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MICROSPECTROPHOTOMETRY OF NUCLEAR DNA IN LYMPHOID CELLS OF LYMPH NODES IN SYSTEMIC BLOOD DISEASES

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Because of difficulty in the diagnosis of histological preparations of lymph nodes from patients with systemic blood diseases when ordinary methods of investigation are used, an attempt was made by microspectrophotometry to study the nuclear DNA content of lymphoid cells from patients with chronic lymphatic leukemia, myelomatosis, and a malignant lymphoma of non-Hodgkin type, and the results are given below [2-4].

EXPERIMENTAL METHOD

Microspectrophotometry was carried out on a scanning integrating digital microspectrophotometer, an improved version of the instrument which scanned an enlarged image of the object in two mutually perpendicular directions [1], and which differs from the prototype by scanning the image along lines 0.5 μ wide in frames measuring 5 × 5, 10 × 10, 15 × 15, and 20 × 20 μ^2. Scanning of the image along lines was linear; its sweep could be changed in discrete steps corresponding to the frame chosen by the investigator from the control panel. The frame was scanned in strips in stages with a 0.5-μ step. The scanning time was 5-20 sec. The signal from the photomultiplier for each line was led to a matching preamplifier, then to a logarithmic converter, converting optical transmission into optical density. From the output of the logarithmic converter the signal was led to an integrator, integrating optical density for each line, and at the end of scanning, giving out the total signal, proportional to the integral optical density of the biological object analyzed in the frame, and corresponding to the quantity of test material in it. The analog signal from the output of the integrator was transformed by means of an analog-to-digital converter into a digital signal. Unlike other known scanning devices, the instrument provides for automatic compensation of

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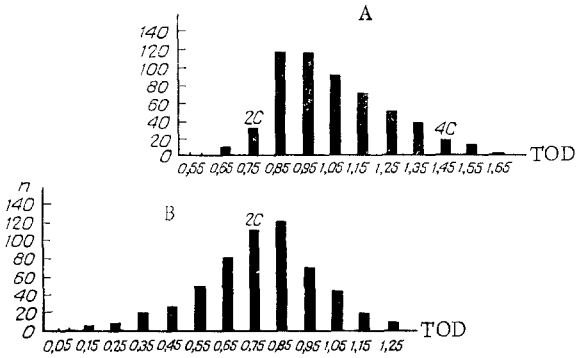

Fig. 1. Histograms of distribution of TOD of nuclear DNA in lymphoid cells of intermediate zone of lymph nodes. A) Malignant lymphoma of non-Hodgkin type, B) myelomatosis. Abscissa, TOD of lymphoid cells; ordinate, number of lymphoid cells. 2c) Ploidy of nuclei of lymphoid cells of two units.

the substrate in respect of each line of scanning. If scanning begins outside the cell (or nucleus) or is interrupted, the optical density in these areas is automatically compensated to zero and memorized. In this way, by contrast with the plug method, the true value of the optical density of the object analyzed can be obtained and the intensification effect can be avoided. Manual compensation of the optical density of the substrate is also provided for, with reduction of its value on the display panel to zero. The well stabilized source of light provides a constant monochromatic beam.

Histological sections 5-7 μ thick, cut from the superficial cervical lymph nodes of patients dying at the age of 30 to 75 years from chronic lymphatic leukemia (21), myelomatosis (six), and malignant lymphoma of non-Hodgkin type (six) during the first 24 h after the patients' death, were used for investigation. Material was fixed in 10% formalin and the sections were stained with hematoxylin and eosin and by the Feulgen method [5, 6]. The total optical density (TOD) of the nuclear DNA investigated in the frame was determined in conventional units (CU). To determine the ploidy of the lymphoid cell nuclei, the DNA content in nuclei of spermatozoa in histological preparations of ejaculate and from the epididymis of healthy subjects and from persons dying accidentally at the age of between 25 and 45 years was determined as the control. The mean DNA content in nuclei of the spermatozoa was 0.3576 CU ($m = \pm 0.002$), rounded off to 0.36 CU. This value was equivalent to a ploidy of one unit (1c). Since the histological pattern of the lymph nodes was obliterated in all cases, three zones were distinguished as a rough guide: subcapsular, intermediate, and periportal. The subsequent investigation was aimed at studying lymphoid cells of the intermediate zone. The nuclear DNA content was measured in 30 small, medium-sized, and large lymphoid cells in a $5 \times 5\ \mu^2$ frame (altogether 2970 measurements). The weighted arithmetic mean was then determined for each type of lymphoid cell, and also the mean value for all cells. Differences between the values obtained were analyzed at a 95% level of significance.

EXPERIMENTAL RESULTS

By microspectrophotometry it was possible to distinguish between malignant lymphoma of non-Hodgkin type, chronic lymphatic leukemia, and myelomatosis on the basis of the nuclear DNA content of the lymphoid cells. In malignant lymphoma of non-Hodgkin type (Fig. 1) the histogram of distribution of the nuclear DNA content in the lymphoid cells was shifted very slightly to the right (relative to a ploidy of 2c), and the maximal number of cells (the modal class) had a ploidy of 2.36c; the left-hand side of the histogram (relative to the modal class), moreover, sloped more gently whereas the right-hand side was steeper. The mean content of nuclear DNA in the lymphoid cells was 2.12 ± 0.02c. The histogram of distribution of the nuclear DNA content in the lymphoid cells in myelomatosis was shifted considerably to the right (relative to a ploidy of 2c), and the ploidy of the modal class of cells was 2.64c; the left-hand side of the histogram (relative to the modal class), moreover, was steeper and the right-hand side sloped more gently. The mean nuclear DNA content in the lymphoid cells corresponded to a ploidy of 2.85 ± 0.02c. In chronic lymphatic leukemia (Fig. 2) the histogram of distribution of the nuclear DNA content in the lymphoid cells was rather closer to the

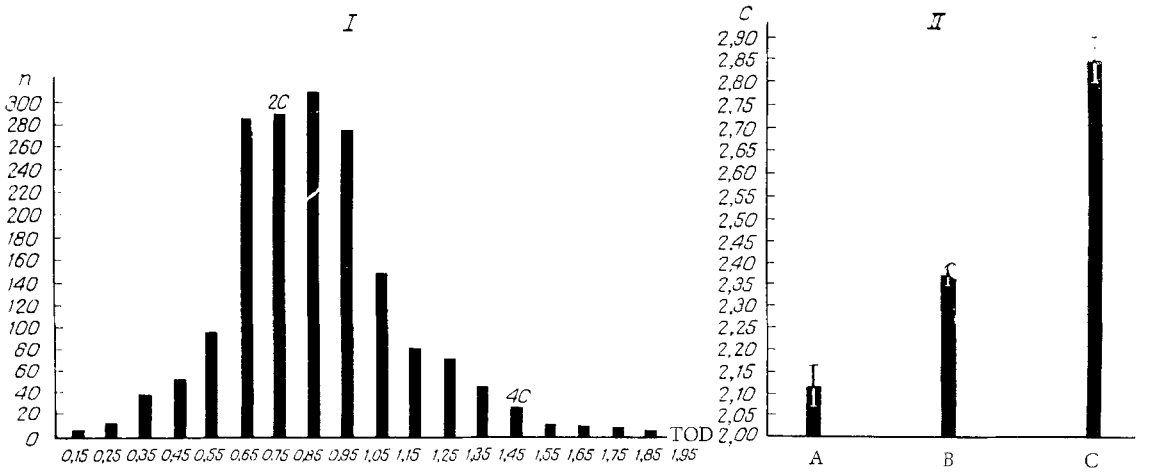

Fig. 2. Distribution of TOD of nuclear DNA of lymphoid cells in intermediate zone of lymph nodes in chronic lymphatic leukemia (I) and mean total content of nuclear DNA of lymphoid cells of intermediate zone of lymph nodes (II). 1) Abscissa, TOD of lymphoid cells; ordinate, number of lymphoid cells. 2c) Ploidy of lymphoid cell nuclei equal to two units. II) Abscissa, malignant lymphoma of non-Hodgkin type (A), myelomatosis (B), chronic lymphatic leukemia (C); ordinate, nuclear DNA content in lymphoid cells (in ploidy units — c). Vertical line shows twice the sampling error.

ormal distribution (relative to a ploidy of 2c). The ploidy of the modal class of cells was 2.36c. The left- and right-hand sides of the histogram (relative to the modal class) had approximately the same character of distribution. The mean nuclear DNA content in the lymphoid cells was 2.37 ± 0.01c.

By studying the nuclear DNA of lymphoid cells from the intermediate zone of the lymph nodes by this microspectrophotometric method it is thus possible to differentiate between malignant lymphoma of non-Hodgkin type, chronic lymphatic leukemia, and myelomatosis both by the character of their histograms and by their nuclear DNA content (Fig. 2). As will be clear from Fig. 2, the minimal content of nuclear DNA is found in malignant lymphoma of non-Hodgkin type (2.12 ± 0.02c), the maximal content in myelomatosis (2.85 ± 0.02c), and an intermediate content in chronic lymphatic leukemia (2.37 ± 0.01c).

The results show that this method can be used as an additional technique in case of difficulty in the diagnosis of these diseases.

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