

Immunoglobulin Classes and Biological Functions of *Campylobacter (Vibrio) fetus* Antibodies in Serum and Cervicovaginal Mucus

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Serum and cervicovaginal mucus (CVM) antibodies from heifers after genital infection or systemic immunization with *Campylobacter (Vibrio) fetus* were classified according to their immunoglobulin class, antigenic specificities, and biological functions. Only immunoglobulin (Ig) A antibodies, specific both for O and superficial, heat-labile, whole-cell (W) antigens, were detected in CVM of convalescent animals. After systemic immunization, antibodies in serum were directed principally to W antigens and were located in IgG₁, IgG₂, and IgM classes; CVM antibodies of the same specificity were detected only in the IgG subclasses. Functional tests revealed that antibodies of W specificity, whether of the IgA or IgG class, were capable of immobilizing the organism. However, IgG antibodies immobilized with clumping, whereas IgA antibodies immobilized single organisms within the 3-min period. None of the antibody preparations was bactericidal in the presence of homologous complement when the infecting strain was used as the target organism, but a bactericidal effect was observed when the target strain was rough and non-encapsulated. Both serum and CVM from systemically immunized animals opsonized *C. fetus* organisms, but CVM from locally immunized animals containing IgA antibodies was not opsonic. It is hypothesized that functions of immobilization for IgA and IgG and of opsonization for IgG are important features of protective immunity in venereal vibriosis.

Host defense mechanisms against venereal diseases are of current interest in both domestic animals and man. Bovine venereal vibriosis is a useful model for the study of the functions of antibody in protecting the genital tract for several reasons. The etiological agent, *Campylobacter (Vibrio) fetus venerealis* is a gram-negative monotrichous rod (29) with a characteristic cell wall (25, 39, 42) and a microcapsule or equivalent structure which prevents agglutination of whole cells by O antiserum (24). When organisms are introduced cervicovaginally, the infection is confined to the genital tract with no increase in serum agglutinins. Bacteria are cleared first from the uterus and uterine tubes and then from the cervicovaginal area over the next several months (28). The infection results in a period of infertility which correlates well with the 3- to 5-month duration of endometritis (28). Convalescent immunity with regained fertility develops frequently in the face of persistent vaginal infection. Agglutinins are present in the cervicovaginal mucus (CVM) of infected and convalescent animals. Wilkie et al. (33) have

reported the agglutinins to be of the immunoglobulin (Ig) A class and of O specificity, whereas Pedersen et al. (20) found anti-campylobacter antibodies in IgG, IgM, and IgA classes during early stages of infection. In animals immunized systemically with bacterin in complete Freund adjuvant (CFA), on the other hand, infection never became established after challenge (34). Antibodies were of the IgG class and of whole-cell specificity (33). Recently conducted passive transfer experiments (R. L. Berg, B. D. Firehammer, M. Border, and L. L. Myers. Abstr. 55th Annu. Meet. Conf. Res. Workers Anim. Dis., 1973) indicate that these antibodies are protective.

With this background, we decided to investigate some of the biological functions of campylobacter antibodies in convalescent and systemically immunized animals. Functions were compared with immunoglobulin class and antigenic specificity of antibodies.

MATERIALS AND METHODS

Bacterial strains. Two *C. fetus venerealis* strains were used. Strain 1016 was isolated originally from

an infertile cow by J. H. Bryner (National Animal Disease Laboratory, Ames, Iowa) in 1965 and lyophilized. In 1972 it was used to infect several heifers (no. 23, 37, 43, and 60) (G. D. Schurig, M.Sc. thesis, Cornell University, Ithaca, N. Y., 1974). Strain 1016-43b was isolated from animal 43, which required seven breedings before pregnancy occurred. It was maintained in the laboratory by passage in Albimi (Pfizer Co.) semisolid medium (41) at 2- to 3-week intervals. For functional tests, subcultures of strain 1016-43b were made on cystine heart agar (Difco) blood plates (41) and grown for 2 to 3 days in an atmosphere of 2.5% O₂, 10% CO₂, and 87.5% N₂. Only encapsulated organisms possessing O antigen were used. The presence of capsular antigen was established by insuring inagglutinability of cells by homologous O antiserum. Possession of O antigen (39) was based on the presence of colonies with smooth morphology (4), since a correlation of these properties has been established in *C. fetus* (37, 40).

C. fetus venerealis strain UM, which belongs to the same O serogroup as 1016, has been characterized previously (39). It is unencapsulated as evidenced by agglutinability with O antiserum (A. J. Winter, unpublished data.) The smooth form (UM-S) was subjected to rapid serial passage in order to select a variant (UM-R) producing colonies with rough morphology.

Animals and sampling procedures. Virgin Holstein heifers approximately 2 years of age were used. Two animals (31 and 61) were immunized by subcutaneous injection of strain 1016-43b bacterin in CFA on two occasions 5 weeks apart (34). Samples used in this study were taken before immunization and from 10 to 17 weeks after beginning immunization.

Animals 23, 37, and 60 were infected cervicovaginally at estrus with 2×10^8 to 4×10^8 organisms of strain 1016. Samples were collected for the present study 7 to 9 months later, by which time all three had become culturally negative.

Serum samples were inactivated at 56 C for 30 min and stored at -20 C. Those from systemically immunized animals were diluted for use in functional tests (compare agglutination titers in Table 1 with those in Tables 3 to 7). CVM was aspirated from the cranial area of the vagina with infusion pipettes. The total amount collected was extracted overnight in 5 ml of physiological saline at 4 C after vortexing with glass beads. Sample weights ranged from 0.3 to 2.3 g. The suspension was then centrifuged at $48,000 \times g$ for 30 min, and the supernatant was filter sterilized and stored at 4 C. Extraction was not done on a weight-volume basis because great changes in hydration of mucus occur during the estrus cycle. Weight changes, therefore, are due principally to differences in water content rather than solids including antibody protein.

Agglutination tests. Whole (W) cells were obtained by suspending 3-day growth from blood plates in formalized saline (0.3% formaldehyde solution), and were used without further washing. Similarly grown cells which had been steamed for 2 h in distilled water to remove heat-labile antigens and expose the O antigen are referred to as O cells. After steaming they were washed twice and stored in formalized saline.

Both W and O cells were diluted in formalized saline to an optical density (OD) of 0.6 at 525 nm and incubated with equal volumes of doubling dilutions of CVM extracts or serum samples at 50 C for 24 h. The titer was expressed as the reciprocal of the highest dilution which gave 50% clearing.

Immobilization tests. *C. fetus* organisms from 2-day plate cultures, which exhibited excellent motility, were suspended in McCoy tissue culture medium at an OD of 0.37 at 525 nm (approximately 10^8 cells/ml) and warmed to 37 C. Samples of serum or CVM, also prewarmed, were mixed with the bacteria in equal volume. Two loops of the mixture were placed on a warm slide, covered with a warm cover slip, and examined by phase-contrast microscopy. Preparations were scored for percentage of cells, single or clumped, which exhibited motility and for the percentage of cells clumped at 3 min.

Bactericidal tests. Bovine complement was used throughout since preliminary experiments had demonstrated that guinea pig complement had no greater activity in these tests and recent evidence indicates that homologous complement is sometimes fixed where heterologous complement is not (10, 21). Bovine complement was prepared from normal bovine blood by a modification of the method of Mayer (16). Two absorptions at 0 C with *C. fetus* O and W cells were necessary to remove natural antibodies (8, 38). Portions were stored at -70 C. The hemolysin titer was 64 by the method of Barta and Barta (1).

The suspending medium in all cases was McCoy 5A plus 20% fetal calf serum (Grand Island Biological Co.), 4 U of bacitracin, and 4 μ g of novobiocin per 1.0 ml of medium. The concentrations of antibiotics used do not affect the growth of *C. fetus*. All tests were performed in plastic snap-cap tubes (Falcon Plastics) containing 0.1 ml of CVM or serum, 0.1 ml of complement, 0.1 ml of a 2-day culture of *C. fetus* at OD₅₂₅ of 0.37, and 1.2 ml suspending medium. This mixture was incubated for 3 h at 37 C on a tumbler at 15 rpm. A 1:10 dilution was made into sterile distilled water in a tube containing 10 glass beads (2-mm diameter). This was vortexed for 2 min to break up clumps and serial dilutions in distilled water were performed. Viable cell counts were done by the drop method (17), with colonies being counted after 3 days of incubation. Controls consisted either of mixtures containing organisms and 1.4 ml of medium (see Table 5) or of antibody and organisms plus 1.3 ml of medium but without complement in order to correct for lowered counts due to agglutination (see Table 6). Viability is expressed as a percentage of the control value and was calculated as follows:

$$\frac{(\text{viable count with antibody + complement})}{(\text{viable count of control})} \times 100$$

Opsonization. Macrophages were obtained by a modification of the method of Outteridge et al. (19). The right front quarter of the udder of a dry cow was infused with 25 to 50 ml of 0.25 M sucrose containing 5 to 10 μ g of lipopolysaccharide extracted from *Escherichia coli* (Difco). Secretions were washed out of the quarter with 0.25 M sucrose at 4 to 6 days. Cells were

suspended in McCoy 5A tissue culture medium containing fetal calf serum and antibiotics as above. Cells were allowed to settle in Leighton tubes with cover slips, the nonadherent cells were washed off with Dulbecco phosphate-buffered saline (DPBS), and fresh medium was added. Medium was changed as indicated by pH changes and at the beginning of the opsonization experiment.

The opsonization procedure consisted of adding to the Leighton tube 1 ml of the above medium with 0.1 ml of serum or CVM and 0.1 ml of *C. fetus* suspended at OD_{525} of 0.37. Tests were done both with and without 0.1 ml of bovine complement. The mixture was incubated for 3 h at 37 C to allow phagocytosis to occur and then washed three times with DPBS; the cover slip was fixed in methanol before staining with May-Grünwald-Giemsa. Slides were examined under phase contrast and scored for the number of organisms in the cytoplasm of each of 50 macrophages per cover slip.

Indirect fluorescent antibody (IFA) assays. Bovine immunoglobulins were isolated and antisera were prepared in guinea pigs as previously described (7). Guinea pig antiglobulin was prepared in rabbits by injecting guinea pig IgG in CFA into footpads, followed in 3 weeks by an intravenous booster. The rabbit antibodies were conjugated with fluorescein isothiocyanate by the method of The and Feltkamp (27). The IFA test was performed according to Duncan et al. (8) with slight modifications. Briefly, fixed smears prepared from whole or steamed *C. fetus* cell suspensions were overlaid with bovine serum or CVM, incubated for 45 min at room temperature, and washed for 20 min in three changes of phosphate-buffered saline, pH 7.2. Slides were blotted and the smears were overlaid with the guinea pig anti-bovine immunoglobulin G₁, G₂, M, or A. Incubation and washing steps were repeated as before. After blotting, the smears were overlaid with the fluorescein-labeled rabbit anti-guinea pig IgG, incubated, and washed. The blotted slides were overlaid with a 1% solution of eriochrome black T for 5 s as suggested by Fey (11) to minimize background and nonspecific staining. After mounting, the slides were examined with a Leitz Ortholux microscope equipped with an Osram HB 200 mercury vapor lamp, heat-absorbing and BG12 transmitting filters, and blue- and ultraviolet-absorbing barrier filters.

The specificity of antisera to IgG₁, IgG₂, and IgM was determined in the IFA test using standards prepared from hyperimmune serum of heifer 61 following the methods previously described (7). Only anti-IgM was positive with the first peak from a Sephadex G-200 column, and only anti-IgG₂ was positive with the first peak from diethylaminoethyl (DEAE)-Sephadex A50. Anti-IgG₁ did not react with the first peak from DEAE but did react with the second, which contained both IgG₁ and IgG₂. Anti-IgA reacted with a CVM sample that was negative for IgM, IgG₁, and IgG₂. In each case several absorptions of the anti-immunoglobulin with insolubilized, purified immunoglobulins and with W and O cells was necessary to render the reagents specific in the IFA test. All reagents were used at two dilutions or more below that dilution at which brightness of fluores-

cence began to diminish.

Statistical treatment of bactericidal data. The original observations were taken to be from the Poisson law with large mean value, thus approximating the normal distribution. Analysis of variance techniques and F tests were used in judging treatment versus control differences. The data were summarized by presenting treatment effect as a percentage of the control. In some cases, 95% fiducial limits were calculated for those ratios by appealing to Fieller's theorem (12).

RESULTS

After systemic immunization of heifers 31 and 61, serum agglutinins were directed almost wholly against W antigens. In fact, O agglutinin titers had dropped from preimmunization levels (Tables 1 and 3). This phenomenon has been noted once before in our laboratory after immunization of cattle with *C. fetus* antigens in CFA (J. R. Duncan, unpublished data) and is presently unexplained. In CVM, no antibodies were detected in preimmunization samples, and only whole-cell agglutinins were detected after immunization. Serum antibodies were distributed among both IgG subclasses and IgM (Table 1), whereas those in CVM were restricted to the IgG subclasses (Table 2). In contrast, agglutinins in CVM of convalescent heifers 23, 37, and 60 were of varying specificities and only antibodies of the IgA class were detected (Table 2). The IFA tests were completed 5 to 6 months after samples were collected, by which time the agglutination titers of CVM from convalescent animals had fallen, whereas those from systemically immunized animals had not (compare Tables 2 and 3). Since only IgA antibodies were detected in CVM from the former and only IgG antibodies were detected in CVM from the latter, it is assumed that agglutination was due to antibodies of those classes and that IgA was more labile than IgG.

The ability of antibodies to immobilize the organism was studied and related to their agglutinating properties and immunoglobulin class. Serum antibodies of O specificity (natural antibodies [38]) produced a minimal decrease in motility with only slight clumping, whereas antibodies of whole-cell specificity, mainly of IgG₁ and IgG₂ subclasses, immobilized most of the cells with clumping (Table 3). Similar results were obtained with CVM antibodies from systemically immunized heifer 31 (Table 3). CVM antibodies from heifer 61 did not immobilize, probably due to the low levels of antibody. CVM antibodies from convalescent animals produced a high degree of immobilization without significant clumping when antibodies against W antigens were present but not when only O antibodies were detected (Table 3).

TABLE 1. Titration of immune serum antibody activity for whole and steamed cells of *C. fetus*

Animal	Agglutination test ^a		Indirect fluorescent antibody test ^b							
			IgG ₁		IgG ₂		IgM		IgA	
	O	W	O	W	O	W	O	W	O	W
31	40	1280	64	512	64	512	128	32	- ^c	-
61	<40	1280	256	4096	128	1024	128	32	-	-

^a O, Steamed *C. fetus* cells; W, whole *C. fetus* cells used as antigens.

^b Specificity of guinea pig anti-bovine immunoglobulin antisera.

^c -, Undetectable in undiluted sample.

TABLE 2. Titration of cervicovaginal mucus extract antibody activity for whole and steamed cells of *C. fetus*

Animal	Immune status ^a	Agglutination test ^b		Indirect fluorescent antibody test ^c							
				IgG ₁		IgG ₂		IgM		IgA	
		O	W	O	W	O	W	O	W	O	W
31	SI	<4	16	1	8	1	8	- ^d	-	-	-
61	SI	<4	4	-	8	-	4	-	-	-	-
23	LI	8	16	-	-	-	-	-	-	2	8
37	LI	16	16	-	-	-	-	-	-	4	8
60	LI	16	<4	-	-	-	-	-	-	2	2

^a SI, Systemically immunized; LI, locally immunized by infection.

^b O, Steamed *C. fetus* cells; W, whole *C. fetus* cells.

^c Specificity of guinea pig anti-bovine immunoglobulin sera.

^d -, Undetectable in undiluted sample.

TABLE 3. Effect of serum and cervicovaginal mucus extracts on motility and clumping of *C. fetus* in 3 min^a

Animal	Anti-body source	Immune status ^b	Agglutination titer ^c		Motility ^d	Clump-ing ^d
			O	W		
31	Serum	PI	128	<4	++++	++
31	Serum	SI	<4	32 ^f	++	+++
31	CVM ^e	SI	<4	16	++	+++
61	Serum	PI	64	<4	++++	++
61	Serum	SI	<4	32 ^f	++	++++
61	CVM	SI	<4	<4	++++	±
23	CVM	LI	64	32	±	±
37	CVM	LI	32	256	±	+
60	CVM	LI	32	<4	+++	±

^a Average of three replicate experiments.

^b PI, Preimmunization sample; SI, systemically immunized; LI, locally immunized by infection.

^c O, Steamed *C. fetus* cells; W, whole *C. fetus* cells.

^d Symbols for approximate percentages of cells motile or clumped at 3 min are as follows: -, 0%; ±, 5%; +, 5 to 10%; ++, 10 to 50%; +++, 50 to 75%; +++++, >75%.

^e CVM, Cervicovaginal mucus.

^f See Table 1 for titers of undiluted serum.

Since there seemed to be a correlation between whole-cell agglutination titer and ability to immobilize organisms, selected antibody preparations were diluted to produce a uniform whole-cell agglutination titer of 16. On this basis, CVM antibodies from convalescent animals (IgA) were equally or more efficient in immobilization than serum or CVM antibodies from systemically immunized animals (IgG₁ and IgG₂) (Table 4). Also, samples which contained IgA antibodies immobilized single organisms without clumping them.

Bactericidal effects of serum and CVM samples on strain 1016-43b are reported in Table 5. Statistical analysis indicated that none of the viable counts differed significantly from the control. When preimmunization serum samples containing predominantly O antibodies were tested against strain 1016-43b and against smooth and rough variants of strain UM, a statistically significant proportion of UM-R organisms was killed, whereas UM-S and 1016-43b organisms were not susceptible with one minor exception (Table 6). Complement alone was equally effective in killing UM-R

TABLE 4. Motility and clumping effects of serum and cervicovaginal mucus extracts at a standardized agglutination titer of 16^a

Animal	Anti-body source	Immune status ^b	Agglutination titer ^c		Motility ^d	Clump-ing ^d
			O	W		
31	Serum	SI	<4	16	+++	+++
61	Serum	SI	<4	16	++	+++
31	CVM ^e	SI	<4	16	+++	++
23	CVM	LI	16	16	±	±
37	CVM	LI	<4	16	+	-

^a Average of two replicate experiments.

^b PI, Preimmunization sample; SI, systemically immunized; LI, locally immunized by infection.

^c O, Steamed *C. fetus* cells; W, Whole *C. fetus* cells.

^d Symbols are as in Table 3.

^e CVM, Cervicovaginal mucus.

TABLE 5. Complement-mediated bactericidal effect of serum or cervicovaginal mucus extracts^a

Animal	Antibody source	Immune status ^b	Agglutination titer ^c		Viability ^d
			O	W	
31	Serum	SI	<4	16	97.3
61	Serum	SI	<4	16	79.1
31	CVM ^e	SI	<4	16	75.0
61	CVM	SI	<4	4	86.4
23	CVM	LI	64	64	81.8
37	CVM	LI	8	320	92.0
60	CVM	LI	32	<4	130.6

^a Average of three replicate experiments.

^b SI, Systemically immunized; LI, locally immunized by infection.

^c O, Steamed *C. fetus* cells; W, whole *C. fetus* cells.

^d Expressed as percent of control. Values were not significantly different from the control at $\alpha = 0.05$.

^e CVM, Cervicovaginal mucus.

(Table 6). Observations on the colony morphology of surviving UM-R organisms revealed that most had reverted to the smooth form. A small percentage of cells treated with antibody alone or complement alone also reverted, and reversions in low frequency were occasionally observed in stock cultures.

The effects of antibodies on phagocytosis of *C. fetus* organisms by macrophages are depicted in Table 7. In the absence of antibody, 0 to 20 intracellular *C. fetus* organisms were seen per macrophage; therefore, the phagocytic index was reported as the percentage of macrophages with more than 20 intracellular *C. fetus*. Both serum and CVM antibodies from systemically immunized animals (IgG₁ and IgG₂) were very efficient opsonins. CVM antibodies (IgA) from convalescent animals, on the other hand, exhib-

ited no appreciable opsonizing effect. Complement alone or with antibody appeared to enhance phagocytosis only slightly.

DISCUSSION

Little is known about the function of antibodies in protecting the female genital tract against bacterial infections. Therefore, the present study was designed to investigate this question, as well as to distinguish effects of the various immunoglobulin classes. On local immunization of women with candida (30) or polio antigen (18), antibodies were mainly of the IgA class as in our study, but antibodies of other classes have been detected in the immune response of the human female genital tract to spermatozoa (31) or during the early stages of bovine vibriosis (20; L. B. Corbeil, manuscript in preparation). Thus it appears that variations in antigens and in stages of immunization can

TABLE 6. Complement-mediated bactericidal effect of *O* antisera on different *C. fetus* strains^a

Strain	Viability ^b after incubation with:		
	Serum 31 ^c + C	Serum 61 ^c + C	C alone
1016-43b	97.1	65.2	68.9 ^d
UM-S	85.1	69.8	70.1
UM-R	29.5 ^d	31.5 ^d	10.7 ^d

^a Average of three replicate experiments.

^b Expressed as percent of control. C, Complement.

^c Agglutination titer: O = 16, W = -.

^d Significantly different from control at $\alpha = 0.05$.

TABLE 7. Effect of bovine antibodies on macrophage phagocytosis of *C. fetus*^a

Animal	Antibody source	Immune status ^b	Agglutination titer ^c		Phago-cytic index ^d
			O	W	
N ^e					0
31	Serum	SI	<4	16	96
61	Serum	SI	<4	32	98
31	CVM ^f	SI	<4	16	97
61	CVM	SI	<4	4	94
23	CVM	LI	32	16	3
37	CVM	LI	8	320	9
60	CVM	LI	32	<4	1

^a Average of two replicate experiments.

^b SI, Systemically immunized; LI, locally immunized by infection.

^c O, Steamed *C. fetus* cells; W, whole *C. fetus* cells.

^d Percentage of macrophages with more than 20 intracellular organisms each.

^e N, Negative control.

^f CVM, Cervicovaginal mucus.

influence secretory as well as systemic immune responses.

Functional tests revealed several differences between CVM from locally immunized animals containing mainly IgA and CVM from systemically immunized animals containing IgG. Antibodies of both classes were able to immobilize organisms if the specificity was against whole-cell superficial antigens, but initial tests suggested that IgA samples were more efficient than IgG samples (Table 3). An attempt was made to determine the efficiency of immobilization by antibodies of the various immunoglobulin classes by standardization of the whole-cell agglutination titer at 16, although it was recognized that antibodies of differing classes vary in their ability to agglutinate. In fact, Eddie et al. (9) have shown that IgA is about 10 times more efficient in agglutination than IgG on a weight basis. Even so, at a whole-cell titer of 16, CVM from locally immunized animals immobilized more effectively than IgG-containing samples from systemically immunized animals (Table 4). Therefore, the efficiency of IgA in immobilization can be calculated to exceed that of IgG by a comparable factor.

Another difference between immobilization by samples containing IgA or IgG was that IgG immobilized organisms while clumping them, whereas IgA immobilized mostly single organisms. The basis for this difference is unexplained but may reside in differences between the IgA and IgG antibody populations in valence, antigenic specificities, or functional properties of the Fc components. DiPierro and Doetsch (6) have shown that peritrichous *E. coli* were immobilized singly by hyperimmune serum, whereas monotrichous *Pseudomonas fluorescens* were immobilized only when several were linked together. Our results with IgG agree with their observations, but IgA was able to immobilize single monotrichous organisms. The concept that immobilization occurs exclusively by cross-linkage of separate filaments (3) may hold for IgG antibodies but not necessarily for those of polymeric IgA and, presumably, IgM classes.

Immobilization was not due to complement-dependent killing since complement was not present under test conditions. Furthermore, it was found that the infecting strain was not killed by complement together with either serum or CVM antibodies (Table 5). Carrier and Kramer (5) concluded from their studies that immune bovine serum was moderately bactericidal for *C. fetus*. However, statistical significance of the difference between the controls and treated samples was not reported, and

the fall in colony counts could be accounted for by clumping of organisms due to agglutinins in the serum. The antibodies we studied were directed principally toward superficial heat-labile antigens (Table 5), and it has been demonstrated that capsular antibodies may not be effective in mediating complement-dependent killing (15). O antibodies mediate complement killing of gram-negative organisms, but many species, even though unencapsulated, are resistant (26). Data in Tables 5 and 6 indicate that *C. fetus* is protected from the bactericidal effect of antibody and complement by its O side chains, possibly in combination with the capsule. Thus, only a rough variant (UM-R) of an unencapsulated strain was killed, and complement alone, presumably activated through the alternate pathway (2, 13), was sufficient for this purpose (Table 6). It is notable that most of the survivors had reverted to the smooth state. The explanation for reversion of a cloned, rough population of cells in the presence of antibody and complement is speculative. Since a small percentage of cells reverted in complement-free controls and in stock cultures, the effect of antibody and complement may have been one of selection.

The major lesion in venereal vibriosis is a mononuclear infiltration of the mucosa in which mononuclear phagocytes as well as lymphocytes and plasma cells are present. Therefore, one function of CVM antibodies could be to enhance phagocytosis. It has been demonstrated (Table 7) that opsonizing activity was good in serum and CVM samples containing IgG antibodies but was essentially absent in CVM samples in which only IgA antibodies were detectable. These findings are consistent with those of several investigators (9, 36, 43), although there are a few reports of IgA being opsonic (23, 32). Complement fixation by antibody was not important for opsonization in this system, consistent with Reynolds and Thompson's studies of opsonization in bronchial secretions (22, 23).

From these results, inferences are possible regarding some mechanisms whereby antibodies may exert protective functions in bacterial infections of the female genital tract. Since encapsulated smooth strains of *C. fetus* are resistant to the bactericidal effects of antibody and complement, direct killing of the organisms is considered unimportant. Opsonization by IgG antibodies may have a significant role in clearance of organisms from the genital tract, especially in systemically immunized animals in which infection fails to become established (34). Immobilization may be a key factor in limiting invasiveness and, if so, would constitute a

particularly important attribute of secretory IgA, which is highly efficient in this function. In venereal vibriosis, motility is likely to be of importance in penetration of the cervix and of the mucus layer covering the mucosa so immobilization could prevent both entry of organisms into the uterus and colonization of the uterine mucosa. This is consistent with observations during the carrier state of the disease, at which time antibodies are present in CVM and organisms are restricted to the cervix and vagina (28).

Although IgA is the predominant immunoglobulin class in most external secretions, few of its antibacterial functions have been described. These include enzyme and toxin inhibition (14) and inhibition of bacterial adherence to epithelial cells (35). The observation that IgA antibodies can immobilize *C. fetus* adds another potentially protective function to this short list.

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