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The distribution of complement-fixing antibody and growth-inhibiting antibody to *Mycoplasma hominis*

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Mycoplasma hominis is a common inhabitant of the human genital tract. This organism can be isolated frequently from patients with genital infections and from those with no evidence of such infection (Harkness, 1950; Randall, Stein & Ayres, 1950). It is likely that *M. hominis* is a commensal and in general may be isolated more frequently from women than men (Nicol & Edward, 1953). The organism may also be a potential pathogen and the cause of genital and puerperal infections accompanied by the appearance of complement-fixing antibody in the serum of the patient (Stokes, 1959; Lemcke & Csonka, 1962). Mufson *et al.* (1965) have shown that *M. hominis* is capable of producing exudative pharyngitis in experimentally infected volunteers.

The growth of mycoplasms can be inhibited by specific antibody and Taylor-Robinson, Purcell, Wong & Chanock (1966) have developed a sensitive metabolic inhibition test which allows the measurement of growth-inhibiting antibody in human sera. An investigation was made into the occurrence and distribution of growth-inhibiting antibody to *M. hominis*, compared with the distribution of complement-fixing antibody in the same sera.

MATERIALS AND METHODS

Sera

The material used for this study was the sera received with 3200 consecutive requests sent to the Central Serology Laboratory, Manchester, between 1 and 9 February 1966.

Procedure

The sera were inactivated at 56° C. for 30 min. Using a Multiple Pipetting and Diluting Machine,* a 1/5 dilution of each serum was prepared and 0·1 ml. volumes of each dilution distributed in two series of MRC plastic trays. One series of trays was used for the screen complement-fixation test and the other series for the screen growth-inhibition test. The sera were stored at −20° C. until testing was completed. Trays containing doubling dilutions for the titration of sera giving positive results at 1/5 dilution were prepared in a similar manner.

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Media

The basic medium for growing *M. hominis* consisted of 7 parts Difco PPLO broth, 2 parts unheated human serum, 1 part boiled blood extract, 1/2000 thallium acetate and 1000 units penicillin/ml. For the inhibition tests the basic medium was used but with added arginine (1 %), phenol red (0·002 %), and fresh unheated guinea-pig serum (7 %), with the final pH adjusted to 7·0.

Growth-inhibition test

The test was performed in the manner of Taylor-Robinson, Purcell, Wong & Chanock (1966) but modified by replacing the glucose in the medium with arginine (Taylor-Robinson, Purcell & Chanock, 1966). The metabolism of arginine by the mycoplasms produces an increase in the pH of the medium resulting in a colour change. For convenience the test was performed in MRC plastic trays using 0·1 ml. as a unit volume. Preliminary chessboard titrations with a rabbit antiserum and different doses of organisms, prepared from 48 hr. broth cultures of *M. hominis*, were performed. The challenge dose of organisms chosen for the single row test was 10^5 colony-forming units per ml.; this allowed the results to be read after 30 hr. incubation at 34° C. The test in the final form consisted of adding 0·3 ml. of a mixture of basic medium, indicator, arginine, guinea-pig serum and organisms to 0·1 ml. of a dilution of patient's serum. All sera were tested at 1/5 dilution; any sera showing inhibition of colour change were later titrated. The tests were read when the end-point of the positive control serum was readable at its known titre (1/6000) and in this way the sensitivity of each batch of tests was kept constant. On further incubation the organisms tended to 'grow through' in the presence of small amounts of antibody. During incubation the plastic plates were covered with celluloid covers to avoid evaporation losses. The colour change was read against a white background with overhead fluorescent illumination.

Complement-fixation test

The complement-fixing antigen and immune rabbit serum were prepared by the method of Card (1959). Veronal buffer was used for the preparation of dilutions and reagents. The haemolytic system contained $2\frac{1}{2}$ % sheep red cells sensitized with 5 M.H.D. of rabbit anti-sheep haemolysin.

Preliminary experiments confirmed that the test was most sensitive when incubated overnight at 4° C. The optimal working dilution of the antigen was estimated by a chessboard titration against immune rabbit serum and using minimum haemolytic doses of complement estimated by incubating the antigen and complement dilutions with negative serum under the normal test conditions of time and temperature. The complement dose for the test proper was estimated in the same way. A single pool of guinea-pig serum preserved by Richardson's method was used throughout the experiment at a constant dilution. All reagents were added to the prepared trays of dilutions with a Donaldson's dropper delivering 0·1 ml. volumes. The trays were shaken 15 min. after addition of sensitized cells and the test read after a further 45 min. at 37° C.

Analysis of results

The routine reporting at the Central Serology Laboratory utilizes a punch-card installation.* Analysis of cards produced during the period of the study permitted the analysis of the patients by age, sex and type of department attended. This information, together with the results of the two tests, was punched on a further card for each patient giving a positive reaction with one or both tests. These cards were then sorted into the required groups and totalled or reproduced as required.

RESULTS

Homologous rabbit serum

In a preliminary experiment various dilutions of *M. hominis* suspension were titrated against the homologous rabbit serum by the growth-inhibition technique. The highest dilution of serum that inhibited the colour change was 1/6000. The complement-fixation titre of this serum, with the batch of antigen used for this investigation, was also 1/6000. Serum was again collected from the rabbit 4 months after the post-immunization sample and re-examined. The complement-fixation titre had fallen to 1/400 whereas no growth-inhibiting property was detectable.

Human sera

A total of 3163 sera were tested by both techniques: in 13·5 % the complement-fixation test was positive and in 4·2 % the growth-inhibition test was positive. Six sera were found with a positive growth-inhibition test and a negative complement-fixation test. When these were divided on the basis of sex, 2265 sera were from female patients and 16·2 % contained complement-fixing antibody and 4·7 % growth-inhibiting antibody, while of sera from 560 male patients, 10 % were positive in the complement-fixation test and 4·6 % positive by the growth-inhibition technique. The results obtained with sera from each sex were then analysed separately in respect of age and titre of antibody and divided into various patient groups. In the remaining 338 sera, the sex or origin of the patient was not known and these results were not analysed further.

Female patients

The percentage distribution of complement-fixing antibody is shown in Table 1*a*. In the 0 to 5 year age group, antibody was present in one baby under 3 months old and was probably of maternal origin. One patient (who was pregnant) under the age of 15 also had antibody. With these exceptions the incidence of antibody increases through the sexually active years, the increase continues during the decade after the menopause and then the incidence declines. Assuming that the higher titres of 1/40–1/80 are likely to indicate more recent or severe infection there is the same increase in incidence of these titres up to the age of 55.

The percentage distribution of growth-inhibiting antibody is shown in Table 1*b*. Again there is a steady increase in incidence with age but with a sharper increase

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Table 1*a*. *Complement-fixing antibody for* Mycoplasma hominis *type I in* 2265 *female patients*

Age (years)	Complement-fixation titre 80	40	20	10	5	Tr.	(+)	Total per-centage	No. exam-ined
> 65	.	.	.	5·3*	.	.	.	5·3	57
55–65	.	.	6·2	4·1	.	2·0	.	12·4	48
45–55	2·4	9·5	2·4	4·8	.	4·8	2·4	26·2	42
35–45	3·3	3·9	6·1	4·4	2·8	1·1	0·5	22·1	181
25–35	1·8	3·2	4·0	4·3	0·5	1·3	.	15·2	618
15–25	1·9	3·4	3·3	2·1	1·5	1·7	0·6	14·4	953
5–15	.	.	.	10	.	.	.	10	10
0–5	.	.	.	.	.	.	2·7	2·7	37
Unspecified	1·9	3·1	6·3	4·7	2·2	0·9	0·3	19·4	319

* Percentage positive.
(+), Positive result at 1/5 dilution but insufficient serum for titration.
Tr., Weak fixation at 1/5 dilution.

Table 1*b*. *Growth-inhibiting antibody for* Mycoplasma hominis *type I in* 2265 *female patients*

Age (years)	Growth-inhibiting titre 80	40	20	10	5	Tr.	(+)	Total per-centage	No. exam-ined
> 65	.	.	.	.	.	.	.	.	57
55–65	.	.	.	4·1*	.	2·0	.	6·1	48
45–55	.	.	2·4	4·8	4·8	4·8	.	16·7	42
35–45	.	0·5	0·5	3·9	1·6	.	.	6·6	181
25–35	.	0·3	1·0	1·0	1·1	0·5	0·2	4·1	618
15–25	0·2	0·1	0·5	1·2	1·3	0·6	0·3	4·2	953
5–15	.	10	.	.	.	.	.	10	10
0–5	.	.	.	.	.	.	2·7	2·7	37
Unspecified	.	0·9	0·6	.	2·5	0·3	.	4·4	319

* Percentage positive.
(+), Positive result at 1/5 dilution but insufficient serum for titration.
Tr., Weak neutralization at 1/5 dilution.

Table 2. *General hospital patients*

Age (years)	Female: Total no. examined	Female: Percentage positive by CFT	Female: Percentage positive by GIT	Male: Total no. examined	Male: Percentage positive by CFT	Male: Percentage positive by GIT
> 65	56	3·6	0	54	9·3	7·4
45–65	80	16·3	10·0	120	5·0	0·8
25–45	92	15·2	4·3	75	6·7	5·3
15–25	33	15·1	3·0	27	0	0
5–15	7	0	0	3	0	0
Unspecified	44	13·6	2·3	64	6·3	3·1
Total all ages	312	12·8	4·5	343	5·8	3·2

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in the post-menopausal decade. The titres of growth-inhibiting antibody were generally lower than those obtained in the complement-fixation test. In female general hospital patients (Table 2) the incidence of antibody rises with age to a

Table 3. *Ante-natal patients*

Age (years)	Total no. examined	Percentage positive by CFT	Percentage positive by GIT
35–45	133	21·8	6·8
25–35	548	14·1	3·5
15–25	865	12·3	3·5
Unspecified	274	17·5	4·0
Total all ages	1820	14·4	3·8

Table 4. *V.D. clinic patients*

	Female			Male		
Age (years)	Total number examined	Percentage positive by CFT	Percentage positive by GIT	Total number examined	Percentage positive by CFT	Percentage positive by GIT
> 65	1*	+	−	1*	+	+
45–65	8	37·5	25·0	23	13·0	8·7
25–45	18	44·5	11·1	146	10·3	2·1
15–25	38	23·1	7·7	76	7·9	3·9
5–15	1*	+	+	0	0	0
Total all ages	66	33·3	12·1	246	10·2	3·7

* Positive result from the single patient in these groups.

Table 5. *Female inmates of H.M. prisons*

Age (years)	Total no. examined	Percentage positive by CFT	Percentage positive by GIT
25–45	10	30·0	30·0
15–25	19	26·3	26·3
Total all ages	29	27·6	27·6

Table 6. *Female patients of general practitioners*

Age (years)	Total number examined	Percentage positive by CFT	Percentage positive by GIT
25–45	31	9·7	3·2
15–25	32	12·5	3·1
Unspecified	40	17·5	7·5
Total all ages	103	13·6	4·9

maximum in the 45–65 age group and then declines. In antenatal patients (Table 3) the incidence of antibody also increases with age and complement-fixing antibody is approximately three times as common as growth-inhibiting antibody at all

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ages. Female patients attending venereal disease clinics (Table 4) have a much higher incidence of antibody than the previous groups. The maximum incidence is in the 25–45 age group although the numbers examined in each category are small. A small group of female inmates of H.M. prisons (Table 5) showed a similar high incidence of antibody and in this group the results from both tests were the same and showed no significant age differential. The distribution of antibody amongst female patients of general practitioners, shown in Table 6, was very similar to that in general hospital patients.

Table 7. *All patient groups, males and females*

	Female			Male		
		Percentage positive by			Precentage positive by	
Age (years)	Total no. examined	CFT	GIT	Total no. examined	CFT	GIT
> 65	57	5·3	0	56	10·7	5·4
55–65	48	12·4	6·1	76	6·6	2·6
45–55	42	26·2	16·7	74	5·4	1·4
35–45	181	22·1	6·6	94	3·4	1·1
25–35	618	15·2	4·1	142	12·7	4·2
15–25	953	14·4	4·2	104	6·7	1·9
5–15	10	10·0	10·0	7	0	0
0–5	37	2·7	2·7	21	4·8	4·8
Unspecified	319	19·4	4·4	76	7·9	3·9
Total all ages	2265	16·2	4·7	560	10·0	4·6

Male patients

In general complement-fixing antibodies to *M. hominis* are less common in men than women, although the overall incidence of growth-inhibiting antibodies is about the same. The age distribution of antibodies differs between the sexes (Table 7). In men, as with the women, there is a steady increase in incidence with age. The higher incidence in the 25–35 year age group in this series is due to the disproportionately large number of V.D. clinic patients in this group. In men we find that the incidence of antibody continues to increase in the over-65 group whereas in women of this age antibody is less common. Male patients of the general hospital group (Table 3) show this difference particularly and a direct comparison is possible with the similar female group. Male V.D. clinic patients (Table 4) have a higher than average incidence of antibody amongst the males but much lower than the comparable female group.

Children

Forty-one specimens of serum were from children under the age of five. Two children, one male and one female, had both growth-inhibiting and complement-fixing antibody, both were less than three months old and it was assumed that these antibodies were of maternal origin.

DISCUSSION

In the population examined, growth-inhibiting antibody to *M. hominis* was rarely found in the absence of complement-fixing antibody and was less common. With a few exceptions the complement-fixation titres were higher than the growth-inhibition titres. The growth-inhibition test may be less sensitive than the complement-fixation test, although the converse has been shown to hold for other *Mycoplasma* spp. by Taylor-Robinson, Purcell, Wong & Chanock (1966). We think that growth-inhibiting antibody probably appears in smaller amounts only in the more prolonged or severe infections, and disappears more quickly than complement-fixing antibody. That this may be so is suggested by the behaviour of these antibodies in the hyperimmune rabbit. Personal observation of a small number of patients with puerperal *M. hominis* infections also supports this hypothesis.

Little evidence has been accumulated about the frequency of antibody to *M. hominis* in the general population. Studies that have been made have usually been on a small number of people and often with no reference to age or sex. Lemcke & Csonka (1962) found antibody in 4% of 109 female blood donors and compared this with the antibody incidence of 50% amongst 51 patients with salpingitis. Shepard (1954) has pointed out that, for comparison, groups need to be matched for age, sex, social class and also sexual promiscuity. This is clearly difficult but in this study we have taken a comparatively very large sample and picked out various easily defined groups to see what differences there are.

Antibody to *M. hominis* is uncommon below the age of 15 years, obvious exceptions being in very young babies with maternal antibody and in an unusually promiscuous female patient. After this age, the presence of antibody becomes more frequent with increase in age, although important differences are to be seen between the sexes. In women, apart from a generally higher incidence of complement-fixing antibody, we find that antibody reaches a peak in the post-menopausal decade, and is uncommon after the age of 65. This is in contrast to the men where the incidence of antibody rises sharply over the age of 65. This difference is possibly due to the genito-urinary troubles that beset men at this age and *M. hominis* may play a part in prostatic inflammation. Why the post-menopausal decade in women should be the period of highest incidence of antibody also requires explanation. It is possible that as the acidity of the vagina becomes less, this allows *M. hominis* to proliferate.

The incidence of growth-inhibiting antibody in men and women is about the same (4·6–4·7%) but complement-fixing antibody is commoner in women (16·2%, compared with 10% in men). This may mean that women tend to suffer from trivial infections that fail to generate neutralizing antibody, whereas in men, when *M. hominis* infection does occur, both neutralizing and complement-fixing antibodies are likely to appear.

The only other recent survey of antibody to *M. hominis*, of any size, was reported by Taylor-Robinson *et al.* (1965). These workers examined a total of 256 sera from patients of all ages using an indirect haemagglutination technique which was much more sensitive than the complement-fixation test in their hands. These

patients, who were not divided into sexes, showed a general increase in the incidence of antibody with age, reaching a maximum in the 40 to 49-year group. They were able to detect some antibody in a few children between 5 and 14 years of age.

The age incidence of complement-fixing antibody to *M. pneumoniae*, which is an accepted respiratory pathogen, is not the same as we find with *M. hominis*. Andrews (1965), who also examined sera collected in N.W. England, and Grayston, Alexander, Kenny & Clarke (1965), working in America, have shown that antibody to *M. pneumoniae* is present in children and reaches a maximum incidence in early adult life. This distribution is quite unlike that of *M. hominis* antibody, which we find to be uncommon in young people and which reaches a maximum incidence in later life. This suggests that *M. hominis* does not behave as a respiratory pathogen or that, if it does, young people are not commonly infected.

Ante-natal patients, patients from general hospitals and patients of general practitioners are alike in antibody distribution. In comparison, antibodies to *M. hominis* are three times as common in female V.D. clinic patients and prison inmates. This is in accord with the results of other workers and may reflect the sexual promiscuity of these women. The overall incidence of growth-inhibiting antibody in male V.D. clinic patients and male hospital patients appears similar. This is partly explained by the difference in age distribution in the two groups and the high rate of positive results in hospital patients over 65.

SUMMARY

Sera from 3163 patients were examined for growth-inhibiting and complement-fixing antibody to *Mycoplasma hominis*. The results were analysed in respect of the age and sex of the patients. Antibodies were found to be uncommon in young people but increased in frequency with age; they were present in old men but were less common in women over sixty-five. Patients attending V.D. clinics and prison inmates had a much higher incidence of antibody than hospital and ante-natal patients. Complement-fixing antibody was approximately three times as common as growth-inhibiting antibody in the sample examined; it was also more common in women than men but the overall incidence of growth-inhibiting antibody was the same in each sex. It was concluded that possibly growth-inhibiting antibody is produced in more severe infections and that it disappears from the serum more quickly than complement-fixing antibody.

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