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An *in vitro* comparison of the effect of some antibacterial, antifungal and antiprotozoal agents on various strains of *Mycoplasma* (pleuropneumonia-like organisms: P.P.L.O.)

By AUDREY G. NEWNHAM and H. P. CHU

Department of Animal Pathology, School of Veterinary Medicine, Madingley Road, Cambridge

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INTRODUCTION

Over the last 20 years many workers, mainly in the United States, Canada, Britain, Scandinavia and Australia, have sought suitable drugs to inhibit growth of organisms of the Pleuropneumonia group (P.P.L.O.) or Mycoplasmataceae. Most of the work has been carried out *in vitro* on the mycoplasmata of bovine, caprine, rodent or human origin, and *in vitro* or *in ovo* on those of avian origin. Summaries of the findings of this previous work are given in Tables 1 and 2.

The purpose of the present study was twofold: first, to discover whether representative collections of *Mycoplasma gallisepticum* strains from Britain and other countries were similar in their sensitivity to drugs; and, secondly, to find out whether *M. gallisepticum* drug sensitivities differed significantly from drug sensitivities of some other more typical members of the mycoplasmata; such differences might reflect differences in the structure of the various strains of *Mycoplasma* at present included in the single genus by Edward & Freundt (1956).

Our particular interest has lain in the apparent differences between the non-pathogenic avian mycoplasmata and the pathogenic coccobacilliform bodies of Nelson (1936*a–d*) which were named *Mycoplasma gallisepticum* by Edward & Kanarek (1960). Strain X 95 of Markham was taken as the type-species of this latter group, and so far all pathogenic strains tested in this laboratory have belonged to this distinct serological group (Chu & Newnham, 1959). Up to 1962 this was the only serotype known to agglutinate erythrocytes of avian and mammalian origin. But Yoder & Hofstad (1962) in the United States, and Roberts (1963) in Britain, have since described two new serotypes (distinct from each other and from *M. gallisepticum*) which may also agglutinate avian erythrocytes and which were isolated from air-sac lesions in chickens and turkeys.

M. gallisepticum strains differ from the more typical members of the mycoplasmata mainly in morphology, but Adler (1964) has listed a number of other differences. To gain more fundamental information on their supposed or actual differences, a study of this kind should be associated not only with studies in biochemistry and biophysics (Leach, 1962; Razin, 1963*c*; Razin, Argaman & Avigan, 1963; Morowitz *et al.* 1962), but also with serology, immunochemistry (Fowler, Coble, Kramer & Brown, 1963; Lemcke, 1964) and detailed cytology

Table 1. *Summary of earlier reports of inhibition of* Mycoplasma *by drugs* in vitro

Authors and date	No. of strains	Name and/or origin of strains	Type of medium used	Minimum inhibitory concentration	Minimum lethal concentration	Sensitivities expressed as μg. of drugs per ml. of medium: Streptomycin	Dihydrostreptomycin	Tetracycline	Chlortetracycline	Demethylchlortetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Spiramycin	Tylosin	Kanamycin	Sodium aurothiomalate	Nitrofurans	Nystatin	Polymixin
Paine *et al.* 1948*a*	1	Human G.U.	Liquid	—	√	—	—	—	0·25	—	—	—	—	—	—	—	—	—	—	—
Hatch, 1949	6	Human G.U.	Liquid	—	√	25	—	—	25	—	—	—	—	—	—	—	—	—	—	No action
	1	Rat lung	Liquid	—	√	No action	—	—	25	—	—	—	—	—	—	—	—	—	—	No action
Leberman *et al.* 1950	7	Human G.U.	Liquid	√	—	0·1–15	15– > 200	—	25– > 200	—	—	15– > 100	—	—	—	—	—	—	—	—
Leberman *et al.* 1952	7	Human, G. U.	Liquid	√	—	—	—	—	—	—	0·1–0·5	—	—	—	—	—	—	—	—	—
Robinson *et al.* 1952	28	Human G.U.	Liquid	—	√	128–4096	—	—	8– > 256	—	4–256	16–512	—	—	—	—	16–128	—	—	—
			Solid	C.L.C.	—	2–128	—	—	2–16	—	0·25–2	4–16	—	—	—	—	16–128	—	—	—
		Rat arthritis (L 4) *M. arthritidis*	Liquid	—	√	2048	—	—	> 256	—	256	512	—	—	—	—	2048	—	—	—
			Solid	C.L.C.	—	128	—	—	16	—	1·0	8	—	—	—	—	2048	—	—	—
Melén 1952	20	Human G.U.	Liquid	√	—	5–10	20– > 80	—	0·32–1·25	—	0·16–0·63	2·5–10	—	—	—	—	—	—	—	—
Keller & Morton, 1953	3	Human G.U.	Liquid	√	—	—	—	—	—	—	—	—	> 200	—	—	—	—	—	—	—
Harkness & Bushby, 1954	6	Human G.U.	Solid	√	—	10–40	—	1·5–3·0	25–50	—	0·4–1·5	12–25	100–150	—	—	—	—	—	—	—
Blyth, 1958	47	Human G.U. *et al.*	Solid	√	—	4–32	—	0·25–0·5	0·5–4	—	0·5–1·0	4–8	> 64	2 16	—	—	—	—	—	—
Nasemann & Röckl, 1960	**	Human G.U.	Liquid	√	—	Good effect	—	—	0·5	—	—	Good effect	—	—	—	—	—	—	—	—
				—	√				5–10											
Robinson *et al.* 1956	**	Human G.U.	HeLa and conjunctival cell lines	—	? √	256	—	—	—	—	4	—	—	—	—	—	—	—	—	—
Kuzell *et al.* 1949	1	Rat arthritis (L 4) *M. arthritidis*	Liquid	√	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
			—	—	√	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
Hearn *et al.* 1959	**	T.C.C.	Solid (disks)	—	—	Resistant	—	—	Sensitive	—	Sensitive	Less sensitive	—	—	—	—	—	Less sensitive (NF)	—	—
			Human and mammalian cell lines	—	√	—	—	—	100–200	—	—	—	—	—	—	—	—	—	—	—
Fogh & Hacker, 1960	**	T.C.C.	Amnion and HeLa cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 200 for 2 weeks	—	—	—	—

Table 1 (*cont.*)

Authors and date	No. of strains	Name and/or origin of strains	Type of medium used	Minimum inhibitory concentration	Minimum lethal concentration	Sensitivities expressed as μg. of drugs per ml. of medium: Streptomycin	Dihydrostreptomycin	Tetracycline	Chlortetracycline	Demethylchlortetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Spiramycin	Tylosin	Kanamycin	Sodium aurothiomalate	Nitrofurans	Nystatin	Polymixin
Pollock *et al.* 1960	15	T.C.C.	Mammalian cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 100 for 2 days to 3 weeks	—	—	—	—
Kenny & Pollock, 1963	4	T.C.C.	Human and mouse cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 400 for 3 days	—	—	—	—
Pollock *et al.* 1963	**	T.C.C.	EE and HE cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 100 for 5 hr.	—	—	—	—
Rouse *et al.* 1963	**	T.C.C.	Human cell lines	—	—	—	—	—	—	—	—	—	—	—	—	Inhib. by 50 for 2–3 weeks	—	—	—	—
Carski & Shepard, 1961	7	6 T.C.C., 1 G.U.	Solid (disks)	—	—	Resistant to 10	—	Sensitive to 30	Sensitive to 30	—	Sensitive to 30	Sensitive to 30	Resistant to 15	—	—	—	—	—	No action	No action
			HeLa cells	✓	—	—	—	0·5	—	—	—	8	—	—	—	—	—	—	—	—
Collier, 1957	**	T.C.C. (human G.U.)	HeLa cells	—	—	Resistant to 80	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nelson, 1960	1	T.C.C.	HeLa cell lines	—	—	Resistant to 100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nelson, 1960	1	Mouse infectious catarrh *M. pulmonis*	HeLa cell lines	—	—	Sensitive to 100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kingston *et al.* 1961	**	Eaton agent *M. pneumoniae*	Monkey kidney cells	✓	—	—	—	—	—	1·5	—	—	—	—	—	—	—	—	—	—
Lecce & Sperling, 1955	1	Pathogenic avian	Solid (disks)	—	—	—	—	'Good effect'	'Good effect'	—	—	'Less effect'	—	—	—	—	—	—	—	—
Yamamoto & Adler, 1956	10	Pathogenic avian	Liquid	✓	—	<6·25–12·5	3·13–>100	<1·56–6·25	1·5 100	—	<1·56–25	—	<1·56–100	—	—	—	—	Inactive (FZ and NF)	—	—
				—	✓	6·25–500	6·25–>500	1·56–>250	6·25–>250	—	<1·56–>250	—	1·56–>250	—	—	—	—	Inactive (FZ and NF)	—	—
Fahey, 1957	1	Turkey sinusitis	Liquid	—	✓	>100	—	—	25–>25	—	5–25	—	—	—	—	—	—	—	—	—
Domermuth & Johnson, 1955	1	Pathogenic avian (A 5967)	—	✓	—	0·01	—	—	0·1	—	0·01	0·1	—	—	—	—	—	0·1 (FZ)	—	—
			—	—	✓	10	—	—	100	—	10	100	—	—	—	—	—	10 (FZ)	—	—

Table 1 (*cont.*)

Authors and date	No. of strains	Name and/or origin of strains	Type of medium used	Minimum inhibitory concentration	Minimum lethal concentration	Sensitivities expressed as μg. of drugs per ml. of medium: Streptomycin	Dihydrostreptomycin	Tetracycline	Chlortetracycline	Demethylchlortetracycline	Oxytetracycline	Chloramphenicol	Erythromycin	Spiramycin	Tylosin	Kanamycin	Sodium aurothiomalate	Nitrofurane	Nystatin	Polymixin
Domermuth, 1958	1	Pathogenic avian (Winchester)	Liquid	√	—	0·1–1·0	—	0·1	—	—	—	0·1–1·0	—	—	—	—	—	0·1–1·0 (FZ)	—	—
				—	√	1000	—	> 10	—	—	—	10	—	—	—	—	—	10 (FZ)	—	—
Domermuth, 1960	1	Pathogenic avian (Winchester)	Liquid	√?	—	1000	—	10	—	—	—	100	1·0	—	—	—	—	—	—	—
				—	√?	10,000	—	100	—	—	—	100	10	—	—	—	—	—	—	—
Olesiuk & van Roekel, 1959	1	Pathogenic avian	Liquid	—	√	—	—	—	—	—	0·1	—	—	—	—	—	—	—	—	—
Osborn *et al.* 1960	1	Turkey sinusitis	Liquid	√	—	3·13	6·25	1·56	1·56	—	0·78	—	100	—	—	—	—	—	—	—
Gross, 1961	1	Pathogenic avian (Winchester)	Liquid	√	—	—	—	100–1000	—	—	—	—	0·01	—	—	—	—	10 (FZ) 1·0 (FT)	—	—
Cook *et al.* 1963	1	Turkey sinusitis	Liquid	√	—	—	—	—	—	—	—	—	—	0·5	—	—	—	—	—	—
Cook & Inglis, 1964	1	Pathogenic avian (A 514)	Liquid	√	—	—	—	—	—	—	—	—	0·06	0·25	—	—	—	—	—	—
Inglis (pers. comm.)	1	Pathogenic avian (A 514)	Liquid	√	—	—	—	—	—	—	—	—	0·06–1·0	0·125–4·0	0·008–0·125	—	—	—	—	—
Adler *et al.* 1956	2	Goat lung (K)	Liquid	√	—	100	> 12·5–100	1·15–50	> 25–50	—	1·56–> 50	—	< 1·56–6·25	—	—	—	—	—	—	—
		Goat arthritis (KS)	Liquid	—	√	100	50–100	50	50	—	> 25–50	—	1·56–50	—	—	—	—	—	—	—
	5	Sheep lung	Liquid	√	—	100	100	3·13–6·25	12·5–25	—	1·56–3·13	—	50	—	—	—	—	—	—	—
				—	√	100	100	6·25–25	50	—	3·13–6·25	—	50	—	—	—	—	—	—	—
Hamdy *et al.* 1957	2	Lamb pneumonia	Liquid	—	√	No action	—	—	No action	—	40–50	—	2500–3000	—	—	—	—	—	—	—
	2	Turkey sinusitis	Liquid	—	√	0·4–1·0	—	—	0·6–1·5	—	0·5	—	0·09	—	—	—	—	—	—	—
Turner, 1960	1	*M. mycoides* var. *mycoides* (V 5)	Liquid	√	—	7·8	—	0·25	15·6	—	7·8	—	3·9	—	—	—	—	12·5 (NF)	—	—
Pak (pers. comm.)	2	Goat pleuro-pneumonia	Liquid	—	√	—	—	5	> 100	—	10	—	—	—	< 0·5–1·0	—	—	—	—	—
Hudson (pers. comm.)	2	*M. mycoides* var. *mycoides*	Liquid	√	—	—	—	—	—	—	—	—	—	—	0·07	—	—	—	—	—
Razin, 1963*b*	2	Saprophytic	Liquid	√	—	—	—	—	—	—	—	—	—	—	—	—	—	—	> 125	—
	1	Human oral																		
	1	Bovine																		
	1	Goat																		
	1	*M. gallisepticum* A 5969																		
Lampen *et al.* 1963	1	*M. gallisepticum*	Liquid	√	—	—	—	—	—	—	—	—	—	—	—	—	—	—	> 100	—

Key. T.C.C., tissue culture contaminant; G.U., genito-urinary tract; C.I.C., concentration causing complete inhibition; **, unknown number; ?, not clear; FZ, Furazolidone; NF, Nitrofurazone; FT, Furaltidone.

Table 2. *Summary of earlier reports on inhibition of* Mycoplasma *by drugs* in ovo

Authors and date of publication	Source and description and no. of strains	Drugs: Streptomycin or Dihydro-streptomycin	Chlortetra-cycline	Oxytetracycline	Chlor-amphenicol	Erythromycin	Kanamycin	Organic arsenicals	Sodium aurothiomalate	Polymixin
Wong & James, 1953	Pathogenic avian	Some action	Some action	Best action	No action	—	—	—	—	No action
Gross & Johnson, 1953	5, pathogenic avian	Prolonged life of embryos	Prolonged life of embryos	Prolonged life of embryos	Little action	—	—	—	—	—
Yamamoto & Adler, 1956	2, pathogenic avian (C and F)	Least action	Some action	Some action	—	Best action	—	No action	—	—
Hamdy *et al.* 1957	2, pathogenic avian	—	—	Some action	—	Best action	—	—	—	—
Adler *et al.* 1956	2, caprine (K: pneumonia; KS: arthritis)	—	Prolonged life of embryos	Prolonged life of embryos	—	Prolonged life of embryos	—	Prolonged life of embryos (KS only)	—	—
Switzer, 1953	*M. hyorhinis* (porcine)	50 mg./ml. protected 3/12 embryos	50 mg./ml. protected 10/12 embryos	5 mg./ml. protected 12/12 embryos	—	—	—	—	—	—
Nasemann & Röckl, 1960	Human genito-urinary	—	Inhibited by 0·5 μg./egg; killed by 5 μg./egg	—	—	—	—	—	—	—
Eaton, 1950	Mac and De strains of Eaton agent (*M. pneumoniae*)	—	Reduction of agent in yolk sacs	—	—	—	—	—	—	—
Eaton *et al.* 1951	Mac & De strains of Eaton agent (*M. pneumoniae*)	—	—	—	1 dose of 5 mg. 1 hr. after infn. inhibited multiplication	—	—	—	—	—
Eaton & Liu, 1957	Mac and FH strains of Eaton agent (*M. pneumoniae*)	MIC for Mac = 1000 μg. MIC for FH = 125 μg.	—	—	—	—	—	—	—	—
Marmion & Goodburn, 1961	Hetter (FH) strain of *M. pneumoniae*	—	—	—	—	—	—	—	1 dose of 25–30 mg. reduced no. of organisms	—
Goodburn & Marmion, 1962	Hetter (FH) strain and Bethesda PI 898 strain of *M. pneumoniae*	—	—	—	—	—	50 mg. inhibited specific antigen formation	—	1 dose of 25–30 mg. reduced no. of organisms	

combined with electron microscopy. A start has already been made in this laboratory using electron microscopy and agar-gel diffusion techniques and publication of our findings will follow in due course.

MATERIALS AND METHODS

Culture medium

The basal medium used throughout the work was Brucella broth and Brucella agar prepared by Albimi Laboratories Inc., Brooklyn, New York. The liquid medium was modified in the following way: 28 g. of the powder was dissolved in 100 ml. of distilled water and the solution was dialysed against 900 ml. distilled water. After 48 hr. the dialysate was made up to a final volume of 1 l. with distilled water and the pH adjusted to 7·0. To each 100 ml. of the medium was added 0·2 ml. penicillin containing 100,000 units per ml. and 1·0 ml. of a 1 in 80 solution of thallium acetate. Finally, Andrade's indicator was added together with 0·1% glucose and 15% unheated sterile horse serum. The complete medium was subsequently sterilized by passage through a Seitz-EK filter. The manufacturer's instructions for reconstituting the solid medium were followed exactly except for the addition of glucose, horse serum and the same concentration of antibiotics as used in the liquid medium.

Origin of strains of Mycoplasma, *etc.*

Twenty coccobacilliform strains of *M. gallisepticum* were tested together with sixteen strains of classified and unclassified mycoplasmata from various sources in Britain and other countries. L 1, the stable L-form of *Streptobacillus moniliformis*, obtained from the Lister Institute, was included for comparison. Summaries of details of the strains are presented in Tables 3 and 4.

Drugs and antibiotics tested

The antibacterial, antifungal and antiprotozoal agents used in this study are listed below:

Tylosin tartrate (Tylan)
Demethylchlortetracycline hydrochloride (Ledermycin)
Chlortetracycline hydrochloride (Aureomycin)
Tetracycline hydrochloride (Achromycin)
Oxytetracycline hydrochloride (Terramycin)
Spiramycin adipate (Rovomycin)
Erythromycin lactobionate (Erythrocin)
Chloramphenicol (Chloromycetin)
Streptomycin sulphate
Kanamycin sulphate (Kannasyn)
Ethidium bromide
Prothidium bromide
Antrycide methyl sulphate
Furazolidone

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Table 3. *Strains of* Mycoplasma gallisepticum

Name of strain	Source	Origin	Date of origin	Remarks and references
A 197 A 101 A 202 A 303	Infectious turkey sinusitis	H. P. Chu (Cambridge)	1956 1956 1957 1958	Chu & Newnham (1959) A 301: Chu & Newnham (1959)
BMO A 733	Infectious turkey sinusitis	A. G. Newnham (Cambridge)	1961 1961	— Newnham, Ostler & Chu (to be published)
A 108 A 141 A 333	Sinus exudate (Nelson's Fowl Coryza)	H. P. Chu	1956 1957 1958	Chu & Newnham (1959)
A 514 A 752	Sinus exudate (Nelson's Fowl Coryza)	H. P. Chu & A. G. Newnham A. G. Newnham	1958 1961	From same farm as A 333: Newnham (1963) —
A 5969	Chicken with 'C.R.D.'	H. van Roekel (Massachusetts)	1951	Jungherr, Luginbuhl & Jacobs (1953)
X 95	Tracheal and air sac tissues of chickens with 'C.R.D.'	F. S. Markham (U.S.A.) via D. G. ff. Edward	1953	Type-species: *M. gallisepticum*. Edward & Kanarek (1960)
S 6	Brain of turkey with torticollis and sinusitis	D. V. Zander via H. E. Adler (California)	1954	Adler & Yamamoto (1956), Zander (1961)
F 293	Trachea of chicken with 'C.R.D.'	D. V. Zander via H. E. Adler (California) J. Taylor via J. Fabricant (Cornell)	1956 1956	Adler & Yamamoto (1956) Calnek & Levine (1957)
SV	Turkey sinus exudate	H. E. Adler (California)	1957	Adler & Yamamoto (1957)
D	Turkey sinusitis exudate	J. E. Fahey via J. F. Crawley (Canada)	1953	Fahey (1954); Fahey & Crawley (1954). Called 'Crawley' by Chu & Newnham (1959)
BLOK	Air-sacs of chicken with 'C.R.D.'	M. E. Stumpel (Holland)	1958	Stumpel (1959)
'T'	Probably an egg contaminant isolated while passaging human NGU material	M. C. Shepard (U.S.A.) via E. Klieneberger-Nobel	1956	Shepard (1958); E. Klieneberger-Nobel (1962); R. Lemcke (1964)

Table 4. *Strains of classified and unclassified mycoplasma*

Name of strain	Serological group	Source	Origin	Date of origin	Remarks and references
Iowa 695	'9th' avian serotype	Air-sacs of 'pipped' turkey embryos	H. W. Yoder and M. S. Hofstad (Iowa)	1962	Agglutinates avian red cells. Yoder & Hofstad (1962)
A 36	Corresponding to Kleckner's group D	Trachea of chicken with primary infectious bronchitis (IBV)	H. P. Chu (Cambridge)	1955	'Fried-egg' type of colony. Chu & Newnham 1959)
A 326	Corresponding to Kleckner's group C	Sinus of chicken also containing *M. gallisepticum*	A. G. Newnham (Cambridge)	1958	'Fried-egg' type of colony
A 564	Corresponding to Kleckner's group C	Air-sacs of chicken also containing *M. gallisepticum*	A. G. Newnham (Cambridge)	1958	
Tu	Kleckner's group C (Kleckner, 1960)	Turbinates of 'normal' chicken	H. E. Adler (California)	1956	Non-pathogenic; Group II of Adler. (Ann. Meeting Amer. Vet. Med. Ass., Cleveland, Ohio, 1956–57)
Fowl	*M. gallinarum* corresponding to Kleckner's group B	Trachea of chicken with primary fowl pox	H. P. Chu (Cambridge)	1953	Non-pathogenic. Chu (1954); Edward (1954); PG. 16 (Edward & Freundt, 1956)
B 733		Trachea of chicken with mild coryza and fowl pox	F. T. W. Jordan (Liverpool)	1959	'Fried-egg-type of colony
A 64179		Trachea of 'normal' chicken	Y. V. Pereira (Connecticut)	1956	'Fried-egg' type of colony. Chu & Newnham (1959)
Laidlaw	*M. laidlawii*	Sewage	W. J. Elford and P. P. Laidlaw via L. Dienes (Boston)	1936	Saprophytic. Laidlaw & Elford (1936)
TC 7277	*M. laidlawii*	Tissue culture contaminant	E. S. Murray via L. Dienes (Boston)	1955–57	Isolated by Murray *et al.* (1957) from human conjunctival cell cultures. (Personal communication L. Dienes)
Bovine 'K'	*M. mycoides* var. *mycoides*	Contagious bovine pleuropneumonia	Type-culture (Colindale)	Unknown	Serologically identical with strain 403 (Hudson, Melbourne) and Bovine 'PI'
Bovine 'PI'	*M. mycoides* var. *mycoides*		Type-culture (Pasteur Institute)	Unknown	Classified as PG. 1 by Edward & Freundt (1956)
G 1/61	Unnamed	Lung of goat with pleuropneumonia	C. P. Pillai (Khartoum)	1961	Serologically indistinguishable from *M. mycoides* var. *mycoides*. Cottew & Hudson (personal communication), Lemcke (1964)
Goat	*M. mycoides* var. *capri*	Pleural fluid of goat with pleuropneumonia	H. P. Chu and W.L.B. Beveridge (Ankara and Cambridge)	1950	Edward (1953; 1954); classified as PG 3 by Edward & Freundt (1956)
Agalactiae	Corresponding to *M. agalactiae*	Infected goat's milk from V. Zavagli (Italy)	E. Klieneberger-Nobel (Lister Institute)	1953	Lemcke (1964)
2098/61	Corresponding to *M. pulmonis*	Rat lung pneumonia	I. Brewer & D. E. Stevenson (Tunstall Laboratory, Sittingbourne)	1961	Serologically indistinguishable from L 3 (Klieneberger, 1938)

Nitrofurazone
Neoarsphenamine (Neosalvarsan)
Nystatin (Mycostatin)
Polymixin B sulphate (Aerosporin)
Sodium aurothiomalate (Myocrisin)

Experimental method

Two drops of a 2- to 3-day broth culture of each strain were inoculated into a series of tubes containing 2 ml. of broth and falling concentrations of the drug under test. When the drugs were water-soluble, the concentrations were 1000, 200, 40, 8, 2, 0·5, 0·1, 0·02 and 0·004 μg./ml. broth. A broth control without drugs was included in the series. When testing against Furazolidone, Nitrofurazone and antrycide, the concentrations lay between 200 and 0·004 μg./ml., while with prothidium bromide the concentrations lay between 40 and 0·004 μg./ml. Erythromycin and antrycide were dissolved in a little methanol before adding to the broth, and Furazolidone was similarly dissolved in a little dimethylformamide. Concentrations of nystatin and polymixin are given in units/ml., the concentrations of nystatin lying between 200 and 0·004 units/ml. The solutions were made up just before the tests.

After inoculation the tubes were incubated at 37° C. for 7 days and a record kept of acid production as indicated by Andrade's indicator which was used as an index of growth. At the end of 1 week a loopful from the tube containing the highest concentration of the drug to show acid production was plated on to agar and the plates incubated for 2–4 days, after which they were examined for colonies by means of a dissecting microscope of $\times 35$ magnification and using oblique, transmitted light. Many of the tests were repeated at least once and results varying by more than one tube were rarely encountered. *M. agalactiae* and the three strains of *M. gallinarum* differed from the rest by not fermenting glucose; with these organisms growth was estimated by plating from each tube on to solid medium from the second day onwards.

RESULTS

The sensitivities *in vitro* of the thirty-six strains of *Mycoplasma* and the L-form of *Streptobacillus moniliformis* in liquid medium are presented diagrammatically in Tables 5–7.

The results obtained with sodium aurothiomalate (Myocrisin) are not included in the tables owing to the frequent occurrence of a zone of inhibition with growth at both higher and lower drug concentrations when testing strains of *M. gallisepticum*. Thirteen strains of *M. gallisepticum* were tested repeatedly, some in triplicate on the same day and some on different days (to a maximum of five occasions). Variable results were obtained—sometimes no 'zoning' occurred and the strains grew in all concentrations from 0·004 to 1000 μg./ml.; sometimes the maximum concentrations permitting growth varied from 0·004 to 40 μg./ml.; but on most occasions, from about the fourth day onwards, acid production was

Table 5. *Sensitivities* in vitro *of avian mycoplasmata other than* Mycoplasma gallisepticum *to drugs*

Drugs	Concentration of drug (μg./ml. broth)								
	0·004	0·02	0·1	0·5	2	8	40	200	1000
Tylosin	○	▲	○ ● △ ○	● ●	—	—	—	—	—
Demethylchlortetracycline	—	○	▲ △ ○ ● ○	—	●	—	—	—	—
Chlortetracycline	—	—	○	△ ○	● ○	▲ ●	●	—	—
Tetracycline	—	—	● △	● ●	—	—	—	—	—
Oxytetracycline	—	—	—	○	○ △	● ● ▲ ● ○	—	—	—
Spiramycin	—	—	—	○ ○	▲ △ ○	● ● ●	—	—	—
Erythromycin	—	—	—	—	—	●	○ ○ ▲ ○ ● △	—	● +
Chloramphenicol	—	—	—	—	▲ ○ ○ ● △ ○ ●	—	●	—	—
Streptomycin	—	—	—	—	—	—	● △	● ○ ○ ○	▲ ● +
Kanamycin	—	—	—	—	△ ○ ○ ○	● ● ●	—	—	▲ +
Ethidium bromide	—	—	—	—	○ ○	▲ ○ ● △	—	—	—
Prothidium bromide	—	—	—	—	● ●	△	▲ ● ● +	—	—
Antrycide	—	—	—	—	—	—	△ ○ ○ ○	▲ ● + ● ●	—
Furazolidone	—	—	—	▲	●	● ○	● ○ △ ○	—	—
Nitrofurazone	—	—	—	—	△	○	△ ● ● ●	○ ○ +	—
Neoarsphenamine	—	—	— —	— —	—	— —	▲ ○ ● ○ ○	○ ● ●	—
Nystatin*	—	—	—	—	—	—	—	● ● ○ △ + ● ○ ○ ▲	—
Polymixin*	—	—	—	—	—	—	—	—	● ● ○ △ + ● ○ ○ ▲

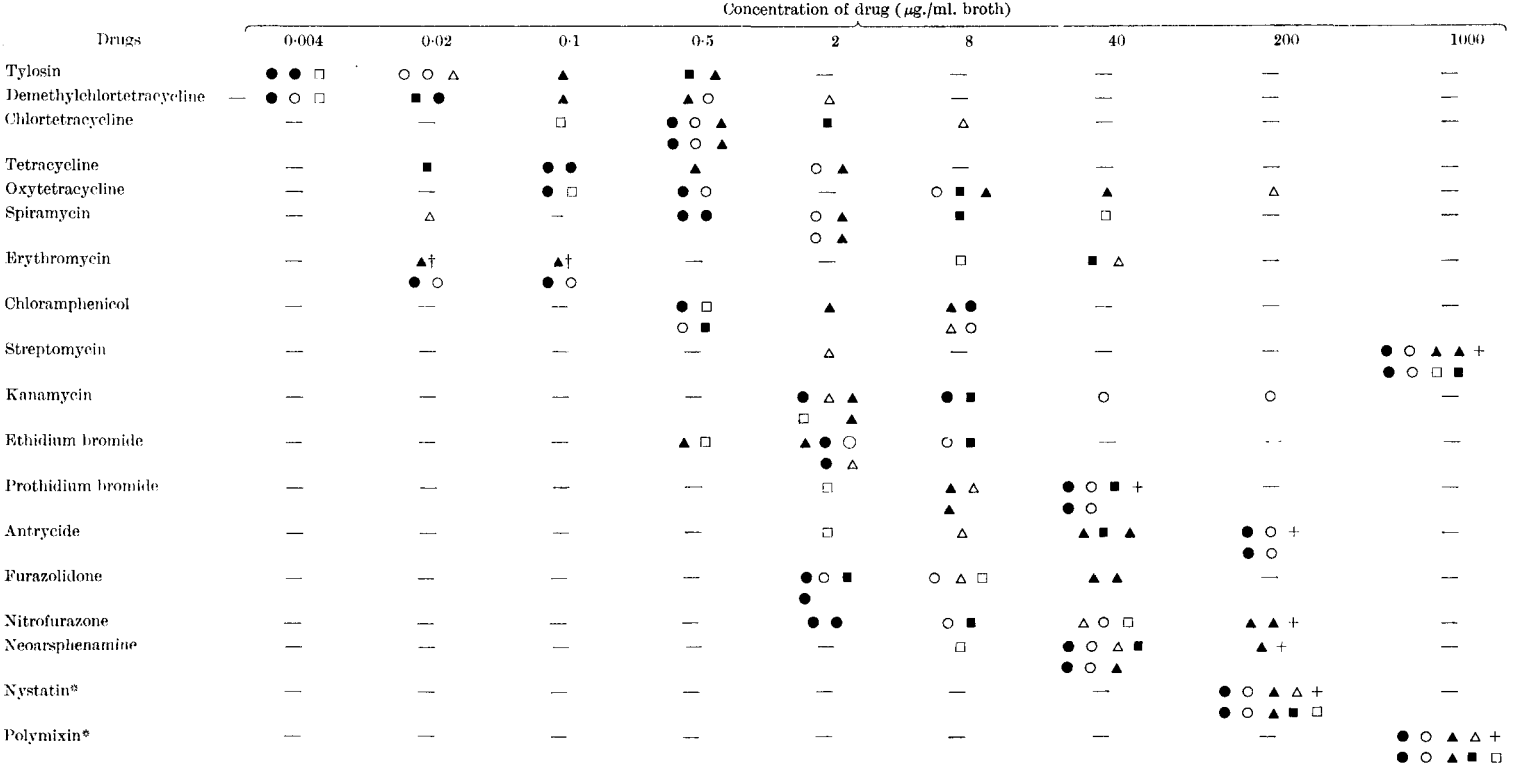
Key. Each symbol represents a single strain of mycoplasma; its position indicates the maximum concentration of drug permitting its growth. ●, 'Fowl', B 733; A 64179 (*M. gallinarum*; Kleckner's Group B); ○, TU, A 326, A 564 (Kleckner's group C); △, A 36 (Kleckner's group D); ▲, Iowa, 695; +, Highest concentrations tested; * concentrations in units/ml.

Table 6. *Sensitivities* in vitro *of* Mycoplasma gallisepticum *to drugs*

Drugs	Concentration of drug (μg./ml. broth)								
	0·004	0·02	0·1	0·5	2	8	40	200	1000
Tylosin	●●●○ ●●●○	●○○△ ●○▲	●	—	—	—	—	—	—
Demethylchlortetracycline	●● ●○	●●●○○ ●●○○	●▲ ●○	—	—	—	—	—	—
Chlortetracycline	—	—	●●●○ ●●○△	●▲○ ●○	●○ ●○	—	—	—	—
Tetracycline	—	●	●●○ ●●△	●●▲ ●○	—	—	—	—	—
Oxytetracycline	—	●	●● ●●	●●○ ●○○	●●○△ ●○○	▲	—	—	—
Spiramycin	—	●	●●●○ ●●○	●●○▲ ●●○△	●○ ○○	—	—	—	—
Erythromycin	●	●●●○ ●●●○	●●○○ ●○○△	●○▲	—	—	—	—	—
Chloramphenicol	—	—	●○	●○○	●●●●●○△ ●●●●○▲	○	—	—	—
Streptomycin	—	—	—	●●	●●▲	●●●○○ ●●●○△	●○ ○	—	○○+
Kanamycin	—	—	—	●	●○	●●○○△ ●○○○	●●○ ●●▲	—	—
Ethidium bromide	—	—	○●○ ○ ○	●●○△ ●●○	—	—	—	—	—
Prothidium bromide	—	—	—	—	●●●○○△ ●●○○○	●○	—	—	—
Antrycide	—	—	—	—	○	●●○○ ●●○	●○	—	—
Furazolidone	—	—	—	●●●	●●○○○△ ●●○○▲	●○	—	—	—
Nitrofurazone	—	—	—	●	●●●○ ●●	●○○△ ●○○	—	—	—
Neoarsphenamine	—	—	—	●○	●○△ ●○	●●●○○ ●●○○	—	—	—
Nystatin*	—	—	—	—	—	—	—	●●●●○○▲+ ●●●○○○△	—
Polymixin*	—	—	—	—	—	—	—	—	●●●○○○+ ●●●○○○△

Key. Each symbol represents a single strain of mycoplasma; its position indicates the maximum concentration of drug permitting its growth. ●, British strains; ○, North American strains; ▲ Dutch strain; △, 'T' strain; +, highest concentration tested; *, concentrations in units/ml.

Table 7. *Sensitivities* in vitro *of L*1 *and mammalian and saprophytic mycoplasmata to drugs*



Key. Each symbol represents a single strain of mycoplasma; its position indicates the maximum concentration of drug permitting its growth. ●, strains from bovine pleuropneumonia; ■, *M. agalactiae*; ▲, saprophytic strains; ○, strains from caprine pleuropneumonia; □, rodent strain (2098/61); △, L1:L-form. *, concentrations in units/ml.; †, see text; +, highest concentration tested.

observed in concentrations of 0·004 and 0·02 μg./ml., and again at 8 μg./ml., with a zone of inhibition lying between 0·1 and 2 μg./ml., and then from 40 to 1000 μg./ml. This inhibition might remain for the whole 7 days (or longer), or only one tube would finally show inhibition, or 'zoning' would have disappeared altogether by the end of the test period.

This phenomenon was not observed when testing the other strains of *Mycoplasma* against sodium aurothiomalate. The maximum concentration permitting growth of avian non-pathogenic strains varied from 2 to 200 μg./ml., while Iowa 695, the two saprophytic strains, the rodent strains and the two goat pleuropneumonia strains grew in all concentrations up to 1000 μg./ml. *M. agalactiae* and Bovine 'PI', however, did not grow in over 40 μg./ml., and Bovine 'K' was inhibited by 8 μg./ml.

DISCUSSION

Differing techniques, different media, the use of solid or liquid medium, the decrease in activity of some drugs in solution over different test periods, the comparison of different species and of different strains within a species, and the ready emergence of resistant strains, could all contribute to the varied results obtained by independent workers.

It was because of the wide differences observed between the sensitivity to erythromycin of human genito-urinary mycoplasmata and strains of *M. gallisepticum*, both *in vitro* and *in vivo*, that this comparative study of drug sensitivities was initiated. Reports from all workers studying human strains stressed the almost complete lack of sensitivity of the strains to the drug *in vitro* (Keller & Morton, 1953; Harkness & Bushby, 1954; Blyth, 1958) and *in vivo* (Rubin, Somerson, Smith & Morton, 1954). Carski and Shepard (1961) also reported the insensitivity of their tissue culture contaminant (? human) strains to 15 μg./ml. of the drug.

A few workers have found erythromycin sensitivities of other mammalian mycoplasmata which compare well with those of human urethritis strains, although there are exceptions (see Table 1). These findings contrasted with reports of high sensitivity, both *in vitro* and *in ovo*, of most pathogenic avian mycoplasmata (see Tables 1 and 2). Inglis (pers. comm.) observed variations in sensitivity of strain A 514 of from 0·125 to 1·0 μg./ml. after 7 days' incubation, depending on the concentration of organisms in the inoculum.

In our experiments, twenty strains of *M. gallisepticum* were inhibited by 2 μg./ml. or less of erythromycin, the maximum concentration permitting growth varying between 0·004 and 0·5 μg./ml. The non-pathogenic avian strains, however, were capable of growth in 8 to 1000 μg./ml. *M. agalactiae* and 2098/61 were also relatively insensitive.

It will, however, be seen that the results obtained with the two saprophytic strains resemble those with *M. gallisepticum*, and an unusual result was observed with the goat and bovine pleuropneumonia strains. After 5 days' incubation the maximum concentrations permitting growth were recorded as lying between 0·004 and 0·5 μg./ml. for Bovine 'K' and G 1/61, and between 0·02 and 2 μg./ml. for

Bovine 'PI' and 'goat'. After 7 days, however, growth of all four strains had occurred up to much higher concentrations—8–1000 μg./ml. This result was probably due to the loss of activity of the drug after 5 days, the drug being bacteriostatic against these strains, but bactericidal against all other strains tested. Compared with the tetracyclines, however, erythromycin seems in general to be more stable in solution. Unlike chlortetracycline, its inhibitory action increases with increased alkalinity (Haight & Finland, 1952*a*). Blyth (1958) studied in detail the loss of activity of erythromycin, neomycin, tetracycline, oxytetracycline, chloramphenicol, spiramycin, streptomycin and some other drugs over a test period of 5 days. Although erythromycin (and neomycin) were the only two drugs whose action had not decreased over 5 days in agar at 37° C., Haight & Finland (1952*a*) reported a progressive deterioration in activity of all solutions of the drug in broth over 4–7 days at 37° C. and at room temperature. They also stated (Haight & Finland, 1952*b*) that the drug exerted its effect best on multiplying bacteria and that its action could be either bacteriostatic or bactericidal depending on the sensitivity of the organisms concerned and the concentration of the antibiotic.

Tetracycline, together with the derivative forms of this antibiotic, have been tested extensively against mycoplasmata *in vitro* and *in vivo*. In our experiments it inhibited thirteen strains of *M. gallisepticum* at concentrations varying from $< 0{\cdot}1$ to < 2 μg./ml., and similar concentrations were recorded for most of the other mycoplasmata. This compares favourably with the findings of most other workers (see Table 1). Wide variations found by Yamamoto & Adler (1956) when testing ten different avian strains, and by Domermuth (1958), were probably due to selection of resistant organisms, as Blyth (1958) later reported that the M.I.C. for tetracycline against his human mycoplasmata increased from 0·5 to 16 μg./ml. over twenty subcultures in the presence of the drug.

Selection of resistant strains and loss of activity of the drug is of special significance when examining the effect of tetracyclines on growing organisms. This is particularly true in the case of chlortetracycline, which is the least stable of the tetracyclines particularly in solution at incubator temperatures, in an alkaline pH (Lepper, 1956) or when in contact with serum or ascitic fluid (Paine, Collins & Finland, 1948*b*). At pH 2·5 the half-life of chlortetracycline is about 14 days, while at pH 8·5 it is only about 4 hr. Thus perhaps also in our experiments a false picture is given, where, if the test period had been shorter, the figures presented would have been somewhat lower. Blyth (1958) exposed his mycoplasmata to the drug for only 2 days, but found that the activity of chlortetracycline had greatly decreased after only 1 day's incubation at 37° C. The variation in results obtained with different avian mycoplasmata might also have been partly due to prolonged tetracycline therapy of infected birds before isolation of the organism again resulting in emergence of resistant strains (Fahey, 1957; Osborn & Pomeroy, 1958; Osborn, Mataney & Pomeroy, 1960; Newnham, 1963).

Figures given by other authors for the inhibition of mammalian mycoplasmata were considerably higher than those recorded in our experiments (see Table 1). The same was true of *M. gallisepticum*, where our seventeen strains were inhibited

at concentrations between 0·1 and 8 μg/ml., figures, in general, somewhat lower than those reported by previous workers, although Gross (1961) did comment that the drug would have long since been inactivated over his test period of 4 weeks.

Considerable variations in sensitivity were obtained by all workers with oxytetracycline. It was one of the first antibiotics to be tested, with favourable results *in vitro* and *in vivo*, against human genito-urinary mycoplasmata and non-gonococcal urethritis. Robinson, Wichelhausen & Brown (1952), testing twenty-eight strains from human rheumatic and genito-urinary diseases, observed that more than half of their strains were completely inhibited by 1·0 μg/ml. or less. They commented that this drug was superior to chloramphenicol, chlortetracycline, streptomycin and sodium aurothiomalate, perhaps because of its greater stability, although there was apparently some loss of activity over the test period. They noted great differences between the minimal inhibitory concentrations of the drug and the minimal lethal concentrations for most strains, sometimes as great as 32-fold or occasionally 256-fold.

Results obtained by other workers with oxytetracycline against mammalian mycoplasmata seem, in general, to indicate a lesser sensitivity than the human strains (see Table 1), but in our experiments inhibition was obtained at quite low concentrations of the drug. Strains of *M. gallisepticum* were generally inhibited at an even lower concentration than were the other mycoplasmata (0·1–8 μg./ml.), figures which compare favourably with those of most other workers.

The results with demethylchlortetracycline in our experiments showed that its activity against most of the thirty-three mycoplasma strains was higher than that of the other tetracyclines. All strains except one were completely inhibited at concentrations ranging from less than 0·02 to 2 μg./ml. The apparent superiority of this tetracycline may have been due to its much greater stability in solution over 7 days at 37° C. (Finland & Garrod, 1960).

Chloramphenicol, a broad-spectrum antibiotic commonly used in the past against mycoplasma infections, was tested but was found somewhat less inhibitory than the tetracyclines. Again very varied results were obtained by previous workers. Maximum concentrations of the drug permitting growth of our strains ranged from 0·5 to 40 μg./ml. although nineteen strains of *M. gallisepticum* were inhibited between 2 and 8 μg./ml.

The results obtained with streptomycin differed widely, not only between the *M. gallisepticum* group and the other mycoplasmata, but also amongst the strains of *M. gallisepticum* themselves. The majority of the heterogeneous group were comparatively insensitive to the drug and grew in concentrations of from 40 to 1000 μg./ml. Two strains of *M. gallisepticum*, however, both from North America, were also able to grow in 1000 μg./ml. and three strains (from Britain and the U.S.A.) were capable of growth in 40 μg./ml. It is perhaps worth noting that the British strains were in general more sensitive to the drug than those from the U.S.A. and Canada, where streptomycin may have been more widely used in the past for treatment of avian respiratory mycoplasmosis.

The sensitivities reported here were comparable with those of previous workers

who also found great variability depending on the origin of the strains concerned, and on whether the minimal inhibitory or minimal lethal concentrations of the drug were recorded. As with our findings, the pathogenic avian strains were in general more sensitive to streptomycin than the human, mammalian and tissue culture strains tested.

With streptomycin the problem of very rapid 'one-step' resistance must be considered (Blyth, 1958; Domermuth, 1960). According to the work of Blyth, using human genito-urinary strains, this resistance was permanent and remained after twenty-seven passages in drug-free medium.

Spiramycin at low concentrations was effective in inhibiting many strains of *Mycoplasma* in these experiments and, in particular, twenty strains of *M. gallisepticum* which were inhibited at concentrations between 0·02 and 8 μg./ml. This is comparable with the findings of Inglis (pers. comm.), who, using a test period of 7 days in broth, reported the M.I.C. of strain A 514 as 0·125–4 μg./ml., depending on the number of organisms in the inoculum. He reported a similar relative range of activity (0·008–0·125 μg./ml.) for tylosin against strain A 514. Of the nineteen drugs tested in our experiments, tylosin appeared to be the most active under our test conditions. The drug prevented growth of fifteen out of sixteen strains of *M. gallisepticum* at concentrations between 0·02 and 0·1 μg./ml., findings similar to those of Inglis.

Few workers have tested tylosin against mycoplasmata isolated from disease in mammals. Pak (pers. comm.), in Turkey, however, found a minimal inhibitory concentration of 0·5–1·0 μg./ml. when testing two goat pleuropneumonia strains, and Hudson (pers. comm.) in Australia found that the bacteriostatic dose of tylosin against two strains from bovine pleuropneumonia was 0·07 μg./ml. Promising results with *in vivo* work have been reported by a few workers on avian and mammalian mycoplasmal diseases, but further investigation is necessary to determine the true efficacy of this drug *in vivo* after encouraging *in vitro* results.

Kanamycin has been widely used against mycoplasma contamination of tissue cultures. Successful eradication has been reported by most workers (see Table 1), but concentrations of the drug and application time have varied considerably. Emergence of resistant strains has apparently not yet become a problem, although Gourevitch *et al.* (1958*b*) were able to produce resistant strains of bacteria without difficulty.

Although not very active in comparison with the other commoner antibiotics, in our experiments kanamycin had a range of activity of 2–200 μg./ml. against all thirty-four strains tested. Only two strains ('goat' and Iowa 695) were capable of growth in 200 and 1000 μg./ml. respectively. L 1 was also completely inhibited at 40 μg./ml.

Kanamycin has advantages over the earlier antibiotics (penicillin, streptomycin, tetracycline, erythromycin, etc.) in that it is active against organisms which have become resistant to the other antibiotics, although a slight incomplete cross-resistance was found with neomycin and streptomycin by Gourevitch, Hunt & Lein (1958*a*). Gourevitch *et al.* (1958*b*) stated that at sufficiently high concentrations this antibiotic was bactericidal; this concentration being twice the bacterio-

static concentration against *Staphylococcus aureus*. Its main advantage when used against *Mycoplasma*, however, is in tissue culture work, where kanamycin can be used at very high concentrations (up to 400 μg./ml.) without detrimental effect on the tissue culture cell-systems themselves (Pollock, Kenny & Syverton 1960; Smith, Lummis & Grady, 1959).

In vitro results obtained in our experiments and in those of previous workers showed that both Furazolidone and Nitrofurazone had a somewhat greater activity against *M. gallisepticum* than against the other species of *Mycoplasma* tested. The maximum concentrations of Nitrofurazone and Furazolidone permitting growth of *M. gallisepticum* varied between 0·5 and 8 μg./ml., concentrations similar to those reported by Gross (1961). Domermuth & Johnson (1955) and Domermuth (1958) found considerable differences between the minimal inhibitory and minimal lethal concentrations of Furazolidone against two pathogenic avian strains (A 5967 and Winchester), the M.I.C. varying between 0·1 and 10 μg./ml., and the M.L.C. being 10 μg./ml. for both strains.

In contrast to these findings, most of the strains other than *M. gallisepticum* were still capable of growth in concentrations of 0·5 to more than 200 μg./ml. of Furazolidone and 2 to more than 200 μg./ml. of Nitrofurazone.

Ethidium bromide, prothidium bromide and antrycide have for some years been used in the treatment of bovine trypanosomiasis in African countries, but they have not been commonly used against mycoplasmal diseases. The only previous work on the action of any of these drugs *in vitro* on organisms of the mycoplasma group has been reported by Nasri (1963). Using Dafaalla's medium (Dafaalla, 1961) he tested four strains of *M. mycoides* var. *mycoides* against ethidium bromide and found that the drug had a bactericidal effect only at 1000 μg./ml. after 6–24 hr. exposure. This is in contrast to our findings, where the two strains of *M. mycoides* var. *mycoides* and G 1/61 were inhibited at between 2 and 8 μg./ml. *M. mycoides* var. *capri* and *M. agalactiae* were both inhibited at between 8 and 40 μg./ml., and 2098/61 was inhibited by 0·5–2 μg./ml. The results obtained with mammalian mycoplasmata were very similar to those found with the non-pathogenic avian mycoplasmata, although twelve strains of *M. gallisepticum* were considerably more sensitive, all being inhibited at between 0·1 and 2 μg./ml.

Results obtained with prothidium bromide and antrycide also showed that strains of *M. gallisepticum* were generally more sensitive than the other strains of *Mycoplasma*. Antrycide appeared to have very little inhibitory effect on the mammalian or non-pathogenic avian strains, most strains being capable of growth in 40–200 μg./ml. Nine out of eleven strains of *M. gallisepticum*, however, were inhibited between 8 and 40 μg./ml. Figures obtained with prothidium bromide lay, in general, between those obtained with ethidium bromide and antrycide, *M. gallisepticum* again being rather more sensitive than the other strains of *Mycoplasma*.

Neoarsphenamine and other polyvalent organic arsenicals have been tested against mycoplasmata *in vitro*, *in ovo* and *in vivo*, sometimes with considerable effect. In our experiments neoarsphenamine was distinctly more active against the sixteen strains of *M. gallisepticum* than against the group of heterogeneous mycoplasmata.

The maximum concentrations of drug permitting growth of the former group varied from 0·5 to 8 μg./ml., whereas for the latter group figures of 8–200 μg./ml. were obtained. Turner (1960) found that the V 5 strain of *M. mycoides* var. *mycoides* was inhibited by 125 μg./ml. neoarsphenamine or 62·5 μg./ml. oxyarsphenamine, figures comparable with those we obtained against two similar bovine strains, where growth was completely inhibited between 40 and 200 μg./ml. Turner regarded the lack of sensitivity to organic arsenicals as unexpected for they are known to be superior to inorganic arsenicals as bacteriostatic agents.

The 'Zone Phenomenon' found when testing sodium aurothiomalate against *M. gallisepticum* in our experiments was also observed by Robinson *et al.* (1952), although they were testing human and rodent strains. They reported minimal lethal concentrations of the drug as 16–128 μg./ml., and commented that, unlike the tetracyclines, the inhibitory and lethal concentrations of this drug did not lie far apart. In our experiments with strains other than *M. gallisepticum*, very varied results were obtained, some being comparable with those of the above authors.

Explanations for the 'Zone Phenomenon' are not readily forthcoming. It is possible that a complex is formed by sodium aurothiomalate with constituents of the culture medium, which, at certain concentrations, is inhibitory for some mycoplasmata (Nineham, pers. comm.). The varying results obtained with the same strains on different occasions might then be due to different batches of medium and, in particular, to horse serum from different horses.

Polymixin allowed the growth of L 1 and 29 strains of *Mycoplasma* tested in a concentration as high as 1000 units/ml. (approximately 167 μg./ml.). No difference in sensitivity was observed between the *M. gallisepticum* group and the heterogeneous group. These findings were similar to those of Hatch (1949), who found that polymixin at 50 μg./ml. was ineffective against eight strains of human and rodent *Mycoplasma.* Carski & Shepard (1961) also found that their seven tissue culture strains were insensitive to the drug, and Wong & James (1953) reported the lack of inhibition by polymixin of a few strains of *M. gallisepticum* in chick embryos.

The suggested mode of action of polymixin has been reviewed by Newton (1956). He, and other workers, used Gram-positive and Gram-negative bacteria as test organisms. It is thought that this drug acts primarily on the protoplast membrane and/or cell wall by combining with the phospholipid components and this results in the disorganization of the osmotic barrier (Gale, 1963). As mycoplasmata do not possess the normal type of cell wall, it is perhaps not surprising to find that this drug exerts no inhibitory action on the strains tested.

When tested against the fungicide, nystatin, all thirty-one mycoplasma strains and L 1 grew actively in concentrations up to 200 units/ml. This is equivalent to approximately 66·7 μg./ml. Razin (1963*b*). using strains *M. laidlawii*, *M. mycoides* var. *mycoides*, *M. mycoides* var. *capri* and *M. gallisepticum*, also found no inhibition up to 125 μg./ml. over a test period of 48 hr. This test period was superior to ours in that nystatin is known to lose approximately 40–50% of its antifungal activity in 5 days when in organic solvent–water preparations, even at room temperature.

Similar lack of activity was reported by Lampen, Gill, Arnow & Magana-Plaza (1963). Using strain A 5969 of *M. gallisepticum* they found that growth was not inhibited over 5 days up to a concentration of 100 μg./ml.

Both sets of workers none the less found that the mycoplasmata absorbed a considerable quantity of nystatin. These results were unexpected in the light of previous findings that nystatin-sensitive fungal cells and protoplasts bound considerable amounts of the drug while nystatin-resistant bacterial protoplasts and eubacteria failed to do so significantly (Lampen, Morgan, Slocum & Arnow, 1959; Lampen, Arnow, Borowska & Laskin, 1962; Kinsky, 1962). Eubacteria contain only traces of sterols, or none at all (Fiertel & Klein, 1959) whereas mycoplasmata, like the nystatin-sensitive fungi, algae, protozoa and animal cells (Lampen *et al.* 1962) contain, especially in the cell membrane, considerable quantities of cholesterol (Smith & Rothblat, 1962; Razin, 1963*a*). Thus Razin (1963*b*) suggested that the differences in capacity of various organisms to bind such polyene antibiotics does not account satisfactorily for the selective toxicity of the drug.

SUMMARY

A study was made in liquid medium over 7 days at 37° C. of the inhibitory action of nineteen antibacterial, antifungal and antiprotozoal drugs on twenty strains of *M. gallisepticum*, eight other avian mycoplasmata, six mammalian mycoplasmata, two saprophytic mycoplasmata and the L-form of *Streptobacillus moniliformis* (L–1).

The twenty strains of *M. gallisepticum* from Britain and other countries showed a similar range of drug sensitivity except where resistant strains were included. Tylosin and demethylchlortetracycline appeared to have the highest inhibitory action, followed by erythromycin, spiramycin, tetracycline, chlortetracycline, oxytetracycline and ethidium bromide. A 'Zone Phenomenon' frequently occurred with sodium aurothiomalate, inhibition often being observed between 0·1 and 2·0 μg./ml. Polymixin and nystatin had no inhibitory effect on the growth of any mycoplasmata tested. With the exception of erythromycin and streptomycin in some cases, the pattern of sensitivity observed with the mycoplasmata of diverse origin was similar to that of *M. gallisepticum*, most strains, however, being somewhat more resistant than *M. gallisepticum* to many of the drugs.

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REFERENCES

ADLER, H. E. (1964). A comparison of some characteristics of *Mycoplasma mycoides* var. *mycoides* and *Mycoplasma gallisepticum*. *Amer. J. vet. Res.* **25**, 243–5.

ADLER, H. E. & YAMAMOTO, R. (1956). Preparation of a new pleuropneumonia-like organism antigen for the diagnosis of chronic respiratory disease by the agglutination test. *Amer. J. vet. Res.* **17**, 290–3.

ADLER, H. E., YAMAMOTO, R. & CORDY, D. R. (1956). The effect of certain antibiotics and arsenicals in inhibiting growth of pleuropneumonia-like organisms isolated from goats and sheep. *Cornell Vet.* **46**, 206–16.

ADLER, H. E. & YAMAMOTO, R. (1957). Pathogenic and nonpathogenic pleuropneumonia-like organisms in infectious sinusitis of turkeys. *Amer. J. vet. Res.* **18**, 655–60.

BLYTH, W. A. (1958). An investigation into the aetiology of non-gonococcal urethritis with special reference to the role of pleuropneumonia-like organisms. Thesis for the Degree of Doctor of Philosophy, University of London.

CALNEK, B. W. & LEVINE, P. P. (1957). Studies on experimental egg-transmission of pleuropneumonia-like organisms in chickens. *Avian Dis.* **1**, 208–21.

CARSKI, T. R. & SHEPARD, C. C. (1961). Pleuropneumonia-like (Mycoplasma) infections in tissue culture. *J. Bact.* **81**, 626–45.

CHU, H. P. (1954). The identification of infectious coryza associated with Nelson's coccobacilliform bodies in fowls in England and its similarity to the chronic respiratory disease of chickens. *Proc. Xth World Poultry Congr., Edinburgh*, II, 246.

CHU, H. P. & NEWNHAM, A. G. (1959). What is chronic respiratory disease of chickens (C.R.D.)? *Proc. XVIth Int. Vet. Congr., Madrid*, **1**, 163–93.

COLLIER, L. H. (1957). Contamination of stock lines of human carcinoma cells by pleuropneumonia-like organisms. *Nature, Lond.*, **180**, 757–8.

COOK, J. K. A. & INGLIS, J. M. (1964). Comparison of *in vitro* activity of spiramycin and erythromycin against *Mycoplasma gallisepticum*. *J. comp. Path.* **74**, 101–7.

COOK, J. K. A., INGLIS, J. M. & PARKER, W. G. C. (1963). Spiramycin adipate in the treatment of mycoplasmosis in turkeys. *Vet. Rec.* **75**, 215–18.

DAFAALLA, E. N. (1961). Solid media for the growth of *Asterococcus mycoides*. *J. comp. Path.* **71**, 259–67.

DOMERMUTH, C. H. (1958). *In vitro* resistance of avian PPLO to antibacterial agents. *Avian Dis.* **2**, 442–9.

DOMERMUTH, C. H. (1960). Antibiotic resistance and mutation rates of Mycoplasma: *Avian Dis.* **4**, 456.

DOMERMUTH, C. H. & JOHNSON, E. P. (1955). An *in vitro* comparison of some anti-bacterial agents on a strain of avian pleuropneumonia-like organisms. *Poult. Sci.* **34**, 1395–9.

EATON, M. D. (1950). Action of aureomycin and chloromycetin on the virus of primary atypical pneumonia. *Proc. Soc. exp. Biol., N.Y.*, **73**, 24–9.

EATON, M. D. & LIU, C. (1957). Studies on sensitivity to streptomycin of the atypical pneumonia agent. *J. Bact.* **74**, 784–7.

EATON, M. D., PERRY, M. E. & GOCKE, I. M. (1951). Effect of nitro-compounds and aldehyde semicarbazones on the virus of primary atypical pneumonia. *Proc. Soc. exp. Biol., N.Y.*, **77**, 422–5.

EDWARD, D. G. FF. (1953). Organisms of the pleuropneumonia group causing disease in goats. *Vet. Rec.* **65**, 873–4.

EDWARD, D. G. FF. (1954). The pleuropneumonia group of organisms: a review together with some new observations. *J. gen. Microbiol.* **10**, 27–64.

EDWARD, D. G. FF. & FREUNDT, E. A. (1956). The classification and nomenclature of organisms of the pleuropneumonia group. *J. gen. Microbiol.* **14**, 197–207.

EDWARD, D. G. FF. & KANAREK, A. D. (1960). Organisms of the pleuropneumonia group: their classification into species. *Ann. N.Y. Acad. Sci.* **79**, 696–702.

FAHEY, J. E. (1954). A haemagglutination-inhibition test for infectious sinusitis of turkeys. *Proc. Soc. exp. Biol., N.Y.*, **86**, 38–40.

FAHEY, J. E. (1957). Infectious sinusitis of turkeys caused by antibiotic resistant pleuropneumonia-like organisms. *Vet. Med.* **52**, 305–307.

FAHEY, J. E. & CRAWLEY, J. F. (1954). Studies on chronic respiratory disease of chickens. IV. A haemagglutination-inhibition diagnostic test. *Canad. J. comp. Med.* **18**, 264–72.

FIERTEL, A. & KLEIN, H. P. (1959). On sterols in bacteria. *J. Bact.* **78**, 738–9.

FINLAND, M. & GARROD, L. P. (1960). Demethylchlortetracycline. *Brit. med. J.* ii, 959–63.

FOGH, J. & HACKER, C. (1960). Elimination of pleuropneumonia-like organisms from cell cultures. *Exp. Cell Res.* **21**, 242.

FOWLER, R. C., COBLE, D. W., KRAMER, N. C. & BROWN, T. McP. (1963). Starch gel electrophoresis of a fraction of certain of the pleuropneumonia-like group of microorganisms. *J. Bact.* **86**, 1145–9.

GALE, E. F. (1963). Mechanisms of antibiotic action. *Pharmacol. Rev.* **15**, 481–530.

GOODBURN, G. M. & MARMION, B. P. (1962). A study of the properties of Eaton's primary atypical pneumonia organism. *J. gen. Microbiol.* **29**, 271–90.

GOUREVITCH, A., HUNT, G. A. & LEIN, J. (1958*a*). Antibacterial activity of kanamycin. *Antibiotics Chemother.* **8**, 149–59.

GOUREVITCH, A., ROSSOMANO, V. Z., PUGLISI, T. A., TYNDA, J. M. & LEIN, J. (1958*b*). Microbiological studies with kanamycin. *Ann. N.Y. Acad. Sci.* **76**, 31–40.

GROSS, W. B. (1961). The effect of chlortetracycline, erythromycin and nitrofurans as treatment for experimental 'Air-Sac' disease. *Poult. Sci.* **40**, 833–41.

GROSS, W. B. & JOHNSON, E. P. (1953). Effect of drugs on the agents causing the infectious sinusitis of turkeys and chronic respiratory disease (air-sac infection) of chickens. *Poult. Sci.* **32**, 260–3.

HAIGHT, T. H. & FINLAND, M. (1952*a*). The antibacterial action of erythromycin. *Proc. Soc. exp. Biol., N.Y.*, **81**, 175–83.

HAIGHT, T. H. & FINLAND, M. (1952*b*). Observations on mode of action of erythromycin. *Proc. Soc. exp. Biol., N.Y.*, **81**, 188–93.

HAMDY, A. H., FERGUSON, L. C., SANGER, V. L. & BOHL, E. H. (1957). Susceptibility of pleuropneumonia-like organisms to the action of antibiotics erythromycin, chlortetracycline, hygromycin, magnamycin, oxytetracycline and streptomycin. *Poult. Sci.* **36**, 748–54.

HARKNESS, A. H. & BUSHBY, S. R. N. (1954). *World Hlth Org. Rep.* W.H.O./V.D. T. 117.

HATCH, M. H. (1949). Studies on some characteristics of the pleuropneumonia group of organisms. *A Symposium on Current Progress in the Study of Venereal Disease*, U.S. Govt. Printing Office, p. 183.

HEARN, H. J., OFFICER, J. E., ELSNER, V. & BROWN, A. (1959). Detection, elimination and prevention of contamination of cell cultures with pleuropneumonia-like organisms. *J. Bact.* **78**, 575–82.

JUNGHERR, E. L., LUGINBUHL, R. E. & JACOBS, R. E. (1953). Pathology and serology of air sac infection. *Proc. Amer. vet. med. Ass.* p. 308.

KELLER, R. & MORTON, H. E. (1953). Susceptibilities of Kazan, Nichols and Reiter strains of Treponema and Pleuro-pneumonia-like organisms to the antibiotic erythromycin. *Amer. J. Syph.* **37**, 379.

KENNY, G. E. & POLLOCK, M. E. (1963). Mammalian cell cultures contaminated with pleuropneumonia-like organisms. I. Effect of pleuropneumonia-like organisms on growth of established cell strains. *J. infect. Dis.* **112**, 7–16.

KINGSTON, J. R., CHANOCK, R. M., MUFSON, M. A., HILLEMAN, L. P., JAMES, W. D., FOX, H. H., MANKER, M. A. & BOYERS, J. (1961). 'Eaton agent pneumonia'. II. Treatment with demethylchlortetracycline. *J. Amer. med. Ass.* **176**, 118.

KINSKY, S. C. (1962). Nystatin binding by protoplasts and a particulate fraction of *Neurospora crassa*, and a basis for the selective toxicity of polyene antifungal antibiotics. *Proc. nat. Acad. Sci., Wash.*, **48**, 1049.

KLECKNER, A. L. (1960). Serotypes of avian pleuropneumonia-like organisms. *Amer. J. vet. Res.* **21**, 274–80.

KLIENEBERGER, E. (1938). Pleuropneumonia-like organisms of diverse provenance: some results of an enquiry into methods of differentiation. *J. Hyg., Camb.*, **38**, 458–76.

KLIENEBERGER-NOBEL, E. (1962). *Pleuropneumonia-like Organisms (PPLO): Mycoplasmataceae*. London and New York: Academic Press.

KUZELL, W. C., GARDNER, G. M. & FAIRLEY, D. L. M. (1949). Aureomycin in experimental polyarthritis with preliminary trials in clinical arthritis. *Proc. Soc. exp. Biol., N.Y.*, **71**, 631–3.

LAIDLAW, P. P. & ELFORD, W. J. (1936). A new group of filterable organisms. *Proc. Roy. Soc.* B, **120**, 292.

LAMPEN, J. O., MORGAN, E. R., SLOCUM, A. & ARNOW, P. M. (1959). Absorption of nystatin by microorganisms. *J. Bact.* **78**, 282–9.

LAMPEN, J. O., ARNOW, P. M., BOROWSKA, Z. & LASKIN, A. I. (1962). Location and role of sterol at nystatin-binding sites. *J. Bact.* **84**, 1152–60.

LAMPEN, J. O., GILL, J. W., ARNOW, P. M. & MAGANA-PLAZA, I. (1963). Inhibition of the pleuropneumonia-like organism *Mycoplasma gallisepticum* by certain polyene antifungal antibiotics. *J. Bact.* **86**, 945–9.

LEACH, R. H. (1962). The osmotic requirements for growth of Mycoplasma. *J. gen. Microbiol.* **27**, 345–54.

LEBERMAN, P. R., SMITH, P. F. & MORTON, H. E. (1950). The susceptibility of pleuropneumonia-like organisms to the *in vitro* action of antibiotics: aureomycin, chloramphenicol, dihydrostreptomycin, streptomycin and sodium penicillin. *J. Urol.* **64**, 167–73.

LEBERMAN, P. R., SMITH, P. F. & MORTON, H. E. (1952). Susceptibility of pleuropneumonia-like organisms to the action of antibiotics. II. Terramycin and neomycin. *J. Urol.* **68**, 388–402.

LECCE, J. G. & SPERLING, F. G. (1955). Chronic respiratory disease. III. The effect of treatment on the pleuropneumonia-like organisms flora of avian tracheas. *J. Amer. vet. med. Ass.* **127**, 54–6.

LEMCKE, R. M. (1964). The serological differentiation of *Mycoplasma* strains (pleuropneumonia-like organisms) from various sources. *J. Hyg., Camb.*, **62**, 199–219.

LEPPER, M. H. (1956). *Aureomycin (Chlortetracycline) Antibiotics Monograph*, no. 7. New York: Medical Encylopaedia Inc.

MARMION, B. P. & GOODBURN, G. M. (1961). Effect of organic gold salts on Eaton's primary atypical pneumonia agent and other observations. *Nature, Lond.* **189**, 247–8.

MELÉN, B. (1952). The susceptibility of pleuropneumonia-like organisms to the *in vitro* action of some antibiotics. *Acta path. microbiol. scand.* **30**, 98.

MOROWITZ, H. J., TOURTELLOTTE, M. E., GUILD, W. R., CASTRO, E., WOESE, C. & CLEVERDON, R. C. (1962). The chemical composition and submicroscopic morphology of *M. gallisepticum;* avian PPLO A 5969. *J. molec. Biol.* **4**, 93.

MURRAY, E. S., CHANG, R. S., BELL, S. D., TARIZZO, M. L. & SNYDER, J. C. (1957). Agents recovered from acute conjunctivitis cases in Saudi Arabia. *Amer. J. Ophthal.* **43**, 32.

NASEMANN, T. & RÖCKL, H. (1960). Pleuropneumonia-like organisms; their effect on chicken chorioallantoic membrane and their resistance to antibiotics. *Ann. N.Y. Acad. Sci.* **79**, 588–92.

NASRI, M. EL (1963). A note on the action of Ethidium bromide on *Mycoplasma mycoides*. *Vet. Rec.* **75**, 812–13.

NELSON, J. B. (1936*a*). Studies on an uncomplicated coryza of the domestic fowl. V. A coryza of slow onset. *J. exp. Med.* **63**, 509–13.

NELSON, J. B. (1936*b*). Studies on an uncomplicated coryza of the domestic fowl. VI. Coccobacilliform bodies in birds infected with the coryza of slow onset. *J. exp. Med.* **63**, 515–22.

NELSON, J. B. (1936*c*). Studies on an uncomplicated coryza of the domestic fowl. VII. Cultivation of the coccobacilliform bodies in fertile eggs and in tissue cultures. *J. exp. Med.* **64**, 749–58.

NELSON, J. B. (1936*d*). Studies on an uncomplicated coryza of the domestic fowl. VIII. The infectivity of foetal membrane and tissue culture suspensions of the coccobacilliform bodies. *J. exp. Med.* **64**, 759–69.

NELSON, J. B. (1960). The behaviour of murine pleuropneumonia-like organisms in HeLa cell cultures. *Ann. N.Y. Acad. Sci.* **79**, 450–7.

NEWNHAM, A. G. (1963). Antibiotics in the eradication of avian respiratory mycoplasmosis: a review of the literature together with the results of laboratory trials using chlortetracycline and demethylchlortetracycline. *Res. vet. Sci.* **4**, 491–505.

NEWTON, B. A. (1956). The properties and mode of action of the polymixins. *Bact. Rev.* **20**, 14–27.

OLESIUK, O. M. & VAN ROEKEL, H. (1959). The effects of antibiotics on experimental chronic respiratory disease in chickens. *Avian Dis.* **3**, 457–70.

OSBORN, O. H. & POMEROY, B. S. (1958). The effect of antibiotics on the infectious sinusitis agent of turkeys: Part I. Egg-transmission. *Avian Dis.* **2**, 180–6.

Osborn, O. H., Mataney, C. F. & Pomeroy, B. S. (1960). The effect of antibiotics on the infectious sinusitis agent of turkeys: the *in vivo* development of antibiotic-resistant strains of Mycoplasma. *Ann. N.Y. Acad. Sci.* **79**, 581–7.

Paine, T. F., Collins, H. S. & Finland, M. (1948*a*). Bacteriologic studies on aureomycin. *J. Bact.* **56**, 489–97.

Paine, T. F., Collins, H. S. & Finland, M. (1948*b*). Laboratory studies with aureomycin. *Ann. N.Y. Acad. Sci.* **51**, 228–30.

Pollock, M. E., Kenny, G. E. & Syverton, J. T. (1960). Isolation and elimination of pleuropneumonia-like organisms from mammalian cell cultures. *Proc. Soc. exp. Biol., N.Y.*, **105**, 10–15.

Pollock, M. E., Treadwell, P. E. & Kenny, G. E. (1963). Mammalian cell cultures contaminated with pleuropneumonia-like organisms. *Exp. Cell Res.* **31**, 321.

Razin, S. (1963*a*). Structure, composition and properties of the PPLO cell envelope. *Recent Progress in Microbiology, VIII.* (ed. N. E. Gibbons), pp. 526–34. Toronto: University Press.

Razin, S. (1963*b*). Binding of nystatin by Mycoplasma (Pleuropneumonia-like organisms). *Biochim. biophys. Acta*, **78**, 771–3.

Razin, S. (1963*c*). Osmotic lysis of Mycoplasma. *J. gen. Microbiol.* **33**, 471–5.

Razin, S., Argaman, M. & Avigan, J. (1963). Chemical composition of Mycoplasma cells and membranes. *J. gen. Microbiol.* **33**, 477–87.

Roberts, D. H. (1963). The isolation of a previously unreported avian *Mycoplasma* serotype and some observations on the incidence of Mycoplasma in poultry. *Vet. Rec.* **75**, 665–7.

Robinson, L. B., Wichelhausen, R. A. & Brown, T. McP. (1952). Sensitivity studies on human pleuropneumonia-like organisms. *J. Lab. clin. Med.* **39**, 290–302.

Robinson, L. B., Wichelhausen, R. A. & Roizman, B. (1956). Contamination of human cell cultures by pleuropneumonia-like organisms. *Science*, **124**, 1147.

Rouse, H. C., Bonifas, V. H. & Schlesinger, R. W. (1963). Dependence of adenovirus replication on arginine and inhibition of plaque formation by pleuropneumonia-like organisms. *Virol.* **20**, 357–65.

Rubin, A., Somerson, N. L., Smith, P. F. & Morton, H. E. (1954). The effects of the administration of erythromycin (Ilotycin) upon *Neisseria gonorrhoeae* and pleuropneumonia-like organisms in the uterine cervix. *Am. J. Syph.* **38**, 472–7.

Shepard, M. C. (1958). Growth and development of T strain pleuropneumonia-like organisms in human epidermoid carcinoma cells (HeLa). *J. Bact.* **75**, 351–5.

Smith, C. G., Lummis, W. L. & Grady, J. E. (1959). An improved tissue culture assay. II. Cytotoxicity studies with antibiotics, chemicals and solvents. *Cancer Res.* **19**, 847–52.

Smith, P. F. & Rothblat, G. H. (1962). Comparison of lipid composition of pleuropneumonia-like organisms and L-type organisms. *J. Bact.* **83**, 500–6.

Stumpel, M. E. M. (1959). Relation between chronic respiratory disease (C.R.D.) and chronic coryza in chickens. *Tijdschr. Diergen.* **84**, 741–50.

Switzer, W. P. (1953). Studies on infectious atrophic rhinitis of swine. I. Isolation of a filterable agent from the nasal cavity of swine with infectious atrophic rhinitis. *J. Amer. vet. med. Ass.* **123**, 45–7.

Turner, A. W. (1960). Growth-inhibition tests with *Mycoplasma mycoides* as a basis for chemotherapy and selective culture media. *Aust. vet. J.* **36**, 221–4.

Wong, S. C. & James, C. G. (1953). The susceptibility of the agents of chronic respiratory disease of chickens and infectious sinusitis of turkeys to various antibiotics. *Poult. Sci.* **32**, 589–93.

Yamamoto, R. & Adler, H. E. (1956). The effect of certain antibiotics and chemical agents on pleuropneumonia-like organisms of avian origin. *Amer. J. vet. Res.* **17**, 538–42.

Yoder, H. W. & Hofstad, M. S. (1962). A previously unreported serotype of avian Mycoplasma. *Avian Dis.* **6**, 147–60.

Zander, D. V. (1961). Origin of S6 strain Mycoplasma. *Avian Dis.* **5**, 154–6.