FEULGEN-DNA VALUES IN MEGAKARYOCYTES

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INTRODUCTION

The prevailing trend in modern hematology considers the megakaryocyte as an originally diploid cell in which the chromatin material has undergone successive duplications without concomitant cytoplasmic partition (1–3). No agreement has been reached, however, concerning the maximum degree of ploidy reached by the megakaryocytic nucleus before the cytoplasm breaks up into platelets (4–6). Our cytophotometric findings, which have been reported previously (7), suggest a polyploid sequence from 2N to 64N. The aim of the present study is to submit the aforementioned data to further statistical analysis in order to judge the validity of the proposed pattern of development.

MATERIAL AND METHODS

The techniques and methods employed have already been discussed in detail (8). Bone marrow from both femurs of an adult male rabbit was smeared, air dried, fixed in a methanol-formalin-glacial acetic mixture, and stained with the Feulgen reaction according to Stowell (9). A total of one hundred and forty-five megakaryocytes were measured in a Pollister-type microspectrophotometer (10, 11), using the two-wavelength method developed by Ornstein (12) and Patau (13). Leucocytes and metamyelocytes were also studied in order to obtain diploid reference values (7).

RESULTS

Fig. 1 shows the histogram of megakaryocytes (extinction times area in μ^2) with the abscissae in logarithmic scale. Due to the latter, the areas representing 1 per cent of relative frequency become progressively smaller for each cycle. There are net peaks for the 2N, 4N, 16N, 32N, and 64N classes, while the 8N peak is missing, due, probably, to the technical problems involved: at this stage it is difficult to distinguish between a cluster of four reticular cells and an octoploid nucleus. If each datum is divided by the haploid Feulgen-DNA value (9 arbitrary units), only the degree of ploidy per nucleus is left. In order to get the mean class values, two methods can be applied:

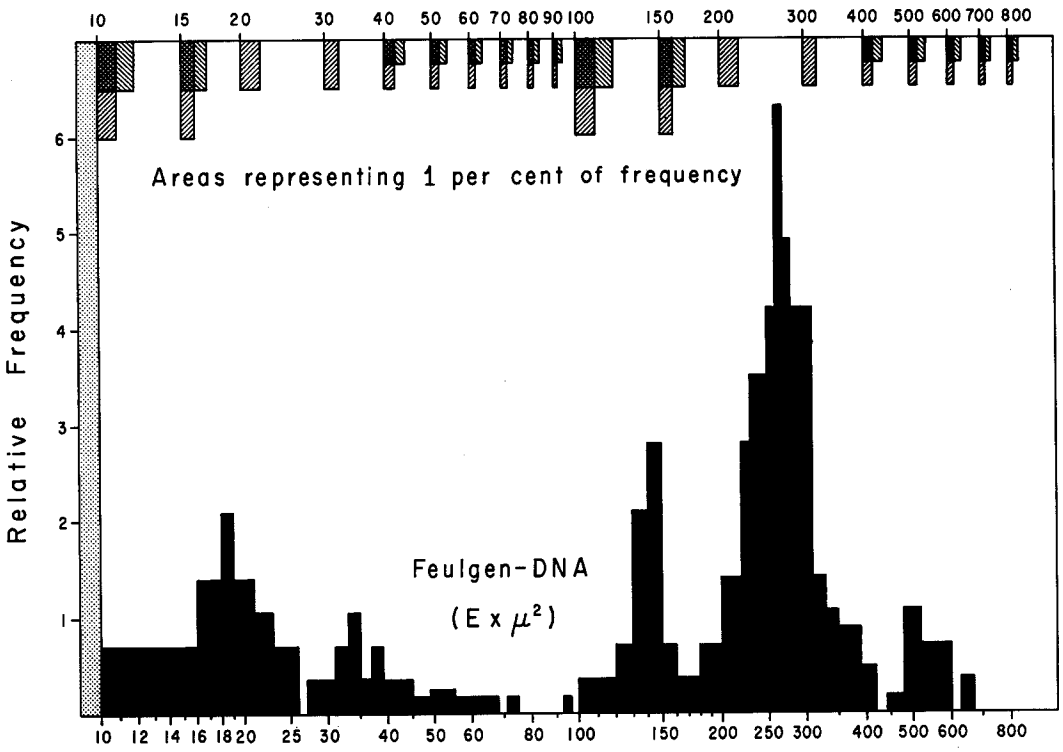

FIGURE 1 Frequency distribution of Feulgen DNA-values in rabbit megakaryocytes. Abscissae: DNA values in "extinction times area" units. Ordinates: relative frequency. Due to the logarithmic scale used in the plotting, the areas representing 1 per cent of frequency (top of the figure) become progressively smaller for each decimal fraction of any cycle.

TABLE I

Distributional Model

$(1.5)\ 2^{i-1}N$ ↓ Lower class limit	$< 2^iN$ ↓ Mean (log. distrib.)	$< (2^i + 2^{i-3})N$ ↓ Mean (random distrib.)	$< (1.5)2^iN$ ↓ Upper class limit
1.5	2	2.25	3
3	4	4.5	6
6	8	9	12
12	16	18	24
24	32	36	48
48	64	72	96
96	128	144	192

(*a*) the histogram is divided according to the different modes (18, 36, 132, 278, and 545 units); (*b*) the intervals are selected as shown in Table I. If 2^i represents a given power of 2 and N is the haploid Feulgen-DNA content, the mean of such a class should be 2^iN, provided the distribution is logarithmic; its lower and upper limits should be, respectively, $1.5(2^{i-1})N$ and $1.5(2^i)N$. If, however, the growth is not logarithmic but at random (*e.g.* by fusion of cells), for the same limits as above, the mean would shift from 2^iN to $(2^i + 2^{i-3})N$. In brief, if the distribution agrees with the powers of 2, these should become the mean class values (2N, 4N, 8N, 16N, etc.), whereas if it is at random the means should become progressively higher than the powers of 2 (2.25N, 4.5N, 9N, 18N, 36N, etc.).

As shown in Table II, the mean degrees of ploidy, selected by either of the two methods, are practically the same and fall within the powers of 2 (N, representing the haploid DNA content, has been dropped for simplicity). As additional information, two of the main variables involved in cytophotometry (variation due to the apparatus and variation among the Feulgen-stained nuclei of a certain class) have been separated by statistical evaluation and are expressed in Table II,

both as standard deviation and coefficient of variation. The methods for analysis of variance have been reported elsewhere (14).

DISCUSSION

Rothlin and Undritz (6) have outlined the following steps in the process of megakaryocytic maturation: (1) One 2N nucleus; (2) one 4N or two 2N nuclei; (3) four diploid nuclei or one big stage (sixteen nuclei) is the upper limit reached by these cells. Except for some variation in relative class frequencies due to differences in methodology, our findings are in agreement with those of Japa. The histogram and the mean class values draw clear lines between the 16N, 32N, and 64N classes, supporting the view of nearly simultaneous replications of chromosomal sets. This agreement certainly excludes a random manner of growth

TABLE II

Feulgen-DNA Values in Rabbit Megakaryocytes

Class	Mean Ploidy		SD Apparatus		SD Cells		C.V. Apparatus		C.V. Cells	
	V	P	V	P	V	P	V	P	V	P
2N	2.03	2.19	0.51	0.55	0.31	0.00	25.3	25.3	15.3	0.0
4N	4.04	4.15	0.64	0.63	0.41	0.55	15.8	15.1	10.0	13.4
8N	6.67	8.08	1.88	1.84	0.00	1.75	28.2	22.8	0.0	21.7
16N	14.65	18.34	1.22	2.48	1.76	3.14	8.3	13.5	12.0	17.1
32N	30.84	31.74	3.65	3.67	4.80	4.13	11.8	11.6	15.6	13.0
64N	60.61	60.61	4.74	4.74	5.67	5.67	7.8	7.8	9.4	9.4

V, Visual method. Classes selected from histogram.
P, Preset classes.
C.V., Coefficient of variation.

octoploid nucleus; (4) the 16N stage, called promegakaryocyte, which evolves without further growth into the adult megakaryocyte. Japa (4) studied human bone marrow using acetocarmine-stained squashes. By means of nuclear counts he concluded that megakaryocytes are multinucleate cells which develop by normal mitotic division without simultaneous cytoplasmic segmentation. He described five stages along the thrombopoietic line: (1) binucleate (2.5 per cent); (2) tetranucleate (25.5 per cent); (3) octonucleate (53 per cent); (4) cells with sixteen nuclei (18 per cent); and (5) cells with thirty-two nuclei (1 per cent). Cells with larger amounts of nuclei were not seen nor did their number fall out of the expected powers of 2.

More recently Kinoshita and Ohno (5), who followed the regeneration of rabbit bone marrow microcinematographically, contend that the 32N by cytoplasmic fusion of histiocytes or reticular cells.

SUMMARY

The analysis of the Feulgen-DNA content of megakaryocytes measured by cytophotometry confirms previous work indicating that growth of the nuclei occurs by self-duplication: 2N, 4N, 16N, 32N, and 64N peaks could be detected, suggesting that the development occurs by successive replications of DNA (or DNP) content and that the 64N stage is the upper limit reached by the cell before platelet production.

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