Nuclei from Normal and Leukemic Mouse Spleen. III. The Desoxypentosenucleic Acid Content per Nucleus Calculated from Total Cell Counts*

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In a study of the nucleic acid content of isolated nuclei, the average desoxypentosenucleic acid (DNA) content of nuclei from transplanted leukemic spleen (line 9421) was found to be increased to 1.45 times the normal value (6). Since only 5–10 per cent of the nuclei were recovered, however, the possibility arose that these nuclei were not representative of the total population. Since all the DNA is in the nucleus, the average DNA per cell or per nucleus can also be calculated from the total DNA content of any tissue if cell counts can be made on the whole organ (8). Previous total cell counts were unreliable (6), because many nuclei were trapped in networks of connective tissue fibers. This difficulty has now been overcome by a more thorough homogenization of the material, and reliable total cell counts have been obtained. The DNA content per cell has been calculated for normal spleen and for spontaneous and transplanted leukemic spleen. New data on isolated nuclei have also been obtained. The average DNA contents per nucleus obtained by the two methods are in close agreement. As had been anticipated from the lower DNA content of whole leukemic spleen, the number of cells per gram of spleen is considerably reduced in leukemia.

MATERIALS AND METHODS

Mice of the AK strain (2) were used. A spleen was considered normal if it was small in size and dark red in color and showed no enlargement of the splenic lymph buds. The normal spleens averaged 148 mg. (91–167) in weight. The spleens from the mice with spontaneous leukemia were very large, averaging 850 mg. (445–1,630) in weight, and varied from dark to light red. Two lines of transplanted leukemia were used: 9421 leukemia, which has been studied extensively in this laboratory (4–6), and AK4 leukemia (1). Leukemic spleen minced in physiological saline was injected intraperitoneally in the amount of 10^6 cells per mouse. Advanced leukemia developed in 8 or 9 days for the 9421 line, and the spleens averaged 352 mg. (308–420) in weight. In the AK4 line it took 6 or 7 days for the leukemia to develop, and the spleen weights averaged 417 mg. (308–500). In both lines of leukemia the spleens were light red in color, and the splenic lymph buds were greatly enlarged.

The mice were sacrificed by spinal fracture. Their spleens were quickly excised, cleaned free of visible fat, chilled, and weighed. One gm. of spleen from freshly killed mice was used for each experiment. The entire homogenization procedure was carried out at about 5° C. The tissue was homogenized for 2 minutes in a Potter-Elvehjem homogenizer (7) in 9 cc. of 0.88 M sucrose containing 0.0018 M calcium chloride for the normal spleens and 0.0023 M calcium chloride for the leukemic spleens (6, 9). The homogenizer was attached to an electronically controlled motor rated at 685 r.p.m. This homogenate was then transferred to a heavy-walled round-bottomed test tube, 27 mm. in diameter, containing 0.05 cc. of *n*-capryl alcohol. The homogenizer tube was rinsed several times, with a total volume of 10 cc. of 6 per cent acetic acid containing 0.0018 M calcium chloride for the normal spleens and 0.0023 M calcium chloride for the leukemic spleens. This mixture was homogenized for 2 minutes by means of a notched stainless steel blade, 1.4 cm. in diameter, attached to a shaft which was rotated by a Dumore motor rated at 16,000 r.p.m. for 110 v. For this homogenization the motor was run at 40 v.

The DNA was determined by the modified Schneider (10, 11) procedure previously described (4).

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Samples of the final homogenate were diluted 1:200 in red blood cell pipettes with 5 per cent acetic acid containing 0.06 per cent methyl green (8) and counted in a hemocytometer. Examination of the entire ruled area revealed only unclumped, clearly separated cells and nuclei. The contents of the four corner squares (a total volume of 0.4 c. mm.) were counted; this total multiplied by 10^7 gave the number of cells per gram of spleen. The results of 10 counts were then averaged to give the mean value for each experiment. The probable error of the mean ranged from 1–3 per cent.

When smears made from the final homogenate were stained with Wright's stain and examined microscopically under oil immersion at a total magnification of 1,225, the nuclei and cytoplasm could be clearly differentiated. In most instances the cytoplasm had been stripped from the nuclei. Although a great many of the nuclei looked ragged, none of them were broken in such a manner that two or more pieces of the same nucleus would have been counted separately as individual cells in the hemocytometer.

Normal and leukemic spleen nuclei were isolated and analyzed for DNA by the procedures previously described (6, 9). By reducing the diameter of the flannelette filters to 5 cm., the yield of nuclei was increased from 10 per cent to 30 per cent. Samples for counting were diluted 1:100 with the acetic acid-methyl green described in this paper. A volume of 0.4 c. mm. was counted, and 5 counts were averaged to give the mean value for each experiment.

RESULTS AND DISCUSSION

The analytical results are shown in Chart 1. The top row shows the mg. of DNA per gram of spleen. The average value for normal spleen, 15.0, agrees with previous values found in this laboratory (4). The average value for spontaneous leukemia is 12.7; for AK4 leukemia, 11.6; and for 9421 leukemia only 10.6 mg. per gram of spleen.

The second row shows the number of cells per gram of spleen for the same tissues. The average for normal spleen is 22.9×10^8 cells per gram; for spontaneous leukemia, 17.5×10^8; for AK4 leukemia, 15.7×10^8; and 14.1×10^8 cells per gram of spleen for 9421 leukemia.

The results for both DNA and number of cells per gram of normal spleen show considerably more scatter than do the values obtained on the two lines of transplanted leukemia. The values for spontaneous leukemia show an intermediate degree of scatter. The variation in the amount of scatter is not due to the technic, since the probable error of the mean is the same for all the cell counts. It is probably due to the fact that the transplanted leukemic spleens are subject to much less biological variation than are the other two types of spleen tissue. The transplanted leukemias are initiated in mice of the same age by the injection of a known number of cells, and the disease develops at a consistent rate for each line of leukemia. The variation in the spontaneous leukemic material is somewhere between that of the normal and the transplanted leukemias. Here two factors probably contribute to the scatter of the results: the variable rate at which the disease develops and the use of only one or two spleens for each experiment. The normal results show the most scatter of all, presumably because of the great variability of the normal material.

The DNA per cell, calculated from the above results, is shown in the bottom row, together with the values found for the isolated nuclei of the present study. The normal average value, 6.6×10^{-9} mg., agrees closely with the values found for the isolated nuclei—both the average of 6.4 found in the present study and that of 6.5 reported previously (9). A lower value, 6.0, has been reported for rat spleen nuclei isolated in citric acid (3).

For spontaneous leukemia the DNA content per cell, averaging 7.3×10^{-9} mg., is higher than the normal value. This is in close agreement with the average value of 7.4 found for isolated nuclei in the present study, but higher than the value of 6.3 found previously for isolated nuclei (6).

In the AK4 leukemia an average value of 7.4×10^{-9} mg/cell was obtained by analysis of whole tissue; no nuclei were isolated. In the 9421 leukemia the average values were 7.5×10^{-9} for whole tissue analysis and 7.2 for the isolated nuclei, again a reasonable agreement. These values are much lower than the average of 9.4 found previously for isolated nuclei (6). This decrease does not seem to be the result of an inconsistency in technic, since the DNA per nucleus found for normal spleen was 6.4 in the present study and 6.5 in the previous report (9). Furthermore, in earlier work (4) the total DNA content of the leukemic spleen was about 14 mg/gm of tissue, while in the present study it averaged only 10.4 mg/gm. This also implies that the low values found for DNA in the 9421 leukemia are the result of a change in the leukemia itself over the past 2 years (about eighty transplant generations), rather than of any technical error. A study of the correlation between the average DNA per nucleus in normal and transplanted leukemic spleen and the frequency of mitotic figures, myelocytes, megakaryocytes, and other cells with more than the diploid amount of DNA will be reported elsewhere.[1]

The close agreement between the DNA values obtained in the present study on isolated nuclei and on the whole tissue illustrates two points: first, that, within the experimental error, there is no loss of DNA during the isolation of the nuclei; and second, that the isolated nuclei are a representative sampling of the total nuclei, at least insofar as their DNA content is concerned.

In view of the decided difference in the number

[1] J. J. Biesele, N. A. Mixen, and M. L. Petermann, in preparation.

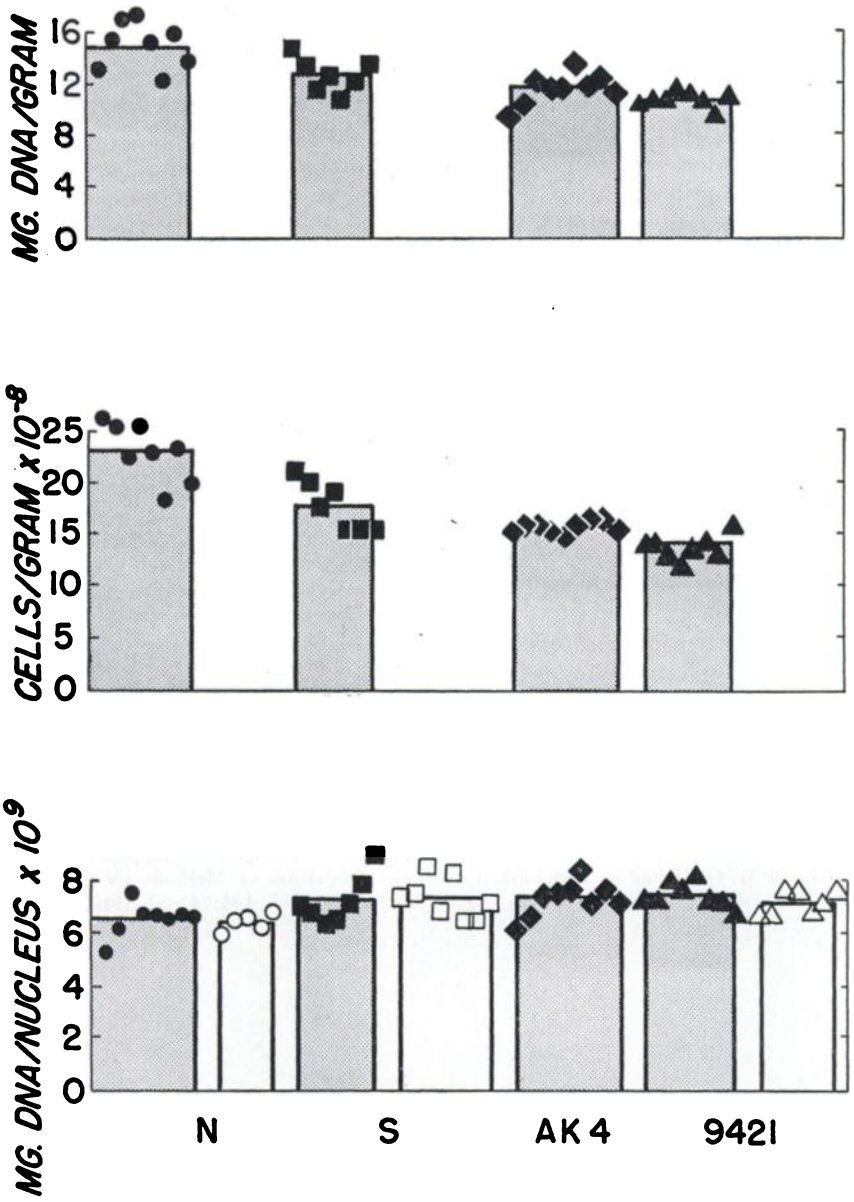

CHART 1.—Top row, mg of DNA/gm of spleen. Middle row, cells/gm of spleen $\times 10^{-8}$. Bottom row, mg of DNA/nucleus $\times 10^{9}$. ● = normal spleen; ■ = spontaneous leukemia; ◆ = AK4 leukemia; and ▲ = 9421 leukemia. The cross-hatched columns (closed symbols) show the values obtained on whole tissue, and the open columns (open symbols) show the values obtained on isolated nuclei. The height of each column corresponds to the average for each group of experiments.

of cells per gram of spleen found for normal and transplanted leukemic spleen, it is of interest that, when tissue homogenates are fractionated, the nitrogen distribution between the nuclear fraction, mitochondria, submicroscopic particles, and supernatant has always been the same for normal and leukemic spleen ([4] and unpublished[2]).

SUMMARY

1. A procedure for making total cell counts on mouse spleen has been described.

2. The desoxypentosenucleic acid (DNA) contents per nucleus calculated from the DNA contents and cell counts on the whole tissues were 6.6×10^{-9} mg. for normal spleen; 7.3 for spontaneous leukemia; 7.4 for transplanted leukemia, line AK4; and 7.5 for line 9421.

3. The same values were obtained for the DNA content per nucleus either by analysis and cell counts of whole spleen or by analysis and counts of isolated nuclei. This suggests that, when nuclei are isolated in a neutral sucrose-calcium chloride medium, no DNA is lost during the isolation procedure and that the isolated nuclei are representative of the total population.

4. The number of cells per gram of spleen was 22.9×10^8 for normal spleen; 17.5×10^8 for spontaneous leukemia; 15.7×10^8 for AK4 leukemia; and 14.1×10^8 for 9421 leukemia.

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[2] M. L. Petermann and M. G. Hamilton, unpublished results.