

Correlation between Gestational Age and Buccal Cytology

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Buccal smears of 100 newborns have been examined after simple methylene blue staining on the first day of life. Gestational age of the infants varied from 24 to 44 weeks. A close inverse correlation between pycnotic index and gestational age was found ($r = -0.777$). Determination of the pycnotic index in the first day smear permits an approximative assessment of gestational age.

Since the small for date or dysmature newborn represents a welldefined clinical and therapeutical entity, many attempts have been made to find a simple method for reliable differentiation between premature infants and those with intrauterine growth retardation. However, besides certain physical and neurological signs which may be useful in estimating gestational age at birth, only a few laboratory methods seem to help in distinguishing true prematures from dysmature babies [8, 9, 11]. Unfortunately, even these methods are complicated, time consuming and because of a wide scattering, reliable only in typical cases.

Based on the observation of EIDENBENZ [2], a remarkable difference in the sex chromatin frequency of full-term and premature newborns was demonstrated by us [6] and by HOMMA and KAJII [4]. Although more detailed studies suggested that the variation of sex chromatin frequency

in the first days of life was due to incorrect selection criteria [1, 5, 7], it seemed interesting to study the possible cytological differences between buccal smears of newborns of various gestational age.

MATERIAL AND METHOD

In a preliminary pilot study buccal smears were taken from 30 newborns within 12 hours after birth. Gestational age of the babies varied from 27 to 41 weeks. A gestational age of 37 completed weeks was chosen as a dividing line between full-term and preterm infants. Newborns whose birth weight fell below the 10th percentile of our local standard [3] were rated as growth retarded for their gestational age. The smears were immediately fixed and stained in several ways including Papanicolau's method and thionine for sex chromatin. The analysis of this material suggested a conspicuously decreasing tendency in the ratio of pycnotic nuclei with increasing gestational age.

This is why in the second part of the study only the pycnotic index was exam-

ined. Buccal smears of 100 consecutively admitted newborns were taken, irrespective of sex, birth weight and maturity. Only those children were excluded where lacking exact information about the time of the last menstruation, the gestational age could not be determined. The smears were obtained within 24 hours post partum.

For determining the pycnotic index a simple quick method was elaborated.

The smears were fixed in alcohol for 30 minutes, hydrolyzed in 5N HCl for 5 minutes at room temperature, thoroughly washed in distilled water and stained in 5% neutral methylene blue. After staining and washing the slides in

Evaluation of the smears was carried out as follows. The smears were coded, the examiners did not know their origin. 200 cells without any selection were scored in each smear. Only small, dark, obviously shrunk nuclei were regarded as pycnotic, the percentage incidence of such nuclei was expressed as pycnotic index.

RESULTS

Buccal smears of 100 newborns (38 males and 62 females) were examined on the first day after birth. Gestational age of the infants varied from

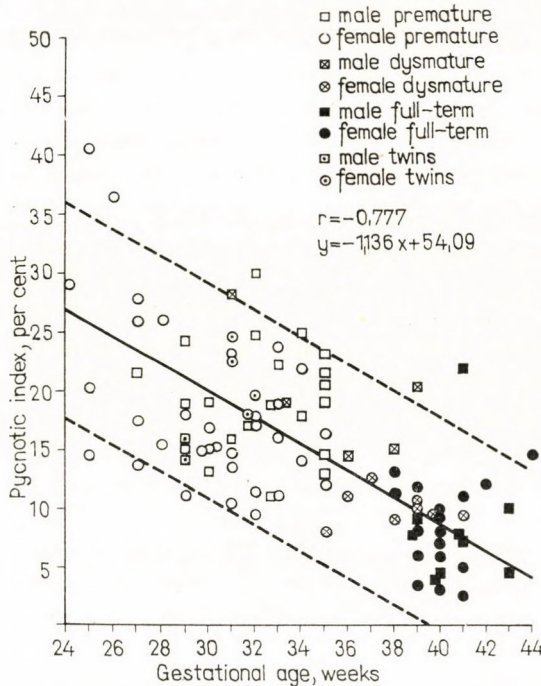


FIG. 1

water, they were let to dry and covered with Canada balsam.

The cytoplasm is not stained with this method. Normal nuclei are large and pale-blue with a fine intranucleic chromatin structure, while pycnotic nuclei appear as small homogeneously dark globules.

24 to 44 weeks. As shown in Fig. 1, a close inverse correlation between pycnotic index and gestational age was observed. No sex difference was found. A highly significant difference could be demonstrated when mean

values for prematures and full-term babies were compared ($p < 0.001$). The values for dysmature infants ranged well with those of prematures and full-terms of the corresponding gestational age. The inverse correlation between pycnotic index and gestational age in weeks was strongly significant, but because of the relatively large variation this method also failed in exact determination of maturity. However, as shown by the double standard deviation lines of the regression, the pycnotic index permits an approximative assessment of the gestational age of the newborn. A value of 5% makes, for example, a gestational age under 35 weeks rather unlikely, while a pycnotic index of 24% strongly suggests a gestational age of less than 35 weeks.

DISCUSSION

Many papers have dealt with the cytological changes in vaginal smears during early postnatal life, considering several possible relations between endocrine and morphological events, as recently reviewed by PILOTTI [10].

There was no reason to compare our results with these studies, since (i) in order to be able to examine also males, buccal smears were taken in the present study;

(ii) the staining method and evaluation applied were simplified procedures, pycnosis was scored only according to size and density of the nuclei, shape and colour, *i.e.* eosinophilia and basophilia of the cytoplasm, were disregarded.

Thus we could not make inferences as to hormonal changes, although it would have been interesting to examine the correlation between the endocrine changes of the last weeks of gestation and the pycnotic values found at birth.

The correlation between pycnotic index and gestational age obtained by our method proved highly significant. However, there was a considerable individual variation. This variation could be explained partly with real individual differences among newborns of the same maturity and partly with methodical errors. The greatest difficulties seem to appear in identifying nuclei with dense, structureless chromatin whose size is still larger than those with complete pycnosis. Incorrect evaluation seems to be the most common source of error, nevertheless, the disparity between pycnotic indices found by the two examiners was surprisingly small in this study.

Our method in its present form seems to be useful for an approximative assessment of gestational age. However, its accuracy is not worse than that of other more complicated procedures, and it has the advantage of being simple, quick, inexpensive and helpful in some instances to decide whether true prematurity or intrauterine growth retardation is responsible for the low birth weight.

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