Table 1. The percentage of erythroblasts with nucleoli and values of the nucleolar coefficient[a]

Cells	With nucleoli		Nucleolar coefficient	
	RNA	Ag	RNA	Ag
Polychromatophylic erythroblasts	**73.5** (8.1)[b]	**96.8** (3.1)*	**1.6** (0.7)	**3.5** (0.9)*
Orthochromatic erythroblasts	**39.1** (7.4)	**71.2** (3.5)*	**1.2** (0.2)	**1.4** (0.6)

a – in percentage
b – **mean** (standard error)
* – significant difference ($P < 0.02$) in comparison with cells stained for RNA using the t-test
RNA – specimens stained for RNA
Ag – specimens stained for AgNOR proteins

Silver-stained micronucleoli were also noted in erythroblasts expelling the nucleus (Fig. 3). The size of micronucleoli was always smaller than that of ring-shaped nucleoli of lymphocytes, i.e. smaller than 1 µm (Smetana, 1980). The incidence of micronucleoli in investigated cells and the number of nucleoli per cell expressed by the values of the nucleolar coefficient are presented in Table 1.

Polychromatophylic erythroblasts

In specimens stained for RNA about 73% of cells possessed nucleoli (Table 1). Almost all of them (97.2%, S.E. 4.2) were represented by micronucleoli, and other nucleolar types such as nucleoli with more or less distinct nucleolonemas or ring-shaped nucleoli (see Smetana, 1980) were practically absent in these cells. The number of nucleoli per cell expressed by the values of the nucleolar coefficient was about 1.6 (Table 1). In specimens stained for AgNOR proteins, the presence of nucleoli was noted almost in all cells and the values of the nucleolar coefficient were also significantly larger – 3.5 (Table 1).

Orthochromatic (late – pycnotic) erythroblasts

In specimens stained for the presence of RNA, nucleoli were noted only in 39% of cells (Table 1) and almost all of them (99.2%, S.E. 0.7) were represented by micronucleoli. In specimens stained for AgNOR proteins, nucleoli were present in a significantly larger number of cells, i.e. in 71% of them (Table 1). The values of the nucleolar coefficient did not show marked differences between cells stained with the silver reaction for AgNOR proteins and those stained for RNA (Table 1).

Discussion

The present study provided an additional information on micronucleoli in advanced stages of cell differentiation and maturation, which were represented in the present study by polychromatophylic and orthochromatic erythroblasts (see Erslev, 1972; Lessin and Bessis, 1972). The last dividing stages of the erythroid lineage – polychromatophylic erythroblasts characterized by the presence of micronucleoli – exhibit significantly larger values of the nucleolar coefficient in specimens stained for AgNOR proteins than in those stained for RNA. In addition, these last dividing as well as terminal non-dividing nucleated stages of the erythroid lineage – orthochromatic erythroblasts – possessed a significantly much smaller percentage of cells with micronucleoli after staining for RNA than after staining for AgNOR proteins. Thus, both these observations indicate that some micronucleoli do not contain RNA detectable by the light microscopic cytochemistry.

Therefore, based on the above described observations, it seems to be likely that micronucleoli representing the last stages of nucleolar development in maturing cells (Smetana, 1980; Schwarzacher and Wachtler, 1983) further develop and transform to nucleolar remnants or simple nuclear bodies, which lose RNA. Previous studies, comparing the values of the nucleolar coefficient of mature granulocytes and monocytes after visualizing nucleoli with various cytochemical procedures for demonstration of nucleolar proteins and RNA, support such conclusions (Smetana at al., 1997; Likovský and Smetana, 2000). On this occasion it should also be mentioned that few ultrastructural studies on embryonic rat erythroblasts and nucleated mature amphibian or avian erythrocytes also demonstrated that nucleolar remnants in these cells consist mainly of fibrillar centers composed of proteins, but without or only with a limited amount of surrounding RNA-containing structures (Zatsepina et al., 1988; Ochs and Smetana, 1991).

At the end of the discussion and possible interpretation of the above presented observations it should also be mentioned that the presented results confirm recent previous reports according to which the values of the nucleolar coefficient and incidence of nucleolated cells is larger after visualizing nucleoli by cytochemical methods for demonstration of characteristic nucleolar proteins than after those for demonstration of RNA (see Smetana et al., 1997; Likovský and Smetana, 2000).

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