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Evolution of serum C_3, IgG, IgA and IgM levels of healthy mothers and their mature newborns during the early neonatal period

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Susceptibility to infections is most pronounced during the perinatal period. The main characteristic feature of the clinical course is a proneness to generalisation; this can be attributed to immaturity of the immune system.

In addition to a series of laboratory parameters, quick and exact determinations of immunoglobulins and complement fractions repeatedly performed within a short time may be useful in confirming a suspected infection and in following the course of the disease.

In order to establish the normal basal levels and the dynamics of changes 30 mothers, all healthy, having no abnormality during pregnancy or shortly after delivery, and their healthy mature neonates were examined for IgG, IgA, IgM and C_3 serum levels. These basal values are useful in judging the parameters under pathological conditions.

During the perinatal period of life the fetus leaves his isolated environment and enters in, and adapts himself to, an open world carrying stimuli markedly increased both in quality and quantity. No order of importance can be established among the organ systems participating in this adaptation. Among them, the immune system faces extremely augmented tasks from the first second after birth: the organism changes its stimulus-free environment for a world full of antigens and it has to defend its integrity under the new conditions. It can do this only if its ontogenetic development has been undisturbed in the intrauterine environment. Maturation of the defence mechanism does not stop at birth; on the contrary it deepens and markedly speeds up after birth. Studies on the immune system in perinatal life not only elucidate the controlling mechanisms but also lead to a better understanding of the newborn's immunity and defence against infections.

Separate analysis of cellular and humoral immunity is not justified as the distinction between them has methodological rather than theoretical reasons. There is much controversy concerning perinatal immunology owing to the great number of methods and in pathological conditions their interpretation is even more difficult. The aspecific cellular immune functions like microphage activity, chemotaxis, phagocytosis and killing have been elucidated in most details [4, 16]. In newborn babies especially in those exposed to stress

situations, bactericidal activity is decreased mostly due to defects in the hexosemonophosphate shunt and the late oxidation system [36, 37]. In addition, the endogenous anti-oxidant system is less effective, leading to enhanced auto-oxidation and a further impairment of the newborns' defence mechanism [5, 39].

Neonatal proneness to viral and bacterial infections has been ascribed to the impaired function of the lymphoid cell population; some workers found, however, normal lyphocyte competence in newborn babies [13]. Among the lymphocytes there is a higher incidence of 0-cells void of surface markers [7, 18]. The response to various antigens and mitogens is also impaired, pointing to a decreased cellular immunity. In cases with high serum IgM levels the lymphocyte response to C on A is weak [14]. The B-lymphocytes induced in cord blood by PMW, a T-cell dependent activator, and one-week old neonates do not produce immunoglobulins in spite of a normal mitotic index, while T-cell dependent inductors are capable of enhancing IgM production in cells from the same blood samples [3]. The depressed immunoglobulin secretion has been attributed to increased suppressor activity originating from a lymphocyte population binding the monoclonal antibody OKT 8. these cells can be found both among E-rosette forming and non-forming cells, they are also present in the T_{gamma} and $T_{non\text{-}gamma}$ fractions alike [10, 12, 26, 33]. The supernatant of umbilical cord T-cells also shows a suppressive property against PWM stimulation [25].

An inhibitory effect of monocytes has not yet been fully proved but it is certainly a negative factor in IgG and IgM synthesis of newborns. In cell interaction studies primary B-cell immaturity has been demonstrated [11, 24]. Similarly, responses to T-cell mitogens, the NK cell effect and the MLC phenomenon all show a lower activity in newborns than in adults [1, 21, 40]. In the neonate the mitogenic response to Gram-negative bacteria is weaker than to Gram-positive ones [34].

Immaturity of cellular immunity is accompanied or followed by incomplete competence of the humoural system. The course of maturation of the various serum protein fractions is variable. A careful follow-up of the immunoglobulin levels is not only important for establishing the normal value and its limits but indispensable in the diagnosis of pathological conditions; in fact, it bears therapeutic consequences. At birth, only the IgG levels are equal to adult values; they fall to a minimum by the third month even in healthy infants. This is due to the well-known gap between transplacentar transfer and initiation of endogenous synthesis of IgG [8, 9, 38].

Recent work has shown that the transfer through the placenta is promoted by an active process showing increasing activity during pregnancy. This is the reason why the IgG concentration is low in immature babies.

In some mature newborns levels twice higher than the maternal value can be demonstrated. There is a linear correlation between gestational age and placentar transfer of IgG [30]. The placenta is impermeable for IgA and IgM [2].

IgG, IgM and IgA concentrations in the duodenal juice are constant from the second week to the nineteenth year of life [20]. The mammary secretion of neonates contains 0.03 times less IgA than the milk of their mother [40]. Newborns undergoing surgery for gastrointestinal anomalies and fed parenterally have markedly higher IgM and IgA and lower IgG levels than normal babies [31]. The maturation curves of C_3, C_4 and immunoglobulins obtained by nephelometry closely correspond to earlier findings [17]. Among the components of the complement system C_{1q}, C_3, C_4, C_5 and the total haemolytic complement (CH_{50}) have a reduced level during the neonatal period. Some other humoural factors such as properdin and interferon are also deficient [6, 32]. Alpha-fetoprotein, which has an immunosuppressive effect, exhibits a level increasing with the degree of immaturity [22, 27].

During the perinatal period even the mature organism has difficulties in surmounting viral and bacterial infections. Sepsis is often diagnosed late, although prevention and early diagnosis are the best weapons against sepsis. Birth and the subsequent few days are the most vulnerable period in this respect. Studies of the IgG, IgA, IgM and C_3 levels, the dynamics of their changes and interactions between these parameters during that period are thus justified.

The basis of our investigations were estimations of the C_3, IgG, IgA and IgM levels in maternal blood, in cord blood and blood taken from the newborn 72 hours after birth.

Material and Methods

Thirty mother-infant pairs were selected for the study. Pregnancy was uneventful and uncomplicated in all mothers, the history revealed no infection or drug effect. Delivery was uncomplicated, all newborn babies were mature and healthy. Blood for blood chemistry was taken by vein puncture during delivery, from the cord by the dropping method after dissection of the umbilical cord, and from a peripheral vein of the newborn infants 72 hours after birth. One ml blood was necessary for the determinations. All newborn babies were exclusively breast-fed. No infection was detected during the neonatal period, neither maternal nor neonatal.

Of the babies 16 were boys and 14 were girls. The mean duration of gestation was 38.7 weeks with a range from 37 to 41 weeks. Mean birthweight was 3150 g, the highest value was 3850 g, the lowest 2400 g (Table I). The mentioned determinations were performed in all three samples of each mother-infant pair. The measurements were carried out by rate nephelometry in the Beckman Immunochemistry System (ICS, Beckman Instruments, Inc., Fullerton, CA 92634). All dilutions and the solution for nephelometry were prepared by the use of a Beckman dilutor and dispenser.

All antisera, calibration mixtures, buffers and solutions were special Beckman kits; all the prescriptions of the makers

TABLE I

Number and data of mothers and newborn infants

n	Male/female	Gestational age week	Birth weight gram
30	16/14	38.5±1.5	3150±480

were strictly observed. The mean duration of one measurement was 1 minute. The levels were expressed in gram/liter units.

The maternal and newborn values were then compared; comparison was also made between the cord blood value and the 72 hour value in each case. The individual percentual changes or differences were calculated as follows

$$\Delta c\% = \frac{c_2 - c_1}{c_1} \times 100$$

where c_1 is the initial maternal resp. cord-blood concentration and c_2 the cord-blood resp. 72-hour concentration of the protein.

Statistical analysis comprised calculation of the means ($\bar{x}$) with scatter (s); the linear regression equation was constructed ($y = ax + b$), the correlation coeffient (r) was calculated. The levels of statistical significance were estimated by Student's *t*-test.

RESULTS

Marked differences were found in the C_3 level of the maternal and infant blood samples; the mean cord-blood level was 50% of the maternal level (cord-blood value, 0.88 g/l), it sharply increased by the third day of life (mean, 1.24 g/l) (Fig. 1).

The percentual average increase was 42%, however even the 72 hour value was significantly lower than the adult, maternal level (Table II). The IgG concentration of the cord-blood was higher than the maternal level but the difference was not significant statistically. It showed no change over the three days. Pair analysis of the maternal versus infant values revealed, however, deeper re

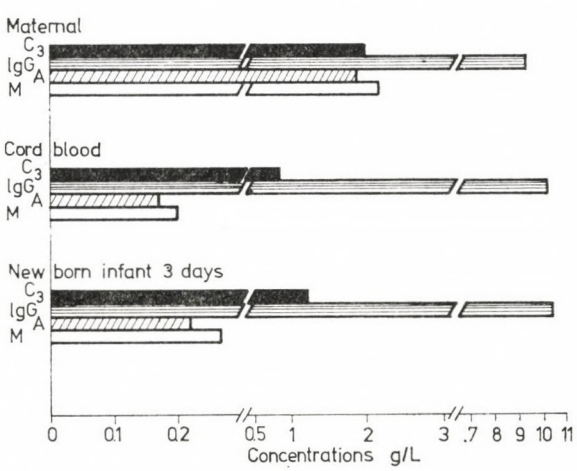

FIG. 1. Serum C_3, IgG, IgA and IgM in mothers, in cord-blood and in neonatal blood samples taken 72 hours after birth

TABLE II

Serum concentrations, g/L

	C_3		IgG	IgA	IgM
Maternal	2.04±0.45*	++	9.32±2.31	1.91±0.82	2.23±0.87
Cord-blood	0.88±0.30	++ ++	10.26±2.14	0.17±0.06 +	0.201±0.12 +
Newborn infant 3 days	1.25±0.35	++	10.39±2.76	0.22±0.09	0.22±0.08

* Values are (x ± SD) g/L

+ $p < 0.05$
++ $p < 0.001$

lationships which will be discussed in detail later.

The mean value of maternal IgA was nearly 2 g/l. There was no correlation between the maternal level and the cord-blood value.

After 72 hours the mean neonatal value was slightly but statistically

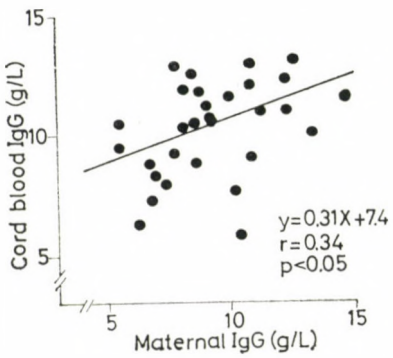

FIG. 2. Correlation between maternal and cord-blood IgG concentrations

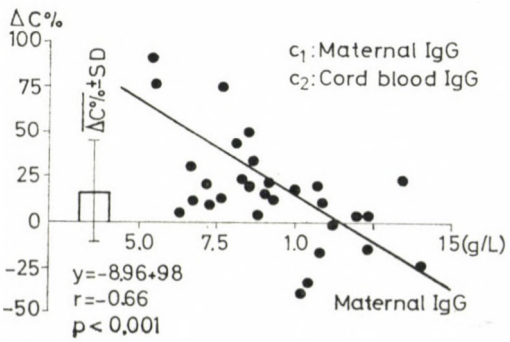

FIG. 3. Percentual difference between the cord-blood IgG level and the maternal IgG level plotted against the absolute value of the maternal IgG level

significantly increased. A value exceeding 0.1 g/l represents a markedly high level in healthy neonates of this age.

IgM showed a similar behaviour, a significant increase was seen by the third day of life. Here, a value higher than 0.2 g/l can be taken as a markedly increased level.

The following correlations were found.

There was a positive correlation between the maternal IgG level and the corresponding cord-blood value (Fig. 2). The infant's values were generally higher than the corresponding maternal value, the correlation was significant statistically.

A negative correlation was found between the percentual mother-infant difference and the maternal level (Fig. 3). In the infants of mothers with a low level the cord-blood value was about double while there was no increase or even a slight decrease occurred whenever the maternal IgG level was above the average. This correlation was significant statistically. The mean IgG level of the cord-blood samples and those taken 72 hours

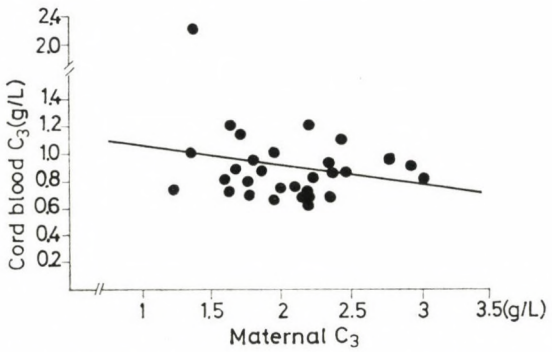

FIG. 4. Correlation between maternal and cord-blood C_3 levels

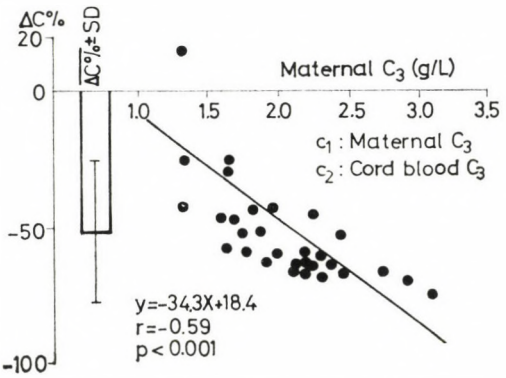

FIG. 5. Percentual difference between the cord-blood C_3 level and the maternal C_3 level plotted against the absolute value of the maternal C_3 level

after birth did not differ significantly.

In spite of considerable variations in the maternal C_3 level the cord-blood concentration showed a narrow scatter (Fig. 4). With a low maternal value the percentual deviation of the newborn value was small, i.e. a

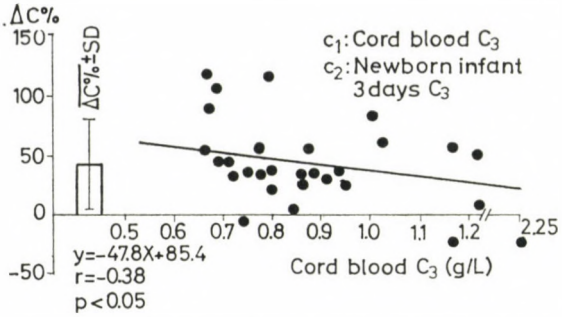

FIG. 6. Percentual increase in C_3 concentration in the same newborn during the first three days plotted against the absolute cord-blood value

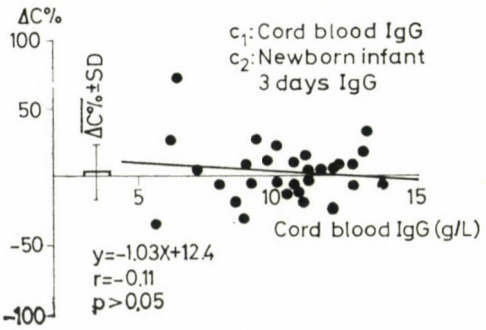

FIG. 7. Percentual increase in IgG concentration in the same newborn during the first three days plotted against the absolute cord-blood value

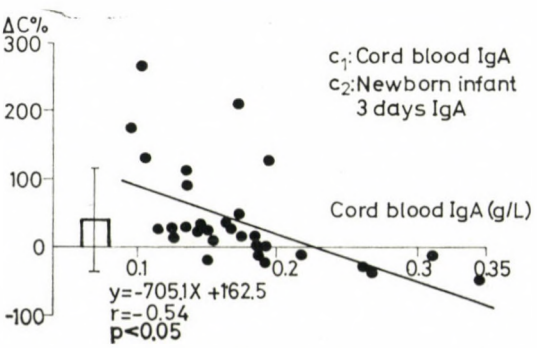

FIG. 8. Percentual changes in IgA in the same newborn during the first three days, plotted against the absolute cord-blood value

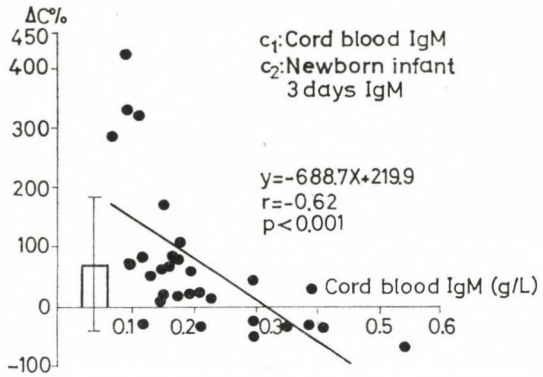

FIG. 9. Percentual changes in IgM concentration in the same newborn during the first three days plotted against the absolute cord-blood value

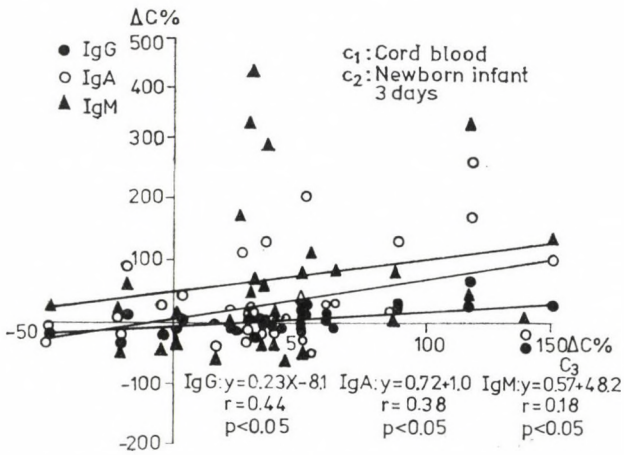

FIG. 10. Percentual changes in IgG, IgA and IgM concentrations plotted against percentual changes in C_3 concentration during the first three days of life

relative stability of the newborn level is secured (Fig. 5). The lower the cord-blood value the larger the increase by the end of the third day of life (Fig. 6). This correlation was also significant statistically.

Generally, the immunoglobulin levels increased within the first 72 hours of life, the increase was, however, different for the individual Ig classes. The IgG hardly changed (Fig. 7). The percentual increase of IgA was steeper in cases with an initially low IgA level (Fig. 8), and a similar trend was found with IgM (Fig. 9). Both correlations were significant.

A positive, significant correlation could be established between the percentual increase of C_3 and that of IgG and IgA. A similar trend between the increments of C_3 and of IgM was

seen but the degree of correlation did not attain statistical significance (Fig. 10).

The higher the maternal C_3 level, the higher were the IgG and the IgM levels in the same mother. High IgG levels were linked with high IgA and IgM in the same mother. A positive correlation was found for C_3—IgG and IgG—IgM in newborns at 72 hours of age. No other statistically significant relationship was observed, and the sex of the newborn had no effect on any parameter.

Discussion

In Hungary, 19% of the perinatal mortality was due to infection during the early seventies [19]. Since then, the situation has not changed significantly. Therefore, rapid and exact methods are necessary for the early detection of infection. Among others, leucocyte count, blood smear, erythrocyte sedimentation rate, C-reactive protein, haptoglobin, antithrombin III are of use, and determination of immunoglobulins and complement components and bacteriological cultures are of outstanding importance [28, 29, 35].

Most newborns infected during the perinatal period show a marked increase in IgM and IgA with a concomitant decrease in IgG [23]. In animal experiments, low activity of the complement system may lead to fatal bacterial infection. A follow-up of the C_3 level is of primary importance since it is a central link of both the classical and the alternative pathways. A low or absent value may critically affect the mechanisms of defence [15].

The method used for the determination of IgG, IgM and IgA and C_3 is rapid and reproducible. They are not only ancillary aids but elementary tools in screening and detection of neonatal infection.

Our data help in establishing the normal mean values and limits of these parameters in newborn babies. In addition, interesting relationships have been detected. The active transport responsible for the materno-fetal transport of IgG works more efficiently in the presence of a low maternal level. This secures an invariably high level in the mature infant. This in turn does not change during the first three days provided no infection or other pathological conditions occur.

Similarly, a slightly low but rather uniform level of C_3 is found in the cord-blood in spite of considerable variations of the maternal level. In other words, the newborn's level is rather independent of that of the mother. During the first 72 hours intensive synthesis occurs, especially if the initial value was lower than average.

IgM shows the steepest increase during the first 72 hours of life. This points again to the fact that the primary immune response is perfectuated by IgM production. In the newborn, all environmental antigens elicit a primary immune response. For similar reasons, the course of

IgA changes during the first days of life is only natural.

A common anabolic capacity may be the cause of certain correlations between the course of changes in various immunoglobulin levels; a steeper increase in C_3 is accompanied by a more marked increase in IgA and IgG. This development can be hindered by any derangement in the newborn's metabolism. In normal populations, a positive correlation exists between C_3 and immunoglobulin levels.

Serial determination of these parameters during the newborn period of life may be of great help in the early diagnosis and classification according to severity of infection. It may also be useful in evaluation of substition therapy carried out by administration of blood, plasma, immunoglobulins, leucocyte or platelet and complement preparations.

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