SHORT ARTICLES

ISOLATION OF MYCOPLASMS AND OTHER ORGANISMS FROM THE PLACENTA AFTER CAESAREAN SECTION

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MYCOPLASMS are among the organisms that form the normal vaginal flora, and those occurring most frequently in this situation are the T-strain mycoplasmas and the "large-colony" species *Mycoplasma hominis*. Whether the T-strain mycoplasmas ever cause disease is not yet known with certainty, although attempts have been made to establish them as a cause of non-specific urethritis (Ford, Rasmussen and Minken, 1962; Csonka, Williams and Course, 1966).

M. hominis is a common commensal in the female genital tract, but reports of its isolation from the bloodstream of patients in the puerperium indicate that it can also cause infection (Slingerland and Morgan, 1952; Stokes, 1955; Tully *et al.*, 1965; Tully and Smith, 1968). Antibody to *M. hominis* is more frequent in patients after delivery or abortion than before (Jones, 1967*a* and *b*) and *M. hominis* has been isolated from the foetal tissues after abortion or stillbirth (Hayflick and Stanbridge, 1967; Jones, 1967*a*; Pease, Rogers and Cole, 1967). Harwick *et al.* (1967) reported a case of septicaemia due to *M. hominis* associated with abortion and were able to isolate the organism both from the maternal bloodstream and from the foetal tissues. In this case, and in some of those described by Jones (1967*a*), placentitis was observed as part of the pathological process. It is difficult to estimate the frequency of such infections; Tully and Smith took blood cultures 24 hr after delivery from 50 unselected patients and found one case of septicaemia due to *M. hominis*.

In the study reported here, we have taken cultures from a series of placentas obtained at Caesarean section under conditions in which the chance of contamination by the vaginal flora was minimal. We report the isolation of bacteria and mycoplasmas from the amniotic surface of these placentas and the subsequent progress of the patients from whom they were obtained.

MATERIALS AND METHODS

Patients. One hundred and twenty patients undergoing elective or emergency Caesarean sections were studied. After delivery of the placenta through the abdominal wall, a swab was taken from the amniotic surface. A specimen of blood was also collected from the umbilical cord for antibody and immunoglobulin estimations.

Methods. For bacterial cultures, horse blood agar incubated aerobically and anaerobically and Brewer's medium were used. Mycoplasmas were cultured in PPLO broth (Difco) with 20 per cent. yeast extract, 10 per cent. human serum, thallium acetate 1 in 4000 and penicillin 1000 units per ml. The T-strain mycoplasmas were cultured in tryptone broth (Oxoid), pH 6.8, containing 1 per cent. urea, penicillin 1000 units per ml and 0.02 per cent. phenol red. The mycoplasma broths were subcultured on agar media containing similar constituents.

The methods of performing the complement-fixation test and the metabolic inhibition test have been described previously (Jones and Sequeira, 1966). *M. hominis* strain H27 was used for the preparation of the complement-fixing antigen and in the metabolic inhibition tests. Immunoglobulins were estimated by the radial diffusion method in plates supplied by Hyland Laboratories.

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RESULTS

The amniotic surfaces of 95 of the 120 placentas yielded no growth of micro-organisms. From each of a further 9 placentas a few colonies of coagulase-negative staphylococci were grown but were regarded as contaminants. From 16 placentas a variety of other bacteria and mycoplasmas were isolated. Table I shows the number of times each organism was isolated. Mycoplasmas were grown from 7 placentas (T-strains, 5; *M. hominis*, 2).

The clinical histories of the 16 patients from whom micro-organisms of possible significance were cultured, and of their babies, were reviewed. Particular reference was made to the length of time the membranes had been ruptured before delivery, whether there was post-partum fever, and whether the baby showed any signs of infection (table II).

TABLE I
Organisms grown from 120 placental swabs

Organism	No. of isolations
T-strain mycoplasmas	5
<i>Mycoplasma hominis</i>	2
Lactobacilli	3
<i>Pseudomonas aeruginosa</i>	2
<i>Staphylococcus aureus</i>	1
<i>Klebsiella aerogenes</i>	1
<i>Haemophilus influenzae</i>	1
Enterococci	1
Anaerobic streptococci	1
α -Haemolytic streptococci	1

Fever occurred in the puerperium in the patients from whom the following organisms had been isolated: *M. hominis* (2 out of 2 cases); T-strain mycoplasmas (3 out of 5 cases); anaerobic streptococci (2 out of 2 cases); lactobacilli (3 out of 3 cases); *Staphylococcus aureus* (1 case); *Klebsiella aerogenes* (1 case). No fever was observed in patients whose placenta cultures yielded *Pseudomonas aeruginosa* (2 cases), *Haemophilus influenzae* (1 case) and enterococci (1 case). Bacteria isolated from the placenta were also grown from septic lesions in 2 babies (*Staph. aureus* 1, *H. influenzae* 1) and from 2 mothers (*Staph. aureus* 1, *K. aerogenes* 1).

The histories of this group of patients were compared with the histories of 99 of the 104 patients from whom no organisms were grown. In the "culture-positive" group 10 patients (62 per cent.) and in the "culture-negative" group 24 patients (24 per cent.) had fever after operation. The length of time the membranes had been ruptured in the patients in each group is shown in table III.

The immunoglobulins were estimated in cord sera from 80 babies. No correlation was found between raised levels of IgM or IgA and either a positive placental culture or a prolonged time since membrane rupture. These sera were also examined for antibody to *M. hominis*, and in 16 (20 per cent.) complement-fixing antibody was present (mean titre 1 in 5) and in 5 (6 per cent.) metabolism-inhibiting antibody was also present (mean titre 1 in 4).

Case histories of the patients with placental infection due to M. hominis

Case no. 6. This patient was a single girl, aged 26 yr and pregnant for the first time. She was admitted in early labour and with the membranes intact. She did not progress in labour and a Caesarean section was performed 21 hr after the rupture of the membranes. *M. hominis* was isolated from the placenta at operation and also from vaginal swabs taken in the puerperium. On the day following the operation, her temperature was 104°F, and she remained febrile for 3 days during treatment with ampicillin. The baby did not show

TABLE II

Details of patients with positive placental cultures

Case no.	Organism	Interval since membrane rupture (hr)	Duration of fever (days)	Condition of mother	Condition of baby
1	T-strain mycoplasm	48	4	...	normal
2	T-strain mycoplasm	55	none	...	asphyxia at birth
3	T-strain mycoplasm; α -haemolytic streptococci	24	1	wound infection (anaerobic streptococci)	diffuse rash
4	T-strain mycoplasm	65	none	<i>Trichomonas</i> present in vagina	"bubbly" and cyanosed at birth
5	T-strain mycoplasm	24	3	...	liquor purulent; limp at birth; slightly sticky eyes
6	<i>Mycoplasma hominis</i>	21	4	production of antibody to <i>M. hominis</i>	normal
7	<i>Mycoplasma hominis</i>	60	4	production of antibody to <i>M. hominis</i>	raised IgM
8	Lactobacilli; anaerobic streptococci	48	3	...	normal
9	Lactobacilli	0	3	pregnancy with intra-uterine contraceptive device <i>in situ</i> ; threatened abortion in early pregnancy	raised IgM and IgA levels
10	Lactobacilli	48	1	...	asphyxia livida
11	<i>Pseudomonas aeruginosa</i> (enrichment culture)	24	none	wound infection (anaerobic streptococci)	normal
12	<i>Pseudomonas aeruginosa</i> (enrichment culture)	4	none	...	prolapse of cord, otherwise normal
13	<i>Staphylococcus aureus</i>	26	3	wound infection (<i>Staph. aureus</i>); <i>Staph. aureus</i> also isolated from vaginal swab	septic spots, sticky eyes (<i>Staph. aureus</i>)
14	<i>Klebsiella aerogenes</i>	16	2	wound infection (<i>K. aerogenes</i>)	normal
15	<i>Haemophilus influenzae</i> (enrichment culture)	8	none	...	sticky eye (<i>H. influenzae</i> and <i>Staph. aureus</i>)
16	Enterococci	31	none	...	neonatal pneumonia

any signs of infection, and both mother and baby were discharged 9 days after operation. Samples of serum from both mother and baby were examined for antibody to *M. hominis* (table IV).

TABLE III

Relation between length of time since rupture of the membranes and result of culture of placenta

Time since rupture of membranes (hr)	Number of patients with	
	placenta culture negative	placenta culture positive
0	66	1
4	13	1
4-12	7	1
12-24	6	5
over 24	7	8
Total	99	16

TABLE IV

Antibody response to Mycoplasma hominis infection

Time after delivery	Amount of antibody in serum of				
	mother		baby		
	CFT*	MIT†	CFT*	MIT†	IgM
<i>Case no. 6</i>					
0	—	—	< 5‡	< 2‡	6§
7 days	10‡	< 2‡	—	—	—
10 days	—	—	< 5	< 2	20
8 weeks	160	8	—	—	—
26 weeks	20	< 2	—	—	—
<i>Case no. 7</i>					
10 days	640	16	20	< 2	100
4 weeks	320	128	10	< 2	24
20 weeks	80	< 2	5	—	28

* CFT = Complement-fixation test.

† MIT = Metabolic inhibition test.

‡ = Reciprocals of titres.

§ = Mg per 100 ml.

Case no. 7. This patient, who was aged 25 yr, had 4 previous pregnancies and was admitted in early labour following spontaneous rupture of the membranes. On the day following admission she became pyrexial and felt shivery, and treatment with ampicillin was begun. Because of failure to progress in labour and the possibility of intra-uterine infection, she was delivered by Caesarean section. She remained pyrexial for 4 days and until tetracycline was given instead of ampicillin. The baby did not show any signs of

infection, but there was some difficulty in establishing respiration at birth. Both mother and baby were discharged 12 days after operation. Serum samples were examined for antibody to *M. hominis* (table IV).

DISCUSSION

The flora of the naturally delivered placenta is essentially that of the vagina (Slotnick *et al.*, 1964). In our series, culture of the placenta at Caesarean section showed that organisms from the vaginal flora reached the amnion in a small proportion of patients. In some cases there was subsequent evidence of infection, with the isolation of the appropriate organism, either in the mother or the baby (table II). In 26 patients the membranes had been ruptured for more than 12 hr, and half of these had positive placenta cultures. The membranes had been ruptured for less than 12 hr in 89 patients and only three of these had positive cultures. Patients with a positive placenta culture had fever after operation more often than did patients in the "culture-negative" group. This association between premature rupture of membranes and sepsis is well known, although in some other investigations coliform bacteria were the most common organisms isolated (Webb, 1967).

The organisms most frequently isolated in this series (5 cases) were the T-strain mycoplasmas. These organisms are frequent inhabitants of the female genital tract (Csonka *et al.*, 1966). Whether they caused any infection in the patients studied is uncertain, although on the basis of previous experience (Jones and Tobin, 1968, and unpublished observations), it is possible that the sticky eye of the baby in case no. 5 might have been due to mycoplasmas. In all five of these cases there was a prolonged interval after membrane rupture, 3 babies had some respiratory distress at birth, and 3 mothers were febrile after delivery, but without the demonstration of a serological response to the organism there is little evidence to incriminate these T-strain mycoplasmas as a cause of any of the symptoms. The frequency of isolation of these organisms in this study may merely reflect the frequent occurrence of T-strain mycoplasmas in the vagina. The occurrence of 2 cases of infection with *M. hominis* in this series adds to the growing evidence that this organism causes puerperal infection. From observation of these and other cases, it appears that the infection is usually transient in that the patient may become afebrile after a day or so without specific treatment. Ampicillin, frequently prescribed for post-partum infections, is without effect on mycoplasmas. Occasionally, however, the fever is prolonged and treatment with tetracycline or some other suitable antibiotic is needed, as in case 7 in the series.

The antibody responses to *M. hominis* in cases 6 and 7 are of interest. In patient no. 6 (table IV), there was probably no pre-existing antibody to *M. hominis* at the time of delivery, as none was detected in the cord sample of blood from the baby. Seven days after delivery, antibody was detectable in the mother by the complement-fixation test; later, a higher titre was observed and some metabolism-inhibiting antibody was detected. Twenty-six weeks after the infection only complement-fixing antibody remained. No evidence of an immune response by the baby was detectable. In case 7, there was a similar sequential rise and fall of antibody titres with only complement-fixing antibody persisting. The brisker antibody response in this patient was probably a result of previous antigenic experience of *M. hominis*, the antibody in the cord blood reflecting the maternal levels at the time of delivery. The raised level of IgM in the serum from the baby 10 days after delivery may represent a response to *M. hominis*, although the titre of complement-fixing antibody in this serum was not significantly changed by treatment with 2-mercaptoethanol, indicating that the complement-fixing antibody was of maternal origin. The general pattern of antibody response to *M. hominis* in these patients is similar to that detected by Tully and Smith (1968) by means of an immunofluorescence technique. Our results demonstrate that in *M. hominis* infections complement-fixing antibody is produced earlier than metabolism-inhibiting antibody and to a higher titre.

The frequency of complement-fixing and metabolism-inhibiting antibody in the 80 cord sera collected from these babies was essentially the same as the frequency in unselected antenatal patients tested in early pregnancy (Jones and Sequeira, 1966). This indicates that most of the maternal antibody to *M. hominis* is able to cross the placenta, and is also in

conformity with the finding that any increase in incidence of these antibodies tends to occur in the puerperium rather than during pregnancy (Jones, 1967b).

SUMMARY

Swabs taken from the amniotic surface of the placenta in a series of 120 Caesarean sections were cultured for mycoplasmas and bacteria; organisms of possible significance were isolated from 16 patients. In most of the patients, the membranes had been ruptured for more than 12 hr before Caesarean section, and in 10 of them there was pyrexia after the operation. Some of the babies showed evidence of neonatal infection.

The most frequently isolated micro-organisms in the series were T-strain mycoplasmas. Two cases of infection by *Mycoplasma hominis* were encountered, and the clinical and serological findings in them are described. The possible importance of mycoplasmas as a cause of infections in the puerperium is discussed.

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