev.	0.1 cc. undiluted	>500
Clostridium novyi	068	Dr. Novy, U. of M.	0.1 cc. undiluted	>500
Clostridium novyi	04309	N. I. H.	0.1 cc. undiluted	>500
Clostridium perfringens (*welchii*)	01400	Dr. DeKruif, U. of M.	0.1 cc. undiluted	>500
Clostridium perfringens (*welchii*)	02122	Dr. Reed, Kingston, Ont.	0.1 cc. undiluted	>500
Clostridium septicum	01587	Univ. of Calif.	0.1 cc. undiluted	>500
Clostridium septicum	01759	K. S. A. C.	0.1 cc. undiluted	>500
Clostridium tetani	03724	N. Y. Dept. of Health	0.1 cc. undiluted	>500
Clostridium tetani	04770	Mueller variant	0.1 cc. undiluted	<0.1
Corynebacterium diphtheriae	036	P. D. Stock	1–10,000,000	0.5
Corynebacterium diphtheroides	04464	P. D. Stock	1–10,000,000	1.0
Corynebacterium pyogenes	01536	Bovine uterus	1:10	2.5
Diplococcus pneumoniae Type I	04385	Purulent chest fluid	1–10,000,000	1.0
Diplococcus pneumoniae Type III	04645	P. D. Stock	1–10,000,000	2.5
Diplococcus pneumoniae Type XII	04338	N. I. H.	1–5,000,000	2.5
Escherichia coli var. *communior*	04420	Pyelitis, Rt. kidney	1–20,000,000	2.5
Escherichia coli var. *communior*	04256	Blood cult. uterine infection	1–20,000,000	2.5
Escherichia coli var. *communis*	04474	Kidney infection, urine	1–10,000,000	2.5
Escherichia coli var. *communis*	04508	Blood culture	1–10,000,000	2.5
Escherichia coli var. *communis*	04508	Blood culture	1–20,000,000	2.5
Escherichia coli var. *communis*	04746	Urine sp.	1–20,000,000	2.5
Escherichia coli var. *communis* streptomycin-resistant	01495 04814	Frequent transfer P. D. Stock	1–10,000,000 1–10,000,000	2.5 0.5
Escherichia coli sp.	—	Urine (F)	1–10,000,000	2.5
Escherichia coli sp.	—	Urine (O)	1–10,000,000	2.5
Escherichia coli sp.	—	Pre-treatment sample of urine	1–10,000,000	2.5
Gaffkya tetragena	0584	Pure culture, U. of Iowa	1–10,000,000	2.5
Hemophilus pertussis (Phase I) (12)	04692	Dr. Kendrick, Mich.	10^4 organisms	0.2
Hemophilus pertussis (Phase I) (12)	04688	Clinical case	10^4 organisms	0.2
Hemophilus pertussis (5 isolates) (12)	—	Clinical cases	10^4 organisms	0.2–0.3
Klebsiella pneumoniae	04012	Sputum	1–10,000,000	0.75
Klebsiella pneumoniae	04172	Blood culture	1–10,000,000	2.5
Klebsiella pneumoniae	04184	Spinal fluid	1–10,000,000	0.75
Klebsiella pneumoniae	04299	Sputum	1–10,000,000	0.5
Klebsiella pneumoniae	04389	Throat	1–10,000,000	0.5
Klebsiella pneumoniae	04483	Sputum	1–10,000,000	0.75
Klebsiella pneumoniae	04682	F. D. A.	1–500,000	0.5
Klebsiella pneumoniae	04682	F. D. A.	1–1,000,000	0.5
Klebsiella pneumoniae	04682	F. D. A.	1–10,000,000	0.5
Klebsiella pneumoniae	04682	F. D. A.	1–20,000,000	0.5
Klebsiella pneumoniae (smooth)	04812	F. D. A.	1–20,000,000	0.5
Klebsiella pneumoniae (opaque)	04812	F. D. A.	1–10,000,000	0.5
Klebsiella pneumoniae	04812	Frequent transfer	1–20,000,000	0.5
Klebsiella pneumoniae	04544	Frequent transfer	1–10,000,000	1.0
Klebsiella pneumoniae I	—	Urine	1–10,000,000	2.5

TABLE II—*Continued*

Organism	P-D Culture Bureau No.	Source	Inoculum	Inhibiting conc.
				μg./ml.
Klebsiella pneumoniae II	—	Urine	1–5,000,000	15.0
Malleomyces mallei	01828	P. D. Stock	1:2000	37.5
Micrococcus citreus	0394	Boil on head	1–10,000,000	2.5
Micrococcus citreus	03481	Gonorrheae	1–10,000,000	5
Micrococcus pyogenes var. *albus*	04460	Female gonorrheae	1–10,000,000	2.5
Micrococcus pyogenes var. *albus*	04475	Pus	1–10,000,000	5
Micrococcus pyogenes var. *albus*	04513	Pustule	1–10,000,000	2.5
Micrococcus pyogenes var. *albus*	—	Ear infection (M)	1–5,000,000	1.0
Micrococcus pyogenes var. *aureus*	04816	F. D. A.	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04778	F. D. A.	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04662	U. S. D. A.	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04598	U. S. D. A.	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04546	N. I. H.	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04540	F. D. A.	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04518	Blood culture	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	04509	Boil	1–10,000,000	10
Micrococcus pyogenes var. *aureus*	—	Ear (M)	1–5,000,000	5
Micrococcus pyogenes var. *aureus*	—	Urine (Mc)	1–5,000,000	5
Micrococcus pyogenes var. *aureus*	04778	Frequent transfer	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	—	Frequent transfer	1–10,000,000	5
Micrococcus pyogenes var. *aureus*	02482	Frequent transfer	1–10,000,000	10
Mycobacterium phlei	02145	P. D. Stock	1–10,000,000	25
Mycobacterium tuberculosis (13) (human virulent, 12 strains)	—	Fresh isolates	—	6.25 to 12.5
Neisseria catarrhalis	03447	Trachea at autopsy	1–10,000,000	0.5
Neisseria catarrhalis	03995	Tonsillar abscess	1–5,000,000	0.5
Neisseria catarrhalis	04388	Cough plate	1–5,000,000	2.5
Neisseria meningitidis	04107	N. I. H.	1–5,000,000	2.5
Pasteurella avicida	01673	Heart blood of chicken	1:2000	0.5
Pasteurella avicida	04587	Liver of goose	1:2000	0.25
Pasteurella avicida	04588	Heart blood of chicken	1:2000	0.25
Pasteurella bollingeri	02855	Colorado Agric. Coll.	1:2000	0.25
Pasteurella bollingeri	04042	Calf's lung	1:10	0.75
Pasteurella bubalseptica	02217	Buffalo strain	1:2000	0.5
Pasteurella cuniculicida	01345	Sinus of rabbit	1:2000	0.5
Pasteurella oviseptica	02854	Colorado Agric. Coll.	1:2000	2.5
Proteus vulgaris	0924	P. D. Stock	1–10,000,000	2.5
Proteus vulgaris	02164	N. I. H.	1–10,000,000	5
Proteus vulgaris	02165	N. I. H.	1–10,000,000	2.5
Proteus vulgaris	04262	N. I. H.	1–10,000,000	1.0
Proteus vulgaris	04736	Urine	1–10,000,000	2.5
Proteus vulgaris	04736	Frequent transfer	1–10,000,000	2.5
Proteus species No. 1	—	—	1–10,000,000	25
Proteus species No. 2	—	—	1–10,000,000	10
Proteus species No. 3	—	—	1–10,000,000	5
Proteus species No. 4	—	Urine	1–10,000,000	25
Pseudomonas aeruginosa	04126	Eye	1–10,000,000	50
Pseudomonas aeruginosa	04248	Swab from left arm	1–10,000,000	37.5
Pseudomonas aeruginosa	02133	Horse	1–10,000,000	75
Pseudomonas aeruginosa	04591	Abdominal cavity of chicken	1–10,000,000	75
Pseudomonas aeruginosa	04625	Turkey	1–10,000,000	50
Pseudomonas aeruginosa	04629	Bovine	1–10,000,000	>200
Pseudomonas aeruginosa	2430	Frequent transfer	1–10,000,000	37.5
Pseudomonas aeruginosa	01925	Stool culture	1–10,000,000	37.5
Pseudomonas aeruginosa	01925	Frequent transfer	1–10,000,000	37.5
Pseudomonas aeruginosa	01926	Eye	1–10,000,000	25
Pseudomonas aeruginosa	01931	Stool culture	1–10,000,000	25
Pseudomonas aeruginosa	04728	Urine	1–10,000,000	100
Pseudomonas aeruginosa	—	Urine (Mc)	1–5,000,000	50
Pseudomonas aeruginosa	—	Urine (C)	1–10,000,000	50
Pseudomonas aeruginosa	—	Urine (Mc)	1–10,000,000	15
Pseudomonas aeruginosa (rough type)	—	Urine (Mel)	1–5,000,000	50
Pseudomonas aeruginosa (smooth type)	—	Urine (Mel)	1–10,000,000	15
Pseudomonas aeruginosa	—	Urine (S)	1–10,000,000	10
Pseudomonas aeruginosa	—	Urine (L)	1–5,000,000	25
Rhodospirillum rubrum	01348	P. D. Stock	1–10,000,000	0.25
Salmonella enteritidis	0613	P. D. Stock	1–10,000,000	0.75
Salmonella enteritidis	01799	P. D. Stock	1–10,000,000	2.5
Salmonella gallinarum	04564	Chicken origin	1–10,000,000	2.5

TABLE II—*Continued*

Organism	P-D Culture Bureau No.	Source	Inoculum	Inhibiting conc.
				µg./ml.
Salmonella gallinarum	04574	Turkey	1–10,000,000	2.5
Salmonella paratyphi	01179	N. I. H.	1–10,000,000	0.75
Salmonella paratyphi	02156	N. I. H.	1–10,000,000	0.75
Salmonella schottmuelleri	01180	N. I. H.	1–10,000,000	2.5
Salmonella schottmuelleri	01180	Frequent transfer	1–10,000,000	2.5
Salmonella schottmuelleri	01181	N. I. H.	1–10,000,000	0.5
Salmonella typhimurium	03869	Rabbit typhoid 104	1–10,000,000	2.5
Salmonella typhimurium	0301	Rat	1–10,000,000	2.5
Salmonella typhimurium	04221	Heart blood of lamb	1–10,000,000	5
Salmonella typhosa	04425	P. D. Stock	1–10,000,000	2.5
Salmonella typhosa	04469	N. I. H.	1–10,000,000	2.5
Salmonella typhosa	04537	N. I. H.	1–10,000,000	1
Salmonella typhosa	04599	N. I. H.	1–10,000,000	2.5
Salmonella typhosa	04683	N. T. C. C.	1–10,000,000	1
Salmonella typhosa	04788	Stool culture	1–10,000,000	1
Salmonella typhosa	04815	Typhoid carrier	1–10,000,000	1
Salmonella typhosa	02481	Frequent transfer	1–10,000,000	0.75
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier (M.C.)*	1–10,000,000	2.5
Salmonella typhosa	—	Typhoid carrier (M.C.)†	1–10,000,000	1.0
Salmonella typhosa	—	Typhoid carrier (J.T.)†	1–10,000,000	2.5
Sarcina aurantiaca	01346	P. D. Stock	1–5,000,000	1
Sarcina lutea	04813	F. D. A.	1–5,000,000	10
Sarcina lutea	04813	Frequent transfer	1–10,000,000	0.5
Serratia marcescens	071	P. D. Stock	1–5,000,000	5
Serratia marcescens	04547	A. T. C. C.	1–10,000,000	2.5
Serratia marcescens	04642	A. T. C. C.	1–10,000,000	2.5
Serratia marcescens	04702	P. D. Stock	1–10,000,000	5
Shigella dysenteriae	01339	P. D. Stock	1–10,000,000	0.75
Shigella dysenteriae	01675	N. I. H.	1–10,000,000	0.75
Shigella madampensis	04021	A. T. C. C.	1–10,000,000	2.5
Shigella madampensis	04022	A. T. C. C.	1–10,000,000	2.5
Shigella paradysenteriae				
(*Bacillus dysenteriae* Flexner I)	03444	A. T. C. C.	1–10,000,000	1
(*Bacillus dysenteriae* Flexner II)	01654	P. D. Stock	1–10,000,000	0.5
(*Bacillus dysenteriae* Flexner II)	0822	P. D. Stock	1–10,000,000	0.5
(*Bacillus dysenteriae* Flexner III)	02904	A. T. C. C.	1–10,000,000	1
(*Bacillus dysenteriae* Flexner VI)	03880	P. D. Stock	1–10,000,000	0.75
(*Bacillus dysenteriae* Flexner VII)	01650	P. D. Stock	1–10,000,000	0.5
(*Bacillus dysenteriae* Flexner VIII)	01652	P. D. Stock	1–10,000,000	0.5
(*Bacillus dysenteriae* var.)	01341	P. D. Stock	1–10,000,000	2.5
Shigella sonnei	02170	P. D. Stock	1–10,000,000	2.5
Shigella sonnei	02171	P. D. Stock	1–10,000,000	2.5
Shigella sonnei	04628	P. D. Stock	1–10,000,000	5
Shigella sonnei	04628	Frequent transfer	1–10,000,000	2.5
Streptococcus hemolyticus	04774	Abscess	1–5,000,000	2.5
Streptococcus hemolyticus	04714	P. D. Stock	1–5,000,000	0.75
Streptococcus hemolyticus	04701	Group A	1–5,000,000	1.0
Streptococcus hemolyticus	04472	Frequent transfer	1–5,000,000	1.0
Streptococcus infrequens	04464	Frequent transfer	1–5,000,000	2.5
Streptococcus lactis	03454	Pasteurized milk	1–5,000,000	0.75
Streptococcus non-hemolyticus	04622	P. D. Stock	1–10,000,000	0.75
Streptococcus non-hemolyticus	04150	Extracted tooth	1–5,000,000	0.25
Vibrio comma	04643	N. I. H.	1:2000	1.0

* Pre-treatment. † After treatment.

processed in the manner described for preparing the suspension of spores and mycelial fragments.

All of the fungi were tested first in a series of dilutions ranging from 50 to 1000 μg/ml. of the drug. The actinomycetes, *Actinomyces bovis* and *Nocardia asteroides*, were retested at lower concentrations and four species, *Trichophyton interdigitale, T. mentagrophytes, T. rubrum* and *Microsporum canis*, at higher concentrations (up to 2500 μg/ml.) of chloramphenicol. In order to keep the drug in solution at the higher concentrations 1.8 ml. of the drug-broth mixture and 0.2 ml. of inoculum were used in each tube instead of 1 ml. of each as in the lower concentration series.

Spirochetes and Protozoa

The authors are indebted to Dr. Paul E. Thompson (24) and Dr. O. M. Gruhzit (25) of the Parke, Davis Research Laboratories for the data included in this section.

In Vitro Tests: Endamoeba histolytica (University of Chicago strain, with mixed bacterial flora) was tested in two media, Egg-Locke diphasic and Balamuth (essentially protein free). Various concentrations of chloramphenicol were incorporated in these media and amebicidal activity determined by microscopic examination for motility after 24 hours at 37° C.

Borrelia novyi and *recurrentis* and *Trichomonas foetus*

TABLE III

Fungi

Fungus	Bureau No.	Source	Inoculum		Inhibition
			Type	*No./ml.*	*μg./ml.*
Actinomyces bovis	04	P. D. Stock	Mycel. Susp.*	—	5
Alternaria sp.	04738	P. D. Stock	Spores	281,000	>1000
Allescheria boydii	04619	N. I. H.	Spores	500,000	>1000
Aspergillus clavatus	04445	Dr. Waksman	Spores	500,000	>1000
Aspergillus flavus	04456	Dr. Thom	Spores	500,000	>1000
Aspergillus fumigatus	04612	N. I. H.	Spores	500,000	>1000
Aspergillus glaucus	04821	P. D. Stock	Spores	500,000	>1000
Aspergillus nidulans	04434	P. D. Stock	Spores	500,000	>1000
Aspergillus niger	01383	P. D. Stock	Spores	500,000	>1000
Blastomyces dermatitidis					
Mycelial phase	04613	N. I. H.	Spores & Mycel.†	500,000	>1000
Candida (Monilia) *albicans*	04600	Bronchus	Spores	500,000	>1000
Cryptococcus neoformans					
(Torula histolytica)	04611	N. I. H.	Spores	500,000	>1000
(Torula histolytica)	04817	Meningitis Dr. Thompson	Spores	500,000	>1000
Epidermophyton floccosum	04624	Tinea cruris	Spores & Mycel.†	375,000	>1000
Helminthosporium sativum	04677	A. T. C. C.	Spores	250	>1000
Histoplasma capsulatum					
Mycelial phase	04799	N. I. H.	Spores & Mycel.‡	—	>1000
Mycelial phase	04800	N. I. H.	Spores & Mycel.	—	>1000
Hormodendrum sp.	04637	Dr. Downing	Spores	500,000	>1000
Microsporum audouini	04606	N. I. H.	Mycel. Susp.§	20,000	>1000
Microsporum canis	04605	N. I. H.	Spores & Mycel.†	100,000	>2500
Microsporum gypseum	04604	N. I. H.	Spores	125,000	>1000
Mucor racemosus	01385	P. D. Stock	Spores	500,000	>1000
Nocardia asteroides	04618	N. I. H.	Mycel. Susp.*	—	20
Penicillium expansum	01386	P. D. Stock	Spores	500,000	>1000
Phialophora verrucosa	04617	N. I. H.	Mycel. Susp.*	—	>1000
Rhizopus nigricans	04529	A. T. C. C.	Spores	500,000	>1000
Rhodotorula sp.	01527	P. D. Stock	Spores	500,000	>1000
Saccharomyces carlsbergensis	04450	Fleischmann	Spores	500,000	>1000
Saccharomyces cerevisiae	01525	P. D. Stock	Spores	500,000	>1000
Saccharomyces ellipsoideus	01524	Mich. State Coll.	Spores	500,000	>1000
Sporotrichum schenckii	04614	N. I. H.	Spores	500,000	>1000
Trichophyton acuminatum	04607	N. I. H.	Spores	485,000	>1000
Trichophyton interdigitale	04452	N. I. H.	Spores	500,000	>2500
Trichophyton mentagrophytes	04781	N. I. H.	Spores	500,000	>2500
Trichophyton rubrum (T. purpureum)	04639	N. I. H.	Spores	500,000	>2500
Trichophyton sabouraudi	04638	N. I. H.	Spores & Mycel.‡	—	>1000
Trichophyton schoenleini	04603	N. I. H.	Mycel. Susp.*	—	>1000
Trichophyton sulfureum	04608	N. I. H.	Spores	500,000	>1000
Trichophyton tonsurans	04609	N. I. H.	Spores	730,000	>1000
Trichophyton violaceum	04676	A. T. C. C.	Mycel. Susp.*	—	>1000

* Suspension of emulsified mycelial material. The number of fragments was not determined.
† Both spores and mycelial fragments are included in the count.
‡ Light suspension of spores and mycelial fragments.
§ Mycelial fragments counted.

were suspended in a suitable menstruum containing varying concentrations of chloramphenicol, as indicated in Table IV, and observed for immobilization. Observations were made after two hours in the case of the Borreliae and after seven hours exposure for trichomonas.

In Vivo Tests: Plasmodium lophurae was tested in ducks (intraperitoneal treatment) and chicks (oral treatment) and the results compared with the activity of quinine.

Rats and dogs were used to evaluate the activity of chloramphenicol against *E. histolytica in vivo*. Treatment was given orally and results expressed as number of animals cleared of infection or degree of suppression of infection.

B. novyi was tested in groups of mice treated by either the intraperitoneal or oral route. Results are expressed as percentage suppression of spirochetemia.

Rabbits infected with *Treponema pallidum* were treated with various concentrations of chloramphenicol twice daily for eight days. Rate of disappearance of treponemes and degree of healing of lesions under treatment were noted. The animals were held to check for relapses in the dosages at which healing under treatment was noted.

RESULTS

Tables I to IV summarize the results obtained with the different micro-organisms that have been tested in the laboratory. Wherever possible the minimal inhibiting concentration of chloramphenicol for the strain, under the conditions tested, is given. Otherwise, the maximum concentration tested or the dose administered, in the case of *in vivo* tests, is entered. The identifying numbers given for the bacteria and fungi are those assigned by the Parke, Davis and Company culture bureau, where a complete history of the strain is available. Strains without assigned numbers are for the most part recent isolates from clinical cases prior to or during treatment with chloramphenicol.

The Emergence of Microbial Resistance

With the finding of considerable variation in susceptibility to chloramphenicol of different cultural lines of certain bacterial species, it became

TABLE IV

Spirochetes and protozoa

In vitro tests

Organism	Medium or menstruum	Time	Conc.	Result
			μg./ml.	
Endamoeba histolytica *	L. E. L.	24 hours	1000	Negative
Endamoeba histolytica *	Balamuth	24 hours	250	Significant
Borrelia novyi *	50% horse serum	2 hours	10 to 50	Immobilization
Borrelia recurrentis *	2.5% rat serum	2 hours	2.5	Immobilization
Trichomonas foetus †	0.7% sodium chloride	7 hours	2000	Negative
Pelomyxa carolinensis ‡	Pace & Kimura buffer	48 hours	2500	Negative
Tetrahymena geleii ‡	2% proteose peptone	48 hours	2500	Negative

In vivo tests

Organism	Host	Treatment			Result
		Dose	Schedule	Route	
		mg./kg./day	*days*		
Plasmodium lophurae *	Ducks	200	b.d.×5	I.P.	Q‖ = <0.05
Plasmodium lophurae *	Chicks	537	5	Diet	Q = <0.1
Endamoeba histolytica *	Rats	583–868	7	Diet	9/14 cleared
Endamoeba histolytica *	Dogs	200	b.d.×10	Oral	Suppression
Borrelia novyi *	Mice	14.9	5	Diet	50% Suppression
Borrelia novyi *	Mice	7.6	×3(4, 22, 28 hrs.)	I.P.	50% Suppression
Treponema pallidum §	Rabbits	25	b.d.×8	I.M.	Negative
Treponema pallidum §	Rabbits	50–100	b.d.×8	I.M.	Spirochetes and lesions cleared 4–5 days, relapsed

* Thompson, P. E., Research Laboratories of Parke, Davis & Company, personal communication and (12).
† Reutner, T. F., Research Laboratories of Parke, Davis & Company and (12).
‡ Pace, D. M., and Russell, D., quoted (12).
§ Gruhzit, O. M., personal communication, Research Laboratories of Parke, Davis & Company.
‖ Quinine equivalent.

TABLE V

Induced resistance to chloramphenicol in bacteria

Organism	P-D Culture Bureau No.	Transfers on chloramphenicol containing medium	Inhibiting conc. chloramphenicol µg./ml.	
			Original	Final
Salmonella typhosa	clinical case	12	10	250
Aerobacter aerogenes	0126	12	5	50
Escherichia coli	01495	12	10	500
Proteus (sp.)	04736	8	500	>4000
Pseudomonas aeruginosa	01925	8	1250	>4000
Klebsiella pneumoniae	04544	12	5	15
Alcaligenes metalcaligenes	clinical case	7	1250	2250

of interest to determine whether increased resistance to chloramphenicol could be induced by laboratory manipulation. Our early observations indicate that certain species of micro-organisms will develop resistance to chloramphenicol when sub-inoculated on media containing increasing concentrations of the drug. The bacterial species tested include *Salmonella typhosa, Aerobacter aerogenes, Escherichia coli, Proteus* sp., *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Alcaligenes metalcaligenes.*

The test was carried out by sub-inoculating 0.1 ml. of the various cultures into Brain-Heart Infusion Broth (Difco) with 10 per cent horse serum added and containing various concentrations of chloramphenicol. The cultures were incubated for 72–96 hours. From the tube containing the highest concentration of chloramphenicol, in which good growth was obtained, 0.1 ml. was sub-inoculated into 2.0 ml. of medium containing the same and higher concentrations of the drug. In Table V the preliminary results of this study are indicated. It can be seen that the susceptibility of these organisms has decreased from approximately 2 to 50 fold in the course of these passages.

The results of tests designed to detect a similar phenomenon for *Rickettsia prowazekii* have so far been negative. One series has been carried through 13 passages, and a second through six passages in chick embryos treated with partially inhibiting doses of chloramphenicol. Comparative tests of the passaged and stock strains have indicated no change in susceptibility to the drug under these conditions.

SUMMARY AND CONCLUSIONS

Sixty-four genera of micro-organisms including 290 species and strains have been tested for their susceptibility to chloramphenicol. The results are recorded in Tables I to IV.

From Table I it appears that all of the Rickettsiae tested are susceptible to chloramphenicol. *Coxiella burnetii* (Q Fever) may be more resistant than the others as larger doses are required to produce corresponding delay in mean death time of treated embryos. Possibly the whole lymphogranuloma venereum-psittacosis group of virus (*Miyagawanella*) will prove to be as susceptible as the type species. There are indications that influenza virus in mice and the virus of Newcastle disease of chickens respond somewhat to comparatively massive doses of the drug. It is possible, however, that the therapeutic effect seen can be explained by the action of the drug upon secondary bacterial invaders. While this hypothesis is apparently borne out by the negative results with chloramphenicol on these viruses in embryonated eggs, further work and possibly controlled clinical trials are indicated.

Tests against the virus of poliomyelitis have proved negative. Dr. J. L. Melnick states as follows: "I have gone over the records of the work done with Dr. Filiberto Ramirez Corria on the use of this material on two strains (Lansing and Y-Sk) of poliomyelitis virus and on Theiler's intestinal virus of mice. When the viruses were titrated using saturated aqueous solutions of Chloromycetin as diluent, there was no effect on the titer even when the viruses were inoculated into mice receiving daily inoculations of the drug (1 cc. of saturated solution intraperitoneally). Further-

more, daily oral administration of Chloromycetin did not appear to have any effect on the intestinal carrier state of mice spontaneously harboring Theiler's virus of mouse encephalomyelitis" (22).

From Table II, which gives the results from tests of over 200 bacterial strains representing 25 genera, it can be concluded that considerable variation in susceptibility is encountered between genera as well as between species and even strains within the species. Under the conditions tested susceptibility to concentrations of 10 μg/ml. or less, indicating possible clinical application, was observed for 21 of the genera. These include *Aerobacter* (2/2),[4] *Bacillus* (6/6), *Brucella* (7/7), *Corynebacterium* (3/3), *Diplococcus* (3/3), *Escherichia* (11/11), *Hemophilus* (7/7), *Klebsiella* (11/12), *Micrococcus* (19/19), *Neisseria* (4/4), *Pasteurella* (8/8), *Proteus* (8/10), *Pseudomonas* (1/19), *Salmonella* (37/37), *Sarcina* (3/3), *Shigella* (16/16), *Streptococcus* (8/8) and *Vibrio* (1/1). *Pseudomonas,* though fairly resistant, is included since it seems to be susceptible to chloramphenicol in concentrations obtainable in the urine. The results given for *Alcaligenes* leave the status of this genus somewhat in doubt. A stock strain of *A. faecalis* was found to be susceptible but three different isolates from the same clinical case of *A. metalcaligenes* infection were found to be highly resistant. A somewhat similar situation is observed for *Proteus vulgaris* where two of ten strains tested required a somewhat higher concentration (25 μg/ml.) for complete inhibition. An interesting finding was encountered with the *Clostridia.* While this group in general was of very low susceptibility, one strain, the Mueller variant of *Cl. tetani,* was very susceptible. This variant does not produce spores in culture, which raises the speculation that possibly the apparent resistance of this group is a reflection of the resistance of the spores rather than of the vegetative forms.

While not within the scope of this paper, *in vivo* laboratory trials with certain of the bacterial agents have been conducted. Gould *et al.* (26) report encouraging results with the cholera vibrio in mice and Gruhzit (25) has found activity in mice against certain of the Gram-positive cocci and the salmonella group. Sarber (27) has treated mice infected with *Hemophilus pertussis* and found chloramphenicol to be an effective chemotherapeutic agent. Clinical trials are in progress against many of these pathogens. To date clinical confirmation has been obtained in typhoid fever (19), brucellosis (20), and certain urinary infections caused by Gram-negative species (21).

Of the forty species or strains of fungi tested for susceptibility to chloramphenicol, Table III, only two were completely inhibited by the drug at the concentrations used in the test. These two species, *Actinomyces bovis* and *Nocardia asteroides,* both of which belong to the actinomycetes, were inhibited by 5 μg/ml. and 20 μg/ml. of the drug, respectively. Since blood levels of this magnitude are easily attained, trial on clinical infections with these agents is indicated.

Growth of four species of ringworm fungi, *Trichophyton mentagrophytes, T. interdigitale, T. rubrum* and *Microsporum canis,* was retarded by 1000 μg/ml. of the drug but complete inhibition could not be obtained even when the drug concentration was increased to 2500 μg/ml.

With the possible exception of the spirochete of relapsing fever (*Borrelia recurrentis*), the results with the spirochetes and protozoa tested were not promising. Doses of 12.5 mg/Kg. twice daily for eight days were ineffective on syphilitic lesions in rabbits. Although similar treatment with 25 or 50 mg/Kg. cleared the lesions, relapses occurred when treatment was stopped.

Attempts to induce increased resistance to chloramphenicol by passage in the presence of the drug have been successful in the case of seven bacterial species, but not for *R. prowazekii* in the embryonated egg. It remains to be seen whether, under practical conditions in the clinic, this finding will be of importance.

[4] Indicates number susceptible strains/number of strains tested.

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