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STUDIES ON NEONATAL INFANTS AND OLDER CHILDREN**

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THE FORMATION OF MACROGLOBULIN ANTIBODIES. II. STUDIES ON NEONATAL INFANTS AND OLDER CHILDREN *

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In another publication (1), the molecular size of antibodies formed in response to immunization with typhoid-paratyphoid (TPT) vaccine was studied in adult humans. In this report premature infants, immunized shortly after birth, were studied in a similar manner in order to compare this aspect of the immune response in infants and adults. It has been shown (2) that the initial antibodies produced in response to TPT immunization are 19S macroglobulins. The sequence of formation of macroglobulin antibody, followed by 7S antibody, has also been observed by Taliaferro, Talmadge, Freter and Stelos (3-7), who showed that in rabbits immunized with sheep or beef red cells the first antibodies formed had an electrophoretic mobility of the γ -1 type and had a sedimentation coefficient of 18S. These were followed, in time, by 6S antibodies associated with the γ -2 globulins. Molecules of antibodies of different size in rabbits, produced in response to single protein antigens, have recently been reported (8).

We were also interested in determining the effect of maternally-transferred antibody on the formation by infants of agglutinins to TPT antigens. In addition, the effect of sulfhydryl compounds on the macroglobulin antibodies produced by the premature infant was investigated. It has been suggested that the agglutinin activity of 19S typhoid H antibody of the neonatal infant differs qualitatively from that of the adult after treatment with 2-mercaptoethanol (9).

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MATERIALS AND METHODS

Forty-two premature infants were studied. Preimmunization blood was obtained from the umbilical cord or from the infant immediately before immunization. In a number of cases both were obtained. Infants were immunized by intramuscular injection into the anterior thigh of 0.25 ml of a TPT A and B vaccine (Lederle) containing 1 billion organisms of *Salmonella typhi* per ml and 250 million organisms of *S. paratyphi* A and B per ml. Thirty-one infants received three injections 1 week apart; eleven received only one injection. The first injection was given within 48 hours after birth and in most instances within the first 24 hours. The first blood sample was obtained 1 week after the third injection in most cases. Additional blood samples were obtained before and after booster injections given at varying times after the initial course of immunization.

Fourteen older children, aged 9 months to 8 years, hospitalized for orthopedic rehabilitation or convalescing from primary pulmonary tuberculosis were immunized with three injections of 0.5 ml of TPT vaccine given intramuscularly, 1 week apart. Sera were obtained before immunization and at various times after the third injection.

Blood samples were allowed to clot at 37° C and the sera removed. Each serum was diluted with an equal volume of distilled water and dialyzed for 2 days against 0.01 M phosphate buffer at pH 8.5. After dialysis, sera were stored at -20° C. The dialyzed sera, usually 1 to 3 ml, were fractionated by column chromatography on diethylaminoethyl (DEAE)-cellulose as previously reported (10, 11). Proteins were eluted with phosphate buffers of increasing concentration and decreasing pH.

Four chromatographic fractions were obtained. The first and second, obtained at pH 7, 0.01 M and 0.025 M, respectively, contained the bulk of the γ -globulin which was entirely of the 7S variety. The third contained most of the albumin and the α - and β -globulins. In the last peak were the 19S γ -globulins in addition to other proteins, with some contamination by 7S γ -globulin. There was almost complete separation of the 7S and 19S γ -globulin, however, and they could be tested for individually.

The sera and chromatographic fractions were tested for agglutinating antibody by the standard serial dilution

TABLE I
Mean agglutinin titers after primary typhoid-paratyphoid (TPT) immunization
of 31 premature infants

Age	Infants	Typhoid O		Typhoid H		Paratyphoid A		Paratyphoid B	
		7S	19S	7S	19S	7S	19S	7S	19S
<i>wks</i>	<i>no.</i>								
3.1 (2-5)	31	0	3 (0-40)	1 (0-20)	323 (10-2,560)	12 (0-40)	490 (40-2,560)	24 (0-160)	523 (20-5,120)
8.0 (6-10)	14	0	0	17 (0-80)	61 (0-160)	49 (0-160)	65 (0-320)	90 (0-320)	47 (0-160)
13.0 (11-15)	10	0	0	3 (0-10)	11 (0-20)	56 (0-160)	11 (0-20)	91 (10-160)	5 (0-20)
18.4 (16-20)	8	0	0	3 (0-10)	4 (0-20)	48 (20-80)	1 (0-10)	130 (40-320)	3 (0-10)

method (12), starting with a dilution of 1:10. Tests were performed for antibody to each of the four antigens administered, *S. typhi* O and H, and *S. paratyphi* A and B.¹ The antigens were used at a 1:10 dilution. Tubes were shaken, incubated at 37° C for 2 hours, and placed in the refrigerator overnight. The titer was recorded as the highest dilution showing definite agglutination.

In order to determine the effect of sulfhydryl agents, the pertinent chromatographic fractions from each of a number of sera with high agglutination titers were tested in duplicate. In one set of tubes, antibody and antigen were diluted in the usual manner in phosphate-buffered saline, pH 7.0. In another set, 2-mercaptoethanol (ME) was added to the phosphate-buffered saline used in the dilution of antibody and antigens. In most cases the final concentration of ME was 0.05 M. The two sets of tubes were treated identically and, except for the ME, the method of testing was the same as that otherwise used.

In addition, one serum was treated with ME under diverse conditions and tested simultaneously with controls lacking ME as follows. 1) A sample of undiluted test serum was added to an equal amount of 0.1 M ME and the mixture allowed to stand at 4° C for 24 hours. Agglutination tests were then performed after dilution of antibody and antigen: a) in phosphate-buffered saline,

and b) in phosphate-buffered saline after the initial 1:10 dilution was made in 0.05 M ME. 2) A sample of undiluted test serum and serum diluted 1:5 with buffered saline were mixed with an equal volume of 0.05 M ME and dialyzed for 24 hours against 0.05 M ME. Agglutination tests were then carried out after serial dilution of the serum in the saline buffer.

RESULTS

The antibody response obtained in a group of premature infants with no preimmunization titer is seen in Table I. One week after the third injection, when the infants were about 3 weeks old, virtually the entire agglutinating titer was found in the 19S antibody fraction. After the initial rise there was a slow fall in titer, which was accompanied by a change in the type of antibody present from primarily 19S to the 7S type. This change occurred most rapidly with paratyphoid B and least rapidly with typhoid H.

Nineteen of the 43 preimmunization sera (usually cord blood) contained agglutinins to one or more of the antigens. In none of the infants was maternally-transferred antibody to typhoid O ob-

¹ Supplied by the Texas State Department of Health Laboratories, Austin, Tex.

TABLE II
Inhibition of antibody response by maternally-transferred antibody

Age	Typhoid O		Typhoid H		Paratyphoid A		Paratyphoid B	
	7S	19S	7S	19S	7S	19S	7S	19S
<i>wks</i>								
0 (cord blood)	0	0	20	0	20	0	0	0
3	0	0	20	0	40	0	40	320
13	0	0	0	0	10	0	80	0
Booster immunization at 13 weeks								
17	0	0	0	0	20	0	160	0

TABLE III
Mean agglutinin titers in 16 infants before and after booster immunization with TPT

	Typhoid O		Typhoid H		Paratyphoid A		Paratyphoid B	
	7S	19S	7S	19S	7S	19S	7S	19S
<i>wks</i>								
Prebooster; average age 16.7 (6-24)	0	0	11 (0-40)	7 (0-20)	83 (0-320)	2 (0-10)	150 (0-320)	7 (0-40)
Postbooster; average age 21.5 (13-29)	0	1 (0-20)	77 (0-320)	25 (0-80)	127 (40-320)	5 (0-20)	342 (40-1,280)	6 (0-40)

served. Maternally-transferred antibodies to other antigens, however, were in all cases of the 7S variety.

In addition, the presence of maternally-transferred antibody appeared to inhibit active antibody synthesis, but only in the case of the specific antigen-antibody system involved. Where preimmunization antibodies were present, they were usually for typhoid H and paratyphoid A or for typhoid H alone. Maternal paratyphoid B antibody was found in only one infant, occurring simultaneously with antibody to typhoid H and paratyphoid A. A pattern typical of infants who had antibody before immunization is shown in Table II. Typhoid H and paratyphoid A agglutinins were present in this serum before immunization. The results indicate that after immunization there was no significant antibody formation in response to typhoid H and paratyphoid A, for which maternally-transferred antibody was present. Where no transferred antibody was present, as in the case of paratyphoid B, high titers of antibody resulted. The antibody formed in this instance was predominantly the 19S type at 3 weeks of age. The failure of antibody to typhoid O to appear in this infant is not unusual, since

only one out of every four immunized infants responded with a measurable titer for this antigen at any time. When typhoid O agglutinins were present they were always of the 19S type, as observed in adults (1), and their titers were always low.

By 13 weeks of age, the titer of maternally-transferred antibody to typhoid H and paratyphoid A had decreased. The 19S paratyphoid B antibody had dropped to titers below 1:10, while the 7S paratyphoid B was essentially unchanged from the level at 3 weeks (one-tube changes were not thought to be significant). The inhibition by maternally-transferred antibody of active antibody synthesis persisted even after a booster immunization given at 13 weeks. This was most clearly demonstrated in the case of typhoid H where there was no detectable antibody present 4 weeks after the booster was given, in spite of a negative prebooster titer. The titer to paratyphoid A also showed no rise. The failure of a significant secondary response to paratyphoid B was similar to that seen in other cases where the prebooster agglutinin titer was rather high.

In the 19 infants with maternally-transferred antibody, this antibody was present 28 times for

TABLE IV
Mean agglutinin titers after primary TPT immunization of 14 older infants and children

Av. time after initial immuniz.	Typhoid O		Typhoid H		Paratyphoid A		Paratyphoid B	
	7S	19S	7S	19S	7S	19S	7S	19S
<i>wks</i>								
3.3 (3-5)	0	26 (0-160)	1 (0-10)	226 (40-640)	12 (0-40)	260 (40-640)	28 (0-160)	234 (80-640)
12.0 (11-17)	0	10 (0-40)	1 (0-10)	66 (10-160)	19 (0-40)	57 (20-160)	29 (0-80)	31 (10-80)

TABLE V
*Effect of 2-mercaptoethanol (ME) on antibody titers in 16 premature infants **

	Typhoid O		Typhoid H		Paratyphoid A		Paratyphoid B	
	7S	19S	7S	19S	7S	19S	7S	19S
Without 2-ME	0	4 (0-20)	4 (0-20)	427 (40-2,560)	39 (0-160)	565 (80-2,560)	77 (0-320)	575 (80-2,560)
With 2-ME	0	0	4 (0-20)	10 (0-40)	21 (0-80)	23 (10-80)	41 (0-160)	24 (10-80)

* Tests run in duplicate. ME was added to the phosphate-buffered saline used to dilute antibody and antigens so that the final concentration was 0.05 M.

one of the antigens, typhoid H or paratyphoid A or B. In 17 of the 28 times, immunization was followed by no increase in the serum titer, and no 19S antibody appeared. However, in the other 11 times, low titers of antibody (1:10 or 1:20) were found in the 19S fraction after immunization; but there was no rise in the titer of the unfractionated serum. These low 19S titers disappeared rapidly and did not recur after booster immunization.

The effect of booster injection on infants 6 to 24 weeks old is illustrated in Table III. For each antigen except typhoid O, no significant increase in the 19S antibody titers was observed. On the other hand, after booster immunization, increased levels of antibodies for paratyphoids A and B and typhoid H were associated with the 7S fraction.

The results obtained after initial immunization of a group of 14 older children between 9 months and 8 years of age are shown in Table IV. Sera

obtained before immunization contained agglutinins for typhoid H in low titer in 9 of the 14 patients. Agglutinins to the other three antigens were seen less frequently. Immune sera were drawn 1 week after the third TPT injection and a second postimmunization serum sample was obtained about 9 weeks later. Comparison of the results obtained in neonatal infants with those in the older children indicates that at about 12 to 13 weeks after initial immunization, the neonates showed a striking change from 19S to 7S paratyphoid A and B agglutinins (Table I). The older children (Table IV) showed a much lower proportion of the total antibody activity in the 7S fraction. This difference between neonates and older children was not apparent in the case of the typhoid H agglutinins, although there was a sharper decline in the amount of typhoid H 19S agglutinin in the neonatal infants.

The effect of 2-mercaptoethanol on antibody

TABLE VI
Effect of 2-mercaptoethanol on 19S γ -globulin (Patient Wi)

Test conditions	Typh. H	PTyph. A	PTyph. B
A. Routine test; no ME	1,280	1,280	2,560
B. ME added to buf. saline used to dilute antigens and antibody; final conc. 0.05 M	10	10	10
C. 0.25 ml serum + 0.25 ml 0.1 M ME; left 24 hrs at 4° C; diluted to 2.5 ml with buf. saline; buf. saline used for dilutions of antigens and antibody	40	20	40
D. Same as C except dilution to 2.5 ml done with 0.05 M ME	20	20	20
E. 0.5 ml 1:5 diluted (buf. saline) serum + 0.5 ml 0.05 M ME; dialyzed 24 hrs against 0.05 M ME; dilutions of antigens and antibody done in buf. saline	20	20	20
F. Same as E except used 0.5 ml of undiluted serum	20	20	20

activities is shown in Table V. There was little effect on the 7S antibody present, the slight fall in average titer being within experimental error. However, a striking decrease in titers with almost complete disappearance of agglutinating activity was observed after treatment of the 19S fraction with 0.05 M ME. One serum (Wi), obtained 1 week after the third injection of the primary series, was tested with and without ME under multiple test conditions. All the antibody for which we tested was of the 19S type. The test conditions and reciprocal agglutinin titers are shown in Table VI. Under all test conditions there was a fall in agglutinating activity to very low levels.

DISCUSSION

The failure of macroglobulin antibodies such as typhoid O to traverse the placenta in significant quantity has been documented (2, 13). No decrease in the infant:mother ratio of total γ -globulin, predominantly 7S in type, has been noted. In fact, the data available suggest that the infant has a slightly higher average serum concentration of γ -globulin than has the mother (14, 15). Thus it appears that 7S protein traverses the placenta and that the protection provided the newborn by antibody passively transferred from the mother would vary according to whether the maternal antibody is predominantly 7S or 19S. Antibody to the O antigen of *Escherichia coli* 0111 appears to pass the placental barrier poorly (16). If this, like antibody to typhoid O, is a 19S macroglobulin, it may well be a characteristic of antibodies to somatic coliform antigens.

The concentration of γ -globulin in the serum of infants gradually falls during the first few weeks of life (14, 17, 18), but there is some question as to whether active γ -globulin synthesis takes place during this period. Although no measurements of total γ -globulin were made in the infants studied, there is no doubt that they were able to produce antibodies in response to the antigens of TPT vaccine. Measurable titers were found in most newborn infants tested 1 week after the first injection, i.e., when the infants were 1 week old. Smith (19) found similar results with typhoid H antigen. Others have observed good response in young infants to different antigens (20-22), although in some cases the response seemed inferior

to that seen in older children. Thus, although the serum concentration of γ -globulin falls in the first weeks of life, the infant is capable of response to multiple antigens.

The presence of maternally-transferred antibody appeared to prevent active antibody synthesis to the specific antigen without apparent interference with the other antigen-antibody systems tested. Thus, in the patient shown in Table II, a passively acquired titer of 1:20 for typhoid H inhibited active antibody formation in response to the antigen, while at the same time a high titer was produced to paratyphoid B antigen for which there was no preimmunization titer. This inhibition seemed to persist after a booster immunization given at 13 weeks, at a time when the typhoid H titer had fallen below measurable levels. Since the passively transferred antibody was always of the 7S type and initially the actively formed antibody was predominantly 19S, it may be concluded that the low molecular weight (7S) antibody globulin inhibited synthesis not only of the 7S antibody but also of the (19S) antibody.

The inhibitory effect of maternal antibody has previously been demonstrated with poliomyelitis (23) and diphtheria-pertussis-tetanus immunization (24). The duration of the inhibition in the present study has not been determined, but active antibody formation was not observed in infants with passively acquired antibody even after booster injection. The latest postbooster serum studied was obtained at 29 weeks of age in an infant who had maternal antibody to typhoid H and paratyphoid A antigens. At the time of boosting, these antibodies had decreased to non-measurable levels, yet there was no antibody response. In the same individual, antibody was produced, both on initial immunization and after booster, to paratyphoid B for which there were no measurable passively transferred agglutinins. Thus, in some cases, inhibition of active synthesis could result in failure to produce protective antibody for several months after birth despite administration of the usual amounts of vaccine. A similar effect was observed by Uhr and Baumann (25) in guinea pigs injected with horse antitoxin at the same time as, or as long as 5 days after, primary immunization with diphtheria toxoid. Inhibition of antibody formation was found with the use of complexes formed in antibody excess (25).

These authors also demonstrated suppression of the anamnestic response to diphtheria toxoid by the administration of heterologous antitoxin and also a smaller suppression by homologous antitoxin (26). These observations in experimental animals may well explain the partial inhibition of TPT antibody formation after secondary immunization of infants who have high titers of antibody prior to the booster injection of vaccine.

Typhoid O agglutinins were exclusively 19S in all infants, older children, and adults (1) tested, whereas the other three antibodies became predominantly 7S to a greater or lesser extent with time. As yet no explanation for this difference is available. In infants, after primary immunization, 19S paratyphoid B agglutinins decreased rapidly, and 7S antibody became the predominant type so that by 3 months of age almost no 19S agglutinins were found. This change in antibody type occurred somewhat more slowly with paratyphoid A, but there was a much slower change in antibody type in the case of typhoid H antigen. The changes in adults occurred at a much less rapid rate (1).

Other studies (19, 27) indicate that the human newborn has few if any plasma cells in the bone marrow, the lamina propria of the ileum and appendix, or in lymph nodes proximal to sites of injection of typhoid-paratyphoid vaccine. However, the fact that antibody can be produced by the newborn during this period suggests that the plasma cell may not be its site of origin. It is possible that the striking differences in the rate of change from 19S to 7S antibody production observed between the infants and adults may well be due to antibody synthesis in different types of cells. The 7S antibodies produced in the infants studied appeared at an age when plasma cells can usually be seen. Since few or no 19S agglutinins were formed after booster immunization at 3 months, the cell type which produced antibody up to 3 weeks of age, and which may be the sole source of this particular antibody, may have declined in number to the point where antibody production can no longer be observed. No evidence is available to support this possibility.

In all groups studied, the agglutinin activity of the macroglobulin antibody was markedly diminished after exposure to ME under a variety of conditions. These results confirm the presence of

19S antibodies. The sensitivity of the 19S agglutinins to ME indicates that the dissociated 7S units of these macroglobulin antibodies have little or no agglutinin activity. The disparity of these results with those reported (19) has not been reconciled.

SUMMARY

1. Premature infants and older children produce high molecular weight antibodies (19S) in response to initial immunization with typhoid-paratyphoid vaccine.

2. Maternally-transferred antibody is always of the low molecular weight (7S) type and appears to inhibit active antibody synthesis for the specific antigen-antibody system involved. In some cases this inhibition persists for many weeks.

3. Typhoid O agglutinins are found only in the 19S fraction in both infants and older children, and no change in molecular size occurs with time or secondary immunization.

4. In premature infants the change from 19S to 7S antibody takes place rapidly. At an average age of 13 weeks paratyphoid A and B antibodies are predominantly 7S, while the changes in typhoid H antibody are somewhat slower. Booster immunization elicits almost exclusively 7S antibody.

5. The type of antibody formation in older children seems to resemble more closely that of the adult than that of the premature infant.

6. The agglutinating activity of 19S antibodies is eliminated by treatment with mercaptoethanol under a variety of conditions.

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