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Lymphocyte subpopulations and development of immune functions in the newborn baby

by

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The distribution and dynamics of T, B and O cell populations were investigated in healthy term and preterm babies of 22–38 weeks gestational age, in relation to gestational age and birth weight, using the single slide method. Children 1-14 years old served as controls.

Development of immunological competence in the fetus destined to ensure a smooth adaptation of the newborn is an important problem. The process of immune response in both intrauterine and extrauterine life is brought about by the interaction of a number of factors. This is well illustrated in the figure of Astaldi et al. [2] (Fig. 1). There are

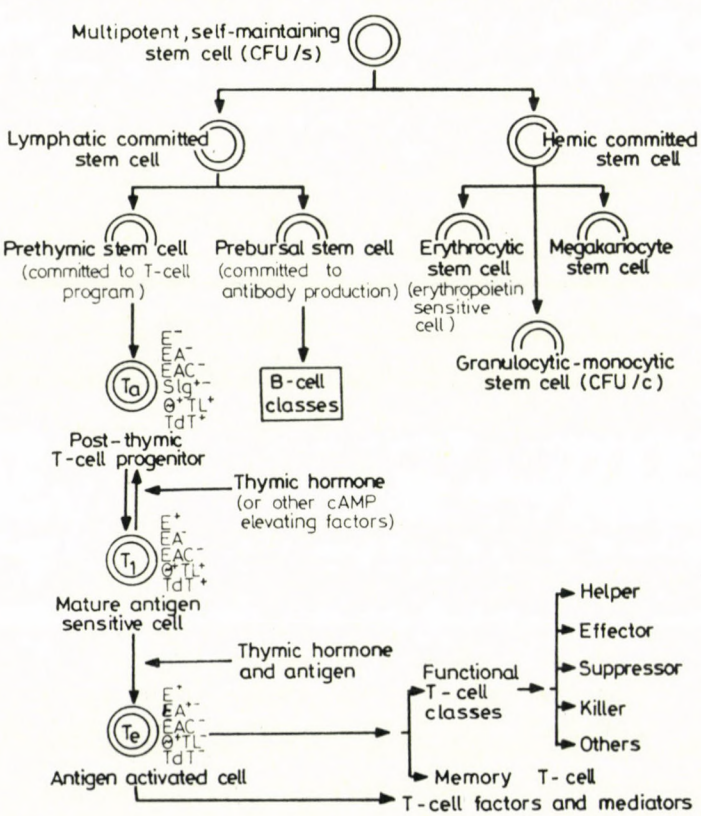


FIG. 1. The mechanism of immune response (After Astaldi et al. 2)

various ways to test the functional capacity of the immune response. The following approaches lend themselves for the purpose.

1) Studies on the maturation and development of phagocyting functions [3, 17, 22, 26, 36];

2) the appearance of circulating immunoglobulins [25, 30, 37, 39, 42];

3) the appearence and dynamics of surface membrane markers [9, 13, 14, 18, 19, 24, 32, 33, 44].

4) changes in enzyme functions affecting the immune functions [1, 15, 16, 23, 28, 38];

5) the effect of various realizing factors [31, 39, 40, 43, 45]; and

6) the isolation of the two lymphocyte subpopulations (T and B cells) on the basis of their electrophoretic mobility [8, 27, 34].

Most of the references cited above were concerned only with part of the development of immune competence, for example the time of the first or measurable reactions, the events following an antigenic stimulus, or the changes in inducibility and response to infections. Since the papers of Sterzl [39, 40] no comprehensive review has been published offering detailed information based on experimental work.

During the past few years we have attempted to study the development of immune competence and its changes during post-natal adaptation by means of complex methods. The present paper reports on some results of this work.

Lymphocyte subpopulations

Part of the methodology developed for the discrimination of T and B lymphocytes in human peripheral blood concerns the characteristics and maturation of surface membrane markers [13, 18, 19, 29].

B-cells carry on their surface receptor structures that derive from immunoglobulin molecules, and can therefore be demonstrated on the surface of these cells with the help of fluorescein isothiocyanate (FITC) conjugated anti-immunoglobulins. In addition, the presence of C_3 receptors can also be shown on these cells 37). B-cells also carry Fc receptors, binding the Fc part of immunoglobulins, that can be detected either with antigen-antibody complexes or with aggregated immunoglobulins [10]. The above methods identify about 25% of the normal adult peripheral blood lymphocytes as B-cells.

T-cells are characterized by the presence on their surface of sheep erythrocyte receptors; therefore, the demonstration of sheep red blood cell (SRBC) rosettes is suitable for the identification of this cell population.

The *total-rosette* technique, where lymphocytes are incubated with the SRBCs for 2 to 24 hr, identifies approximately 70% of normal, adult peripheral blood lymphocytes as T cells.

During a short incubation only the sensitive receptors will bind the erythrocytes. This technique, called the *active-rosette* method will dem-

onstrate approximately 25% T-cells in the peripheral blood. The most widely used rosetting techniques were described by Jondal [18, 19].

Another very sensitive but technically more difficult approach for the identification of T-cells involves the use of specific anti-T-cell sera [10].

On the basis of electrophoretic mobility it was established that T-cells belong to the "fast moving" category, having an electrophoretic mobility of over 1 micron $\cdot$ sec^{-1} $\cdot$ v^{-1} $\cdot$ cm, while B-cells move slower, their mobility being less than 1 micron $\cdot$ $sec^{-1} \cdot v^{-1}$ $\cdot$ cm [8].

Apart from studying the distribution and dynamics of T and B cells in preterm and term newborns, LDH isoenzyme investigations were carried out for differentiation of the two cell types [20].

T and B cells differ not only in their membrane structure but also in other functional properties. T lymphocytes for example have a higher neuraminidase activity but a lower sulphydryl and ribonuclease sensitive phosphatase activity, and they differ in acid alpha-naphthyl-acetate esterase and cholinesterase activity [23, 28]. Freeze-fracturing of their membrane also demonstrates considerable differences [29].

Some of the lymphocytes in human adult peripheral blood do not carry surface markers, and were therefore termed *nil-cells*. Greaves et al. [13]. described a method for their isolation, where sedimented lymphocytes were further separated on a nylon-wool column containing bound anti-immunoglobulin.

Opinions differ about the functional role of nil-cells. Using separated nil-cells obtained from malnourished children, Chandra [5, 6] demonstrated their lytic capacity on xenogeneic not target cells. The mechanism of this spontaneous lymphocytotoxity is not known. The phenomenon which might have important practical consequences was referred to as "in vitro veritas" by Podleski [35]. Chandra [6] described a suppressor effect of nil-cells on the PHA-induced DNA synthesis of T-cells. According to his view, the reduced cellular immune response of malnourished children could be explained on this basis. If this proves true, nil-cells might play an important role in combating infections.

In our studies, the method of Christiansen et al. [9] with minor modifications was used for the identification of the three lymphocyte subclasses. The method involves the use of membrane markers and is assessed on the same slide.

The relationship of lymphocyte populations to gestational age and birth-weight.

Patients and Methods

Patients. All tests were performed on newborns of less than a week of age, hosipitalized at our department. The male : female ratio was 1 : 1. Healthy children between 1 and 14 years of age served as controls.

When determining the relationship of lymphocyte subclasses to gestational age, the following groups were examined.

Less than 28 gestational week,	6 babies
29—36	11 babies
over 37	13 babies
controls	11 children.

There was a similar grouping according to birth-weight,

1001—1500 grams,	10 babies
1501—2500 grams,	10 babies
over 2500 grams,	11 babies
controls	9 children.

Lymphocyte suspension. 2—3 ml peripheral blood was drawn into 500 IU heparin, and sedimented at 1100 *g* for 20 min on a Ficoll-Uromiro gradient of 1072 p/ml specific gravity. Lymphocytes obtained from the interface were washed twice with PBS for 10 min at 200 *g*. Cell concentration was adjusted to 2×10^6/ml. The whole procedure was done at 4 °C.

Sheep red blood cell (SRBC) suspension. Defibrinated sheep blood was washed three times at 800 *g* for 10 min and the suspension adjusted to 2%. Human AB serum inactivated at 60 °C for 10 min was added. Optimal cell concentration was 2×10^8/ml.

FITC-conjugated anti-human IgG and IgM sera. FITC-labelled anti-IgG (Hyland, Antiserum gegen human IgG-Gamma-Ketten spezifisch) and anti-IgM (Hyland, Anti-serum gegen human IgM-μ-Ketten spezifisch) sera were adjusted with PBS to 10 mg/ml concentration.

Lymphocyte labelling. Two parallel studies were started for each newborn. In the first, 100 μl · lymphocyte suspension was incubated with 100 μl anti-IgG serum, in the other with 100 μl anti-IgM serum at 4°C for 50 min. This was followed by washing the lymphocyte suspension twice at 200 *g* for 5 min to remove excess serum and the cells were resuspended in 100 μl volume. 100 μl of SRBC suspension was added to both samples and the mixture was incubated at 37°C for 15 min. The cell suspensions were then centrifuged at 150 *g* for 10 min and further incubated at 4°C for 2 hours. After careful resuspension, results were assessed on a slide.

Evaluation under the fluorescence microscope. A Fluoval fluorescence microscope was used, equipped with a HB 200 light source, B 223 g and G 241 filters, 40/0.95 apochromatic lenses and 6.3 × eyepieces. 200 cells were examined in each sample. Lymphocytes were considered to be T-cells when they bound 3 or more SRBS-s. B-cells were detected as exhibiting surface bound IgG. IgM positive cells were also counted, their number is given separately since their ratio in accordance with other findings, was considerably lower than that of the IgG bearing lymphocytes. Cells that failed to form rosettes and did not carry immunoglobulins on their surface were designed "O"-cells. The ratios of cell subpopulations are

given in percentage. An attempt was made to determine the absolute values per μl blood for the lymphocyte subclasses but this was made unreliable by the finding that the WBC count in newborns shows considerable variation even within a short interval.

Evaluation was done with the two-tailed *t*-test. The Tables show the mean values ($\bar{x}$), number of patients (n), standard deviation (s_x), the *t* values and the level of significance.

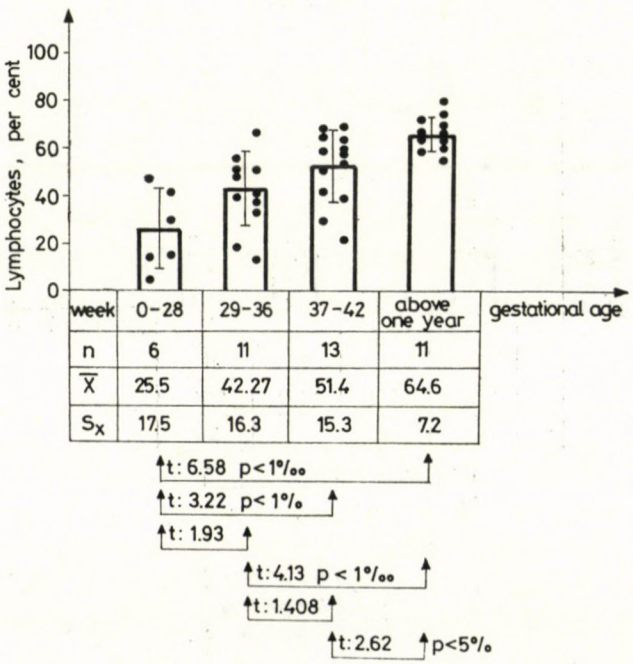
Results

T-cell subpopulation and gestational age. With the increase of gestational age the percentage of T-lymphocytes rises (Table I). Newborns of lower gestational age had lower T-cell values and the difference was significant. There was also a significant difference between any of the alternate groups.

B-lymphocyte subpopulation (IgG positive cells) and gestational age. In contrast to T-cells, the percentage of B-lymphocytes showed a decreasing tendency with gestational age (Table II). The lowest values were found in newborns of over 37 gestational weeks and the controls. However, a significant difference was only found between the control group and those with the lowest gestational age.

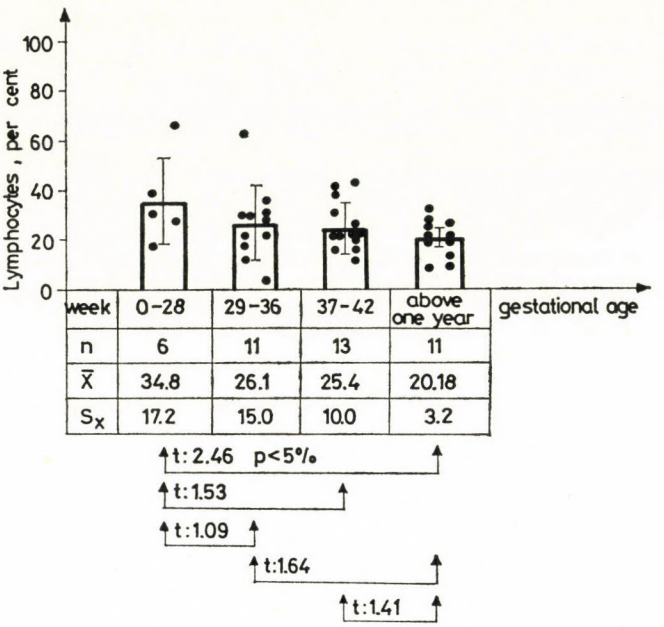
O-cell subpopulation and gestational age. With increasing maturity the number of O cells decreases (Table III). The values in the control group

Table I.



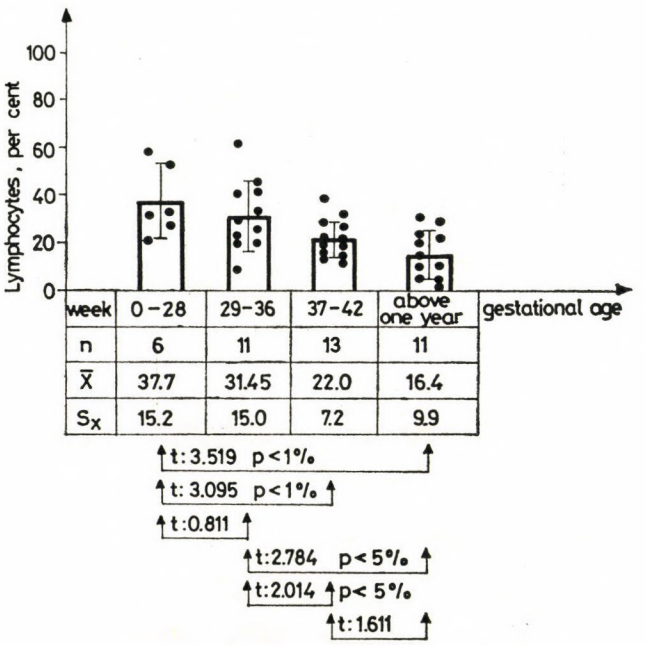
T cell subpopulation and gestational age

TABLE II



B cell subpopulation and gestational age

TABLE III



O cell subpopulation and gestational age

were higher than those reported for adults in the literature. Thus, the decrease continues until adulthood. In spite of the high values, the differences between the four groups were significant in all but two relationships.

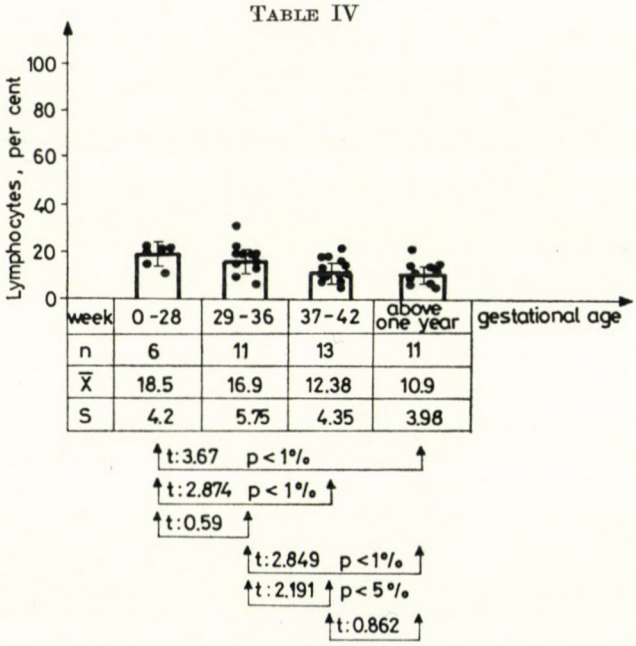
Lymphocyte surface IgM and gestational age. Similarily to IgG, the percentage of IgM bearing lymphocytes decreased with gestational age (Table IV). Since the number of IgM bearing lymphocytes in all the investigated groups was lower than that of the IgG bearing ones, the percentages are given separately for the two types of B-cell.

T-lymphocytes and birth weight. The number of E-rosette forming cells rose parallel with birth weight (Table V), similarily as with gestational age. The differences were significant in relation to the controls as well as between alternate birth weight groups.

B-lymphocytes (surface IgG positive cells) and birth weight. There was no direct relationship in the decrease of B-cells to increasing birth weight, as confirmed by statistical analysis (Table VI). The highest values were found in the 1001—1500 g group, followed not by the 1501—2500 g group but the one with birth weights above 2501 g.

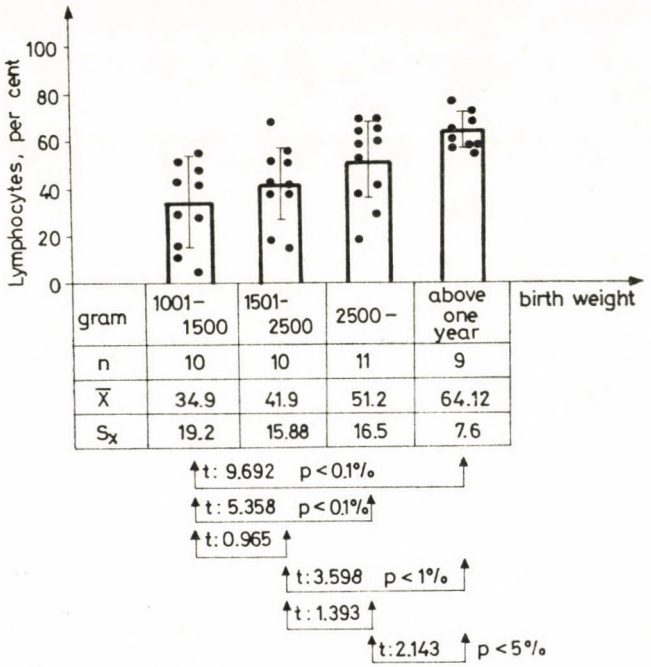
O-cell population and birth weight. The higher the birth weight, the less was the number of O-cells (Table VII). The highest values were found in the 1001—1500 g group, significant differences were, however, only found in two instances.

TABLE IV



Lymphocyte surface IgM and gestational age

TABLE V

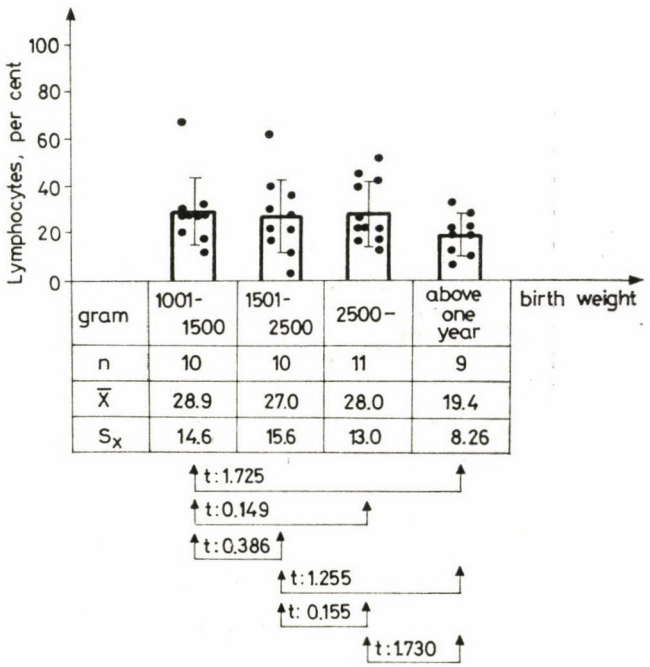


gram	1001–1500	1501–2500	2500–	above one year
n	10	10	11	9
$\bar{X}$	34.9	41.9	51.2	64.12
S_x	19.2	15.88	16.5	7.6

t: 9.692 $p < 0.1\%$
t: 5.358 $p < 0.1\%$
t: 0.965
t: 3.598 $p < 1\%$
t: 1.393
t: 2.143 $p < 5\%$

T cell subpopulation and birth weight

TABLE VI

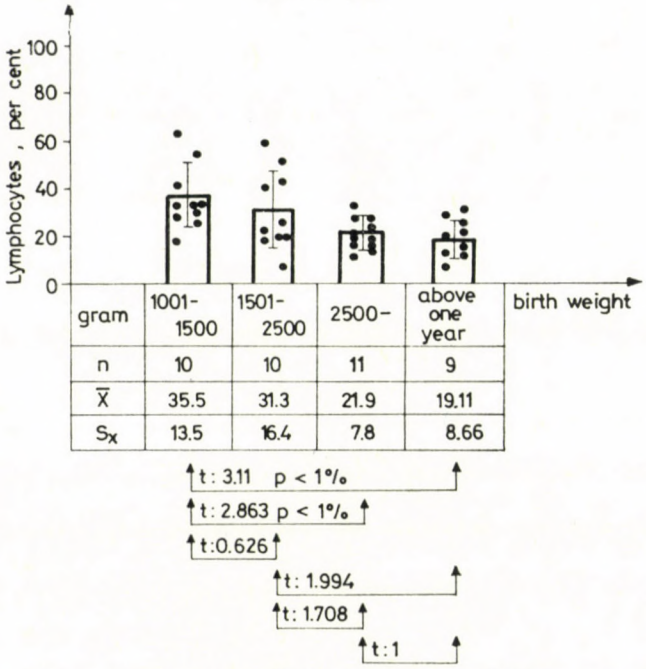


gram	1001–1500	1501–2500	2500–	above one year
n	10	10	11	9
$\bar{X}$	28.9	27.0	28.0	19.4
S_x	14.6	15.6	13.0	8.26

t: 1.725
t: 0.149
t: 0.386
t: 1.255
t: 0.155
t: 1.730

B cell subpopulation and birth weight

TABLE VII

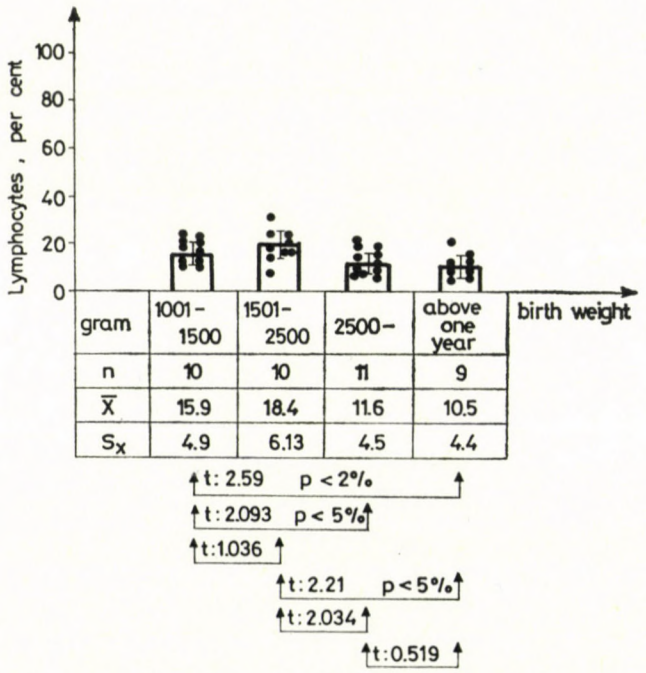


gram	1001–1500	1501–2500	2500–	above one year
n	10	10	11	9
$\bar{X}$	35.5	31.3	21.9	19.11
S_x	13.5	16.4	7.8	8.66

t: 3.11 p < 1%
t: 2.863 p < 1%
t:0.626
t: 1.994
t: 1.708
t:1

O cell subpopulation and birth weight

TABLE VIII



gram	1001–1500	1501–2500	2500–	above one year
n	10	10	11	9
$\bar{X}$	15.9	18.4	11.6	10.5
S_x	4.9	6.13	4.5	4.4

t: 2.59 p < 2%
t: 2.093 p < 5%
t:1.036
t: 2.21 p < 5%
t: 2.034
t:0.519

Lymphocyte surface IgM and birth weight

Lymphocyte surface IgM and birth weight. The distribution of IgM positive cells in the sub-groups was different from that of the IgG bearing cells. The highest values were found in the 1501—2500 g group, followed by the 1001—1500 g group (Table VIII). Significant differences were found in 3 instances.

Discussion

The aim of the present study was not the earliest detection of circulating T, B and O lymphocytes, but the determination of the ratio of immune-competent cells and their dynamics in newborns, with special regard to preterm babies. In other words, we wished to establish the normal values for these age groups. On the basis of 162 tests, carried out from 81 blood samples the following conclusions were drawn. (i) The ratio of T lymphocytes in peripheral blood increases parallel to gestational age and birth weight.

(ii) When analysing B cells, the ratio of IgM bearing lymphocytes was lower than that of the IgG bearing ones. As to their dynamics, both cell types showed a decreasing ratio with advancing gestational age. A similar but less marked change was found in relation to birth weight. We have no explanation for the phenomenon.

(iii) The decrease in the ratio of O-cells was closely related to the increase in gestational age and birth weight. The fact that even the control values were considerably higher than the normal adult values argues for a continuous decrease in this cell type throughout childhood.

The studies were designed to provide normal values for further analysis of the immune-reactivity during the period of post-natal adaptation. They should provide useful information for research workers engaged in this field.

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