RESEARCH INTO THE PRESENCE OF MYCOPLASMA IN SOME OBSTETRIC AND GYNAECOLOGICAL CONDITIONS

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SUMMARY

The Authors report their own experience and data from the literature on the presence of mycoplasma in some obstetric and gynaecological conditions.

Three fundamental stages can be singled out in the development of our knowledge of mycoplasma associated with human disease. They are respectively: *a*) the first isolation of a strain of mycoplasma, obtained in 1937 from an abscess of Bartholin's glands ([14]); *b*) the demonstration of the fact that the Eaton agent (*Mycoplasma pneumoniae*) responsible for primary atypical pneumonia, was not a virus but a mycoplasma ([11]); *c*) the discovery of the T strains of genital mycoplasma, now included in the species of *Ureaplasma urealyticum* ([47, 48]).

Although the first species of mycoplasma (*M. mycoides*) was discovered in 1898 ([43]) in a case of bovine pleuropneumonia, the interest taken by researchers in these micro-organisms may be considered recent history and the abbreviation PPLO (pleuropneumonia-like organisms), which was so successful in the 'forties', contains an implied reference to the above discovery.

By the term « mycoplasma », brought once more to light by Edward and Freundt in 1955, is meant nowadays a group of prokaryotes taxonomically included in the class *Mollicutes,* thus clearly separated from the classes of bacteria (*Schizomycetes*) and viruses (*Microtatobiotes*). It is worth mentioning that this classificatory formulation has reference to various decidedly peculiar biological parameters. These comprise: 1) values of the G+C content expressed as a percentage of the basic totals; 2) the extreme polymorphism, with cells whose diameters vary from 120 nm in the minimum (filtrable) reproductive units, and those in the spherical forms (10 μm); 3) the typical absence of walls, which is directly responsible for the preceding characteristic; 4) the morphology of the colonies, generally of very reduced dimensions and observable only under the microscope.

A further property of mycoplasma, and perhaps the most surprising one, is the

fact, unique in the world of the prokaryotes, that sterols are present as fundamental components of the cell membrane. The present subdivision of the class *Mollicutes* comprises the one order *Mycoplasmatales* and the two families *Mycoplasmataceae* and *Acholeplasmataceae*, composed respectively of the genera *Mycoplasma* and *Acholeplasma*. More than forty species belong to the first of these, among cies belong to the first of these, among which may be mentioned *M. pneumoniae* and *M. hominis* (with 8 serotypes), while the second is made up of 5 species. Finally there are other genera defined as of « incertae sedis », among which, and directly concerned with the present report; is the genus *Ureaplasma*, comprising the sole species *Ureaplasma urealyticum* (with 8 serotypes). If the certainty of the mycoplasmic aetiology of primary atypical pneumonia is excepted, not a few enquiries need to be made into the part played by the mycoplasmas in giving rise to other human diseases. However, it is certainly of interest that these conditions affect the genitourinary apparatus almost exsclusively and essentially revolve around the species *M. hominis* and *U. urealyticum*.

The relevance of *M. hominis* has been discussed in connexion with the aetiology of non-specific urethritis ([16]), salpingitis ([33]), ovarian abscesses ([21]), post-partum fever ([23, 39]), premature labour ([49]), puerperal sepsis ([52]) and septic abortion ([24]).

U. urealyticum has been thought to be correlated with spontaneous abortion ([15]), tubo-ovarian abscesses ([4]), reduced weight of neonates ([6]), puerperal sepsis ([50]), chorioamnionitis ([9]), male and female infertility ([1, 13, 17, 18, 19, 20, 26, 29, 32, 38]) and non-gonococcal urethritis (NGU) ([12, 46, 51, 53, 54]). The latter disease, one of the 14 diseases transmissible by sexual intercourse, has been defined by Shepard as the sixth venereal disease ([25]).

In the present investigations we set ourselves the task of studying, chiefly with epidemiology in mind, the frequency with which mycoplasmas occur in the urogenital tract, both in good health and in the course of certain genital conditions.

MATERIAL AND METHODS

The trials were conducted on 299 patients admitted to the Obstetric and Gynaecological Clinic of the University of Padua or attending its out-patient department, including the Conjugal Sterility Centre. The cases studied were classified in the following categories: *a*) fertile patients with no objective signs of vaginal infection (leucorrhoea, ardor urinae, dysuria, dyspareunia, oedema of the ostium vaginae, etc); *b*) patients with subjective and objective signs of vaginitis; *c*) pregnant women with a negative history due to abortion; *d*) pregnant women with repeated abortion; *e*) infertile males; *f*) infertile females. No chemotherapy or antibiotic therapy had been given to any of the subjects examined at the time when the samples were obtained.

In the *males,* the investigations into mycoplasma were undertaken on seminal fluid obtained by masturbation and collected in sterile containers; a complete semenological examination was also performed on the same sample in parallel. In the *women,* the samples were obtained by means of a sterile vaginal speculum, after visualization of the portio cervicis, from the posterior vaginal fornix, using two swabs utilized respectively for the culture tests and for preparing the specimens for microscopic examination.

The method adopted for isolating and identifying the mycoplasmas, whether from the vaginal exudates or from the seminal fluid, have been described in previous papers ([8, 41]).

Direct microscopic examination. - The specimens prepared from the vaginal exudate and the seminal fluid were stained by the Giemsa's method, and examined in order to study the type and characteristics of the cells present.

Investigation of anti-M. hominis and anti-U. urealyticum antibodies. - A sample of serum was obtained from all the patients with a clinical diagnosis of vaginitis, and was preserved at —25 °C until required for investigation of the anti-mycoplasmic antibodies. This was done by the methods of metabolic inhibition (C-independent) ([56]) and of mycoplasmacidal test (C-dependent), utilizing the same strains isolated from the patient who donated the serum ([34, 35, 36]).

RESULTS

The presence of mycoplasma was demonstrated in 129 (43.1 %) of the 299 samples examined, with a percentage of 42.8 % for *U. urealitycum* and 9.4 % for M. hominis; if it is considered that the association between the two species was observed in 9 %, it will be obvious how very low was the frequency of isolation of *M. hominis* alone (0.4 %).

From an examination of the data obtained, distributed according to the groups previously described (table 1), it substantially appears that:

1) the percentages of isolation of *M. hominis* and *U. urealyticum* were no higher in the sick patients than in the healthy ones. In the clinically healthy women, the pregnant (group *c*) and non-pregnant women (group *a*), these micro-organisms were observed with an only slightly higher frequency as compared to the other groups studied. This difference was more marked especially as regards *M. hominis* and when the two mycoplasmic species were associated.

2) In the 52 patients with an obvious picture of vaginal infection (group *b*), *M. hominis* was demonstrated with a markedly lower frequency as compared to that of the control (group *a*) (3.8 % as compared to 15.4 %).

3) The percentage of positive results among the pregnant women (group *c*) was the highest in absolute figures. In particular, the incidence of *U. urealyticum* was higher (though not showing statistical significance) than in the control group. In this connexion it should be mentioned that these values, when compared with those reported in other investigations (table 2), were higher, and lower only than those obtained by Braun *et al.* ([5]).

4) As regards the 29 pregnant women with a history of at least three repeated abortions, the percentages recorded reached lower values; in aggregate the positive samples amounted to 34.5% against 53.8 % in the controls.

5) In research into the relations between mycoplasma and conjugal infertility, 81 samples of seminal fluid were examined (group *e*) and 38 of vaginal exudate (group *f*), and mycoplasma was observed to be present respectively in 19.75 % and 57.9 % of these. *U. urealyticum* was encountered in 19.75 % of the infertile males, and this value reached 24.2 % if only those patients were considered whose infertility could not be attributed to evident organic or functional causes. It is worth mentioning that, quite often, the colonies of *Ureaplasma* developed from the spermatozoa (photograph 1).

From a general point of view it must be

Table 1.

Groups	N. of samples examined	Mycoplasma po.s. n.	Mycoplasma po.s. %	M. hominis n.	M. hominis %	U. urealyticum n.	U. urealyticum %	M. hom. + U. ureal n.	M. hom. + U. ureal %	Total M. hominis n.	Total M. hominis %	Total U. ureal. n.	Total U. ureal. %
a	52	28	53.8	1	1.9	20	38.5	7	13.5	8	15.4	27	51.9
b	52	24	46.15	0	0	22	42.3	2	3.8	2	3.8	24	46.15
c	47	29	61.7	0	0	23	48.9	6	12.8	6	12.8	29	61.7
d	29	10	34.5	0	0	8	27.6	2	6.9	2	6.9	10	34.5
e	81	16	19.75	0	0	9	11.1	7	8.6	7	8.6	16	19.75
f	38	22	57.9	0	0	19	50	3	7.9	3	7.9	22	57.9
Total	299	129	43.1	1	0.4	101	33.8	27	9	28	9.4	128	42.8

a = control group; *b* = patients with vaginal infections; *c* = pregnant women; *d* = pregnant women with history of repeated abortion; *e* = infertile males; *f* = infertile females.

Table 2. — *Frequency of isolation of mycoplasmas of vagina in healthy pregnant women.*

	U. urealyticum	%	M. hominis	%
Kundsin & Driscol, 1970 [29]	9/40	23	3/40	8
Mardh & Westrom, 1970 [37]	22/50	44	2/50	4
Braun et al. 1970 [5]	403/568	71	22/568	39
Gnarpe & Friberg, 1972 [18]	9/40	23	3/40	8
Romano et al., 1976 [44]	70/143	49	8/143	5.5
our results	29/47	62	6/47	12.86

said that the fact, already mentioned, that *M. hominis* was demonstrated only in one case in this group, shifted the evaluation towards *U. urealyticum,* and, in the second place, towards the association between both species.

If the findings related to the pregnant women are excepted, since these in any case related to the special vaginal situation applicable to pregnancy, [2], the highest colonization by mycoplasma was observed in the infertile females (group *f*), and most of these findings applied to the isolation of *U. urealyticum* alone. It should also be mentioned that only in 2 out of the 13 infertile couples studied were mycoplasmas observed in both partners.

As a supplement to the above data, it is worth emphasizing that the colonies of *Ureaplasma* often originate from the epithelial cells present in the inoculum (photograph 2). This phenomenon is directly connected with the fact that the mycoplasmas are most often adherent to the plasma membrane of the epithelial cells and, though perhaps only rarely, directly localized in side them [27]. The colonies of *M. hominis,* whose dimensions are markedly greater than those af *Ureaplasma,* develop by covering the epithelial cell entirely, or its margins, or they may occur in an isolated manner (photograph 3).

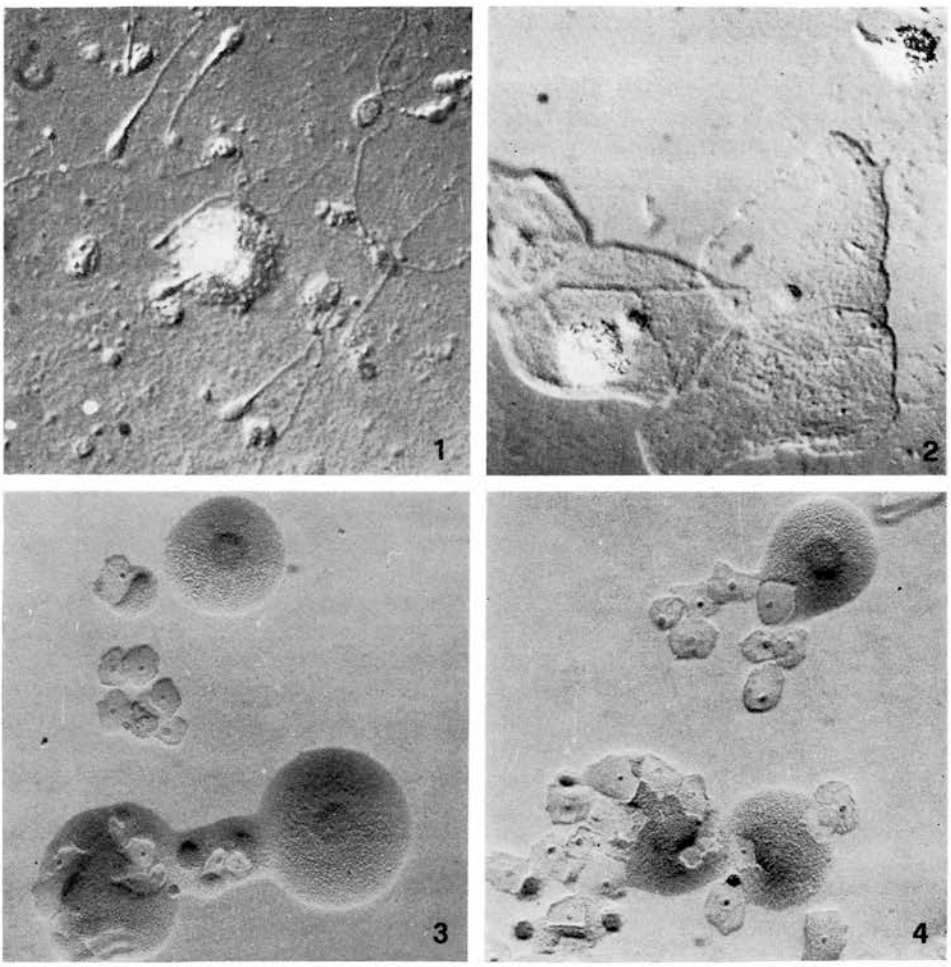
No data of interest, however, emerged from the microscopical examination of the vaginal exudates stained by the Giemsa's method, not even as regards the preparations obtained from the mycoplasmapositive patients. In other words no alterations were found in the type of those described in the cells cultured *in vitro* and contaminated by mycoplasma [27].

It was confirmed by cytological study that there was an association between the presence of the two mycoplasmic species and the isolation of *Haemophilus vaginalis;* in about 80 % of the *M. hominis* + *U. urealyticus*-positive patients it was possible in fact to demonstrate the presence of the « clue cells », characteristic of the vaginal infections sustained by this bacterium.

DISCUSSION

Given that the results stated above constantly indicate a higher percentage of isolation of *U. urealyticum* as compared with that of *M. hominis* (though this was demonstrated almost always in association with the former), no comparative interpretation of the data obtained can be made, unless by direct reference to the groups into which the subjects examined were divided. This also shows that in practice such an interpretation should chiefly be centred upon the problems relating to *U. urealyticum.*

The highest percentage of isolations from « normal » control subjects might in the first instance provide evidence of lack of correlation between the mycoplasmas and the disease. But this conclusion cannot be absolute, since it can neither be



Colonies of mycoplasmas ob served by phase-co ntrast microscopy.

Photo n. 1. — (900 ×) Colony of *U. urealyticum* in sperm culture.
Photo n. 2. — (600 ×) Colonies of *U. urealyticum* in vaginal exudate culture.
Photo nos. 3 & 4. — (165 ×) Colonies of *M. hominis* in vaginal exudate culture.

excluded nor is it improbable that in the gruop of « normal » subjects individuals might in fact be included who were affected by asymptomatic conditions, or at least conditions that could not be demonstrated subjectively or clinically. The fact that we have not observed any significant difference between the isolations relating to the various groups and those relating to the control group still seems important and supports the hypothesis according to which mycoplasmas are commensal microorganisms of the urogenital tract ([47]). As regards this condition, their demonstrability might in any case be influenced by various host-related situations such as the physiological state ([2, 30]), sexual activity ([40]), etc.

The data provided by some authors are in contrast to the above interpretation: they have recorded a higher percentage of isolations in subjects affected by vaginitis, as compared with controls, and these would justify the definition of « low virulence parasites » as applied to the mycoplasmas ([42]). The trials we carried out on 20 samples of serum for the investigation of anti-mycoplasma antibodies, using strains isolated from the same serum donor in reactions of metabolic inhibition and mycoplasmocidal test in the presence of C., have constantly given a negative result. It may perhaps not be excluded that, given the type of infection considered, the immune response might be prevalently represented by the production of secretory IgA, analagously to what has been described for infections of myxovirus and rhinovirus and of *Mycoplasma pneumoniae* ([3]). As has already been stressed, some authors ([7, 10, 15, 17, 28, 45]) claim that the presence of *M. hominis* and of *U. urealyticum* may have marked repercussions upon gestation, and it is precisely because of such observations that we have been led to examine the group composed of pregnant women with a history of repeated abortion. The lowest percentage of isolations both of *M. hominis* and *U. urealyticum* recorded by us in subjects of this group, as compared with normal pregnant women, is not in accordance with the above hypotheses. It should also be added that all the mycoplasma-positive pregnant women belonging either to this group (*d*) or to group (*c*) brought their pregnancies normally to term between the 38th and 40th weeks, and except in one case they gave birth to neonates whose weights ere in perfect agreement with the parameters of the gestrogram of Dunn and Butler, modified by Grella and Fais ([22]). The exceptional case was a pregnant woman of group (*d*) who was ureaplasma-positive and gave birth to a child weighing less than 2500 grams.

The data presented by some authors, who support the importance of mycoplasma as the cause of deteriorated pregnancy or foetal anomalies remain, however, extremely suggestive. Among the observations worthy of special mention are those that have demonstrated that treatment with tetracycline is means as efficacious in bringing pregnancy to term in mycoplasma-positive patients and with histories of spontaneous abortion ([15]).

As already emphasized, the highest percentage of isolations of mycoplasma was recorded by us in group (*c*), composed of pregnant women; and this observation supports the theory that the raising of the vaginal pH, recorded during the course of pregnancy, might encourage colonization of *U. urealyticum,* whose optimum pH during development is about 6.2.

The problem of the relation between mycoplasma and infertility has recently been widely discussed, and this perhaps is the field of investigation towards which the efforts of investigators are at present most directed. *U. urealyticum* has been isolated more often from infertile than from fertile couples; but this difference has not been found, as regards *M. hominis* ([13, 18]). To support the view that mycoplasmas help to give rise to infertility, and a high incidence of infertility has also

been demonstrated in monkeys artificially infected with *U. urealyticum* ([31]). Support for this interpretative trend is also given by the observations of some authors who have reported conception by 30 % of infertile and ureaplasma-positive couples as a result of antibiotic therapy ([17]). In this connexion it must be remembered that it is essential to administer antibiotics to both partners and not to only one of them, with the object of interrupting the « ping pong cycle » of treatment reinfection.

Apart from epidemiological findings that support the hypothesis of a possible relation between mycoplasma and infertility, Gnarpe and Friberg ([20]) have communicated an observation, made on the scanning electron microscope, of ureaplasma adherent to the intermediate piece of the human spermatozoon. Recently ([8]) we have demonstrated by immunofluorescence that *M. hominis* too, whose role in the occurrence of infertility has always been under-estimated, is able to attack human spermatozoa. The union of the mycoplasmas to the spermatozoa has led to hypotheses of interference either with the mobility of the spermatozoon ([1]) or with the fusion of the spermatozoon with the ovum ([51]). The morphology of the spermatozoa seems also to be modified under these conditions. To return to the incidence of isolation of mycoplasmas from infertile couples, significant differences do not exist, according to Taylor-Robinson *et al.* ([55]) and De Louvois *et al* ([13]), even though treatment with doxycycline has, according to Gnarpe and Friberg, resulted in conception in a significantly high number of patients treated. Our results refer to a representative sample of 81 male patients of infertile couples; the percentage of isolation of *U. urealyticum* (19.75 %) is markedly less than that reported in other studies, while that relating *M. hominis* is practically identical with that reported by De Louvois *et al.* ([13]).

If we do not consider the cases of azoospermia, oligospermia and astheno-teratospermia, and assess only subjects with idiopathic infertility, the percentage of isolation of mycoplasma is increased up to 24 %.

Since the fact emerges from the considerations already advanced that the results we obtained are not in accordance with the theory of correlation between the presence of mycoplasma and infertility, vaginitis, and spontaneous abortion, they cannot be considered conclusive.

It cannot, in fact, be forgotten that the data supporting this theory refer to research in which the mycoplasms were isolated from the uterine tube, the foetal membranes and the amniotic fluid; the experiments being formulated, that is, in a way that we were not able to do, and which in the last analysis appears to be more valid from a methodological point of view.

This reasoning not only justifies our proposal to continue the investigations on this topic, examining a larger number of cases, but most of all leads us to confirm what we have already stated in a previous paper ([41]) in connexion with the position of the mycoplasma in vaginal ecology. In our opinion these microorganisms should, in fact, be considered as resident opportunists of the vaginal niche, just as occurs in the case of other species of prokaryotes.

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BIBLIOGRAPHY

1) Alexandre C., Siboulet A., Catalan F., Deubel V.: *Rev. franç. Gynéc., 71,* 539, 1976.
2) Archer J. F.: *Brit. J. Vener. Dis., 44,* 232, 1968.
3) Biberfeld G., Sterner G.: *Acta pathol. scand. Section B, 79,* 599, 1971.
4) Braun P., Besdine R.: *Am. J. Obstet. Gynecol., 117.* 861, 1973.
5) Braun P., Kein J. O., Lee Y-H.: *J. Infect. Dis., 121,* 391, 1970.
6) Braun P., Lee Y-H., Klein J. O.: *N. Engl. J. Med., 284,* 167, 1971.

7) Brunell P. A., Dische R. M., Walker M. B.: *J.A.M.A., 207,* 2097, 1969.
8) Busolo F., Conventi L., Bertoloni G., Meloni G. A.: *Cl. Exp. Obstet. Gynec., 3,* 89, 1976.
9) Caspi E., Herczeg E., Solomon F.: *Am. J. Obstet. Gynecol., 111,* 1102, 1971.
10) Caspi E., Solomon F., Sompolinsky D.: *Isr. J. Med. Sci., 8,* 122, 1972.
11) Chanock R. M., Hayflick L., Barile M. F.: *Proc. Nat. Acad. Sci., 48,* 41, 1962.
12) Csonka G. W., Reo W., Corse J.: *Lancet, 1,* 1292, 1966.
13) De Lavois J., Blades M., Harrison R. F., Hurley R., Stanley V. V.: *Lancet, 1,* 1073, 1974.
14) Dienes L., Edsall G.: *Proc. Soc. Exp. Biol. Med., 36,* 740, 1937.
15) Driscol S. G., Kundsin R. B., Horne H. W., Scott J. M.: *Fert. Steril., 20,* 1017, 1969.
16) Ford D. K.: *Ann. N. Y. Acad. Sci., 143,* 501, 1967.
17) Gnarpe H., Friberg J.: *Nature* (Lond.), *242,* 120, 1973.
18) Gnarpe H., Friberg J.: *Am. J. Obstet, Gynecol., 114,* 727, 1972.
19) Gnarpe H., Friberg J.: *Am. J. Obst. Gynecol., 114,* 963, 1972.
20) Gnarpe H., Friberg J.: *Nature, 245,* 97, 1973.
21) Gotthardson A., Melen B.: *Acta Pathol. Microbiol. Scand., 33,* 291, 1953.
22) Grella P., Fais G.: *Cl. Exp. Obst. Gynecol., 2,* 58, 1975.
23) Harwick H. J., Purcell R. H., Iuppa J. B.: *Obst. Gynecol., 37,* 765, 1971.
24) Harwick H. J., Purcell R. H., Iuppa J. B.: *J. Infect. Dis., 121,* 260, 1970.
25) Horne H. W., Kundsin R. B., Kosasa T. S.: *Fertil. Steril. 25,* 380, 1974.
26) Horne H. W., Kundsin R. B.: *Proc. Soc. Gen. Microbiol., 3,* 145, 1976.
27) Kenny G. E.: *Contamination in tissue culture, Academic Press,* 107, 1973.
28) Klein J. J. O., Buckland D., Finland M.: *N. Engl. J. Med., 280,* 1025, 1969.
29) Kundsin R. B., Driscol S. G.: *Surg. Gynecol. Obstet. 131,* 89, 1970.
30) Kundsin R. B., Parreno A., Kirsch A.: *Brit. J. Vener. Dis., 49,* 381, 1973.
31) Kundsin R. B., Rowell T., Shepard M. C., Parreno A., Lunceford C. D.: *Lab. Animal Science, 25,* 221, 1975.
32) Kundsin R. B., Driscol S. G., Ming P. L.: *Science, 157,* 1573, 1967.
33) Lemcke R., Csonka G. W.: *Br. J. Vener. Dis., 38,* 212, 1962.
34) Lin J-S., Kass E. H.: *Inf. Immunity,* 10, 535, 1974.
35) Lin J-S., Alpert S., Radnay K. M.: *J. Infect. Dis., 131,* 727, 1975.
36) Lin J-S., Kendrick M. I., Kass E. H.: *J. Infect. Dis., 126,* 658, 1972.
37) Mardh P. A., Weström L.: *Acta Pathol. Microbiol. Scand., 78B,* 367, 1970.
38) Matthews C. D., Elmslie R. G., Clapp K. H., Svigos J. M.: *Fertil. Steril, 26,* 988, 1975.
39) McCormack W. M., Lee Y-H., Lin J-S., Rankin J. S.: *J. Infect. Dis., 127,* 193, 1973.
40) Mc Cormack W. M., Almeida P. C., Bailey P. E.: *J.A.M.A., 221,* 1375, 1972.
41) Meloni G. A., Busolo F., Conventi L., Bertoloni G.: *Boll. Ist. Sieroter. Milanese, 56,* 1, 1977.
42) Mendel E. B., Faco G., Rowan D. F., Graham J. H. M., Dellinger D.: *Obst. Gynecol. 35,* 104, 1970.
43) Nocard E., Roux E. R.: *Ann. Inst. Pasteur* (Paris), *12,* 240, 1898.
44) Romano R., Scarlata G., Cadili G., Carollo F.: *Boll. Ist. Sieroter. Milanese, 55,* 568, 1976.
45) Schneider E. L., Standbridge E. J., Epstein C. J., Golbus M., Roddgers G.: *Science, 84,* 477, 1974.
46) Sepetjian M., Thivolet J., Monier J. C., Salussola D.: *Path. Biol., 21,* 949, 1973.
47) Shepard M. C.: *Am. J. Syph. Gon. V. D., 38,* 113, 1954.
48) Shepard M. C.: Lanceford C. D., Ford D. K., Purcell R. H., Taylor-Robinson D., Razin S., Black F. T.: *Int. J. Syst. Bact., 24,* 160, 1974.
49) Shurin P. A., Alpert S., Rosner B.: *N. Eng. J. Med., 293,* 5, 1975.
50) Sompolinsky D., Solomon F., Leiba H.: *Isr. J. Med. Sci., 7,* 745, 1971.
51) Stanbridge E. J.: *Ann. Rev. Microbiol., 30,* 169, 1976.
52) Stokes E. J.: *Lancet, 1,* 276, 1955.
53) Sueltmann S., Allen V., Inhorn S. L., Benforado J. M.: *Health Lab. Sci., 8,* 61, 1971.
54) Taylor-Robinson D., Csonka G. W., Prentice M. J.: *Quant. J. Med., 183,* 309, 1977.
55) Taylor-Robinson D., Rassner C., Furr P. M., Humber D. P., Barnes R. D.: *J. Reprod. Fertil., 42,* 483, 1975.
56) Woode G. N., Mc Martin D. A.: *J. gen. Microbiol., 75,* 43, 1973.