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Clyde W A, Jr. Mycoplasma species identification based upon growth inhibition by specific antisera. *J. Immunology* 92:958-65, 1964.
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Advantage was taken of the fact that mycoplasma growth is inhibited in the presence of species-specific antisera to perfect a new method of speciation. Variables involved were explored and the application to unknown isolates demonstrated. [The SCI® indicates that this paper has been cited in over 610 publications. It is among the ten most-cited papers for this journal.]

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"The identification of Eaton's atypical pneumonia agents as a mycoplasma in 1962[1] led to extensive studies on the role of this organism as a cause of human respiratory disease. Cultures of patients for *Mycoplasma pneumoniae*, as the new organism was named,[2] yielded a variety of other mycoplasma species that had not been identified previously. Due to their small genome size (5×10^8 Daltons), mycoplasmas are much more limited in biochemical characteristics than are other conventional bacteria, and speciation consequently depends upon serological testing. The principal method that had been available was the complement fixation technique, a laborious process plagued by variable degrees of cross reaction.

"Edward and Fitzgerald in 1954[3] had observed that mycoplasma growth was inhibited if homotypic antibody was incorporated into the agar medium. A modification of these studies was presented by Huijman-Evers and Ruys,[4] who placed filter paper discs saturated with antisera on agar plates inoculated by the push-block technique. Further use of these ideas was made to simplify and better control the process, as well as to evaluate it for applicability to mycoplasma species identification.

"Antisera were prepared to eight different mycoplasma species. These organisms in broth cultures were used to inoculate agar plates, and antiserum-impregnated filter paper discs were applied. After incubation for several days to allow growth of the test organisms, zones of inhibition were observed around the homotypic, but not the heterotypic, antisera. The same mycoplasmas and antisera were studied also by the conventional complement fixation method for comparison. The growth inhibition technique proved to be much simpler and more specific as a means of speciation. However, the procedure was relatively insensitive compared to complement fixation and had no role as a serologic test.

"The growth inhibition technique was quickly adopted by other workers in the field of mycoplasmology and has enjoyed extensive application subsequently. This led rapidly to the identification of a wide variety of mycoplasma species which were previously known only by strain abbreviations. A critique of the procedure together with technical variations proposed by others is the subject of a paper from the World Health Organization.[5] A recent description of the growth inhibition technique appears in *Methods in Mycoplasmology*.[6]

"The report in *Journal of Immunology* played a major role in establishing me in a niche in the field. Currently the number of related publications is around 80, but no others have received the same citation popularity. It is of retrospective interest that the definitive experiments on which the work was based required less than one month to accomplish.

"The description of the growth inhibition test for mycoplasma speciation formed the basis of a ten-year Career Development Award from the National Institutes of Health to evaluate the role of mycoplasmas in human diseases. In turn, this work led to editorial board appointments for the *Journal of Bacteriology*, *Infection and Immunity*, *Journal of Clinical Microbiology*, and *Pediatric Infectious Diseases*. Currently, I am chairman-elect of the International Organization for Mycoplasmology, and will become the chief presiding officer following the forthcoming congress to be held June 24-29, 1984, in Jerusalem, Israel."

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MYCOPLASMA SPECIES IDENTIFICATION BASED UPON GROWTH INHIBITION BY SPECIFIC ANTISERA[1]

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The role of mycoplasma in human respiratory tract disease has been established by the demonstration that Eaton's atypical pneumonia agent is a pleuropneumonia-like organism (PPLO) (1–3). Since other mycoplasma can be isolated from the mouth and throat in over half of normal subjects (4, 5), means of differentiating species is essential in assessing the role of various PPLO in disease states. Simple identification procedures would also facilitate study of tissue culture contamination by the mycoplasma.

Mycoplasma species have been classified according to their biologic properties and serologic reactions. With occasional exceptions (6), biologic properties are not unique and do not permit species identification (7). The complement fixation technique has been widely used to study species differences, but the method is laborious and cross-reactions may make uncertain the identity of unknown antigens. Edward and Fitzgerald (8) first described growth inhibition of the PPLO by antiserum, and the phenomenon has been studied more recently by Bailey *et al.* (9, 10). Huijmans-Evers and Ruys (11) have reported a method of inhibiting human genital mycoplasma on solid media by application of antiserum-saturated paper disks; these investigators demonstrated that the inhibition produced was species-specific for the strains studied.

This report describes a technique for identifying mycoplasma species by modification and extension of the method of Huijmans-Evers and Ruys. Eight strains of mycoplasma were studied, including one which has not previously been reported, and the procedure was found to be highly specific. In evaluation of the method, it was determined that the ability of antisera to inhibit mycoplasma was a stable phenomenon which was influenced by the relative proportions of serum and organisms. Use of the growth inhibition technique is demonstrated in the species identification of 155 PPLO strains.

[1] This investigation was conducted under sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported by the Office of The Surgeon General, Department of the Army.

MATERIALS AND METHODS

Mycoplasma species. Seed cultures of the following mycoplasma were obtained from the American Type Culture Collection: *M. laidlawii* A (14-089), *M. laidlawii* B (14-192), *M. hominis* type 1 (14-027) and *M. hominis* type 2 (14-152). Cultures of *M. salivarium* and *M. fermentans* were supplied by Dr. R. M. Chanock, and Eaton's agent (strain Mac) was received from Dr. C. Liu. The PPLO designated herein as strain Patt was first recovered in January, 1962, from pharyngeal cultures of a normal subject in Chapel Hill.

Preparation of antigens. The various organisms were propagated in a medium described by Chanock *et al.* (12), consisting of PPLO broth (Difco), 70%, unheated horse serum, 20%, freshly-prepared yeast extract, 10%, and penicillin, 1000 units/ml. The completed broth was centrifuged at 30,000 × G and passed through a 0.03 Selas candle to remove any particulate matter which could be sedimented with the antigen. Culture vessels consisted of 2-L Erlenmeyer flasks containing agar layers (50 ml) composed of the above medium plus 0.9% Bacto agar, over which was placed 1 L of the broth. After inoculation, flasks were maintained in a humidified 37°C incubator with an atmosphere of 5% CO_2 in air. The PPLO cultures were harvested after 4 days, except for Eaton's organism which was incubated 7 days. The organisms were sedimented at 30,000 × G, and were washed three times in 0.85% saline made with glass-distilled water. The final sediment was resuspended in sufficient saline to provide a 50- to 100-fold concentration of the starting culture. The resulting suspensions were

stored at −65°C for use in immunizing rabbits and as complement-fixing antigens.

Preparation of antisera. Albino rabbits weighing approximately 2 kg were immunized after the manner of Lemcke (13), two or more animals being used for each strain. Each animal received 1 ml of antigen intravenously at 2-day intervals for five injections, followed by 1 ml of antigen suspended in 1 ml of Freund's complete adjuvant intramuscularly. The animals were bled from the heart before immunization and again at 3 to 4 weeks after the last injection. The initial sera of two rabbits were found to inhibit growth of Eaton PPLO, and specimens from these animals were not employed subsequently. All sera were stored at −20°C until needed, and samples were heated at 56°C for 30 min before use in the complement fixation tests.

Complement-fixation technique. All complement fixation titrations were performed with Microtiter equipment (Cooke Engineering Co., Arlington) (14), using two exact units of complement and overnight fixation at 4°C before addition of the hemolytic system. Unitage of antigens and antisera was determined with "checkerboard" titrations. Titration end points were chosen as those serum dilutions producing at least 75% fixation of complement.

Growth inhibition technique. PPLO agar plates (10-cm) were placed open in a 37°C incubator for 1 hr to dehydrate the medium surface, after which each plate was inoculated with 0.1 ml of log-phase broth cultures of mycoplasma. Sterile 6-mm filter paper disks of the type used for antibiotic sensitivity testing were saturated with 0.025 ml of undiluted antisera, using a calibrated loop (Microtiter). Disks were then pressed onto the inoculated agar surface, and the plates were incubated until colonies became visible (1 to 4 days). The plates were examined with a low-power stereomicroscope, and zones of inhibition around disks were measured in millimeters from the disk edge to the beginning of growth. Normal rabbit serum-impregnated disks served as controls on each plate.

RESULTS

Specificity of mycoplasma inhibition with antisera

Eight mycoplasma strains and their homologous antisera were employed to determine the specificity of the growth inhibition technique. Filter paper disks saturated with the undiluted antisera were applied to plates freshly inoculated with broth cultures of *M. laidlawii* A and B, *M. hominis* types 1 and 2, *M. salivarium*, *M. fermentans*, Eaton's PPLO, and PPLO strain Patt. An example of the appearance of these plates after incubation is shown in Figure 1. Inhibition was recorded when no growth, or clearly suppressed growth, occurred beyond 0.5 mm of the disk edge. After the colonies matured, results were unaltered by additional incubation of the cultures. Each strain was inhibited by its homologous antiserum: zones measuring 3 to 7 mm in width were produced in the cultures of *M. hominis* types 1 and 2, *M. salivarium*, *M. fermentans*, Eaton's PPLO, and Patt PPLO. There was no inhibition of these strains by heterologous antisera, nor by normal rabbit serum. Growth of the two Laidlaw strains was suppressed by both their homologous and heterologous antisera, although the zones produced by the heterologous antisera were only half as wide as those around the homologous antiserum disks. It has been shown that the Laidlaw species are closely related biologically and antigenically (7).

The same antisera were titrated for complement-fixing ability against antigens prepared from the various mycoplasma species. Two units of the antigens were allowed to react with serial 2-fold dilutions of each antiserum. The serum-dilution end points obtained are indicated in Table I. Varying degrees of cross-reactivity among the antigens and antisera were evident, the most marked instances being the antisera against the Laidlaw and Eaton organisms with heterologous antigens. The magnitude of relationship among the strains can be more simply expressed through use of a formula applied by Archetti and Horsfall (15) and by Jordan and Gaylin (16) to the study of influenza virus strains:

$$\text{Per cent relationship} = \sqrt{r_1 \times r_2} \times 100,$$

$$\text{where } r_1 = \frac{\text{heterologous titer, antigen 2}}{\text{homologous titer, antigen 1}}$$

$$\text{and } r_2 = \frac{\text{heterologous titer, antigen 1}}{\text{homologous titer, antigen 2}}.$$

Single figures are derived which reflect the degree of interaction between two antigens and two antisera. By definition, a homologous antiserum-antigen reaction receives a rating of 100%. Results of application of this formula to the data in

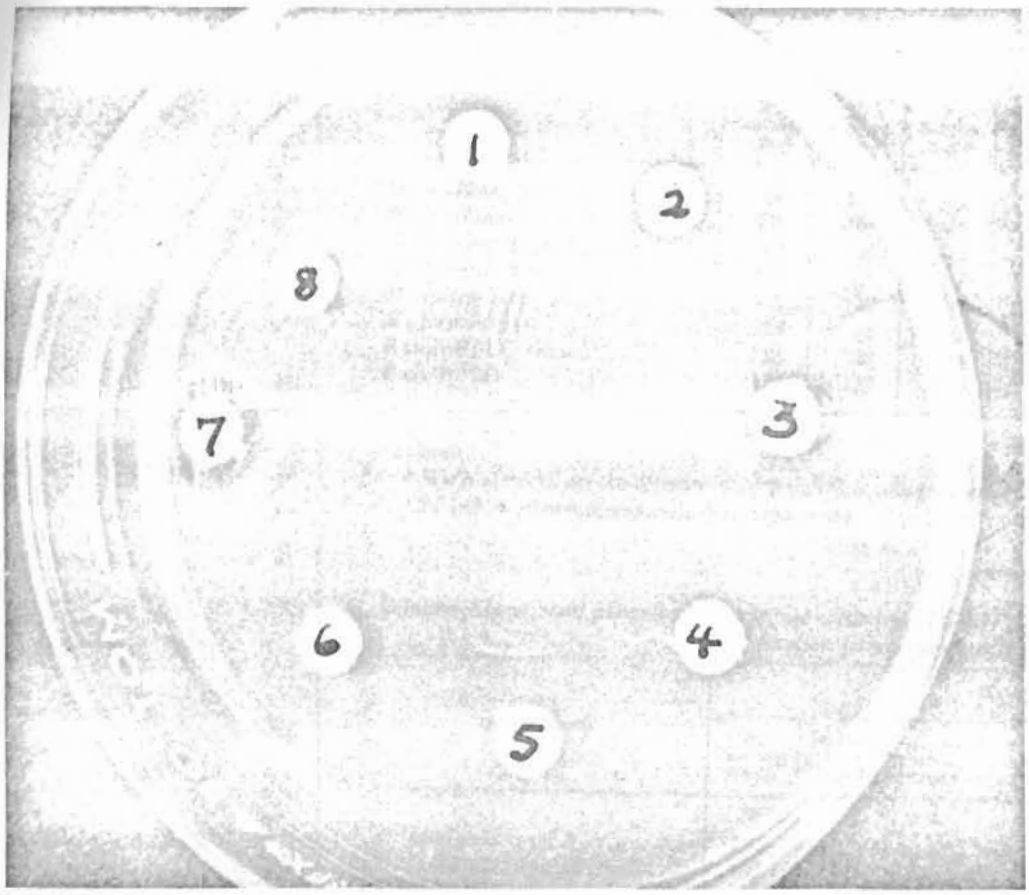

Figure 1. Culture of *Mycoplasma hominis* type 1, 48 hr after inoculation of the agar and application of antiserum-impregnated disks. Antiserum code: (*1*) *Mycoplasma laidlawii* A; (*2*) *Mycoplasma fermentans*; (*3*) Patt PPLO; (*4*) Eaton pleuropneumonia-like organism (PPLO); (*5*) *Mycoplasma salivarium*; (*6*) *Mycoplasma hominis* type 2; (*7*) *Mycoplasma hominis* type 1; (*8*) *Mycoplasma laidlawii* B. To the unaided eye growth of the PPLO appears as a finely granular haze covering the agar surface. A clear zone of growth inhibition surrounds the homologous disk, no. 7, whereas no inhibition is produced by the heterologous antiserum disks.

Table I are presented in Table II. Again it is evident that the Eaton and Laidlaw strains cross-reacted strongly. The remainder of the PPLO were less than 25% related, which was interpreted as evidence for antigenic distinction of these strains. The complement fixation method thus appeared less specific than the inhibition technique, and was more difficult to perform and to interpret.

Sensitivity of the growth inhibition technique

For evaluation of the sensitivity of the inhibition reaction, serial 2-fold dilutions of antisera against the Eaton and Patt PPLO strains were prepared, and disks saturated with each dilution were applied to agar plates freshly inoculated with the homologous PPLO. As indicated in Table III, the width of the inhibitory zones after incubation was directly proportional to the antiserum concentration. Serum dilutions corresponding to 64 complement fixation antibody units for the Eaton organism, and to 32 units for the Patt PPLO, produced inhibition in this experiment. The inhibition procedure as performed appeared to be less sensitive than the complement

TABLE I

Complement fixation titers of mycoplasma antisera against homologous and heterologous antigens

Antigen[a]	Antiserum Titers[b]							
	M. laidlawii type A	*M. laidlawii* type B	*M. hominis* type 1	*M. hominis* type 2	*M. salivarium*	*M. fermentans*	Eaton PPLO[c] (Mac)	Patt PPLO
M. laidlawii type A	*128*	128	32	32	64	<8	256	<8
M. laidlawii type B	256	*256*	32	16	32	<8	256	<8
M. hominis type 1	64	64	*512*	32	32	<8	128	<8
M. hominis type 2	128	128	256	*2048*	64	8	256	32
M. salivarium	128	128	32	32	*2048*	32	256	64
M. fermentans	128	128	32	32	64	*128*	256	<8
Eaton PPLO (Mac)	128	128	64	32	64	<8	*256*	<8
Patt PPLO	128	128	128	64	128	128	128	*2048*

[a] Two antigenic units employed, as determined by "checkerboard" titration with homologous antisera.

[b] Reciprocal of serum-dilution end point (homologous titers in italics).

[c] PPLO = pleuropneumonia-like organisms.

TABLE II

Relationship of eight pleuropneumonia-like organism (PPLO) strains derived from reciprocal complement fixation titrations of antisera

PPLO Strains	Degree of Relationship[b]							
	M. laidlawii type A	*M. laidlawii* type B	*M. hominis* type 1	*M. hominis* type 2	*M. salivarium*	*M. fermentans*	Eaton PPLO (Mac)	Patt PPLO
	%	%	%	%	%	%	%	%
M. laidlawii type A	*100*							
M. laidlawii type B	100	*100*						
M. hominis type 1	18	13	*100*					
M. hominis type 2	13	7	9	*100*				
M. salivarium	18	9	3	2	*100*			
M. fermentans	<25	<18	<7	3	9	*100*		
Eaton PPLO (Mac)	100	70	25	13	9	<25	*100*	
Patt PPLO	<7	<5	<3	2	5	<7	<5	*100*

[a] Calculated from data in Table I. See text.

fixation method for detection or quantitation of antibody.

Variables influencing mycoplasma growth inhibition on solid media

In their work with human genital PPLO, Huijmans-Evers and Ruys indicated that thickness of the agar medium in plates and antiserum storage conditions both influenced the magnitude of growth inhibition produced (11). A series of experiments was therefore designed to evaluate the effect of these and other variables upon inhibition of the eight PPLO strains studied in the present project.

Agar medium thickness. Petri dishes (10-cm) were prepared containing 5, 10, or 20 ml of the PPLO agar medium. Sets of the three types of plates were inoculated with each of the eight PPLO strains, and filter paper disks saturated with undiluted homologous antisera were applied to each plate. After colonies developed, measurement of inhibitory zones indicated inconsistent and minor variations in size which were not related to the amount of agar in the plates. This

TABLE III

Sensitivity of pleuropneumonia-like organism (PPLO) inhibition by antiserum disks

PPLO	Antiserum Dilution[a]							Complement Fixation Titer[b]
	1	2	4	8	16	32	64	
Eaton PPLO (Mac)	2.5	1.0	0	0	0	0	0	128
Patt PPLO	4.0	3.5	3.0	2.5	2.0	1.5	0	1024

[a] Reciprocals of serum dilutions used to impregnate disks. Amount of inhibition recorded in millimeters from edge of disks.

[b] Reciprocals of serum dilution end points determined by titrations with 1 unit of the homologous antigens.

result had not been anticipated, since serum diffusion from the disks would seem to control the size of the inhibitory zones. The medium employed contained only 0.9% agar, and freshly poured plates were used in all experiments. Since the medium was saturated with water, serum penetration may have proceeded too slowly in this instance to influence the zone of inhibition within the effective time period.

Conditions of antiserum storage. The antisera were studied in their ability to produce PPLO growth inhibition after various periods at −20°C, 4°C, and 56°C. The effect of repetitive freezing and thawing of sera stored at −20°C was also evaluated. PPLO agar plates were inoculated with broth cultures of the various strains as before, and disks were applied containing antisera exposed to the conditions listed. These experiments revealed that inhibition of the PPLO was produced by undiluted antisera stored up to 1 year at −20°C; this effect was not altered by additional storage up to 60 days at 4°C. Repeated freezing and thawing of the antisera to a total of 20 cycles also did not affect inhibition, nor did heating the antisera up to 30 min at 56°C. The capacity of the antisera to produce growth inhibition thus appeared to be a relatively stable property.

Culture age. Agar plates were inoculated with the various PPLO strains (excluding the rapidly-growing Laidlaw species), and homologous antiserum disks were applied after varying lengths of time up to 48 hr. The sizes of the inhibitory zones were identical when disks were applied at 0, 4 and 8 hr after plate inoculation. When added 24 hr after the inoculum, the disks produced inhibitory zones averaging 25% narrower than at 8 hr; and at 48 hr, zones 50 to 85% narrower were produced. In the present experiments, antiserum added after maturation of colonies did not cause their dissolution, as was reported by Edward and Fitzgerald (8).

Inoculum size. Bailey *et al.* (10) have demonstrated that in liquid media the degree of PPLO inhibition produced by antiserum depends upon the ratio between number of organisms and antiserum dilution. The effect of antiserum dilution upon a constant inoculum was demonstrated above in the experiments on sensitivity of the disk inhibition method. Corollary experiments

TABLE IV

Relationship between inoculum size and amount of inhibition produced by antiserum-impregnated disks

Pleuropneumonia-like Organism Strain	Colony-Forming Units[a]	Inhibitory Zone[b]
	log$_{10}$	*mm*
M. hominis type 1	4.46	4
	3.46	5.5
	2.46	7.5
	1.46	—[c]
M. fermentans	5.62	6
	4.62	7.5
	3.62	8
	2.62	—[c]
Patt PPLO	6.9	4.5
	5.9	6
	4.9	7.5
	3.9	8

[a] Number of colony-forming units placed on the surface of a 10-cm agar plate.

[b] Width of zone between antiserum disk edge and beginning of growth (averages from three experiments).

[c] Too few colonies for accurate interpretation.

were performed to examine the effect produced by inoculum variations with antiserum constant. Broth cultures of *M. hominis* type 1, Eaton PPLO, and Patt PPLO were diluted with fresh broth in 1-log increments to 10^{-6}. Agar plates were inoculated with 0.1 ml of each dilution of the organisms, and homologous antiserum-impregnated disks were applied to the plates. At the same time, the inoculum dilutions were prepared for colony counting (17). After the colonies matured, inhibitory zones were measured and correlation was made with the number of colony-forming units of PPLO which had been used to inoculate each plate. Results of these experiments are summarized in Table IV. The data indicate that the width of the inhibitory zones around antiserum disks could be related to the number of organisms employed.

The foregoing experiments suggested that although antiserum diffusion into solid medium is a theoretical problem in determining the degree of PPLO growth inhibition, in practice the more important factor was the ratio between the number of organisms present and the amount of antiserum.

Application of the growth inhibition technique in species identification of unknown mycoplasma

The growth inhibition technique was studied in relation to identification of 155 PPLO strains from diverse sources (Table V). Sixty-nine strains were recovered from normal individuals in Chapel Hill, by means of dry cotton swabs of the tonsillar pharynx plated immediately onto PPLO agar. Thirty-one additional strains were obtained in similar fashion from 29 patients having a variety of lower respiratory tract infections, and one strain was recovered from a rabbit kidney cell line. These strains had been adapted to growth in PPLO broth and were stored at −65°C until used. Four isolates from patients with pneumonia in Seattle and four tissue culture-contaminant PPLO were received from Dr. George Kenny. Four isolates of Eaton's PPLO from Marines with pneumonia at Parris Island were obtained from Dr. R. M. Chanock. Forty-one isolates were submitted by Dr. W. P. Glezen (Kansas City), representing 24 specimens from normal individuals and 17 from patients with respiratory tract disease. Agar plates were inoculated with broth subcultures of the various strains, and filter paper disks saturated with the eight PPLO anti-

TABLE V

Identification of 155 pleuropneumonia-like organism (PPLO) strains using disks impregnated with mycoplasma antisera

PPLO Isolates		Species			
Source	No.	*M. hominis* type 1	*M. salivarium*	Eaton PPLO	Patt PPLO
Normal pharynx					
Chapel Hill	69	1	10	0	58
Kansas City	24	0	5	0	19
Patients with pneumonia[a]					
Chapel Hill	29[b]	5	3	5	18
Seattle	4	1	0	3	0
Parris Island	4[c]	0	0	4	1
Kansas City	17	0	1	0	16
Tissue cultures					
Seattle	4	4	0	0	0
Chapel Hill	1	0	0	0	1
Totals	152	11	19	12	113

[a] Except for Eaton PPLO, relationship of isolates to disease is not established nor hereby implied.
[b] One isolate had Eaton and Patt PPLO; one isolate had Eaton PPLO and *M. salivarium*.
[c] One isolate had Eaton and Patt PPLO. These isolations were made initially in tissue culture.

sera described were applied to each plate. After incubation, examination of the cultures was made for evidence of growth inhibition.

The results of these tests are summarized in Table V. Each organism tested was specifically inhibited by a single antiserum. The majority of strains from the normal human pharynx were inhibited by the antiserum against Patt PPLO; isolates were also found which were inhibited by antisera against *M. hominis* type 1 and *M. salivarium*. From the patients with pneumonia, 12 isolates were identified as Eaton's PPLO, 6 as *M. hominis* type 1, 4 as *M. salivarium*, and 35 as Patt PPLO. Four of the tissue culture-contaminant PPLO were inhibited by the *M. hominis* type 1 antiserum, whereas one strain was identical to Patt PPLO. Strains of *M. laidlawii* A and B, *M. hominis* 2, and *M. fermentans* were not represented among the isolates.

DISCUSSION

The specificity of growth inhibition by antisera as a means of identifying PPLO species has not been reported previously, except on a limited scale in studies of human genital strains (9, 11). Serologic identification of PPLO has been accomplished with agglutination, precipitin, and complement fixation procedures, the latter enjoying the widest use. Application of reciprocal complement fixation tests to an unknown PPLO antigen can produce confusing results because of cross-reactions. These cross-reactions may be due to shared antigens, rather than to antibodies against medium components, as recently demonstrated by Taylor-Robinson *et al.* (18). The total absence of demonstrable cross-reactivity by the inhibition technique described suggests that this phenomenon may depend upon properties of the antisera independent of complement-fixing antibodies, although the two factors were related in degree (Table III).

Although it has not been established that the inhibitory substances are specific antibodies, two factors suggest that this is the case. The high degree of specificity in the inhibition experiments demonstrated that the immunized rabbits responded selectively to the eight prototype PPLO used. The stability of the inhibitory factors was also in keeping with the thesis that these substances were antibodies.

The inhibition technique provided rapid and precise identification of 155 strains of PPLO from diverse sources. Variables influencing the success of this procedure appeared to be related chiefly to the amount of antiserum employed and to the size of the PPLO inoculum. These data support the observations of Bailey *et al.* (10), who reported that the inhibition of mycoplasma in liquid media appeared to depend upon the establishment of an equilibrium between the number of organisms and amount of antiserum used. In contradistinction to the work of Huijmans-Evers and Ruys (11), factors related to antiserum diffusion through solid media appeared to play a relatively minor role in these experiments. The technique described was applicable to identification of saprophytic PPLO, organisms recoverable from the human genitalia and oral cavity, and the pathogenic respiratory tract PPLO first described by Eaton (19). The identification of four of five tissue culture-contaminant PPLO as *M. hominis* type 1 lends support to published studies indicating a high frequency of this human species in various tissue cultures (9). A simple species identification procedure, such as that described, may make more feasible studies on the epidemiology of tissue culture contamination with PPLO.

Seventy-three per cent of the strains isolated were inhibited by antiserum against the Patt prototype. Study of the various PPLO antigens and antisera by complement fixation first indicated the unique qualities of the Patt strain, and the growth inhibition studies gave further evidence that this PPLO represented a species not included among the currently classified mycoplasma of human origin (7). This strain is under further study to determine its possible relationship to PPLO recovered by other investigators which have not yet been classified (20). Distinction of the Patt PPLO is important, in that it was frequently recovered from the human pharynx using media suitable for isolation of Eaton's PPLO, and there is a close resemblance between these two strains in colony size and morphology. Eaton and Patt PPLO may be differentiated by biologic means, in that the Patt strain fails to ferment carbohydrates[2] and does not lyse mammalian erythrocytes (21). The frequency with which the Patt organism was isolated from people in both health and disease suggests that this strain may be a previously unrecognized com-

[2] Dextrose, dulcitol, galactose, glycogen, inositol, mannose, maltose, starch, sucrose (21).

ponent of the microbiologic flora of the human throat under normal circumstances.

Acknowledgment. The capable technical assistance of Mrs. Carmen M. Pospesel is gratefully acknowledged.

SUMMARY

A method of mycoplasma species identification is described, based upon inhibition of the PPLO growth on solid media around antiserum-impregnated filter paper disks. In comparison to the classical serologic identification method using complement fixation, the technique described was completely species-specific for identification of unknown antigens, but was less sensitive for detection of antibodies in sera. Examination of several variables influencing this reaction indicated that factors relating to the ratio between the number of organisms used and the amount of antiserum were more important determinants than were factors concerned with antiserum diffusion through the media. The ability of the antisera to produce growth inhibition by the means described was a stable property, being retained after long periods of storage at −20°C and 4°C, and after heating at 56°C.

Application of the inhibition technique permitted successful identification of 150 pleuropneumonia-like organisms (PPLO) isolated from the human pharynx in health and disease, and five strains recovered from tissue cultures. The method was simpler to perform and to interpret than species identification based upon the complement fixation technique.

In the course of these studies, a PPLO isolated frequently from the normal human pharynx was defined which was distinct from the classified mycoplasma species of human derivation by both the complement fixation and growth inhibition methods. This emphasizes the need for recognition of this and other ubiquitous *PPLO* species by investigators studying human respiratory tract disease.

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