Medical PROGRESS

Refer to: Miller ME, Stiehm ER: Phagocytic, opsonic and immunoglobulin studies in newborns (Medical Progress). Calif Med 119:43-63, Aug 1973

Phagocytic, Opsonic and Immunoglobulin Studies in Newborns

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Despite improved diagnostic and therapeutic measures, neonatal septicemia continues to be a major clinical problem. Improvement in the management of the septic neonate should result from application of the increasing information being learned of the neonatal host defense mechanisms. The earlier concept that the neonate was "immunologically null" can now be discarded. Evidence summarized in this review has established that the neonate can marshal an immune response to most antigens and infectious agents.

A further major advance in recent years has been in understanding of the inflammatory response in the neonate, which includes such activities as chemotaxis, phagocytosis and bactericidal killing of ingested bacteria by phagocytes.

UNDERSTANDING OF THE MEANS by which the normal human neonate maintains resistance against micro-organisms has assumed great clinical significance in recent years. This reflects growing understanding of the functional status of neonatal host defense mechanisms. Contrary to earlier beliefs, it is now clear that the normal neonate is not "immunologically null" but has a variety of "specific" and "non-specific" mechanisms available for combatting foreign micro-organisms. In this review, we discuss the current understanding of three important aspects of neonatal host defenses — immunoglobulins, phagocytes and opsonins.

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Supported in part by USPHS Research Grant HD 06463 and AI 10689.

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First let us consider the development of the humoral immune response in the neonatal period, with particular emphasis on alterations of the immunoglobulins in health and disease.

Immunoglobulin Synthesis in the Fetus

In human fetuses synthesis of immunoglobulin M (IgM) has been detected as early as ten and a half weeks,[1] by tissue culture of fetal organs in the presence of radiolabelled amino acids followed by radioimmunoelectrophoresis, primarily in the spleen but in other lymphoid organs as well. IgG synthesis was detected at 12 weeks of gestation and IgA synthesis by 30 weeks.[1]

Van Furth et al[2] detected IgG- and IgM-

fluorescing cells in lymphoid tissue in human fetuses by 21 weeks. Lawton and coworkers found IgM, IgG and IgA surface determinants by immunofluorescence (B cells) in peripheral blood, bone marrow, liver and spleen by eleven and a half weeks.[3] By 14 weeks the distribution of immunoglobulin-staining cells is equivalent to that found in adult tissues. However, cells with cytoplasmic immunoglobulin (those synthesizing immunoglobulin) were not found in early fetuses and do not appear until the time when endogenous immunoglobulin synthesis is occurring.

Although IgG synthesis does not occur until at least 12 weeks, IgG can be detected in the serum of the fetus as early as 38 days, as the result of passive transfer from the mother.[1] Serum IgM is detectable occasionally as early as 17 weeks and is regularly detectable by 30 weeks. Although IgA may be present in the fetal serum in trace quantities as early as six and a half weeks of gestation, it presumably is of maternal origin; IgA may be present more frequently at this time than in the later weeks of gestation, since IgA synthesis is not usually established until close to or after the time of birth.

In sum, the precursor cells of immunoglobulin synthesis (B cells), the synthesizing cells (plasma cells), and the serum immunoglobulins are present in the human conceptus and fetus, and appear in the order IgM, IgG, and IgA, similar to the sequence of the appearance of antibody classes following antigenic stimulation.

Antibody Synthesis in the Newborn

Although the newborn infant has developed the cellular apparatus necessary for active antibody synthesis, it is for the most part dormant at birth. After stimulation, a sluggish response is often noted, particularly to certain antigens; further, the response is often inhibited by maternal transplacental antibody.

Natural Antibodies

Natural antibodies, (antibodies present without active immunization) notably the isoagglutinins, are not synthesized well by the newborn, presumably due to lack of intrauterine antigenic challenge.[4] IgG natural antibodies from the mother will of course be present as a result of transplacental passage. A few investigators have detected endogenously-synthesized natural antibodies in newborn sera. Thomaidis et al[5] found mercerpto-ethanol-sensitive isoagglutinins (and therefore presumably IgM) in 36 of 59 full-term infants, indicating some natural antibody production. Adinolfi[6] found an anti-I cold agglutinin in 14 of 23 cord sera specimens; this was an IgM antibody, indicating endogenous antibody production. Mellbye[7] noted an IgM antibody in all of 20 specimens of cord sera which agglutinated trypsinized-compatible human erythrocytes. Epstein et al[8] found that nearly all cord sera contained an IgM antibody which agglutinated erythrocytes coated with heated Bence-Jones proteins (light chain determinants). All of these IgM antibodies may occur as the result of intrauterine antigenic stimulation which leads to IgM synthesis and some degree of cross-reactivity with these specially-treated erythrocytes.

Inhibition by Maternal IgG Antibody

Transplacentally acquired maternal antibody has a pronounced inhibitory effect on active antibody formation in the newborn period.[9] For examples, infants with passively acquired diphtheria antitoxin or poliomyelitis antibodies have decidedly diminished response to diphtheria toxoid[10] or inactivated poliomyelitis vaccine[11] respectively. Newborn premature and term infants with high levels of passive flagellar agglutinins to Salmonella did not respond at all; infants with moderate titers responded suboptimally; infants without passive agglutinins responded as well as adults to Salmonella vaccine.[12] The specific immunosuppressive effect of maternal antibody is the rationale for delaying measles and rubella vaccination until after six to twelve months of life. For certain powerful antigens (tetanus, for example), the inhibition can be overcome by larger or more frequent immunizations or by the use of adjuvants.[9]

Response to Active Immunization

Administration of antigen to an immunologically immature subject has the potential risk of inducing immunologic tolerance (non-reactivity) rather than immunization. This is exceedingly rare in humans although Provenzano et al[13] noted that 21 of 22 infants given pertussis vaccine during the first 24 hours of life had inadequate response or none at all; further the response to reimmunization in 75 percent of these infants was inadequate up to the age of 15 months, suggesting some degree of tolerance.

Most studies indicate that the newborn responds to injected antigens but to a lesser degree

than older infants and adults. This is true even in the absence of passive maternal antibodies and with a variety of antigens including diphtheria toxoid,[14] inactivated poliomyelitis vaccine,[15] pertussis whole bacterial vaccine[16] and the O antigen of Salmonella typhosa.[12]

There are exceptions to this statement. Smith et al[12] found that the antibody response to the H antigen of Salmonella typhosa was equal in premature and term infants and that their responses were almost as good as in the adults. Osburn et al[17] found that antitoxin levels following the administration of absorbed tetanus toxoid to infants less than two weeks of age were not significantly different from those produced by older infants.

Even when the antibody response of the newborn is adequate, it may differ qualitatively from that of older infants and adults. Smith et al[12] showed that newborns given Salmonella vaccine have IgM flagellar agglutinins exclusively for 20 to 30 days, in contrast to older subjects who have IgM agglutinins during the first week, followed by the appearance of IgG agglutinins.

Extrauterine life stimulates the maturation of the immune system. Dancis et al[18] showed that only one of seven premature and one of eight term newborns responded to diphtheria toxoid; however, seven other prematures immunized at their estimated term birth date (and thus equivalent in age to the term newborns) all responded to diphtheria toxoid. Rothberg[19] showed that the antibody response of premature infants to oral bovine serum albumin was as good as that of term infants, indicating that the interval since birth is more important than the size or somatic maturation.

Inhibition by Breast Milk Antibodies

Maternal breast milk antibodies may inhibit antibody production to orally administered live poliovirus vaccine by inhibiting viral colonization of the gastrointestinal tract.[20] Colostrum may be especially effective, because of its higher immunoglobulin content. This effect may be particularly important in the developing countries, where breast feeding is generally prolonged.

Summary

The newborn infant has the ability to synthesize antibodies when stimulated by antigens, but the response generally is less vigorous than in older infants and adults. Further, the antibody response in newborns may differ qualitatively in prolongation of the IgM response. The age of the infant is better correlated with a strong antibody response than is the maturity of the infant, since the bacterial colonization of the gastrointestinal tract which occurs at the time of birth is the major stimulus for the maturation of the antibody system.

Passive Immunity in the Newborn

The infant passively receives IgG antibodies via the placenta; if breast-fed, he receives secretory antibodies (which are not absorbed) via the oral route. The newborn is protected by IgG maternal antibodies to those disorders in which immunity is associated with long-term persistence of circulating IgG antibody (such as measles, rubella, meningococcal infections, streptococcal disease and H. influenzae infection); some protection against certain other infections (such as vaccinia, varicella, pertussis, tetanus, diphtheria) may also result but, since high titers of protective IgG antibodies do not persist, only recently infected or immunized mothers provide protection to their infants. Certain maternal antibodies to antigens of Gram-negative organisms such as E. coli and Salmonella reside within the IgM class and do not cross the placenta, so that the infant does not receive all of the mother's antibodies to these organisms. Gitlin et al[21] postulated that this deficiency of IgM may be responsible, at least in part, for the unusual susceptibility of newborns to infection with Gram-negative organisms. However, there are IgG antibodies to somatic antigens of these Gram-negative organisms which do cross the placenta and provide sufficient protection for most infants.[22]

In some instances, it is a distinct advantage to have maternal IgM antibodies excluded from the infant; for example, the presence of maternal IgM isoagglutinins (natural anti-A and anti-B) in the infant would result in ABO hemolytic disease in every ABO incompatible maternal-newborn pair.

The passive secretory antibodies ingested by the breast-fed infant inhibit the growth of certain bacteria (for example, E. coli) and viruses (enteroviruses for example) in the gastrointestinal tract. This may have significant survival advantage, particularly in the developing countries. A decreased intake of breast milk may predispose to neonatal septicemia. Winberg and Wessner, in Sweden, noted this;[23] since the decreased intake occurred before the symptomatic period, it was not due simply to poor suckling by sick children.

Immunoglobulin Levels in Fetus, Newborn, and Neonatal Periods

Detailed study of the immunoglobulin levels in health and disease has been greatly facilitated by the development of the radial immunodiffusion method which permits accurate rapid quantitation of serum immunoglobulins from small quantities (less than 1 ml) of serum. Commercial immunodiffusion kits with agar plates and standards are available from several companies.*

IgG

IgG is present in the serum of the human embryo as early as 38 days of gestation despite the absence of IgG-synthesizing cells until three months.[24] IgG levels remain below 100 mg per 100 ml (5 to 10 percent of maternal level) until about 17 weeks of gestation, at which time the IgG increases gradually; at term (40 weeks) the fetal IgG level usually exceeds the maternal IgG level by 5 to 10 percent. Kohler and Farr[25] studied 46 maternal-cord paired specimens and noted a mean maternal IgG of 1,512 mg per 100 ml and a mean cord IgG of 1,260 mg per 100 ml. Gitlin described a rather abrupt onset of maternal-fetal transfer at 22 weeks, so that most fetuses of 25 to 40 weeks had IgG levels equivalent to the maternal level.[1,24] Other investigators,[26-29] including ourselves,[30] have demonstrated a gradual increase of fetal IgG levels, beginning at about 25 weeks; we recorded IgG levels at 26-27 weeks of 330±61 (mean±1 standard deviation), at 32-33 weeks 556±107 mg per 100 ml, at 36-37 weeks 823±135 mg per 100 ml and at term (40-41 weeks) 937±175 mg per 100 ml (Chart 1). This increase allows an estimation of gestational age by the cord blood IgG level. Nearly all of the cord IgG is of maternal origin; however, Martensson and Fudenberg,[31] by assaying genetic gamma-globulin (Gm) types, detected trace quantities of endogenously synthesized IgG in cord blood.

Gitlin has shown that the materno-fetal placental transfer of IgG is under the influence of two distinct mechanisms.[24,32] The first is a passive transfer, in which the fetal IgG level is proportional to the maternal IgG level. This mechanism undergoes maturation with increasing gestational age to permit increased amounts of IgG to cross the placenta with increasing fetal maturation. The second is an enzymatic mechanism which actively transfers IgG from mother to fetus. Because this enzymatic mechanism is inhibited at high maternal IgG levels and is increasingly activated at low maternal IgG levels, it acts as a normalizing influence on IgG levels; infants born to mothers

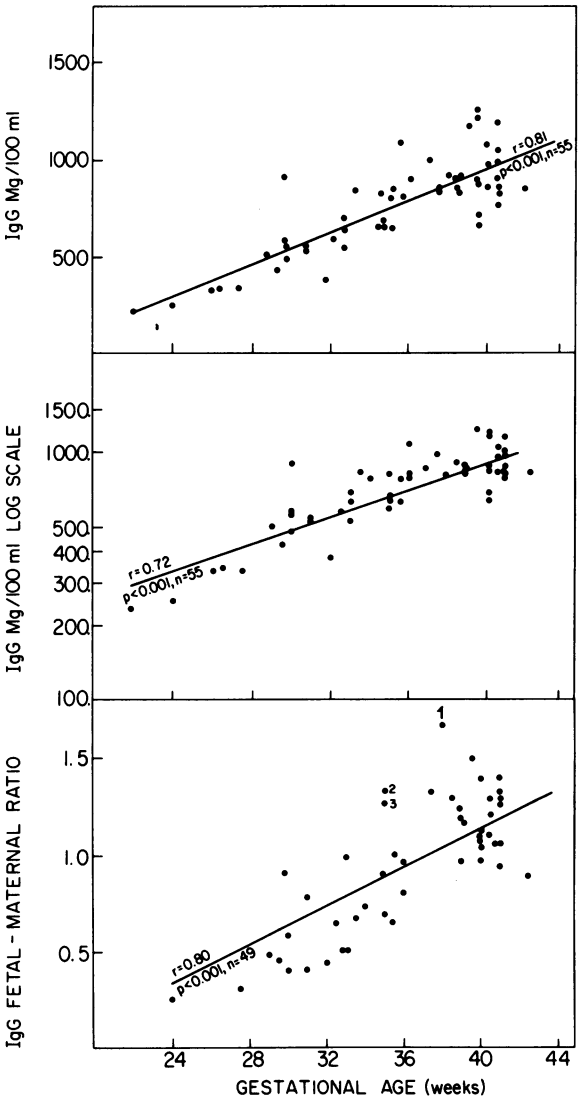

Chart 1.—Correlation of gestational age with (top) serum IgG concentration, (middle) logarithm of serum IgG concentration, and (bottom) fetal-maternal ratio of IgG in appropriate-for-gestational age infants. Correlation of IgG with birth weight was almost as good ($r=+0.791$, $p<0.001$, $n=55$) as was the correlation of fetal-maternal ratios with birth weight ($r=+0.768$, $p<0.001$, $n=49$). Cases 1-3 in the bottom figure illustrate inceased active placental transport of IgG when maternal IgG is low. **Case 1:** maternal IgG 500 mg per 100 ml, fetal IgG 805 mg per 100 ml; **Case 2:** maternal IgG 498 mg per 100 ml, fetal IgG 594 mg per 100 ml; **Case 3:** maternal IgG 498 mg per 100 ml, fetal IgG 632 mg per 100 ml. (Reprinted with permission from Hyvarinen, et al: J Pediatrics 1973, In Press.)

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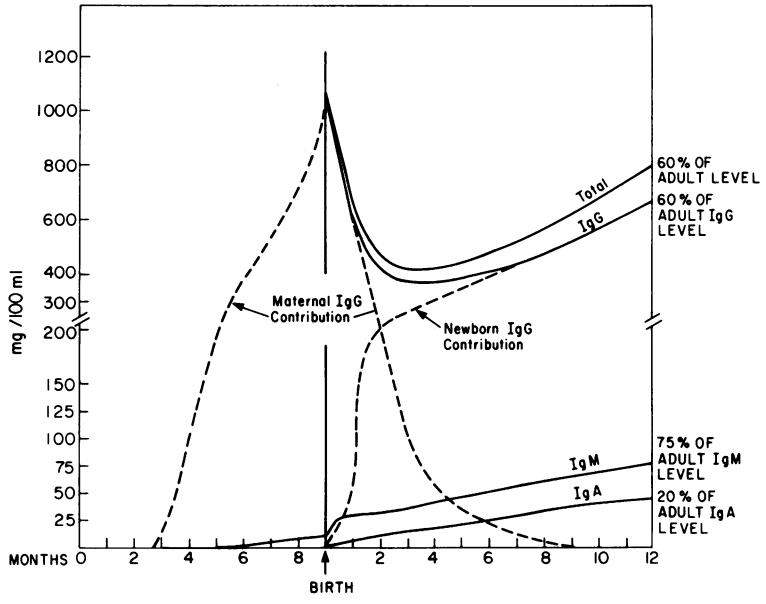

Chart 2.—Immunoglobulin (IgG, IgM and IgA) levels in the fetus and infant in the first year of life. The IgG of the fetus and the newborn infant is solely of maternal origin. The maternal IgG disappears by the age of 9 months, by which time endogenous synthesis of IgG by the infant is well established. The IgM and IgA of the neonate are entirely endogenously synthesized, since maternal IgM and IgA do not cross the placenta.

TABLE 1.—*Levels of Immunoglobulins in Sera of Normal Subjects, by Age*

	IgG		*IgM*		*IgA*		*Total Immune Globulin*	
Age	*mg/100ml*	*Percent of Adult Level*	*mg/100ml*	*Percent of Adult Level*	*mg/100ml*	*Percent of Adult Level*	*mg/100ml*	*Percent of Adult Level*
Newborn ..	1,031±200*	89±17	11± 5	11± 5	2± 3	1± 2	1,044±201	67±13
1- 3 mo ..	430±119	37±10	30±11	30±11	21±13	11± 7	481±127	31± 9
4- 6 mo ..	427±186	37±16	43±17	43±17	28±18	14± 9	498±204	32±13
7-12 mo ..	661±219	58±19	54±23	55±23	37±18	19± 9	752±242	48±15
13-24 mo ..	762±209	66±18	58±23	59±23	50±24	25±12	870±258	56±16
25-36 mo ..	892±183	77±16	61±19	62±19	71±37	36±19	1,024±205	65±14
3- 5 yr ...	929±228	80±20	56±18	57±18	93±27	47±14	1,078±245	69±17
6- 8 yr ...	923±256	80±22	65±25	66±25	124±45	62±23	1,112±293	71±20
9-11 yr ...	1,124±235	97±20	79±33	80±33	131±60	66±30	1,334±254	85±17
12-16 yr ...	946±124	82±11	59±20	60±20	148±63	74±32	1,153±169	74±12
Adults	1,158±305	100±26	99±27	100±27	200±61	100±31	1,457±353	100±24

Values shown above were derived from measurements made in 296 normal children and 30 adults. Levels were determined by the radial diffusion plate method using specific rabbit antisera to human immunoglobulins. Stiehm and Fundenberg, Pediatrics 37:715-727, 1966.

*One Standard Deviation.

with high or low IgG levels tend to have near normal IgG levels.

The postnatal changes in IgG are ilustrated in Chart 2 and Table 1. Levels of IgG fall rapidly for the first three months of life, with a half-life of 25 days. IgG levels reach a low point at age 4 to 6 months (see Table 1). After this, as neonatal synthesis is initiated, IgG levels gradually increase so that the IgG levels by one year of age average about 60 percent of the adult level. Significant neonatal IgG synthesis does not occur until about the third month of life but is well established by age six months.

Since IgG makes up 70 to 80 percent, the total immunoglobulin level largely reflects the IgG level; thus there is a physiologic hypogammaglobulinemia in all infants at age four to six months.

As was previously noted, the level of IgG of cord blood in term infants usually exceeds the maternal level, usually by a factor of 1.2 with a mean maternal-fetal difference of 252 mg per 100 ml.[25] This active transport of IgG may account for the higher level of specific antibodies in the cord sample of paired maternal-cord sera, even in the absence of fetal antibody synthesis.

There are no sex differences in the cord and neonatal levels of IgG or other immunoglobulins.[33,34] Females may have higher levels of IgM after age 8.[34,35]

Fetally synthesized IgG may traverse from infant to mother. Fudenberg and Fudenberg[36] reported the development of an anti-Gm antibody in a woman during pregnancy with specificity toward her infant's Gm type; they postulated that maternal

IgG anti-Gm antibodies in the fetal circulation could result in physiologic hypogammaglobulinemia. Nathenson et al[37] did not find more abnormally low IgG levels in infants of 28 mothers with anti-Gm antibodies; however, only one infant had a significant anti-Gm titer in the cord blood.

IgG Subclasses

There are four subclasses of IgG. They are IgG1, IgG2, IgG3, and IgG4. Each has unique antigenic, metabolic, and antibody properties, and they make up 65, 23, 8 and 4 percent of normal adult IgG respectively.[38] These subclasses were originally defined by antisera to different IgG myeloma proteins, made specific for a particular myeloma IgG by cross absorption. Each IgG subclass contains specific genetic gamma-globulin factors.

Wang et al[39] reported a decrease in the placental transport of IgG2, with the result that neonates have a relative deficiency in this subclass; this would be of clinical significance since IgG2 contains many antibodies to polysaccharide antigens. They also noted an IgG heavy chain not present in adult IgG, and suggested that there was a new subclass of IgG which is specific for the fetus and is endogenously synthesized. They termed this IgF (for fetal gamma-globulin). It must be pointed out that their studies were done on pooled neonatal and adult sera rather than individual specimens.

Morrell et al[40] re-studied the IgG subclass levels on 115 maternal and 128 fetal sera, most of which were maternal-fetal pairs; they did not find a deficiency of IgG2 in cord blood. They noted low fetal:maternal ratios for all four subclasses up to the 16th gestational week, and then a gradual uniform increase with increasing gestational age for all subclasses. In the last weeks of pregnancy, ratios exceed 1:1, in keeping with the findings of Kohler and Farr.[25] They concluded that all four subclasses are transported equally well across the placenta. Further, the presence of IgG2 in cord blood samples was confirmed by assays for Gm (23), a genetic marker for IgG2; in all instances in which the maternal sera had Gm(23) activity, the corresponding cord sera also contained Gm (23) of equal activity.

These investigators[41] also studied the changes of IgG subclass levels in the first two years of life. As can be seen in Table 2, there was considerable heterogeneity among the subclasses. During the first month of life, levels of all the IgG subclasses decrease steeply but IgG3 falls off most rapidly,

TABLE 2.—*Development of the Serum Concentrations of the IgG Subclasses*

Age	No. of Sera	Mean ± 1 Standard Deviation (mg/100 ml) IgG1	IgG2	IgG3	IgG4
10 days ..	7	594±211	256± 52	56±14	29±24
20 days ..	12	521±215	227± 36	39± 8	18± 9
30 days ..	13	425±127	175± 58	30±10	21±14
2 mo ...	11	383±172	153± 53	31±12	11± 5
3 mo ...	12	332±181	91± 44	45±17	13± 8
4 mo ...	10	424±207	110± 39	47±17	10± 6
8 mo ...	10	659±277	147± 56	51±27	12± 9
12 mo ...	6	653±190	160± 75	53±26	7± 4
24 mo ...	14	652±182	166± 41	45±10	25±20
20 yr	108	663±170	322±108	58±30	46*

Modified, with permission, from Morell et al, J Pediatrics 80: 960-964, 1972.

*Distribution assymetric.

reaching a low point by 30 days, in keeping with the more rapid catabolism of this subclass (seven days) compared with the others (20 to 25 days).[42] IgG3 levels then increase rapidly to approach adult levels by age three months. IgG1 levels decrease steeply during the first month and then more slowly for the next two months, indicating the onset of IgG1 synthesis, as early as one month. IgG1 levels reach a low point at age three months, increase significantly by age four months and attain normal adult values by age eight months. IgG2 levels fall at a uniform rate until age three months, and then gradually increase. IgG4 levels show considerble fluctuation during the first year and reach a low point at age 12 months.

IgM

Most newborns beyond 30 weeks of gestation have low but detectable IgM in their cord serum. Since IgM does not cross the placenta, this indicates that IgM synthesis begins early in fetal life, earlier than any other immunoglobulin class. Buckley et al[45] reviewed the results of seven studies of cord blood IgM levels and found that mean levels varied from 5.8 to 15.8 mg per 100 ml and the two standard deviation limits levels varied from 1 to 10 mg per 100 ml (lower limit) to 12.9 to 27.4 mg per 100 ml (upper limit). We utilize a mean of 10 mg per 100 ml with an upper limit of normal (+2 SD) of 20 mg per 100 ml.[44] Small quantities of IgM are found in nearly 100 percent of cord blood specimens from viable infants.[33,45,46] Other observers report no detectable IgM in 6[47] to 25 percent[29] of cord specimens. Differences between the various studies can be attributed in large part to the sensitivity of the method used.

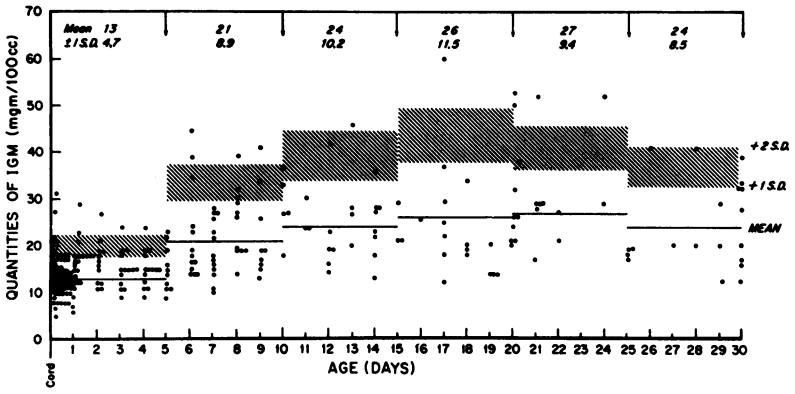

Chart 3.—Serum IgM levels in 175 uninfected newborns during the first 30 days of life. Levels above the shaded area are greater than 2 standard deviations above the mean and are considered abnormally elevated. (Reprinted with permission from Blankenship, et al: J Pediat 75: 1271-1281, 1969.)

The IgM levels in viable premature infants usually are slightly lower than in term infants but not in any predictable fashion.[26,27,29] Further, the proportion of infants with no detectable cord IgM is increased with decreasing gestational age.[29]

Absence of cord IgM has no known clinical significance. In contrast, and as discussed in the next section, an elevated cord IgM level has considerable clinical significance, since it indicates intrauterine antigenic stimulation, often the result of intrauterine infection.

Some of the IgM in cord sera may be in the form of 7S IgM—that is, slowly sedimenting, low molecular weight IgM. Perchalski et al[48] found 7S IgM in six of nine normal cord sera and two of four VDRL-positive cord sera. The contribution to the total IgM and the significance of this 7S IgM is not known; however, 7S IgM has been described in patients with systemic lupus erythematosis, ataxia telangiectasia, dysgammaglobulinemia type I, Waldenstrom's macroglobulinemia, and lymphoma.[49]

IgM synthesis increases rapidly after birth and IgM is the chief immunoglobulin synthesized in the first months of life.[33,34] Buckley et al[43] found that sera from three- and four-day-old infants had significantly higher IgM levels than did cord serum. The second half of the first week of life is a period of rapid rise of IgM levels,[34] probably a result of the antigenic stimulation of gastrointestinal colonization. An exaggeration of this response may occur in the presence of infection acquired at or shortly after birth.[50] As may be noted in Chart 3, IgM levels in normal infants increase rapidly until age 20 to 25 days, then level off or decrease slightly during the next several weeks, only to increase again in the third month of life. The IgM levels at age six months are about 50 to 60 percent of adult levels and 70 to 80 percent by age one year. Adult levels are generally achieved by age two.

IgA

IgA does not cross the placenta and due to a delay in the onset of IgA synthesis, IgA is often not detected by clinical methods in cord blood specimens. Fulginiti et al found IgA in only one of eighteen cord sera specimens by Oudin tube method;[50] Brasher and Hartley found IgA in 30 of 97 specimens of cord sera by immunoelectrodiffusion;[45] Stiehm and Fudenberg found IgA in seven of twenty-two specimens by radial immunodiffusion.[33] When detectable, cord IgA levels usually are 1 to 5 mg per 100 ml. During a maternal-fetal leak IgA levels may be considerably higher (>10 mg per 100 ml), often higher than the IgM level.[44] IgA begins to appear in neonatal sera at about day 15 in most infants (range 5 to 23 days). Levels increase slowly, reaching about 30 mg per 100 ml (15 percent of adult mean) by age six months, and 60 mg per 100 ml (20 to 25 percent of adult mean) by age one year.[32,33] Adult levels may not be attained until adolescence.

IgA may occasionally pass from the mother to the infant via the placenta in trace quantities. Vyas et al[52] described one newborn whose cord serum had anti-IgA antibodies with maternal specificity, suggesting *in utero* sensitization. These same workers found that 15 percent of mothers of recently delivered infants had anti-IgA antibodies, possibly a result of sensitization to fetal IgA following a fetal-maternal leak. Mothers with selective IgA deficiency may be especially susceptible to such *in utero* sensitization.[53]

Secretory IgA

IgA is the chief immunoglobulin of exocrine gland secretions; it is present in a molecular configuration known as secretory IgA which has a larger molecular weight (400,000) than serum IgA and an additional antigenic piece, the secretory component, synthesized by glandular epithelial cells.[54] Since secretory IgA is synthesized

locally rather than transported from the serum, there often is not good correlation between serum and secretory IgA levels.

IgA is first detected in the tears of newborn infants between ten and 20 days of age; it was present in the tears of most infants before the serum had detectable IgA.[55] Haworth and Dilling[56] found IgA appearing in the saliva initially at age two to three weeks, usually a few days before its appearance in the serum. In contrast, IgG is usually present in tears and saliva at birth but its origin (fetal or maternal) is in doubt.[57] IgM is not present in newborn secretions. Both IgG and IgA levels are increased in the secretions in the presence of infection. Neonatal infants receiving exchange transfusion with IgA-rich adult blood or those with elevated serum IgA do not have an earlier appearance of IgA in the secretions.[55,56,58]

Despite the absence of IgA in neonatal secretions, free secretory component regularly is found in the urine and saliva of newborn infants.[59] Remington and Schaefer[60] found free secretory component in the urine of newborn premature infants in the absence of urinary IgA. IgA appeared initially from 40 to 64 days in the urine.

IgD

IgD also does not cross the placenta, and is usually not detected in cord blood. When present, IgD levels are 5 to 30 percent of the normal adult level of 3 mg per 100 ml. Evans et al[29] detected IgD in seven of 84 newborns (8 percent) by a commercial immunoplate system. Berg detected IgD in only one of 64 premature newborns.[27] Maternal IgD levels tend to increase in pregnancy.[61,62]

IgE

IgE and its reaginic activity do not cross the placenta.[62-65] IgE was not detected in 29 of 34 newborns by skin testing with anti-IgE serum;[64] the skin test did become positive during the first month (mean of 21.7 days). In the same study the mean cord IgE level of 13 newborns was 39.1 ng per ml as assayed by radioimmunoassay compared to the maternal level of 177.2 ng per ml in agreement with the findings by Johannson,[66] who found a mean cord IgE level of 36.3 ng per ml compared with a maternal level of 286 ng per ml. IgE skin test reactivity may be related to defective fixation of IgE to neonatal skin. It is of interest that there was a more rapid conversion of the IgE skin test to positive in the bottle-fed than in the breast-fed infants.[64]

In a later study, Bazaral et al,[65] using a modified radioimmunoassay, found cord serum levels of IgE to be 2.1 units per ml (0.45 units IgE was approximately equal to 1 ng) with a range from less than 1 to 7.5 units per ml. The mean maternal IgE was 205.3 ng per ml. No correlation existed between maternal and infant IgE levels. The mean level of IgE at six weeks of age was 5.53 units per ml and at six months of age was 57.6 units per ml. The IgE levels of six-month-old infants from allergic families was 98.6 units per ml, compared with the mean of 29.0 units per ml among infants with a negative family allergy history. Among the six-month-old infants, the three with clinical allergic disease were among the four with the highest IgE levels.

Immunoglobulin Abnormalities Among Selected Groups of Newborns

Premature Infants

Premature infants have lower levels of IgG than do term infants as a result of diminished placental transport during gestation.[26-30] Thus the IgG level of neonatal cord blood is reduced in proportion to the degree of immaturity; small viable infants of 29 to 30 weeks gestational age weighing near 1,300 grams have mean IgG levels of 400 mg per 100 ml, 35 percent of adult and term cord blood levels.[30]

Since the IgG half-life ($T\frac{1}{2}$) is about 25 days at serum levels above 200 mg per 100 ml and the time of onset of IgG synthesis is not significantly altered, the physiologic hypogammaglobulinemia that ensues at age three to six months is more severe and prolonged in the premature. Approximately 50 percent of infants born before the 32nd week of gestation will have IgG levels less than 200 mg per 100 ml from the 16th to the 25th week of life. The difference between individual infants can be attributed to varying times of onset of endogenous gamma-globulin synthesis.

This exaggerated physiologic hypogammaglobulinemia may play an important but as yet undefined role in the increased susceptibility to infection noted among prematures. This is manifested by increased morbidity and mortality rates among prematures[67] and a higher incidence of sudden infant death syndrome.[68] It should be noted that two studies could not demonstrate abnormal immunoglobulin deficiencies among infants succumbing of the sudden infant death syndrome.[69,70]

The mean IgM and IgA levels of prematures

are also decreased due to diminished neonatal synthesis.[26,27,29] Evans et al[29] noted that the mean IgM cord serum level of 24 term infants was 16 mg per 100 ml and that 25 percent had no detectable IgM; in contrast, the mean IgM of 21 infants of 28 to 31 weeks gestational age was 4.4 mg per 100 ml and 71 percent had no detectable IgM. The IgA level of the term newborns was 6.6 mg per 100 ml (and undetected in 71 percent) compared with 2.2 mg per 100 ml in prematures of 28 to 31 weeks (and undetected in 86 percent). Because of considerable scattering, neither IgM nor IgA levels in cord serum can be correlated with gestational age.

Yeung and Hobbs[28] and Thom et al[26] demonstrated a straight-line relationship between the gestational age and the logarithm of the cord IgG level. We noted that gestational age correlated better with the IgG level than with the log IgG level[30] (see Chart 1). There is a lesser but still significant correlation of the cord IgG level with the birth weight.[27,30]

The time of onset of IgM and IgA synthesis is the same in premature infants as in term infants,[71] and they show an equal or near-equal antibody response to injected antigens or infectious agents.[18,19] However, IgM synthesis in prematures may be somewhat less than in term infants. Berg[27] noted significantly lower IgM levels in three-weeks-old premature infants than in three-weeks-old term infants. This in part may be due to lessened exposure to infectious agents inasmuch as prematures are kept in incubators.

The therapeutic use of immune serum globulin (ISG) to prevent infection in prematures has been proposed.[72] Amer et al[73] gave alternate prematures albumin or ISG injections and noted a slightly diminished incidence and severity of infection in the ISG-treated group. In the first month of life, there were five deaths among 136 in the albumin group, and one death among 135 in the ISG group. Further studies must be completed before a definite recommendation can be made.[74]

Small-for-Dates Infants

The IgG levels of 46 of 47 small-for-dates (SFD) newborns (newborns less than two standard deviations below the expected weight for their gestational age) were studied by Papadatos et al,[75] who found that the IgG levels of SFD newborns were less than the IgG of normal newborns of equivalent gestational age, but higher than the IgG of normally-grown infants of equivalent birth weight. They suggested placental dysfunction as a cause for both the intrauterine growth retardation and lower IgG levels. Yeung and Hoggs[28] also found low levels of cord IgG in all 28 SFD infants compared with normal sized infants; 12 had levels less than two standard deviations below their expected mean. Evans et al,[29] however, found that only two of 44 SFD infants had cord IgG levels less than the tenth precentile for gestational age. Eight of the 28 SFD infants studied by Yeung and Hobbs[28] had cord IgM levels over 15 mg per ml, the upper limit of normal for their laboratory. Evans et al[29] found that eight of 44 SFD had elevated (more than 15 mg per 100 ml) IgM levels. IgA levels were not regularly detected in cord blood of SFD infants.[28] Twins, despite being SFD, have normal levels of IgG.[28]

Post-Mature Infants

Yeung and Hobbs[28] found that ten of 12 post-mature babies, two of them SFD, had lower than expected levels of IgG (mean 772 mg per 100 ml compared with control newborns of 1,100 mg per 100 ml). Ackerman et al[76] could not confirm a decreased IgG in 40 post-mature infants (the levels were actually slightly higher than in normal term infants), but they did find that ten of 40 of post-mature infants had detectable IgA. Among those with detectable IgA, the mean concentration was 28 mg per 100 ml in contrast to the trace levels or absence of IgA in term infants. A maternal-fetal bleed was excluded by demonstration of a progressive increase of IgA on serial determination. No abnormalities of IgM were noted.

Infants with Congenital Infections

Elevated IgM in Congenital Infection. Intrauterine infections, primarily but not exclusively those associated with rubella virus, cytomegalovirus, treponema pallidum and toxoplasmosis, may result in easily recognized sepsis in a neonate; in other instances, the infection may be subclinical and not manifest until months or years later.[46,77] Recognition of the condition is important in treatment, prognosis, and isolation procedures.

Stimulated by the observations of Alford[78] and Bellanti et al,[79] who observed prominent immunoelectrophoretic IgM arcs in infants with congenital rubella, Stiehm et al[44] suggested that an elevated IgM level upon quantitative IgM determination of cord serum would be an indicator of congenital infection. This was based on the fact that the infected fetus synthesizes specific IgM antibodies

TABLE 3.—*Incidence of Elevated IgM Levels in Random or Serially Collected Umbilical Cord Sera of Various Groups of Newborns*

Author, Year, Reference	*Population Studied*	*IgM Levels*	
		Number Elevated in Number Studied*	*Percent with Elevation*
Stiehm et al (1966)[44]	Random Newborns (mixed income)	7/129	5.4
Stiehm and Nichol (1968)[87]	Consecutive Newborns (middle income)	2/1100	0.2
Alford et al (1967,1969,1971)[46,77,82]	Consecutive Newborns (mixed income) (1967-1968)	123/2916	4.2
Alford et al (1967,1969,1971)[46,77,82]	Consecutive Newborns (mixed income) (1968-1969)	69/3035	2.3
Hardy et al (1969)[88]	Consecutive Newborns (mixed income)	132/2623	5.0
Sever et al (1969)[85]	Consecutive Newborns (middle income)	14/1768	0.8
Miller et al (1969)[83]	Consecutive Newborns (mixed income)	135/5006	2.7
Lechtig and Mata (1970,1971)[89,90]	Random Newborns, (mixed income from urban underdeveloped country)	1/16	6.3
Lechtig and Mata (1970,1971)[89,90]	Random Newborns, (poor income from rural underdeveloped country)	55/155	48.7
Dent et al (1971)[86]	Consecutive Newborns, (mixed income)	4/100	4.0
Gotoff et al (1971)[81]	Random Newborns, (low income)	41/1659	2.5
Gotoff et al (1971)[81]	Random Newborns, (middle income)	27/2626	1.0

*All the studies have used an IgM of 20 mg per 100 ml or above to be abnormally elevated except for Gotoff et al[81] who used 17 mg per 100 ml, and Hardy et al[88] who used 30 mg per 100 ml.

and immunoglobulins which are readily detectable in cord blood, since maternal IgM does not cross the placenta. Stiehm et al[44] initially used a cord IgM of 20 mg per 100 ml or greater (mean +2 standard deviations) to indicate an abnormally elevated level. Although other investigators have used higher or lower values, most continue to use a cut-off IgM level of 20 mg per 100 ml.[82-86]

The initial study indicated that all infants with congenital infection had elevated cord IgM levels.[44] Subsequent studies[46,77,80-86] confirm that cord IgM is increased in congenital infection but not in all infected infants. Alford[46] found that 38 of 43 infants with clinical congenital infection had pronounced elevation of IgM; however, McCracken et al[84] noted that only 12 of 88 with congenital rubella (18 percent) had elevated IgM.

The degree of elevation of the IgM level in congenital infection is dependent on several factors; in general the more affected the infant is, the more likely he is to have elevated IgM. Further, the type of congenital infection may be important; in our experience infants with rubella have higher IgM levels than other infected infants. The maturity of the infant may be important; a very small premature may have a lessened IgM response than an older infant.

Incidence of Elevated Cord IgM. Because of the simplicity and wide availability of IgM determinations, many investigators have surveyed the IgM levels of various newborn populations in a search for inapparent congenital infection. A summary of several of these studies is given in Table 3. The incidence of elevated cord IgM ranges from 0.2 to 5.4 of cord blood, varying perhaps to some extent on the year but to a major extent on the socio-economic status of the newborn population. A consistent feature is the higher mean IgM level and the higher percentage of infants with IgM elevations in low socio-economic groups. In rural, underdeveloped countries, nearly 50 percent have elevated IgM;[89,90] since the mean IgM is so high an elevated IgM is of considerably less value as an indicator of congenital infection. The increased antigenic stimulation which is occurring in newborns of low socio-economic status may also be an important factor in the increased infant morbidity and mortality in these populations.

Cord IgM Screening Procedure. Despite the above limitations, measurement of cord IgM has proved to be of value in the evaluation of infants suspected of congenital infection. Alford,[46] who has had more experience with this procedure than anyone else, has shown that the infection rate of infants with elevated IgM was much higher than in those with normal values. In his studies shown on Table 3, among the 202 infants with elevated IgM, specific infections were diagnosed in 69. Virtually all of these infants were asymptomatic at birth. Alford also found a higher incidence of central nervous system abnormalities during the follow-up period. This is contrary to the finding of Gotoff et al[81] who noted no difference in the prognosis between those with and those without elevated cord IgM levels. Alford[46]

found the frequency of congenital infection was 0.56 percent among 7,500 general deliveries; in contrast the frequency of congenital infection was 20 percent among 202 infants with elevated IgM. Thus the infection rate is increased 40-fold among infants with elevated IgM levels.

An IgM determination is indicated for any infant suspected of having a congenital infection. Suspicious clinical features include petechiae, hepatosplenomegaly, skin rash, congenital heart defect, microcephaly, inguinal hernia, thrombocytopenia, jaundice and anemia. Further, if the infant is small for gestational age or does not thrive, congenital infection must be suspected. Finally, if the mother is known to have been exposed to rubella or another infectious agent or had a viral syndrome during pregnancy, her newborn infant is suspect.

To be of clinical significance an elevation of IgM must be the result of increased fetal synthesis and not due to maternal blood in the circulation. Indeed, Miller et al[83] felt that in 60 percent of 135 cases of cord IgM elevation, placental leaks were the cause. This may occur in about 1 percent of all deliveries.[91] In the presence of a maternal-fetal bleed the infant's IgA level may exceed the IgM level, reflecting the IgA:IgM ratio in maternal blood. In contrast, in congenital infections, the IgM level exceeds the IgA. For this reason both IgM and IgA levels should be measured routinely on cord blood submitted for IgM analysis.

Further, in the presence of a maternal-fetal bleed, the initially high IgM level will fall significantly in three or four days (because of the short half-life of IgM); in contrast an elevated IgM as a result of congenital infection will remain elevated or increase.

Definitive Diagnosis of Congenital Infection. Two approaches to the definite diagnosis of congenital infection are available. The first is demonstration of the organism by culture of blood, throat, stool or urine. The second is by serological means.

A single positive antibody titer in an infant is of limited significance, since it may only be a reflection of transplacental maternal antibody. Since maternal antibody falls with a half-life of 25 days, a rising or persisting antibody titer, measured on paired specimens drawn at least 30 days apart and run simultaneously, indicates endogenous antibody synthesis and congenital or neonatal infection. A finding of higher antibody titers in the infant's serum in paired maternal and infant specimens (that is, drawn simultaneously) is only significant if the infant's titer is considerably higher (greater than two dilutions) than the maternal titer, inasmuch as mature infants have higher IgG levels than their mothers as a result of active transplacental transport.[25]

A new approach, which permits the immediate diagnosis of congenital infection is the demonstration of IgM-specific antibodies in the newborn serum. Such procedures are available in several laboratories for the rapid diagnosis of congenital rubella,[92,93] syphilis,[94] toxoplasmosis,[95] and cytomegalic inclusion disease.[96] Since IgM does not cross the placenta, the presence of IgM antibodies indicates that the infant is synthesizing the antibody and is or was infected.

Two methods are used to show that the antibody is IgM. The first is to fractionate the infant serum and determine antibody titers on the isolated IgM and IgG fractions.[92] The second is to react the test serum with the antigen, and then identify the immunoglobulin class of the antibody by immunofluorescence using antiserum to human IgM or IgG.[93] These two general methods can be used for the identification of the antibody class to other organisms suspected of causing congenital infection.

Post-Natal Immunoglobulin Studies. Studies of immunoglobulin levels beyond the neonatal period in 22 infants with congenital rubella have been reported by Soothill et al.[97] The initial IgM elevation persisted throughout the first year, often increasing to levels five to ten times normal in nearly all of the 22 infants studied. Most of the IgG values were also high, with lessened physiologic hypogammaglobulinemia; however, six of the 22 infants had decidedly low levels of IgG (in the hypogammaglobulinemic range) and two had symptoms of immunodeficiency.

Infants with Post-Natal Infections

An infection acquired at or shortly after birth will serve as a stimulus for IgM synthesis.[50,98-101] However, since IgM levels normally rise rapidly after birth (probably as a result of bacterial colonization of the gastrointestinal tract), the post-natal IgM level must be significantly greater than that expected physiologically to be of clinical significance.

Blankenship et al[50] reported significantly elevated IgM levels in 27 of 31 infants with viral or bacterial infections in the first 30 days of life. The

IgM levels were measured at three to five day intervals after birth and plotted on a graph such as is shown in Chart 3. The IgM levels began to increase within five days of infection. The IgM levels were generally found to be above the first standard deviation and often above the second deviation for age (see Chart 3). An IgM level above 50 mg per 100 ml at any time in the neonatal period is cause for suspicion of systemic infection.

Panayotou et al[98] reported IgM and IgA elevations in 11 premature newborns with gastroenteritis; ten of them had IgM levels or IgA levels, or both, of 50 mg per 100 ml or greater within the first 30 days of life. The mean levels of IgA of infected newborns were significantly elevated—about twice that of non-infected controls at ages 10, 20 and 30 days. Berg[99] showed that five infants with recurrent (but not severe) infections had significantly higher mean IgM and IgA levels from age six weeks to one year than did ten infection-free infants. IgG levels were the same in both groups.

Khan et al[100] found elevated IgM levels in a significant percentage of 613 infants with various infections occurring at various periods in the first two years of life. For example 29 of 39 neonates with sepsis had IgM levels two standard deviations above the normal level for age. They felt that IgM levels were particularly useful in the diagnostic evaluation of infants suspected of sepsis and previously treated with antibiotics. Nahmias[101] reports that in neonates suspected of sepsis 90 percent are not confirmed by culture; however, in 17 such neonates in whom a significant IgM increase developed, eight had proven infections.

Cord IgM elevations have not been found in neonatal biliary atresia,[44] neonatal hepatitis,[44] mongolism,[102] and congenital malformations,[103] suggesting that congenital infection is not an etiologic factor in these conditions.

Miscellaneous Disorders

Maternal Agammaglobulinemia. Newborns born to mothers with agammaglobulinemia will have low levels of IgG in the cord blood as a result of diminished placental passage.[104,105] Normal levels of IgM and IgA are noted. There are no reports of unusual propensity to infection among such infants.

Antibody Immunodeficiency. Neonates with congenital X-linked agammaglobulinemia usually are not symptomatic until after six months of life, by which time maternal IgG levels have waned.[106] Since there may be some synthesis of IgM and IgA in the first few months, it may not be possible to diagnose this disorder in the first six months. However, a complete absence of IgM and IgA, in the presence of a continuous fall of IgG, is indicative of defective antibody synthesis. Patients with combined immunodeficiency (Swiss agammaglobulinemia) have onset of symptoms in the first three months of life due to their profound cellular immunodeficiency; their immunoglobulins may be in the normal range in this period. In congenital antibody immunodeficiencies with or without cellular immunodeficiency, functional tests of antibody synthesis are indicated.[106]

Mongolism. Miller et al[102] reported that newborns with mongolism have lower levels of cord IgG than do normal controls. Twenty-four newborn Caucasian mongols had mean cord IgG levels of 684 mg per 100 ml, significantly below the IgG level of 1,144 mg per 100 ml in 39 Caucasian control newborns. Eight Negro mongols had mean IgG levels of 931 mg per 100 ml compared with the IgG level of 1,293 mg per 100 ml among 48 Negro control newborns. These differences were highly significant and could not be attributed to prematurity, birth weight, age of collection, maternal age, sex, or other factors.

Intrauterine Transfusion. Hobbs et al[107] reported elevated cord IgM or IgA or both in ten of fourteen infants given intrauterine transfusions for hemolytic disease of the newborn. Three had cord IgM levels exceeding 20 mg per 100 ml, ten had detectable IgA, and five had IgA levels of 10 mg per 100 ml or greater. They believed that these elevations could not be attributed to the transfused blood but instead represented an immune response to the antigenic stimulation of homologous blood.

Phagocytic and Opsonic Studies in Newborns

We consider now several "non-specific" components of neonatal host defenses—phagocytes and opsonins. While the classification of "specific" and "non-specific" immune mechanisms has some utility, it is important to remember the limitations of this distinction. For example, among the opsonins, specific antibodies are highly effective. Is the role of antibody in enhancement of phagocytosis, then, to be considered "specific" or "non-specific"? Similarly, although such processes as chemotaxis and phagocytosis appear to be "non-specific" in basic nature, the role they play in the

elaboration of the specific immune response is at present unclear, and may in future prove to have at least some specificity. The value of the classification is primarily to permit orderly consideration of the rapidly accumulating store of data involving individual processes of the inflammatory-immune response.

Cellular Factors

Among the cellular activities that play a role in protecting the host from infectious agents are movement, phagocytosis and post-phagocytic killing of ingested microorganisms. While each of these will now be considered separately, they are best regarded as a continuum, within which any type of defect may result in increased numbers of infections or defective response to infections.

Polymorphonuclear Leukocyte (PMN) Functions

CELL MOVEMENT

Knowledge about cell movement has increased rapidly over the past few years.[108] It is now recognized that the mechanism(s) by which neutrophils move involve a complex series of interactions between humoral and cellular components. It appears that at least two types of neutrophil movement may be involved in the mobilization of cells in inflammation.[109] The first of these is *chemotaxis,* which refers to specific, directed migration of cells toward an inflammatory focus. The second type of neutrophil movement is *random mobility,* which is characterized by nondirected or Brownian type movement of cells. Chemotaxis is generally measured by some type of modified Boyden assay,[108] the principle of which consists of separating a cell suspension from a source of chemotactically active material(s) by a small-pored filter. The number of cells which traverse the filter in a given period provides an index of chemotactic activity. The mechanisms by which cells are activated to chemotax are not completely understood. Ward and Becker have shown that two cell-bound serine esterases are required,[110] one of which exists in or on the leukocyte in an already activated state. The other is enzymatically inert and becomes activated following interaction with the complement system. Qualitative or quantitative deficiencies of either or both of these esterases might, then, result in defective cell movement.

Miller[111] compared chemotaxis of PMNs from normal human neonates with that of PMNs from normal control subjects, toward a variety of chemotactically active materials. In all cases neonatal PMNs showed significantly decreased chemotactic response. The nature of this deficiency is unknown.

Random mobility of PMNs has been studied by several methods. Current data suggest that random mobility, as measured by the capillary tube migration method, involves different mechanisms of cell movement than chemotaxis.[112] In this type of assay, standardized suspensions of PMNs are placed in micro-hematocrit tubes and placed upon a microscope stage that is then turned to the horizontal. This puts the tubes into a vertical position, as with a sedimentation rate preparation. The tubes are examined through an ocular micrometer, and the maximum distance that cells have moved upward in the tube over a given time is measured. By this method, neonatal PMNs appear to be somewhat less active in random mobility than PMNs from older children and adults.[109] The potential clinical significance of this and other functions of PMNs in the neonatal inflammatory response is considered below.

PHAGOCYTOSIS

The ability of PMNs to ingest, or phagocytize, micro-organisms is a major step in the inflammatory process. The classic studies of Metchnikoff first emphasized the importance of the PMN or "microphage" in body defenses.[113] It was soon suggested, however, that humoral factor(s) played at least an equal role in the phagocytic process.[114] In 1904, the term, *opsonin,* or "purveyor of dainties," was coined.[114] We now recognize that both humoral and cellular factors are of significance in the phagocytic process, and that deficiencies of either can result in clinically significant dysfunction. We consider here the cellular events of phagocytosis, with special reference to the neonate.

Surprisingly few studies of phagocytosis in neonates were performed until the beginning of the last decade. Of those that were, the majority demonstrated a relative deficiency of phagocytic activity in whole blood of newborns as compared with that of older children and adults.[115-120] The studies varied considerably in such factors as the challenge particle used in the assay, the serum concentration, the source of the cells, and a variety of other experimental conditions. The major problem, however, was that the use of whole

blood in the assays made it impossible to evaluate the separate effects of humoral and cellular components.

Data are now available on the individual humoral and cellular aspects of the neonatal phagocytic process. In one group of studies, the phagocytic activity of isolated neonatal leukocytes has been found to be normal.[121-125] Within this group, however, the leukocytes and challenge particles (E. coli, S. aureus 502A, S. marcescens, Strep. pyogenes, D. pneumonia and P. aeruginosa) were incubated in the presence of serum concentrations of 10 percent or more. In *lower* concentrations of serum, however, PMNs from neonates may be less phagocytic.[117] Matoth, in 1952, demonstrated that phagocytosis of starch granules by leukocytes from adults and from cord blood of term neonates was equal in the presence of final concentrations of maternal serum in excess of 10 percent. In the presence of lower concentrations of maternal sera, however, cord blood PMNs were less efficient in phagocytosis than adult PMNs. More recently, the phagocytosis by PMNs from neonates and normal adults of washed, baker's yeast particles has been compared in the presence of varying concentrations of plasma.[126] In final concentrations of 2.5 percent plasma or less, neonatal PMNs ingested far fewer yeast particles than those from adults.

It appears therefore that under certain experimental conditions, neonatal PMNs are deficient in phagocytic activity. Possible *in vivo* support for this observation comes from the data of Forman and Stiehm,[121] who found that PMNs from six of nine term infants with various underlying clinical disorders were sufficient in phagocytosis.

In premature infants, the data are not as clear. Of the few studies which have been performed on this question, the variations of particles and experimental conditions make an overall summary difficult. Miyamoto, using whole blood and killed streptococci,[127] found PMNs from prematures were less phagocytic than those from term infants during the first week of life. These studies, however, did not distinguish between humoral and cellular components of the phagocytic process. Gluck and Silverman[128] found that PMNs of premature infants ingested normal amounts of India ink particles in the presence of adult serum, but diminished numbers in the presence of serum from prematures. Cocchi and Marianelli[129] observed normal phagocytic activity toward viable Pseudomonas aeruginosa in PMNs from premature infants. PMNs from 13 of 14 premature infants studied by Forman and Stiehm[121] phagocytized normal numbers of S. aureus 502A and S. marcescens. Phagocytic activity of PMNs from premature infants in the presence of serum concentrations less than 10 percent has not been studied.

BACTERICIDAL ACTIVITIES

Clinical interest in cellular killing of ingested microorganisms was heightened following the description of Holmes and coworkers of an inborn error of neutrophil function resulting in decreased bactericidal activity of PMNs from patients with chronic granulomatous disease (CGD).[130] There have been a number of investigatons of bactericidal activities of neonatal PMNs, but there are major inconsistencies in these studies, and little consensus information has yet emerged. In studying the fate of viable Pseudomonas aeruginosa within PMNs of premature infants, Cocchi and Marianelli[129] found that bactericidal rates in premature leukocytes were equal to those in controls at 90 minutes, but by three hours were decreased. These differences were unaffected by serum concentrations. Bactericidal activities of PMNs from nine of 25 full-term infants studied by Coen and coworkers were decreased within the first 12 hours of life.[122]

A number of investigators have demonstrated normal bactericidal activities in neonatal leukocytes.[120,125,131] Organisms used in these studies included S. aureus 502A, E. coli, and Pseudomonas aeruginosa. The many inconsistencies and differences in method make it difficult to summarize what has been learned. It appears, however, that the neonatal leukocyte is relatively normal in bactericidal activity.

METABOLIC ACTIVITIES

Interest in the functional activities of PMNs in the neonatal inflammatory process has been accompanied, naturally, by inquiries into the metabolic status of these cells. The problems to which we alluded in the previous section are all the more apparent when one attempts to perform sophisticated metabolic measurements of PMNs. Such variables as pH, temperature, and state of cell, whether resting or phagocytic, are important to the interpretation of results.

In studies on actively phagocytizing PMNs, both Donnell and coworkers[132] and Coen and coworkers[122] found that PMNs from term infants showed less increase of hexose monophosphate shunt

(HMP) pathway following phagocytosis than that seen in adult PMNs.

Park and coworkers[131] found that neonatal PMNs in the resting phase consumed twice as much oxygen as matched maternal PMNs. Following phagocytosis, however, neonatal and maternal PMNs showed similar increases in oxygen consumption and glucose utilization.

Post-phagocytic reduction of nitroblue tetrazolium (NBT) dye to blue formazan has been compared between neonatal and adult PMNs, with conflicting results. Park and coworkers found increased reduction of NBT within neonatal PMNs,[131] while Bellanti and coworkers noted decreased reduction of NBT.[133] McCracken and Eichenwald[125] found normal reduction of NBT within PMNs from 41 infants varying in age from two hours to 71 days.

Rather than attempt to establish one of these studies as being correct to the exclusion of the others, it should be recognized that the differences are probably explained by variation in the conditions of the assays, particularly the time of sampling of the NBT dye reduction. Bellanti and coworkers suggested that activity of PMN glucose-6-phosphate dehydrogenase (G6PD) decays at a significantly greater rate in neonatal than in adult PMNs.[134] They also found a decrease of G6PD and NBT reduction during the first six months of life, followed by a continued increase with age.

Other Cell Types

Little is known of the relative functions in neonates of other cells of the inflammatory response. In the specific immune response, lymphocyte function in neonates is probably normal. As for elaboration of inflammatory mediators, or "lymphokines," little is known of the relative function of neonatal PMNs.

The function of the neonatal eosinophil may be decreased, as suggested by data obtained by the inflammatory cycle technique of Rebuck and Crowley.[135] In this procedure an area of skin is abraded with a sterile scalpel blade, and a cover-slip is placed over the inflammatory site. The cover-slip is changed periodically, replaced with a new slip and is stained and examined microscopically. In a normal adult "skin window" response, PMNs predominate over the first four to twelve hours, followed by a shift to a largely mononuclear cell response.

In studies of the inflammatory response in term neonates, Eitzman and Smith[136] found increased numbers of eosinophils in the second-hour and fourth-hour exudates of newborns over, but not under, 24 hours of age. By contrast, however, Bullock and coworkers[137] were unable to find such a consistent abnormality. In their study, only 13 of 61 babies studied showed significant skin window eosinophilia. They suggested that the group with abnormal eosinophilia represented a subgroup with a possible allergic diathesis. This hypothesis is as yet untested.

Skin Reactivity and Inflammatory Response

Since the interpretation of many of the assays of inflammatory-immune function depends upon the reading of some form of skin response, it should be borne in mind that an abnormal cutaneous response may reflect a basic abnormality of skin reactivity rather than a primary defect of inflammatory or immune nature. An example of errors that may occur unless this warning is heeded is the conclusion that deficient skin test responses in neonates are due to defective cell mediated immunity.

It has long been observed that neonates show poor localization and inflammatory response to dermal infections. This is due in part to certain of the deficiencies of the neonatal inflammatory response (see below), but is probably due also to the fact that neonatal skin is relatively deficient in the manifestation of inflammation. In experimental studies, Seto and Drachman observed the response to dermal infection in weanling and adult rats.[138] Upon dermal injection of pneumococci, the weanling rats had (1) increased incidence of bacteremia and death, (2) delayed migration of neutrophils, (3) delayed migration of mononuclear cells, and (4) poor localization and increased spreading of bacteria.

Eitzman and Smith[136] found that the skin of newborns responded poorly to irritation and failed to localize inflammation when compared with the skin of older children.

Studies of cutaneous inflammation in neonates (by the previously mentioned Rebuck "skin window" method) demonstrated a relatively delayed and less intense shift from the predominately polymorphonuclear to mononuclear phase than in adults.[136,137,139]

Whether the above studies are the result of intrinsic abnormalities in neonatal skin or they simply mirror the observed deficiencies of the inflammatory response is not clear. For example, poor response to irritation and failure to localize

cutaneous infection could result from deficient chemotaxis and phagocytosis. At present the correlations which can be drawn between *in vitro* abnormalities of leukocyte function and clinical significance are limited.[108,109,111] The future should see great interest in definition of these relationships and lead to increased knowledge of the roles these deficiencies play in enhanced susceptibility to infections in the neonate.

Humoral Factors

As has been discussed, it is difficult to adhere closely to a precise classification of the components of the inflammatory-immune response. This is as true with the humoral components as it was with the cellular components. Immunoglobulins, for example, which have been considered in depth as mediators of the "specific" immune response are also among the most potent activators of the complement system, a presumptively "non-specific" mediator of inflammation.

Complement

Appreciation of the wide range of biologic activities mediated by the serum complement system has increased greatly over the past decade. While specific application of this information to the neonate is at a preliminary stage, the field promises to yield applicable information in the not too distant future.

No attempt will be made here to comprehensively review the serum complement system. The reader is referred to several excellent recent reviews.[140,141] In brief, the complement system consists of 11 identified proteins. Upon activation by an appropriate stimulus, such as an antigen-antibody reaction, the activated first component of the reaction, or C1, is formed. C1, as well as subsequently reacting components in the sequence, has enzymatic activity and interacts with the next component in the sequence. At completion, all components have reacted in sequence—C1,4,2,3, 5,6,7,8,9. There are three basic types of biologic activities which are mediated by the complement system:

1. Cytotoxic reactions occur as a result of interaction of all components upon a particular cell surface or membrane. In the classical model of sheep cell hemolysis, for example, the end result of complement reaction upon the sensitized sheep erythrocyte membrane is damage to the membrane, the result of which is an osmotic lysis secondary to the hole which has been "punched" in the membrane.[140] Complement reaction can damage virtually any type of cell, lysis being but one example of such an effect.

2. A second type of complement mediated biologic activity involves changes which occur upon the membrane involved in the complement reaction, as the individual components react in sequence. For example, a pneumococcus which has reacted with antibody and has activated the complement sequence becomes far more readily phagocytized once the first four components have reacted. In other words, total reaction of the system may result in cytotoxic effects, but partial interaction may alter the entire antigen-antibody complex in such a way that phenomena such as opsonization and immune adherence take place. Although the actual mechanisms by which these intermediate steps alter biologic activity is unknown, and may actually prove to be the same as that of the cytotoxic effects of complete reaction, a separate set of biologically significant activities is involved.

3. The third, and potentially most significant mechanism for mediation of biologic activities of the complement system, pertains to low molecular weight "split products" of the native components which are released into the circulation upon activation of the parent molecule. Such materials possess potent activities as histamine releasers (anaphylatoxins) and in the attraction of leukocytes (chemotactic factors). The elaboration of such biologically active materials into the circulation has obvious potential in a wide range of clinical disorders of the inflammatory response.

It now appears that at least two distinct pathways exist by which complement mediated activities can be triggered. The first of these, or the classic pathway, involves, most commonly, activation by an antigen-antibody complex. In the so-called "alternate pathway," however, the initial components of the system are by-passed, and activation takes place directly through the C3 molecule. Among the agents which activate the alternate pathway are zymosan, inulin and endotoxins.

The measurement of complement has provided a number of pitfalls. For many years, it was felt that adequate assessment of complement could be done by measuring total hemolytic activities and immunochemical determinations of C3, or so-called β1C/β1A. It is now clear, however, that such measurements may not provide an adequate estimate of functional activity of a particular compo-

nent. Further, even specific quantitative deficiencies of individual components may not reflect functional status of all activities mediated by that particular component.[142] This means that studies of complement components by immunochemical or hemolytic measures may not adequately reflect biologic activities in newborns.

Synthetic studies have revealed synthesis of the third component (C3) in fetal tissues by immunochemical techniques as early as five and a half weeks of gestation.[1,143] Fireman and coworkers found C3,C4 and C5 levels were decreased in term infants and prematures as compared with maternal and normal adult levels.[144] Propp and Alter found the mean cord levels of C1q,C3,C4 and C5 in sera of term neonates to be 0.752,0.562,0.550, and 0.602 of the respective maternal concentrations.[145] Koch and coworkers found relatively low levels of the first four components in cord blood, as compared with levels in blood from normal adults.[146]

Total hemolytic complement activity in newborns is approximately half of that in matched mothers.[119,144,147-150]

Functional Activities

OPSONIC ACTIVITIES IN PREMATURES AND NEWBORNS

The importance of separate evaluation of humoral and cellular components of the phagocytic process has been emphasized in the preceding section. Complement-derived materials are among the important opsonins in plasma. Measurements of opsonic activity in neonatal plasma have generally revealed a deficiency when compared with that of older children or adults.

As previously noted, the concentration of serum or other source of opsonin may affect the results obtained in a given assay. Miller compared the opsonic activity of neonatal and normal adult plasmas toward baker's yeast particles.[126] Plasma from the neonates had less opsonizing activity toward yeast particles than that from the adult group. The difference was statistically significant, however, at final plasma concentrations only of 10 percent or below.

Opsonic activity of neonatal sera toward various bacteria has been studied by a number of investigators. Dossett and coworkers found neonatal sera were deficient in opsonic activity toward E. coli and serratia marcescens when compared with maternal sera.[124] No difference was found between the opsonic activity of the two groups toward staphylococcus aureus or group B streptococcus. By contrast, Forman and Stiehm found no deficiency of opsonization of neonatal sera of S. aureus 502A or the same strain of serratia marcescens used by Dossett and coworkers.[121] In low birth weight infants (less than 1,925 grams), however, they found deficient opsonization toward both organisms. McCracken and Eichenwald studied opsonic activity of neonatal sera toward S. aureus 502A, E. coli and P. aeruginosa.[125] At serum concentrations of 10 to 25 percent, opsonic activity against all three organisms was diminished in infants under 3,000 grams. The greatest deficiencies were observed against the Gram-negative bacteria.

CHARACTERIZATION OF THE OPSONIC DEFICIENCY

The availability of new data on the process of opsonization has made it necessary to re-think some of our earlier concepts on the process in neonatal sera. For many years, the major thrust of inquiries into the problem centered about the role of antibodies. It was recognized that the major susceptibility to infections in neonates involved Gram-negative bacteria. As we have discussed, it has long been recognized that little, if any, macroglobulin (IgM) is transferred to the fetus. In 1963, Michael and Rosen demonstrated specific antibody to Gram-negative microorganisms in human macroglobulin.[151] Further, they showed that IgM antibodies were a thousand-fold more efficient than IgG antibodies in opsonic and bactericidal activities against Gram-negative organisms in mice.

Gitlin and coworkers showed that bactericidal activities against certain strains of E. coli and Salmonella typhosa 0901 were poorly transferred across the placenta, even when maternal titers were adequate.[21] Bactericidal activity against E. coli was found in only 40 percent of the infants, and against Salmonella in only 20 percent. When bactericidal activity in cord sera was present against either of these organisms, it was found exclusively within the IgG class.

From these studies it was hypothesized that an important factor in the susceptibility of neonates to Gram-negative infections was the deficient placental transfer of IgM antibodies.[21]

It now appears that deficiency of IgM antibodies provides only a partial explanation for the opsonic deficiency in neonatal sera. In the yeast phagocytosis system, it was found that addition of purified IgM to neonatal sera caused little, if any, increase in opsonic activity.[126] Dossett and co-

workers found that complement or other heat-labile factors were necessary to amplify the opsonic potential of both IgG and IgM.[124] The enhancing effect, however, was considerably greater with IgM than with IgG, thus leading them to conclude that IgM antibodies are significant opsonins for E. coli, but that complement is necessary to demonsrate full activity.

As was discussed previously, measurements of individual complement components may not reflect functional deficiencies within the system. Thus, McCracken and Eichenwald found a relationship between C3 levels and opsonic activity against S. aureus and Pseudomonas aeruginosa in their studies, but were unable to demonstrate a similar relationship toward E. coli.[125]

There are few functional studies of complement activities in neonatal sera. In yeast phagocytosis, Miller demonstrated a functional deficiency of the fifth component of complement in neonatal sera:[152] (1) Neonatal sera, which were opsonically deficient toward yeast particles, were restored to normal by the addition of sera from strains of mice with normal amounts of C5 (B10D2 new line), but not by addition of sera from strains of mice deficient in C5 (B10D2 old line). (2) With the use of highly purified human C3 and C5, reconstitution of yeast opsonic activity occurred only when C5 was added (3) Addition of three to five-day-old stored, citrated plasma to neonatal sera was ineffective in reconstitution, while fresh plasma was entirely restorative. The reconstituting effect of five-day-old plasma was *completely* corrected by the addition of C5-containing mouse serum, or by human purified C5, but not by mouse serum deficient in C5.

Little is known of the function of the alternate pathway of complement in neonates. It seems possible, however, that a developmental immaturity of the alternate pathway may play a role in the opsonic deficiency of neonates. Koch and coworkers found low levels of properdin in cord blood as compared with the levels in adult blood.[40]

A provocative, yet unexplained observation is that of Forman and Stiehm, who found that in low birth weight (less than 1,925 grams) infants, decreased levels of IgG appeared responsible for the opsonic defect.[121] In preliminary data, they found that the opsonic defect in six of these infants against their test organism could be corrected *in vivo* by intramuscular injection of Cohn fraction II (largely IgG globulin) or *in vitro* by the addition of IgG as opsonin.

THERAPEUTIC APPLICATIONS

One of the obvious gains to be anticipated from more complete characterization of the opsonic deficiencies of neonatal plasma is improved therapy in neonatal septicemia. While there is as yet insufficient information to establish a recommended therapeutic approach, there are clear trends emerging.

In order to summarize these trends, one must first attempt to reconcile the apparent differences among the various data on neonatal opsonins. The differences in relative opsonic deficiency toward Gram-negative bacteria found by Dossett and coworkers,[124] Forman and Stiehm[121] and McCracken and Eichenwald[125] are probably explicable on the basis of varying sensitivity of assays. The summary of the three studies is that neonatal sera have significant opsonic deficiency toward Gram-negative organisms, and somewhat lesser deficiency toward S. aureus.

The data of Dossett and coworkers showed that the deficiency of neonatal sera involves at least IgM and complement.[124] They therefore suggested the use of fresh plasma infusions in the treatment of neonatal septicemia. In the yeast phagocytic system, Miller demonstrated a relative deficiency of complement, more specifically of C5 in neonatal sera.[152] The defect was corrected *in vitro* by the addition of fresh (less than 24 hours old) citrated plasma, but not by aged plasma. The significance of these data depends upon the correlation between phagocytosis of yeast *in vitro* and phagocytosis of bacteria *in vivo*. Some suggestion that they may be related comes from the similar spectrum of pathogenic agents found in neonatal septicemia and in patients with C5 dysfunction.[142] There are, thus, data from several laboratories which support the use of fresh plasma in the treatment of neonatal septicemia.

Davis and coworkers found that transfusion of adult blood to premature infants (1,000 to 2,000 grams) brought about an increase in opsonic activity of their serum for E. coli.[153] Although the data are encouraging, controlled studies are necessary before justifying the routine use of fresh plasma in the treatment of neonatal septicemia.

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