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The serological differentiation of *Mycoplasma* strains (pleuropneumonia-like organisms) from various sources

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INTRODUCTION

Over a period of several years, strains of *Mycoplasma* from a variety of sources have either been isolated in this laboratory or sent here for examination. Because some of these cultures were associated with disease conditions and it had been suggested that others (e.g. those from tissue cultures) were L-forms of bacteria, it seemed desirable to establish the identity of these cultures by comparing them with *Mycoplasma* cultures of known origin and properties.

Cultural and morphological characters are inadequate for classification, especially as they are readily affected by the type of medium and the conditions of cultivation. Biochemical tests also have a limited value; several species give no reactions and the range of substrates fermented by the others is very similar (Tourtellotte & Jacobs, 1960). Serological methods seem to be the most satisfactory for distinguishing strains.

Agglutination, and sometimes agglutinin absorption have been used for this purpose (Klieneberger, 1938, 1940; Warren & Sabin, 1942; Norman, Saslaw & Kuhn, 1950; Bailey *et al.* 1961). A drawback of this method is that some PPLO suspensions are auto-agglutinable.

A serological method depending on the inhibition of growth by specific antibody was devised by Edward & Fitzgerald (1954). The method had the advantage of being less sensitive than the agglutination test to small antigenic differences between strains of the same species. It was used in conjunction with a complement-fixation test by Huijsmans-Evers & Ruys (1956) to distinguish strains from the human mouth and genital tract, and in a modified form by Bailey *et al.* (1961). However, the growth-inhibition method was found unsatisfactory by Klieneberger-Nobel (1962) who, like Huijsmans-Evers & Ruys (1956), found that some antisera had no effect, even on the homologous strain. She also found that others lost their inhibitory power on storage.

Complement fixation tests were successfully used by Edward (1950), Edward & Fitzgerald (1951), Huijsmans-Evers & Ruys (1956) and Card (1959) to differentiate PPLO from various animal and human sources, and this method was chosen for the present work.

METHODS

Organisms

In all, 82 cultures were examined, 23 of human origin, 16 from tissue cultures and one from Eaton Agent propagated in tissue cultures, 27 from laboratory rats and mice, 2 from sewage, 8 from cattle and goats, 4 from poultry or embryonated eggs and one strain of unknown origin. The designation and source of the strains examined and the worker who isolated them are given in Table 1.

In addition to these 82 strains, antigens were made from three avian cultures, M, Fowl and TU, but were compared only with the other avian strains; no antisera were prepared against them. M (*Mycoplasma iners*) and Fowl (*M. gallinarum*) were recognized as distinct species by Edward & Kanarek (1960); TU, an unnamed non-pathogenic strain isolated in the U.S.A. and assigned by Kleckner (1960) to his group C, was found by Chu & Newnham (personal communication) to be distinct from *M. iners*, *M. gallinarum*, the non-pathogenic A 36 from the U.K. and the pathogenic 'coccobacilliform bodies' or *M. gallisepticum*.

Media and cultural conditions

The liquid medium used for maintaining cultures and growing antigen for the C.F.T. consisted of a tryptic digest of horse meat broth (TDB) supplemented with pooled normal human serum inactivated at 56° C. for 30 min. (10 %, v/v), Oxoid yeast extract (0·5 %, w/v), deoxyribonucleic acid (20 μg./ml.) and penicillin (50 units/ml.). For the living cultures used to immunize rabbits, the human serum was replaced by pooled normal rabbit serum, and for the preparation of the C.F.T. antigen of *M. pneumoniae* by unheated horse serum (20 %, v/v).

Cultures were incubated at 37° C.; all were shaken continuously during incubation with the exception of B 3 and G 2, which grew well only anaerobically, and 823, 826, 837, 844 and BM which grew better as stationary cultures.

For testing glucose fermentation, cultures on ox-heart infusion agar (1·5 %, w/v) with human serum (15 %, v/v), glucose (1·0 %, w/v) and phenol red (0·004 %, w/v) were incubated at 37° C. for 6 days. As controls, plates of the same medium without glucose were inoculated simultaneously and a known glucose-fermenting strain, A 36, was also inoculated on to both media.

Double-diffusion agar precipitation

This technique was used to test antisera for the presence of antibody against constituents of the media. Difco Noble agar (1·0 %, w/v) in normal saline buffered to pH 7·2, with 0·01 M sodium azide as preservative, was poured to a depth of 4 mm. A pattern of seven wells 6 mm. in diameter was used, the centres being 12 mm. apart. Precipitation lines were allowed to form at room temperature.

Reagents for the complement fixation test

These were the same as those described by Card (1959) except that preserved guinea-pig complement and glycerinated rabbit haemolytic serum for sheep corpuscles (Wellcome Research Laboratories) were used.

Table 1. *The* 17 *serotypes established by* C.F.T. *among* 82 *strains of* Mycoplasma

Type or species	No. of strains	Host or source	Designations of strains
ommon human genital (*M. hominis type* 1)	17	Man	H34*, infected abdominal wound; H68, Bartholin's abscess — E. J. Stokes (London); 4387 P*, non-gonococcal urethritis (NGU), R. G. Wittler (Washington); MJW, NGU; M. J. Whittington (London); H27, Reiter's disease (urethra); H33, 587, 731, 105, NGU (urethra); D, E, H, Q, M, O, S, NGU (external genitals); L, primary syphilis (external genitals) — R. M. Lemcke (*London*)
	11	Tissue cultures	ERK, MK2, CH, NCH, Tang, AE, CE, Payne; R. M. Lemcke; 417, 420; L. Hayflick (Philadelphia); HEp2; M. F. Barile (Bethesda)
aton agent (*M. pneumoniae*)	1	Man	FH*, egg-propagated line of Eaton agent; L. Hayflick
luman oral (probably corresponding to *M. salivarium*)	1	Man	B3*, human mouth; D. H. Card (London)
strain (*M. fermentans*)	1	Man	G2*, fusospirillary infection of human genital mucosa; M. Ruiter and H. Wentholt (Groningen)
reviously undescribed	1	Man	Navel*, fusospirillary infection of umbilicus; M. Ruiter and H. Wentholt (Groningen)
reviously undescribed	4	Tissue cultures	823*, 826, 837*, 844; L. Hayflick
	1	—	BM, Eaton Agent propagated in tissue cultures; B. P. Marmion (Melbourne)
at or mouse lung (probably corresponding to *M. pulmonis*)	9	Rats	Kon*, bronchiectasis; E. Klieneberger-Nobel (London); R42L, R45L, R46L, R25NP, RME (1), respiratory tract or middle ear; R42A (1), abscess — R. M. Lemcke; 2098, respiratory tract; D. Stephenson (Sittingbourne); SPF47, respiratory tract; H. C. Bartlema (Rijswick)
	8	Mice	DGE, respiratory tract; D. G. ff. Edward (Beckenham); M1*, M2, 68NP, 72L; respiratory tract; MB*, Peter, 73B; brain — R. M. Lemcke
louse 'rolling disease' (probably corresponding to *M. neurolyticum*)	1	Mouse	KSA*, mouse brain; R. M. Lemcke
at polyarthritis (probably corresponding to *M. arthritidis*) (*M. hominis, type* 2)	5	Rats	Jasmin*, tumour; Baxter, polyarthritis — E. Klieneberger-Nobel; DW, tumour; R. M. Lemcke; LX; L. Dienes (Boston); VII-2, respiratory tract; H. C. Bartlema
	2	Man	Campo*, urethra; L. Dienes; O7, cervix; M. H. Hatch (Baltimore)
	1	Unknown	H606, unknown (Washington)
artially related to *M. arthritidis*	3	Rats	R38*, rhinitis; RME (2), middle ear; R42A (2), abscess — R. M. Lemcke
ontagious bovine pleuropneumonia, *M. mycoides* var. *mycoides*	2	Cattle	pp. cattle*, contagious bovine pleuropneumonia (CBPP); unknown; Gladysdale*, CBPP; J. R. Hudson (Melbourne)
	2	Goats	G1/61*, G11, contagious caprine pleuropneumonia (CCPP) in Sudan; C. Pillai (Khartoum)
ontagious caprine pleuropneumonia	1	Goat	pp. goat*, CCPP in Turkey; W. I. Beveridge and H. P. Chu (Cambridge)
galactia of sheep (probably corresponding to *M. agalactiae*)	1	Goat	Agalactia*, infected goat milk from Italy; E. Klieneberger-Nobel
ovine genital (*M. bovigenitalium*)	2	Cattle	PG11*, genital tract; D. G. ff. Edward; 37, mastitis; I. Davidson and P. Stuart (Weybridge)
aprophytic (*M. laidlawii*)	2	Sewage	Laidlaw A*, Laidlaw B; P. P. Laidlaw and W. J. Elford
	1	Rat	STR; L. Dienes
	1	Tissue culture	TC7277; unknown
on-pathogenic avian (unnamed species)	1	Chicken	A36*, tracheal exudate; H. P. Chu
	2	Embryonated eggs	AM*, embryonated egg material; R. M. Lemcke; PSU4, 'pipped' chick embryo after further egg passage; W. H. Kelton (Iowa)
elson's 'coccobacilliform bodies' (*M. gallisepticum*)	1	*Embryonated egg*	T*, *eggs used to passage NGU exudates*; M. C. Shepard (Camp Lejeune) (identified as 'coccobacilliform body' strain by Klieneberger-Nobel (1962))

* Strains against which test antisera were prepared.

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The preparation of the antisera

At first rabbits were inoculated intravenously (i.v.) with washed saline suspensions harvested from 6-day cultures. Suspensions of several strains, for example *M. hominis type* 1, were found to contain very few if any viable cells after such an incubation period. Later it was found that more potent sera could be obtained with younger, viable cultures and, with the exception of the slow-growing *M. pneumoniae*, cultures 24–72 hr. old, depending on the rapidity of growth of particular strains, were used for injection. *M. pneumoniae* cultures were incubated 11–13 days. Six injections of 1, 1, 2, 2, 4 and 6 ml. were given, i.v., on alternate days, the suspensions for the last 5 being stored at $-30°$ C. until required. Experiments in which, for comparison, 2 rabbits were injected each time with fresh unstored suspensions, showed that such brief storage did not reduce the efficacy of the immunizing antigen. As a rule, two courses of 6 injections were given, separated by an interval of 2–3 weeks. A third series of 3 injections was sometimes given where the serum titre began to drop before the final exsanguination. Blood was taken before immunization and 5–10 days after the final injection of each series.

Antisera were also prepared by subcutaneous (s.c.) injection of suspensions in Freund's complete adjuvant. Washed cells harvested from 250 or 500 ml. of 24–72 hr. cultures were given at one time in several sites. Homologous serum titres were increased 4- to 16-fold if one or more s.c. injections were followed after an interval of 2–3 weeks by a course of 6 i.v. injections, but only low titre sera were obtained if the i.v. were given before the s.c. injections.

To determine whether antibody to traces of medium constituents in the immunizing antigen could account for the low titre cross-reactions observed, 4 rabbits were given, i.v., TDB, the residue centrifuged from TDB and resuspended in saline (TDB residue), Oxoid yeast extract (10 %, w/v) and deoxyribonucleic acid (0·2 %, w/v). Except for the TDB, of which a total of 30 ml. was given, these substances were injected in amounts which were present in 1000 ml. of the complete medium, i.e. the total volume from which immunizing antigen was usually harvested.

Preparation of antigens for complement fixation test

Unheated saline suspensions preserved with merthiolate were prepared as described by Card (1959). However, antigens of G 2, 37, Laidlaw A and Laidlaw B prepared in this way were too anticomplementary for use. Attempts to reduce the anticomplementary effect by sonic treatment and ether extraction failed.

Satisfactory antigens, however, were made from 37 and Laidlaw A by incubating cultures for 15–24 days, instead of the usual 5–6 days. This method did not work with G 2 or Laidlaw B, but satisfactory antigens were obtained by substituting rabbit or horse for human serum in the growth medium and incubating for 15 days.

The complement fixation test

At first titrations were carried out using the method of Card (1959). In subsequent tests, in place of antigens of standard optical density and a concentration of

complement (C′) determined for each antigen, the concentration of each antigen giving the maximum titre with its homologous serum, or with a strongly reacting serum, in the presence of 5HD50 of C′ was determined and then used for all titrations with other sera. With these optimal antigen concentrations, which were invariably less than those of standard turbidity, homologous titres were sometimes higher than in the original test, whereas cross-reactions with heterologous antisera and pre-immunization sera were lower.

In determining the identity of an antigen, its titre with a particular antiserum was compared in the same test with that of the homologous antigen and with that of the corresponding pre-immunization serum. Doubling dilutions of serum starting at 1/20 were used, and the titre expressed as the reciprocal of the highest serum dilution giving at least 50% fixation of complement. Antigens reacting with an antiserum to within $\frac{1}{2}$ or $\frac{1}{4}$ of the homologous titre were considered to be indistinguishable from the homologous strain, since even homologous titres varied to this degree in tests made at different times. Homologous titres are printed in bold type in the tables, those significant of antigenic relationship in ordinary type, and cross-reactions not regarded as significant of relationship, in italic figures.

RESULTS

Establishment of serotypes

As the survey progressed, antisera were prepared against strains that clearly did not belong to the serotypes represented by the antisera already in use. Eventually antisera against 17 distinct serotypes were available (Table 1). Not all the 82 strains were tested against all the antisera, but at least one strain of a given serotype, whether already established or newly discovered, was tested against the full battery of 17 antisera. Within the groups, however, in many cases up to 2 or 3 antisera of the same serotype were tested.

The 17 antisera used for fully cross-testing the serotypes were those against H34, FH, B3, G2, Navel, 823, M1, KSA, Campo, R38, Gladysdale, pp. goat, Agalactia, PG11, Laidlaw A, AM and T (Table 1); all had homologous titres in the range 1280–10,240.

With the exception of R38 and Campo, cross-reactions occurred mostly at $\frac{1}{32}$ or less of the homologous titre. A few occurred at $\frac{1}{16}$ with antisera to FH, B3, pp. goat and Laidlaw A, all of which had the comparatively low titre of 1280; these reactions did not suggest a close antigenic relationship. Only one high-titre serum, KSA, cross-reacted with several heterologous antigens at $\frac{1}{16}$ of the homologous titre (10,240). Gel-diffusion tests (see below) suggested that this may have been due to the presence of non-specific antibody to certain medium constituents.

With R38 and Campo, the heterologous antigen reacted at $\frac{1}{16}$–$\frac{1}{4}$ of the homologous titre, suggesting partial, though not complete relationship.

The nature of the cross-reaction

The modification of the C.F.T. reduced the heterologous titres; it did not eliminate them completely. Low-titre reactions occurred with many of the pre-

immunization sera, but after immunization the reactions with heterologous antigens usually increased two- to fourfold. The occurrence of such persistent cross-reactions suggested either that non-specific antibody to certain constituents of the medium was present in the antisera, or that different species of *Mycoplasma* possessed certain antigens in common.

Of the four medium constituents injected into rabbits, only TDB and 'TDB residue' induced a two- to fourfold rise in titre over the pre-immunization sera against several mycoplasma antigens and TDB residue. The antibody responsible was probably scanty because it produced no precipitate on gel-diffusion plates with the various medium constituents. Nor was any precipitate demonstrable by gel-diffusion between these substances and the antisera used in the C.F.T., except sera against KSA and Gladysdale, which gave diffuse bands both with TDB and the complete medium. It is probable that TDB antibody was responsible for at least part of the cross-reactions which occurred in the C.F.T., and the possibility of removing non-specific antibody by absorbing antisera with horse tissue powders is being investigated.

The observed cross-reactions may not entirely be due to non-specific antibody. The observations of Villemot & Provost (1959) and Taylor-Robinson, Somerson, Turner & Chanock (1963) suggest that different species of *Mycoplasma* have antigens in common. This possibility requires further investigation.

Strains from man

Seventeen strains from the genital tract were tested against antisera to two of them (H34 and 4387P), to a human oral strain, B3, and against an antiserum made by Card (1959) to a human genital strain representative of the single broad serotype which she found among 56 genital strains. Nine of these 17 strains were from the genital tract proper, including 2 strains which had undergone 6–8 passages in chick embryo cell cultures (Csonka & Furness, 1960). The other eight were from the external genitals in patients with non-gonococcal urethritis (NGU) or primary syphilis.

All reacted identically with the four sera, to titre with the three 'genital' antisera and at $\frac{1}{16}$–$\frac{1}{32}$ of the homologous titre with the 'oral' B3 antiserum and may be considered serologically identical with Card's 56 strains. Thus, 73 strains tested by C.F.T. in this laboratory belonged to one broad serological group.

This agrees with the finding of Nicol & Edward (1953), Huijsmans-Evers & Ruys (1956) and Bercovici, Persky, Rozansky & Razin (1962), that the majority of the human genital strains examined belong to one serotype. Edward & Freundt (1956) have named this common human genital type *M. hominis type* 1. Since four cultures of *M. hominis type* 1, PG21, PG23, PG25 and PG26 obtained from Dr D. G. ff. Edward, reacted to high titre with an antiserum against a strain representative of those isolated in this laboratory (Card & Klieneberger-Nobel, unpublished observations) it would seem that our 73 strains belong to this species.

Three other strains of human origin, FH from Eaton's agent of Primary Atypical Pneumonia (*M. pneumoniae*, Chanock *et al.* 1963), B3 (oral) and G2 (*M. fermentans*) were serologically distinguishable from one another and from *M. hominis*

Table 2. *Homologous and cross-*C.F. *titres of* Mycoplasma *spp. from man and rats*

Antigen of strain	Origin	C.F. titre with antiserum: H34 *M. hominis, type* 1	B3 (human oral)	G2 *M. fermentans*	FH *M. pneumoniae*	Navel	Campo (PG27) '*M. hominis, type* 2'	Jasmin *M. arthritidis*
		Homologous titre						
		10240	**1280**	**2560**	**1280**	**5120–10240**	**10240**	**2560**
H34	Man	**10240**	*80*	*80*	*20*	*80*		
B3	Man	*80*	**1280**	*80*	*40*	*80*		
G2	Man	*80*	*80*	**2560**	*20*	*80*	*20–80*	NT
FH	Man	*80*	*40*	*40*	**1280**	*80*		
Navel	Man	*80*	*80*	*40*	*20*	**5120–10240**		
Campo-line PG27	Man							
Campo-line HEM	Man							
O7	Man							
Jasmin	Rats	*40–80*	*40–80*	*40–80*	*20–40*	*40–80*	5120–10240	1280–2560
Baxter	Rats							
LX	Rats							
DW	Rats							
VII-2	Rats							
H606	Unknown							

NT = Not tested.

type 1 (Table 2). This confirms the observations of Nicol & Edward (1953), Dienes & Madoff (1953), Huijsmans-Evers & Ruys (1956), Card (1959), Chanock, Hayflick & Barile (1962) and Taylor-Robinson *et al.* (1963). These 4 serotypes were distinct from a fifth human type represented by the strain Navel (Table 2) and from the 12 other types shown in Table 1.

Navel could be serologically distinguished from all of the other 16 serotypes included in this survey; heterologous antigens all cross-reacted at less than $\frac{1}{32}$ of the homologous titre. The results were quite clear-cut, in contrast to those obtained by Taylor-Robinson *et al.* (1963) in a micro-C.F.T., using antiserum supplied by the present author. These workers obtained very high levels of cross-reaction with antigens of certain oral and genital strains and it was difficult to assess from their results how closely Navel was related to these strains.

Apart from the above report, Navel does not seem to have been compared serologically with other species of *Mycoplasma*. Ruiter & Wentholt (1955), who isolated it from a patient with umbilical dermatitis, noted that it differed from their G-strains (Ruiter & Wentholt, 1952) in its failure to ferment glucose, its non-pathogenicity for mice and its eventual adaptation to growth under aerobic conditions.

Campo, a strain of the so-called '*M. hominis type* 2', was distinct from the other 5 serotypes isolated from man, but indistinguishable from a rat strain Jasmin (Table 2). To verify that the reactions of our line of Campo (PG 27, obtained some years previously from Dr D. G. ff. Edward) were typical of the species, another line of Campo (HEM) and another 'type 2' strain, 07 (Morton, Lecce, Oskay & Coy, 1954), were obtained from Dr H. E. Morton. All these cultures were indistinguishable from 5 rat strains originating in different countries (Table 2). The pathogenicity of two of the rat strains, Jasmin and Baxter, and their similarity to the original L4 (Klieneberger-Nobel, 1960) suggests that they are rat polyarthritis PPLO, the *M. arthritidis* of Edward & Freundt (1956). Strain H 606 also belonged to this group, but whether it was of rat or human origin could not be established.

'Type 2' strains and *M. arthritidis* are reported as failing to ferment glucose (Edward, 1950, 1954; Lecce & Morton, 1954; Tourtellotte & Jacobs, 1960). None of the 'type 2' and rat strains listed in Table 2 produced acid from glucose. Further evidence for the identity of the two species is their ability to produce subcutaneous abscesses in mice (Edward, 1954).

In summary, five serological types were found among mycoplasma strains from man. One, comprising the greatest number of strains, corresponded to the species *M. hominis type* 1, the second to *M. pneumoniae*, the third probably to the oral species, *M. salivarium*, and the fourth to *M. fermentans*. The fifth serotype, comprising only one strain, Navel, has not so far been assigned to any recognized species. Because of its close antigenic relationship to *M. arthritidis*, the position of '*M. hominis type* 2' as a distinct species is in doubt.

Strains from tissue cultures

Sixteen strains of *Mycoplasma* from various mammalian cell cultures were examined. Nine were from HeLa lines, 5 maintained in four London laboratories, 1 from Canada and 3 from one laboratory in the U.S.A. Six cultures came respectively from the cell lines ERK, MK2, mouse sarcoma S180, human pleural D116P, HEp.II and the Fjelde line of human epidermoid carcinoma of skin. ERK was shown to be of human rather than rabbit origin (Coombes, Daniel, Gurner & Kelus, 1961) and both ERK and MK2 are thought to be HeLa lines. Thus, apart from S180, all the cell lines were of human origin. The type of cell culture from which the remaining strain, TC7277, was isolated is unknown: the only information available is that it was isolated in Saudi Arabia from a trachoma culture. A seventeenth strain, BM, may have originated in a tissue culture since it was found in a subculture of a strain of Eaton Agent (line PI898) that had been passaged only in primary monkey kidney tissue cultures.

Eleven of these strains, 10 obtained from cell lines of human origin and 1 from the mouse sarcoma line, S180, reacted to high titre with antisera against *M. hominis type* 1, but gave no significant reaction with the 'human oral' B3 antiserum (Table 3).

Five others, 823, 826, 837 and 844 from the three American HeLa lines and the HEp.II line, and BM from the Eaton Agent strain formed a homogeneous group, as judged by tests with sera against two of them (Table 3). This group was distinct from *M. hominis type* 1, the human oral (Table 3) and 14 other serotypes of *Mycoplasma* (Table 1).

Morphologically, 823 and related strains were highly filamentous when grown in broth. On solid media, all 5 grew better anaerobically; in liquid, growth occurred aerobically but was more profuse if cultures were not shaken. It is not clear whether this preference for a low oxygen tension is an inherent property of this group or whether it is the result of continued propagation in tissue cultures. In this laboratory human genital strains, which usually grow well aerobically, grow very poorly, if at all, on aerobic plates when first isolated from tissue cultures, indicating the need for anaerobic as well as aerobic cultivation when attempting to isolate PPLO from tissue cultures.

TC7277, the strain from an unidentified tissue culture, was not related to *M. hominis type* 1 or to the 823 serotype, but was serologically identical with *M. laidlawii*.

To summarize, most of the tissue culture strains belonged to the species *M. hominis type* 1; one was *M. laidlawii* and the remainder constituted a distinct serological group, unrelated to other known species.

A strain probably derived from embryonated eggs

Fluid from embryonated eggs through which material from a patient with a recurrent skin infection had been passaged was sent to this laboratory by Prof. A. M. Macdonald. The material had produced pock-like lesions about 2 mm. in diameter on the chorio-allantoic membrane (C.A.M.). The pock-producing agent had

Table 3. C.F. *titres of* Mycoplasma *strains from tissue cultures with antisera against* M. hominis type 1, *a human oral and two tissue culture strains*

Antigen of strain	Tissue culture of origin	C.F. titre with antiserum: H34 *M. hominis, type* 1	4387P *M. hominis, type* 1	B3 (human oral)	823	837
		Homologous titre				
		10240	**2560**	**1280**	**10240**	**5120**
ERK	Embryo rabbit kidney (probably HeLa)	2560–5160	640–2560	*40–160*	*40–80*	*40–80*
MK 2	Monkey kidney (probably HeLa)					
CH	HeLa (London, laboratory 1)					
NCH	HeLa (London, laboratory 2)					
Tang	HeLa (London, laboratory 3)					
AE	HeLa (London, laboratory 4, line 1)					
CE	HeLa (London, laboratory 4, line 2)					
Payne	HeLa (Canada)					
H.Ep 2	Human Epidermoid carcinoma (Fjelde line)					
420	Human pleural, D116P					
417	Mouse sarcoma, S180					
823	HeLa (U.S.A.)	*80–160*	*80*	*40–80*	2560–10240	1280–5120
837	HeLa, RB10 (U.S.A.)					
826	HeLa (U.S.A.)					
844	H.Ep II (U.S.A.)					
BM	Eaton agent propagated in tissue culture					

been serially propagated on the C.A.M., producing lesions that contained minute stainable organisms resembling PPLO (Macdonald, personal communication). The present author isolated a mycoplasma strain, AM, from the egg material.

In C.F. tests with antisera of 17 serotypes, AM reacted significantly only with an antiserum against a non-pathogenic avian strain A 36; A 36 also reacted to high titre with AM antiserum (Table 4). Miss A. G. Newnham (Cambridge) confirmed the serological relationship of AM to A 36 by means of an agglutination test. AM differed from A 36, however, in failing to produce acid from glucose.

Table 4. C.F. *titres of various avian* Mycoplasma *strains with* A 36 *and* AM *antisera*

	C.F. titre with antiserum	
Antigen of strain	A 36	AM
A 36 (non-pathogenic avian, U.K.)	**640–1280**	640–1280
AM (embryonated egg)	640–1280	**2560–5120**
PSU 4 (embryonated egg)	640	640–1280
TU (non-pathogenic avian U.S.A.; Kleckner's group C)	*40–80*	*40–80*
M (*M. iners*)		
Fowl (*M. gallinarum*)		
T (*M. gallisepticum*)		

Another strain PSU 4, isolated from a 'pipped' chick embryo after further egg passages, was also related to AM and A 36. These three strains seemed distinct from 4 other recognized avian types or species, TU, *M. iners*, *M. gallinarum* and *M. gallisepticum* (Table 4).

Chu & Newnham (personal communication) have found that A 36 is distinct from the 4 avian types, TU, *M. gallinarum*, *M. iners*, and *M. gallisepticum*, and consider that it probably corresponds to the group D of Kleckner (1960). The present work showed that it is also distinct from 15 serotypes of non-avian origin (Table 1).

The relationship of AM to A 36 suggests that it was derived from the embryonated eggs and not the skin lesions of the patient. This view is consistent with the failure of broth cultures of AM, after 7 transfers on artificial medium, to produce pock-like lesions in the C.A.M. Although it was possible that the *Mycoplasma* had produced the pock-like lesions originally observed and had lost this property during transfer on artificial media, it seems more probable that the pocks were produced by another agent from the human skin infection. The growth of the pock-producing agent on the C.A.M. probably enabled the mycoplasma already present in the eggs to proliferate in the lesions. In experimental animals, analogous situations have been found where latent mycoplasma infections have been made manifest by the inoculation of other infective agents (Klieneberger-Nobel, 1962).

Strains from laboratory rats and mice

Seventeen strains from rats were classified by means of antisera prepared against three of them: Kon, from a lung lesion of a rat with broncho-pneumonia, Jasmin (*M. arthritidis*) and R 38 from purulent rhinitis in a rat. Kon and Jasmin appeared

distinct; there was some antigenic relationship between Jasmin and R38 (Table 5), but it was possible to distinguish between them by the C.F.T.

Eight of these rat strains, R42L, R45L, R46L, R25NP, 2098 and SPF47, all from the respiratory tract, and RME (1) and R42A (1) were indistinguishable from the rat lung strain, Kon (Table 5). RME (1) was isolated from a natural infection in the middle ear and R42A (1) from a subcutaneous abscess produced by the inoculation of R38. Both these organisms may have originated in the respiratory tract; in one rat an identical strain (R42L) was isolated from the lung; in the other (Tuffery, personal communication) there were, on the lung surface, dark spots characteristic of one stage of chronic PPLO bronchopneumonia (Klieneberger & Steabben, 1937, 1940).

Table 5. C.F. *titres of* Mycoplasma *strains from rats with antisera against Kon, Jasmin and R*38

Antigen of strain	Origin of strain	C.F. titre with antiserum: Kon (Homologous titre 1280)	Jasmin (Homologous titre 2560)	R38 (Homologous titre 2560)
Kon, R42L, R45L, R46L, R25NP, 2098, SPF47	Respiratory tract	640–1280	*40–80*	*40–80*
Jasmin	Tumour	*40–80*	1280–2560	320–640
Baxter	Polyarthritis			
DW	Tumour			
LX	(Lesion unknown)			
VII-2	Respiratory tract			
R38	Rhinitis	*80*	320	2560
R42A	Abscess produced by inoculation of R38	640	320	2560
R42A (1)	Isolate from mixed culture R42A	640	*80*	*80*
R42A (2)	Isolate from mixed culture R42A	*80*	320	2560
RME	Infection of middle ear	1280	320	2560
RME (1)	Isolate from mixed culture RME	1280	*80*	*80*
RME (2)	Isolate from mixed culture RME	*80*	320	2560

Two rat strains were indistinguishable from R38. These were R42A (2) which was recovered, together with the lung type R42A (1), from a subcutaneous abscess produced by the inoculation of R38, and RME (2), which was recovered, together with the lung type RME (1), from the middle ear. The presence of 2 distinct strains in the original cultures from the abscess and the middle ear was first shown by the C.F.T.; antigens from these cultures reacted to titre with both Kon and R38 antisera (Table 5). Re-examination of both cultures revealed 2 colony types

and antigens prepared from single colony subcultures showed that both R38 and the lung type were present in the original cultures (Table 5).

Four rat strains, Baxter, DW, LX, and VII-2 were identical with Jasmin in their reactions with antisera against Jasmin, Campo ('*M. hominis type* 2') and R38 (Tables 2 and 5). Another rat strain, STR, which was indistinguishable from *M. laidlawii* may have been present on the rat as a saprophytic contaminant.

Eight strains from mice, DGE, M1, M2, 68NP and 72L from the respiratory tract and MB, Peter and 73B from the brain, were identical with Kon. This was confirmed by titrations with M1 and MB antisera. This serotype has been shown to be associated with respiratory disease in mice (Lemcke, 1961) and probably spreads to the brain when the respiratory tract is heavily infected. Unlike *M. neurolyticum*, another type found in the mouse brain, it is not pathogenic when inoculated intracerebrally (Lemcke, 1961).

Thus, the same serotype is found in the respiratory tract of both rats and mice. Edward (1954) also reported that some PPLO from mice with catarrh had similar pathogenic and cultural properties to L3, the original rat lung type described by Klieneberger (1938). The association of the serotype represented by Kon with rat bronchopneumonia and respiratory disease in mice suggests that it corresponds to the species *M. pulmonis* (Edward & Freundt, 1956).

Another strain, KSA, from the brain of a mouse differed both in colonial morphology and antigenically from Kon and its related strains. It was also distinct from *M. arthritidis*, R38, and 13 other serotypes (Table 1). On primary isolation it produced the symptoms of 'rolling disease', as described by Findlay, Klieneberger, MacCallum & MacKenzie (1938), when inoculated with an agar adjuvant intracerebrally into mice (Lemcke, 1961). A later subculture was less virulent, only one out of nine mice exhibiting the characteristic 'rolling'. The rest survived, developing a pronounced hydrocephalus; one month after inoculation, their serum titre with KSA antigen was 40–80, compared with < 10 in mice inoculated only with agar. The properties of KSA suggest that it is the same as the L5 strain of Findlay *et al.* (1938), the species *M. neurolyticum* of Edward & Freundt (1956).

In summary, all the strains from laboratory rats and mice, with the exception of the saprophyte STR, belonged to four serotypes: 17, mostly from the respiratory tract of both rats and mice, probably corresponded to *M. pulmonis*, 5, from rats, to *M. arthritidis* and one, from mouse brain, to *M. neurolyticum*. A fourth type, represented by R38, R42A (2) and RME (2) from rats with purulent infections, was partially related to *M. arthritidis*.

Strains from cattle and goats

Eight cultures from cattle and goats were compared (Table 6). A 20-year-old strain, pp. cattle, from contagious bovine pleuropneumonia (CBPP) could not be distinguished from Gladysdale (*M. mycoides* var. *mycoides*), a more recently isolated strain from CBPP. A similar observation was made by Yoshida (1961), who found that CBPP strains kept in his laboratory retained their reactivity with rabbit and goat antisera although they no longer reacted with positive sera from cattle infected with the CBPP organism. Some antigenic loss obviously occurs

after prolonged subculture, but it is not apparent in C.F. tests using sera from immunized animals.

Two strains, G 1/61 and G 11, isolated by C. Pillai in the Sudan from goats with contagious caprine pleuropneumonia (CCPP), were indistinguishable from the two CBPP strains (Table 6). Hudson & Cottew (personal communication) also found that Pillai's strain G 1/61 was indistinguishable from *M. mycoides* var. *mycoides*, but distinct from 2 strains reputed to be *M. mycoides* var. *capri*.

Another CCPP strain, pp. goat, isolated by Beveridge and Chu in Turkey, was distinct from *M. mycoides* var. *mycoides* (Table 6). Although this organism is the same as the one described by Edward (1954) as *M. mycoides* var. *capri*, it seems doubtful whether the name is justified in view of the serological difference between it and *M. mycoides* var. *mycoides*, and the fact that strains serologically identical with or very closely related to the latter have been isolated from CCPP.

Of the other three cultures, Agalactia from infected milk of a goat with agalactia and PG 11 (*M. bovigenitalium*) were distinct from one another and from the CBPP and CCPP organisms (Table 6). Agalactia has the cultural and morphological properties described for *M. agalactiae* (Edward, 1954; Freundt, 1957). Strain 37, from bovine mastitis (Davidson & Stuart, 1960), was related to *M. bovigenitalium*. This is in agreement with the findings of Edward & Leach, reported by Stuart *et al.* (1963).

In summary, four species or serotypes were distinguishable among 8 cultures from cattle and goats, *M. mycoides* var. *mycoides*, *M. bovigenitalium*, *M. agalactiae* and a CCPP organism, pp. goat.

Saprophytic strains

Laidlaw's strains A and B were not distinguishable by this test. Earlier reports (Laidlaw & Elford, 1936; Klieneberger, 1940) indicate that A and B are related to some extent antigenically and Edward & Freundt (1956) name only one species, *M. laidlawii*. As already noted, only two cultures besides Laidlaw B, TC 7277 and STR, reacted to high titre with Laidlaw A antiserum.

DISCUSSION

The results from this and other laboratories of typing mycoplasma strains from the human genital tract show that most of them, at least in Europe, belong to one broad serological group, the species *M. hominis type* 1. There may be slight antigenic differences between strains within this species, as suggested by the observations of Nicol & Edward (1953), Card (1959), Oates, Whittington & Wilkinson (1959) and Taylor-Robinson *et al.* (1963). These might account for the differences in sensitivity between the 3 strains H 34, MJW and H 27 in detecting specific antibody in human sera (Lemcke & Csonka, 1962).

Although *M. hominis type* 1 has been isolated from the apparently healthy genital tract (Freundt, 1956; Nicol & Edward, 1953), most of the strains examined in this laboratory were isolated from patients with evidence of genital infection. In some instances (e.g. NGU patients) only PPLO were found; in others, another

Table 6. *Homologous and cross-*C.F. *titres of* Mycoplasma *strains from cattle and goats*

Antigen of strain	Origin of strain	C.F. titre with antiserum: pp. cattle *M. mycoides* var. *mycoides*	Gladysdale *M. mycoides* var. *mycoides*	G 1/61	pp. goat	Agalactia *M. agalactiae*	PG 11 *M. bovigenitalium*
		Homologous titre					
		640	**5120**	**1280**	**1280**	**2560**	**5120**
pp. cattle	Contagious bovine pleuropneumonia (CBPP)	**640**	10240	NT	NT	NT	*80*
Gladysdale	CBPP	640	**5120**	1280	*80*	*20*	*20*
G 1/61, G 11	Contagious caprine pleuropneumonia (CCPP) in Sudan	NT	2560	**1280**	*40*	*< 20*	*20–40*
pp. goat	CCPP in Turkey	NT	*80*	*40*	**1280**	*20*	*40*
Agalactia	Milk of goat with agalactia	*40*	*40*	*20*	*80*	**2560**	*80*
PG 11	Bovine genital tract	*80*	*80*	*40*	*80*	*20*	**5120**
37	Bovine mastitis	*80*	*40*	NT	NT	NT	1280

NT, not tested.

pathogen such as *Trichomonas vaginalis*, *Neisseria gonorrhoeae* or *Treponema pallidum* was present. *M. hominis type* 1 seems to be a potential pathogen which can proliferate in the urogenital tract under certain conditions, especially where tissues have been damaged by some other pathogen or by trauma.

Only one of the human strains, Ruiter & Wentholt's strain Navel, was not related to one of the four types or species *M. hominis type* 1, *M. fermentans*, *M. pneumoniae* and the human oral type. Since it was distinguishable from the 16 other serotypes, it may represent a hitherto unrecognized species.

The antigenic similarity of '*M. hominis type* 2' to *M. arthritidis* suggests that the former should no longer be recognized as a distinct species. *M. arthritidis* is found much more frequently in rats than 'type 2' strains are found in man and it is a recognized rat pathogen, whereas the pathogenicity of 'type 2' strains for man is obscure. It is probable, therefore, that the natural host of this serotype is the rat, and that 'type 2' strains occur in man as commensals or saprophytic contaminants.

There are no reports of the isolation of 'type 2' from the human genital tract in Europe, but in the U.S.A. a few other type 2 genital strains besides Campo and O 7 have been isolated (Norman *et al.* 1950; Bailey *et al.* 1961). There seems little justification, however, for the statement of Corriell, Fabrizio & Wilson (1960), that this is the genital type commonly found in the U.S.A. There has been no survey in America comparable to those carried out in Europe, where a large number of strains from the human genital tract has been identified serologically. Type 1 strains undoubtedly occur in America, as indicated by the identification of the NGU strain 4387P in the present work and the reports of Nicol & Edward (1953) and Bailey *et al.* (1961). They may, therefore, be just as prevalent there as in Europe.

The results of this investigation as well as those of Collier (1957), Corriell *et al.* (1960) and Bailey *et al.* (1961) establish that a large proportion of PPLO from tissue cultures are *M. hominis type* 1. Barile, Malizia & Riggs (1962), using a fluorescent antibody technique, found that 48 strains from tissue cultures were antigenically related, but did not identify the serotype. One of these, H.Ep 2, proved in this present survey to be *M. hominis type* 1—further evidence of the widespread contamination of cell lines by this species. The suggestion of Collier (1957) that this type of *Mycoplasma* was present in the HeLa cells when originally isolated from a carcinoma of the human cervix, seems the most probable of all those proposed to account for the contamination. Cell lines other than HeLa could have become contaminated from HeLa lines maintained in the same laboratory, as the manipulations involved in cultivating cells probably predispose to such cross-contamination.

The isolation of *M. laidlawii* from a tissue culture has not previously been reported. Like the 'type 2' strain found by Bailey *et al.* (1961) it may just be an isolated instance.

The group of 5 tissue culture strains represented by 823 did not belong to any of the species of known origin with which it was compared, so that there is no indication of their origin. Culturally and morphologically they had the characters

of PPLO, not of authentic L-forms. All 5 strains were either isolated in the U.S.A. or came from material which had been passaged in tissue cultures there. The contamination of the cell lines involved might therefore be traceable to a common source, possibly a single supplier of cell cultures.

As the majority of PPLO from tissue cultures clearly belong to recognized species of *Mycoplasma*, it seems unlikely that they are, as has been frequently suggested, L-forms derived from contaminating bacteria under the influence of antibiotics in the cell-culture medium.

As regards avian strains, the isolation of AM and PSU 4 from embryonated eggs and their antigenic relationship to A 36 indicate that non-pathogenic avian strains can pass into the egg. Kleckner (1960) reported that non-pathogenic avian PPLO could be egg-transmitted, but did not specify which types. It is established that the PPLO associated with chronic respiratory disease (C.R.D.) in poultry, *M. gallisepticum* or 'Nelson's coccobacilliform bodies', can be transmitted through the egg (Fahey & Crawley, 1954). Isolation of the C.R.D. organism during the passage of infective agents or pathological material through embryonated eggs has been reported several times (Van Herick & Eaton, 1945; Klieneberger-Nobel, 1962; Marmion & Hers, 1963).

The demonstration in embryonated eggs of another serotype, A 36, besides *M. gallisepticum* emphasizes the necessity of identifying serologically any strain of *Mycoplasma* isolated from infective material passaged through eggs. The same applies to tissue culture passage, since BM, from a line of Eaton Agent maintained in tissue cultures proved to be antigenically related not to *M. pneumoniae*, but to a type of *Mycoplasma* previously found only in tissue cultures. Failure to identify strains properly could result in the erroneous implication, as pathogens in man or animals, of innocuous strains of avian or tissue culture origin. A similar mistake could be made with PPLO from animals, since strains resembling the saprophytic *M. laidlawii* are occasionally found, like STR from a rat and the bovine genital 'S' strains found by Edward (1950).

The widespread occurrence of mycoplasma infection in laboratory rats and mice is illustrated by the fact that the cultures examined came from 12 different stocks or colonies in 5 different countries. Most of the PPLO isolated from rats and mice seemed to belong to species recognized as pathogens of these animals, *M. pulmonis*, *M. arthritidis* and *M. neurolyticum*. The partial antigenic relationship to *M. arthritidis* of a fourth type, R 38, suggests that it is not a distinct species but an antigenic variant of *M. arthritidis*. It is distinguishable from *M. arthritidis* by its lesser pathogenicity in the rat (Klieneberger-Nobel, 1960; Lemcke, 1961).

From the anomalous behaviour in the C.F.T. of antigens prepared with what proved to be mixed cultures, the existence in two rat lesions of more than one serotype was detected. This demonstrates the sensitivity of the C.F.T. used in these investigations, and emphasizes the importance both of establishing clones from single colonies for tests and of isolating more than one colony from the primary plate, if mixed infections are to be detected. Mixed infections may indeed occur more frequently than is suspected. Immunofluorescence techniques, as applied to *Mycoplasma* (Malizia, Barile & Riggs, 1961; Chanock, Hayflick & Barile, 1962;

Clark, Bailey, Fowler & Brown, 1963; Marmion & Hers, 1963) should afford a method of detecting mixtures of serotypes in primary agar cultures. The tendency of mycoplasma species to participate in mixed infections is well illustrated in rats as well as in human genital infections. Two *M. arthritidis* strains, DW and LX, were isolated in association with a bacterium, *Streptobacillus moniliformis*, from lesions in rats.

The similarity of the two contagious bovine pleuropneumonia strains is in accord with observations which suggest that there is only one species, *M. mycoides* var. *mycoides*, associated with contagious pleuropneumonia of cattle (Klieneberger-Nobel, 1962). There is, however, some confusion among the goat strains. Unless the disease was wrongly diagnosed in either case, two serotypes, one of which is indistinguishable from *M. mycoides* var. *mycoides*, were isolated from contagious pleuropneumonia in goats. Moreover, organisms identical with or related to either *M. mycoides* or *M. agalactiae* have been found in disease conditions different from classical CCPP and agalactia (Hudson, personal communication). Thus, recognized species appear to be associated with more than one type of disease in goats. Without more information about the types of *Mycoplasma* in different goat diseases, it would be premature to attribute a particular goat disease, such as CCPP, to any one species.

It is clear that much confusion can be avoided in regard both to the aetiological significance of mycoplasma species in disease conditions and to the taxonomy of the group when cultures are identified by suitable serological techniques. More detailed knowledge of the antigenic structure of the Mycoplasmataceae is obviously desirable. Extraction and purification of the antigens should result not only in the improvement of existing serological methods, like the C.F.T., for diagnosis and identification, but in the development of precipitin techniques. Coupled with immunodiffusion methods, the use of purified extracts should also elucidate both the finer apparent antigenic differences between strains, like those between strains of *M. hominis type* 1, and the partial antigenic relations observed between rat strains such as R 38 and *M. arthritidis*.

SUMMARY

A complement fixation test with rabbit antisera was used to differentiate 82 cultures of *Mycoplasma* from man, mammalian cell cultures, laboratory rats and mice, cattle, goats, poultry, embryonated eggs and sewage.

Seventeen serotypes were distinguished, 5 from man, 1 from mammalian cell cultures, 4 from rats and mice, 4 from cattle and goats, 2 from poultry and one saprophytic. Most of these corresponded to recognized species of *Mycoplasma*, but 1 of human origin (represented by 1 strain, Navel), and 1 from tissue cultures (5 strains), may represent new species. R 38, one of the serotypes from rats, could be distinguished from the species *M. arthritidis*, but is probably an antigenic variant rather than a distinct species. Two species hitherto recognized as distinct, *M. arthritidis* and *M. hominis type* 2, could not be distinguished and appear to constitute a single species. These findings illustrate the necessity, from the viewpoint of taxonomy, of comparing mycoplasma strains by serological methods.

The serotypes of human and animal origin were largely host-specific. Exceptions were the inclusion of *M. arthritidis* from rats and *M. hominis type* 2 from man in a single serotype, the finding of a bovine organism among the strains isolated from goats and of a saprophytic strain in a rat.

In relation to the aetiology of disease in man and animals, the isolation of an endogenous *Mycoplasma* from embryonated eggs used to passage infective material illustrates the importance of identifying these organisms serologically. The demonstration of mixed mycoplasma infections in lesions in two rats shows the necessity of adequately purifying all cultures of *Mycoplasma* before examination.

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REFERENCES

Bailey, J. S., Clark, H. W., Felts, W. R., Fowler, R. C. & Brown, T. McP. (1961). Antigenic properties of pleuropneumonia-like organisms from tissue cell cultures and the human genital area. *J. Bact.* **82**, 542.

Barile, M. F., Malizia, W. F. & Riggs, D. B. (1962). Incidence and detection of pleuropneumonia-like organisms in cell cultures by fluorescent antibody and cultural procedures. *J. Bact.* **84**, 130.

Bercovici, B., Persky, S., Rozansky, R. & Razin, S. (1962). Mycoplasma (pleuropneumonia-like organisms) in vaginitis. *Amer. J. Obstet. Gynec.* **84**, 687.

Card, D. H. (1959). PPLO of human genital origin. Serological classification of strains and antibody distribution in man. *Brit. J. vener. Dis.* **35**, 27.

Chanock, R. M., Dienes, L., Eaton, M. D., Edward, D. G. ff., Freundt, E. A., Hayflick, L., Hers, J. F. ph., Jensen, K. E., Liu, C., Marmion, B. P., Morton, H. E., Mufson, M. A., Smith, P. F., Somerson, N. L. & Taylor-Robinson, D. (1963). *Mycoplasma pneumoniae*. Proposed nomenclature for atypical pneumonia organism (Eaton agent). *Science*, **140**, 662.

Chanock, R. M., Hayflick, L. & Barile, M. F. (1962). Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. *Proc. nat. Acad. Sci., Wash.*, **48**, 41.

Clark, H. W., Bailey, J. S., Fowler, R. C. & Brown, T. Mc.P. (1963). Identification of Mycoplasmataceae by the fluorescent antibody method. *J. Bact.* **85**, 111.

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

COOMBES, R. R. A., DANIEL, M. R., GURNER, B. W. & KELUS, A. (1961). Species-characterizing antigens of 'L' and 'ERK' cells. *Nature, Lond.*, **189**, 503.

CORRIELL, L. L., FABRIZIO, D. P. & WILSON, S. R. (1960). Comparison of PPLO strains from tissue cultures by complement fixation. *Ann. N.Y. Acad. Sci.* **79**, 574.

CSONKA, G. W. & FURNESS, G. (1960). A study of the aetiology of non-gonococcal urethritis and Reiter's disease. *Brit. J. vener. Dis.* **36**, 181.

DAVIDSON, I. & STUART, P. (1960). *Vet. Rec.* **72**, 766 (Letter).

DIENES, L. & MADOFF, S. (1953). Differences between oral and genital strains of human pleuropneumonia-like organisms. *Proc. Soc. exp. Biol., N.Y.*, **82**, 36.

EDWARD, D. G. FF. (1950). An investigation of pleuropneumonia-like organisms isolated from the bovine genital tract. *J. gen. Microbiol.* **4**, 4.

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

EDWARD, D. G. FF. & FITZGERALD, W. A. (1951). The isolation of organisms of the pleuropneumonia group from dogs. *J. gen. Microbiol.* **5**, 566.

EDWARD, D. G. FF. & FITZGERALD, W. A. (1954). Inhibition of growth of pleuropneumonia-like organisms by antibody. *J. Path. Bact.* **68**, 23.

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

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

FAHEY, J. E. & CRAWLEY, J. F. (1954). Studies on CRD of chickens. III. Egg transmission of a PPLO. *Canad. J. comp. Med.* **18**, 67.

FINDLAY, G. M., KLIENEBERGER, E., MACCALLUM, F. O. & MACKENZIE, R. D. (1938). 'Rolling disease'; new syndrome in mice associated with a pleuropneumonia-like organism. *Lancet*, ii, 1511.

FREUNDT, E. A. (1956). Occurrence and ecology of *Mycoplasma* species (pleuropneumonia-like organisms) in the male urethra. *Brit. J. vener. Dis.* **32**, 188.

FREUNDT, E. A. (1957). In *Bergey's Manual of Determinative Bacteriology*, 7th ed. London: Baillière, Tindall and Cox.

HUIJSMANS-EVERS, A. G. & RUYS, A. C. (1956). Micro-organisms of the pleuropneumonia group (Family of Mycoplasmataceae) in man. II. Serological identification and discussion of pathogenicity. *Leeuwenhoek ned. Tijdschr.* **22**, 377.

KLECKNER, A. L. (1960). Serotypes of avian pleuropneumonia-like organisms. *Amer. J. vet. Res.* **21**, 274–280.

KLIENEBERGER, E. (1938). Pleuropneumonia-like organisms of diverse provenance. *J. Hyg., Camb.*, **38**, 458.

KLIENEBERGER, E. (1940). The pleuropneumonia-like organisms: further comparative studies and a descriptive account of recently discovered types. *J. Hyg., Camb.*, **40**, 204.

KLIENEBERGER, E. & STEABBEN, D. B. (1937). On a pleuropneumonia-like organism in lung lesions of rats, with notes on the clinical and pathological features of the underlying condition. *J. Hyg., Camb.*, **37**, 143.

KLIENEBERGER, E. & STEABBEN, D. B. (1940). On the association of the pleuropneumonia-like organism L3 with bronchiectatic lesions in rats. *J. Hyg., Camb.*, **40**, 223.

KLIENEBERGER-NOBEL, E. (1960). Pathogenicity and immunology of organisms of the pleuropneumonia group. *Ann. N.Y. Acad. Sci.* **79**, 615.

KLIENEBERGER-NOBEL, E. (1962). *Pleuropneumonia-like organisms (PPLO) Mycoplasmataceae*. London: Academic Press.

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

LECCE, J. G. & MORTON, H. E. (1954). Metabolic studies on three strains of PPLO isolated from man. *J. Bact.* **67**, 62.

LEMCKE, R. M. (1961). Association of PPLO infection and antibody response in rats and mice. *J. Hyg., Camb.*, **59**, 401.

LEMCKE, R. M. & CSONKA, G. W. (1962). Antibodies against pleuropneumonia-like organisms in patients with salpingitis. *Brit. J. vener. Dis.* **38**, 212.

Malizia, W. F., Barile, M. F. & Riggs, D. B. (1961). Immunofluorescence of pleuropneumonia-like organisms isolated from tissue cell cultures. *Nature, Lond.*, **191**, 190.

Marmion, B. P. & Hers, Ph. J. F. (1963). Proceedings of the conference on newer respiratory viruses. Washington, October, 1962. *Amer. Rev. resp. Dis.* **88** (3), Part 2, 198.

Morton, H. E., Lecce, J. G., Oskay, J. J. & Coy, N. H. (1954). Electron microscope studies of pleuropneumonia-like organisms isolated from man and chickens. *J. Bact.* **68**, 697.

Nicol, C. S. & Edward, D. G. ff. (1953). Role of organisms of the pleuropneumonia group in human genital infections. *Brit. J. vener. Dis.* **29**, 141.

Norman, M. C., Saslaw, S. & Kuhn, L. R. (1950). Antigenic similarity of five human strains of pleuropneumonia-like organisms. *Proc. Soc. exp. Biol., N.Y.*, **75**, 718.

Oates, J. K., Whittington, M. J. & Wilkinson, A. E. (1959). A note on the results of cultural and serological tests for pleuropneumonia-like organisms in Reiter's disease. *Brit. J. vener. Dis.* **35**, 27.

Ruiter, M. & Wentholt, H. M. M. (1952). The occurrence of a pleuropneumonia-like organism in fuso-spirillary infections of the human genital mucosa. *J. invest. Derm.* **18**, 313.

Ruiter, M. & Wentholt, H. M. M. (1955). Isolation of a pleuropneumonia-like organism from a skin lesion associated with a fuso-spirochetal flora. *J. invest. Derm.* **24**, 31.

Stuart, P., Davidson, I., Slavin, G., Edgson, F. A. & Howell, D. (1963). Bovine mastitis caused by *Mycoplasma*. *Vet. Rec.* **75**, 59.

Taylor-Robinson, D., Somerson, N. L., Turner, H. C. & Chanock, R. M. (1963). Serological relationships among human Mycoplasmas as shown by complement-fixation and gel diffusion. *J. Bact.* **85**, 1261.

Tourtellotte, M. E. & Jacobs, R. E. (1960). Physiological and serologic comparisons of PPLO from various sources. *Ann. N.Y. Acad. Sci.* **79**, 521.

Van Herick, W. & Eaton, M. D. (1945). An unidentified pleuropneumonia-like organism isolated during passages in chick embryos. *J. Bact.* **50**, 47.

Villemot, J. M. & Provost, A. (1959). Recherches immunologiques sur la peripneumonie. V. Relations antigeniques entre *Mycoplasma mycoides* var. *mycoides*, *M. mycoides* var. *capri* et d'autres micro-organismes du genre *Mycoplasma* (souches génitales bovines et humaines). *Rev. Élev.* **12**, 251.

Warren, J. & Sabin, A. (1942). Some biologic and immunologic characteristics of a pleuropneumonia-like organism of human origin. *Proc. Soc. exp. Biol., N.Y.*, **51**, 24.

Yoshida, T. (1961). Antigenicity of cell components of antigenic variants of contagious bovine pleuropneumonia organisms in serological tests. *Nat. Inst. Ann. Health Quart.* (*Tokyo*), **1**, 199.