and is consistent with the suggestion that tumours in older children have slower growth rates.

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Appearance of Specific Colostrum Antibodies after Clinical Infection with Salmonella typhimurium

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Summary

Colostrum and serum antibodies to *Salmonella typhimurium* have been found in three patients after clinical gastrointestinal infection during pregnancy. High levels of colostrum IgA agglutinins were directed specifically against both the flagellar and somatic antigens of the infective organism. The levels of colostrum agglutinating activity exceeded those found in the patients sera, while control colostrum gave negative results.

Introduction

The mechanisms by which resistance to infection may be passed from mother to infant are not fully understood. It is established that breast-fed infants have a lower incidence of enteric *Escherichia coli* infection (Hinton and MacGregor, 1958; Mata and Urrutia, 1971) and septicaemic illness (Winberg and Wessner, 1971) than bottle-fed infants. Human milk is rich in defence factors, including a growth enhancer of lactobacilli, an anti-staphylococcal agent, immunoglobulins, certain complement components, lysozyme, lactoperoxidase, lactoferrin, and macrophages and lymphocytes (Goldman and Smith, 1973); however, relatively little is known about their effects on the infant. Nevertheless, attention has been drawn to the possible protective role of passively transferred maternal antibodies present in the colostrum and milk.

Specific and non-specific antibodies to a wide range of micro-organisms have been found in these secretions (Shearman *et al.*, 1972; Goldman and Smith, 1973), and in the few studies involving determinations of immunoglobulin classes these antibodies have been shown to be principally of the IgA type (Adinolfi *et al.*, 1966 a; Mouton *et al.*, 1970; Bennich and Johansson, 1971; Parkin *et al.*, 1973 Zipursky *et al.*, 1973).

Colostrum antibodies are not thought to be absorbed from the intestinal lumen of the newborn infant, and thus their protective influence is likely to be a local one in the alimentary tract (Nordbring, 1957; Amman and Stiehm, 1966). The known survival of maternal agglutinating antibodies to salmonella H antigens in the neonatal gut (Schubert and Grünberg, 1949) has recently been attributed to the intrinsic resistance of colostrum IgA to digestion by trypsin (Brown *et al.*, 1970; Parkin *et al.*, 1973). In addition to the early clinical studies of Schubert and Grünberg (1949) indicating the transfer of specific agglutinating colostrum antibody from mother to offspring after intradermal vaccination with formalin-killed salmonellae, Eddie *et al.* (1971) showed that rabbits fed living, but not killed, *Salmonella typhimurium* developed milk antibodies to the infective organism. The question remained: Could infection with a pathogenic salmonella by the oral route result in similar colostrum antibody production in man? We have studied the immunological reactions of three pregnant women infected with *S. typhimurium*.

Patients and Methods

Three women were selected for further investigation after clinical infection during pregnancy with *S. typhimurium* (phage type 465) as shown by stool cultures. None of them had received an inoculation of TAB vaccine before their illness. Negative stool cultures were obtained from cases 1, 2, and 3, 8, 10, and 36 days respectively after diagnosis. Widal tests were not performed because at the time there was a

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local epidemic of gastroenteritis which had been traced to the specific salmonella organism mentioned above. All three pregnancies proceeded normally.

COLLECTION AND TREATMENT OF COLOSTRUM AND SERUM

Colostrum was collected aseptically within the first 36 hours post partum from the three patients and three control donors. The colostrum was diluted with an equal volume of sterile phosphate-buffered (0·06 mol/l.) saline (0·15 mol NaCl/l.) at pH 7·6 and defatted by centrifugation at 20,000 *g* for 30 minutes at 4°C. The defatted control colostrum samples were pooled before storage with the individual test samples at −20°C. In addition serum was collected from two of the patients (cases 1 and 2) 24 to 36 hours post partum and from all three patients four to six months after delivery. Storage conditions for all serum and colostrum samples were standard.

ANTIBODY ASSAYS

The presence of antibodies in the colostrum and serum preparations was assessed by standard bacterial agglutination and indirect immunofluorescent antibody procedures (Weir, 1965 a). Somatic (O) and flagellar (H) antigen preparations of *S. typhimurium* were produced for use in the two antibody assay systems according to the method described by Weir (1965 b).

The indirect immunofluorescent antibody staining tests were carried out using Behringwerke fluorescein isothiocyanate conjugated rabbit antihuman serum immunoglobulin antisera previously tested by immunoelectrophoresis against normal human serum to ensure class specificity.

The pooled control colostrum had previously been shown to possess agglutinating antibody of the IgA class directed against 48 out of 52 aerobic intestinal bacteria tested (Parkin *et al.*, 1973). In this study the bacterial agglutination activity of the control pool and individual test colostrum samples was assessed using the following heat-killed organisms, which provided a range of bacterial somatic antigens (Edwards and Ewing, 1972): *S. typhimurium* (phage type 465), *S. bovismorbificans*, *S. newport*, *S. kentucky*, *S. paratyphi C*, and *E. coli*.

Bactericidal action of doubling dilutions of test colostrum in the presence of excess preserved guinea-pig complement (Burroughs Wellcome, Beckenham, Kent) or fresh human jejunal juice was assessed by *S. typhimurium* (phage type 465) growth in glucose broth tubes containing phenol red indicator and by subsequent plating of the inoculum on to blood agar plates after 18 hours of incubation at 37°C.

Results

Bacterial Agglutination.—The agglutination titres of test and control colostrum and sera for *S. typhimurium* somatic and flagellar antigens as well as those for control bacteria are shown in table I. Colostrum from all and serum from two of the three patients were shown to possess agglutinins directed specifically against both O and H antigens of the infecting organism. No specific agglutinins to other salmonellae were shown in these women and the pooled control colostrum was negative in this regard. In each of the patients the titre of colostrum anti-*S. typhimurium* agglutinating antibody exceeded that of serum. In addition colostrum antibodies directed against both *E. coli* somatotypes were shown in each of the salmonella-infected mothers, while serum collected on day +1 from cases 1 and 2 failed to agglutinate either of these bacteria. Bacterial agglutination assays performed on subsequent samples of serum indicated the disappearance of *S. typhimurium* agglutinating antibody and the emergence of agglutinins reacting against *E. coli* O1 in cases 2 and 3 and *E. coli* O11 in case 2.

Antibody Class.—The classes of colostrum and serum antibodies directed against the O and H antigens of *S. typhimurium* in the infected mothers as assessed by indirect immunofluorescent staining are shown in table II. The colostrum antibodies were principally IgA. In case 2, however, traces of IgG and IgM antibodies to test organism somatic and flagellar antigens were detected at a colostrum dilution of 128. Serum antibody activity was mainly confined to the

TABLE I—*Bacterial Agglutination by Colostrum and Serum Antibodies*

Case No.	Day of Infection (Delivery = Day 0)	Sample	Day	Agglutinin Titre shown with Stated Antigen				
				S. typhimurium		*E. coli* 0 1	*E. coli* 0 11	Control Salmonellae*
				O	H			
1	−168	Colostrum	+ 1	128	160	32	128	0
		Serum	+ 1	32	60	0	0	0
		Serum	+ 90	0	0	0	0	0
2	−146	Colostrum	+ 1	512	320	256	64	0
		Serum	+ 1	256	20	0	0	0
		Serum	+104	0	0	256	32	0
3	−105	Colostrum	+ 1	128	128	128	128	0
		Serum	+155	0	0	128	0	0
Control pool		Colostrum	+ 1	0	0	128	16	0

*See text.

TABLE II—*Determination of Colostrum and Serum Antibody Class by Indirect Immunofluorescent Staining*

Case No.	Day of Infection (Delivery = Day 0)	Sample*	Day	Antibodies to *S. typhimurium*					
				O Antigen			H Antigen		
				IgA	IgG	IgM	IgA	IgG	IgM
1	−168	Colostrum	+ 1	+++	0	0	+++	0	0
		Serum	+ 1	0	+	0	0	+	0
		Serum	+ 90	0	0	0	0	0	0
2	−146	Colostrum	+ 1	+++	±	±	+++	0	0
		Serum	+ 1	0	+	+	0	±	0
		Serum	+104	0	0	0	0	0	0
3	−105	Colostrum	+ 1	+	0	0	+	0	0
		Serum	+155	0	0	0	0	0	0
Control pool		Colostrum	+ 1	0	0	0	0	0	0

*Antibodies in test colostrum were detected at a dilution of 1/128.

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IgG class, though day +1 serum from case 2 also contained IgM activity against *S. typhimurium* somatic antigen. No antibody against the infective organism could be detected in the control colostrum pool by indirect immunofluorescence at dilutions of from 4 to 128. In general the results of the two immunological assay procedures were comparable in that antibody activity was consistently present in samples from the patients, while the control colostrum pool was shown to be lacking in specific *S. typhimurium* antibody activity by both methods, though its activity against a wide range of enteric bacteria had previously been demonstrated.

Bactericidal Activity.—No bactericidal or bacteriostatic effects were noted when *S. typhimurium* organisms were cultured in the presence of test colostrum with specific antibody activity and either guinea-pig complement or fresh jejunal juice. Bacterial growth in the glucose broth culture tubes progressed unimpaired regardless of the colostrum dilution used. This finding was confirmed by the observation of subsequent bacterial colony formation on blood agar plates.

Discussion

The results of these investigations clearly show that specific antibodies are present in colostrum after gastrointestinal infection with *S. typhimurium*. These findings are in general agreement with those of many other workers who have shown antibody to a great range of micro-organisms present in colostrum (Shearman *et al.*, 1972; Goldman and Smith, 1973) and are closely related to those of Schubert and Grünberg (1949).

Failure to detect raised levels of antibody in test colostrum or sera against salmonellae other than the infecting strain indicates a specificity of production. The presence of antibodies to both *E. coli* strains in test and control colostrum may reflect immunological experience independent of salmonellosis. On the other hand, the presence of these antibodies in the infected mothers may indicate immunization by enteric *E. coli* after salmonella-mediated damage to the mucosal barrier of the gut or the non-specific increase of antibacterial antibody levels after *S. typhimurium* infection.

The clearly illustrated difference in the distribution of colostrum and serum antibodies between the major classes of immunoglobulins support the Tomasi and Bienenstock (1968) concept of local secretory immunoglobulin production. It also supports current views indicating that the kinetics and control of colostrum and serum antibody production may differ considerably.

The mechanism through which the biological function of secretory IgA is mediated remains not clear. Zipursky *et al.* (1973) have shown that 11S human secretory IgA lacks opsonization potential. On the other hand, Anderson (1972) suggested that in piglets passively acquired colostrum antibodies combine with injected antigen to form eosinotactic complexes, thus resulting in phagocytic removal of the antigen. Attention has been drawn to the fact that though IgA haemagglutinins are unable to lyse red cells in the presence of complement (Adinolfi *et al.*, 1966 a) it has been suggested that colostrum IgA can lyse *E. coli* in the presence of lysozyme and a heat-labile serum factor (Adinolfi *et al.*, 1966 b; Burdon, 1973). This effect, however, has not been shown in the rabbit system (Eddie *et al.*, 1971) and our results fail to extend these findings, since in the presence of colostrum antibody and complement or jejunal juice *S. typhimurium* growth was unimpeded.

In conclusion these studies clearly show the production of specific colostrum antibody in women after natural gastrointestinal infection with a defined organism. This observation strongly suggests a possible link between maternal gastrointestinal infection and the transmission of specific potentially protective passive immunity to the neonate. In addition, these findings raise the important question: How is a gastrointestinal immunological experience transmitted to the breast? The presence of specific IgA antibody in breast secretions after gastrointestinal bacterial infection may be the result of localized immunoglobulin production in breast tissue; alternatively it is possible that circulating antibodies are selectively concentrated before export by an as yet unidentified mechanism. Assuming that specific colostrum antibody is locally produced one is forced to postulate either that antigen finds its way from the gut to immunoglobulin producing precursor cells in the breast or that specifically sensitized lymphocytes home to that organ. These possibilities and their underlying mechanisms remain open to further investigation.

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