Complement Components of Paired Mother-Cord Sera.* (26731)

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It is well established that the human is particularly susceptible to a variety of infectious agents during the newborn period. The diminished complement activity of cord serum, compared with that in the maternal circulation(1,2,3,4), may emphasize the importance of this substance as a nonspecific humoral factor in resistance(5).

The present work represents an attempt to define the factors which may play a role in the reduced total hemolytic complement activity of cord serum. A study was made of the major complement components (C'1, C'2, C'3, C'4) present in mother and cord sera. The latter were also tested for the possible presence of anticomplementary substances.

Materials and methods. Blood samples were obtained from 16 pairs of mother and offspring at time of delivery. All were normal, full-term, single birth pregnancies. Birth weights ranged from 2,450-3,820 grams; no cases of fetal distress or perinatal complication were observed. Mothers' ages ranged from 16-36 years; anesthesia, when used, was local. Two samples of blood from the umbilical cord and one from a maternal vein were collected and allowed to clot at room temperature for one hour. The maternal and one cord (Ca) specimens were refrigerated 3-4 hours; the second cord specimen (Cb) for 8-23 hours. The sera were separated, frozen and stored at –20°C until tested. Classical "R" reagents for titrating complement components were prepared from pooled fresh human sera by the method described by Wedgwood(6). All reagents were prepared at the same time, frozen and stored at –20°C in aliquots suitable for individual titrations. Complement activity of each aliquot was determined on the day of component titration with no significant variation in the titers of the reagents noted. In attempting to separate complement components of guinea pig serum by continuous flow paper electrophoresis, it was found that none of the separated fractions would lyse sensitized sheep erythrocytes. Hemolytic activity was restored to those fractions in the α-β region following addition of heated serum, R3, but not R4. Therefore, C'4 appeared to be the missing factor. These findings were independent of buffer (veronal, phosphate, borate), pH (7.4-8.6), ionic strength (0.02-0.07). An ionic strength of 0.05 was found to be less deleterious to complement than 0.02. We could not elute C'4 from the curtain. Further testing of the fractions with R1 and R2 indicated that C'1, C'2, and C'3 migrated as a peak in the α-β globulin region. An R4 reagent prepared in this manner appeared to be more specific than the classical ammonia-treated material, for C'4 was completely undetectable within the peak of the other 3 components. The same phenomenon occurred with human serum, but recovery because of dilution was low. Therefore, the last 4 paired sera were tested with the electrophoretically prepared guinea pig R4. Hemolytic complement titers were expressed as the reciprocal of the dilution required to lyse 50% of a standardized sheep erythrocyte suspension in a reaction volume of 5.0 ml. This technic has been described by Taliaferro and Taliaferro(7) for titration of hemolysin. All serum constituents were placed in an ice bath until admixture was complete, sensitized cells added, the tubes centrifuged following incubation at 37°C for one hour, and the hemoglobin concentration of the supernatant portion measured by a Klett-Summerson colorimeter. The 50% endpoint was determined by the method of Taliaferro and Taliaferro (7). Total protein, based on a modification of the Folin-Ciocalteu method(8,9), and electrophoretic patterns (Spinco Model R and Analytrol) were determined when sufficient serum was available. Concentration of the various serum fractions was calculated by division of the analytrol graph first at the

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TABLE I. Comparison of Total and Component Activities of Complement Obtained from Maternal and Corresponding Cord Sera.

Source*	Time between collection and removal of serum (hr)	Time stored at −20°C (days)	Hemolytic complement (C'H50 units/ml)				
			Total	C'1	C'2	C'3	C'4
M 1	4		120	315	144	190	
Ca	4	35	57	120	60	79	
Cb	8		52	112	55	100	
M 2	4		102	345	87	144	
Ca	5	35	40				
Cb	23		31				
M 3	5		59	120	32	83	>900
Ca	5	36	37	79	<19	79	>600
Cb	19		32	79	<19	63	>600
M 4	5		63	123	44	114	>900
Ca	5	36	32	74	<19	55	>600
Cb	19		25	66	30	46	>600
M 5	4		63	114	72	114	>800
Ca	4	42	29	50	10	50	>900
Cb	18		21				
M 6	4		76	181	60	125	>800
Ca	5	40	35	63	<15	63	>900
Cb	9		33				
M 7	4	35	63	144	17	100	>1600
Ca	4		25	44	16	42	> 900
M 8	4		100	44	104	144	>3200
Ca	4	57	42	250	87	79	>2400
Cb	7		45				
M 9	4		65	122	109	131	>3200
Ca	4	57	34	173	56	44	>2400
Cb	20		22				
M 10	4		72	131	79	125	>3200
Ca	4	50	58	330	83	89	>2400
Cb	22		44				
M 11	4		87	177	117	198	>3200
Ca	4	55	63	125	131	134	>2400
Cb	22		57				
M 12	4		89	104	138	100	>3200
Ca	4	47	38	83	81	87	>2400
Cb	22		32				
M 13	5	7	109				1500†
C 13	5		56				650†
M 14	5	6	93				870†
C 14	5		50				480†
M 15	5	2	109				1145†
C 15	5		60				575†
M 16	5	2	131				1470†
C 16	5		63				790†

* M, maternal serum; C, cord serum; Ca and Cb differ only in time of serum removal. Numbers indicate sets.

† Tested with electrophoretically prepared guinea pig reagent.

origin of the gamma-globulin curve, next at the base of the albumin peak, with the distance between these reference points again equally divided. Thus an arbitrary but relatively standard separation of the albumin and alpha-, beta-, and gamma-globulins was achieved. Possible anticomplementary activity of cord sera was determined in 2 ways: by delaying the removal of serum from one sample (Cb) and allowing it to stand for a longer period than Ca(1); secondly, in 3 cases, by addition of cord aliquots to both

homologous and heterologous maternal sera and testing the resultant mixture for total hemolytic activity.

Results. Total complement and component concentrations of each maternal serum were compared only to that of the corresponding umbilical cord sample. The results are shown in Table I. Maternal titers ranged from 1.2-2.6 times that of their infants with an average 2-fold difference (79.9 *vs* 40.8 units).

The titer of the first component (C'1) in 9 mothers varied from 1.2-3.3 times that of their infants; in 3 cases, tested on the same day, cord sera content was greater than maternal. Maternal C'2 activity ranged from 0.9 to approximately 7 times that of the infant. The third component (C'3) of maternal serum was, without exception, more active (1.1-3.0) than that of cord sera.

In testing the first 12 pairs of sera for the fourth component (C'4), a 50% endpoint was not determined; the highest dilution used in each case produced more than 50% hemolysis. For this reason, the 100% hemolysis endpoints are given (Table I). It was observed that total lysis occurred in the maternal samples to a higher dilution than with the cord. The last 4 pairs were tested with the guinea pig reagent. The maternal 50% hemolysis titers were higher (1.8-2.3) than the infants. The titers with human R4 were greater than those found with the guinea pig reagent. This could be due to incomplete compatability with the latter material, although Ecker(10) states that apparently all components of human and guinea pig sera are mutually interchangeable. However, the ratios within each pair of sera were reproducible and probably represent valid differences.

Results of the tests for anticomplementary activity of cord sera are shown in Table I. The Cb specimen of each set, allowed to stand for a longer period of time between collection and separation of the serum, appeared to lose complement activity in almost every case. In the one exception, the titers were approximately the same. This is in contrast to the observations of Nattan-Larrier *et al.*(1) who proposed a decrease in an anticomplementary substance with the resultant increase in titer if these procedures were followed. Furthermore, it appeared (Table II) that mixing equal volumes of cord and maternal sera resulted in a complement titer only slightly less than that expected. This would indicate that anticomplementary activity of fetal serum is not a major factor in the lower titers observed in these sera relative to the corresponding maternal sera.

Protein concentration and electrophoretic distribution of the various fractions was determined on 7 pairs of sera. The results are shown in Table III. Total protein varied from 6.6-9.0 g % in maternal serum, and from 6.4-7.9 g % in the umbilical cord specimens. No constant maternal/cord ratio was apparent. In general, the concentration of albumin was greater in cord serum, whereas the reverse situation occurred with the alpha- and beta-globulins. The concentration of gamma-globulin was variable with inconstant differences between the pairs of sera.

Discussion. The lower total hemolytic complement activity of cord serum is thought by some(4) to be a reflection of incomplete equilibration by placental transfer. Others (1) suggest the level is influenced by the presence of anticomplementary substances which mask the full activity of cord serum. However, the possibility of complement synthesis by the fetus has not been ruled out. The results of the present studies, although not warranting definite conclusions on either passage or synthesis, tend to support the former concept. In terms of total activity, ratios of cord to maternal sera were consistently similar. Within paired specimens, the ratio of individual cord complement components to corresponding maternal com-

TABLE II. Total Complement Hemolytic Activity of Equal Volume Mixtures of Maternal and Cord Sera.

Specimens	Complement titer (C'H50 units/ml)			
	Cord	Maternal	Mixture	Expected
C 14 + M 13	50	109	79.0	79.5
C 14 + M 14	50	93	66.0	71.5
C 15 + M 13	60	109	75.5	84.5
C 15 + M 14	60	93	72.0	76.5
C 16 + M 16	63	131	70.5	95.5

TABLE III. Total Protein and Electrophoretic Analyses of Maternal and Cord Sera.

Sera	Total protein (g Folin Pro-Sol* equiv./100 ml)	Albumin		α-Globulin		β-Globulin		γ-Globulin	
		%†	g %	%	g %	%	g %	%	g %
M 3	7.8	56	4.36	15.7	1.23	16.8	1.31	11.5	.90
C 3	7.9	76	6.00	4.3	.34	7.9	.63	11.8	.93
M 5	7.7	53	4.16	10.3	.79	23.8	1.83	12.9	.99
C 5	6.4	78	5.00	5.3	.34	7.0	.48	9.7	.62
M 6	7.4	49	3.62	16.5	1.22	22.5	1.66	12.0	.89
C 6	7.4	61	4.46	10.8	.80	13.1	.97	15.6	1.15
M 7	8.8	42	3.70	18.5	1.62	21.6	1.90	17.9	1.57
C 7	6.7	55	3.68	8.9	.60	12.9	.87	23.6	1.58
M 10	8.4	52	4.40	15.4	1.29	18.6	1.56	13.5	1.13
C 10	7.5	66	4.81	8.6	.64	11.5	.86	14.9	1.11
M 11	6.6	34	2.24	23.0	1.52	30.0	1.98	13.0	.86
C 11	7.2	61	4.39	9.4	.67	12.2	.88	17.4	1.25
M 12	9.0	45	4.05	15.0	1.35	22.4	2.02	17.6	1.58
C 12	6.2	65	4.03	7.3	.45	8.4	.52	18.3	1.13

* Pro-Sol—standard protein solution obtained from Bernhard-Scher Co., New Jersey.
† % obtained from analytrol (see text).

ponents corresponded to the ratio of total activity. If the titer of cord blood represented the activity of components synthesized, it seems likely that the component ratio would exhibit greater variation than that found with one or more of the constituents possibly affected. To our knowledge, these represent the first attempts to examine the decreased level of cord complement activity in terms of component parts.

Although the present data do not support the suggestion that full complement activity of cord serum is reduced by the presence of anticomplementary material, such a possibility cannot be ruled out. Delaying the removal of serum from the clot may actually have reduced such a substance as described by Nattan-Larrier *et al.*(1). If so, inactivation of complement under the conditions of our experiments must have occurred at a greater rate, since Cb titers (Table I) were less than Ca. In addition, the failure of cord serum to reduce the complement level of maternal serum (Table II) could result from complete utilization of an inactivating substance by the cord specimen, leaving none to be active on maternal blood. However, the relatively consistent differences in the paired sera suggest that factors other than anticomplementary activity determine the level of complement in cord sera.

Consistent differences between mother and cord serum specimens were also noted in their electrophoretic patterns (Table III). These observations are in complete agreement with those of Moore *et al.*(11). The major variation appeared in the alpha- and beta-globulin region with the maternal samples containing a higher concentration of both fractions. The migration of complement within this region and the lower activity of cord sera may be related to the deficiencies in concentration of the alpha- and beta-globulins in cord serum. The fact that the fetal liver and placenta can produce these proteins(12), lends indirect evidence supporting the concept of complement synthesis. More study is necessary before a final conclusion concerning the origin of this substance in cord blood can be made.

Summary. Complement hemolytic activities of sera, obtained from umbilical cords at birth, were compared to those obtained from the corresponding mothers. The lower total activity of the cord specimens was correlated with a parallel decrease in all components. Anticomplementary substances could not be detected in cord samples. The reduced concentration of complement components in cord sera was correlated with reduced levels of alpha- and beta-globulins as determined by paper electrophoresis. It may be more than coincidental that 3 of the 4 components also migrate in this region.

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