

CYTOPHOTOMETRY OF DNA IN SERIAL SECTIONS

F. E. Vishnevetskii

UDC 612.398.145.1-087.852.4

A photometric (with the SPM scanning photometric microscope and the Wang-720C computer) investigation of DNA in lepra cells was carried out in series of paraffin sections stained by the Feulgen method. Considerable (up to 30-35%) fluctuations of the extinction values were found in individual sections in the same series. The aggregated results for the series were relatively uniform. It is concluded that to determine the DNA content reliably it is necessary to undertake photometric investigations on series of sections.

KEY WORDS: cytophotometry of DNA; serial sections; lepra cells.

The study of DNA by the Feulgen reaction is widely used in medical and biological investigations. In recent years various photometric systems have been used to estimate the intensity of this reaction, so that the DNA content in cells and tissues could be expressed quantitatively. The most accurate results have been obtained by the cytophotometric examination of squash preparations and films with cells arranged in a monolayer. However, in many investigations it is impossible to make films (because of the large number of fibrous structures, the firmly united cells, and so on), and the investigation must be carried out on histological sections, usually after embedding in paraffin wax. In this case, the intensity of the Feulgen reaction (including the photometric intensity) is estimated as a rule, on the basis of the study of single histological sections. The object of the present investigation was to determine the representativeness of a study of the DNA reaction in single sections and to ascertain how the actual DNA content in cells was reflected in the results so obtained.

EXPERIMENTAL METHOD

A macrophage from a focus of lepromatous infiltration, i.e., a lepra cell, was chosen as the test object. According to visual investigations [2, 3], during regression of the disease under the influence of treatment a gradual decrease in the DNA content in the lepra cell is observed. Biopsy material from cutaneous lesions in four patients with the lepromatous type of leprosy before treatment and from six patients with definite morphological signs of regression of lepromatous leprosy, namely vacuolation and destruction of macrophages, with disintegration and the almost total disappearance of the intracellular mycobacteria, was studied. Paraffin sections 5 μ thick, stained by the Feulgen method, were investigated. Two series, each of 10 sections, were cut from each block at different depths of the biopsy specimen. Photometry was carried out on single cells and fields consisting of lepra cells. The measurements were made with an SPM scanning photometric microscope (from Opton, West Germany), with a magnification of 1250 times, with an immersion objective. Before photometry, the dispersion curve of the absorption spectrum for paraffin sections stained by the Feulgen method was derived by a special program of lambda-scanning. The lowest values for transmission of light, i.e., highest optical density, were obtained with the monochromator giving a wavelength of 540 nm, which agreed almost completely with the results of testing the photometric microscope on standard preparations of mouse testis (560 nm) by the method of Agroskin et al. [1]. During photometry the intensity of the reaction was determined by comparison with an adjacent visual field, not containing DNA-fuchsin granules, for which the light transmission index was taken as 100%. The extinction index was calculated as the logarithm of the intensity of light transmission. The results were analyzed on a Wang-720C computer, coupled with the photometric microscope.

Research Institute for the Study of Leprosy, Ministry of Health of the USSR, Astrakhan'. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 86, No. 12, pp. 742-744, December, 1978. Original article submitted April 12, 1978.

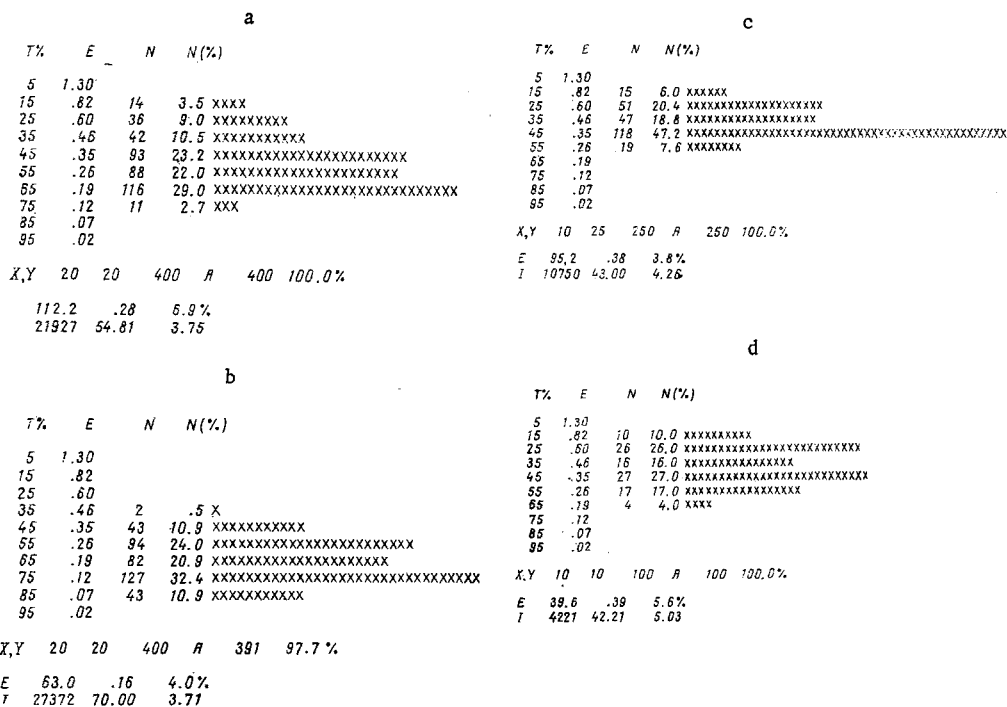


Fig. 1. Histogram of DNA; a) in young lepra cell; b) in fragmented lepra cell; c) in lymphocyte; d) in fibroblast.

TABLE 1. Mean Extinction Values During Photometry of DNA in Serial Sections from Zone of Infiltration

Section number	Fields of young lepra cells in biopsy material from untreated patients				Fields of destructively changed lepra cells in biopsy material from patients receiving effective antileprosy treatment					
	clinical cases (biopsy specimens)									
	1	2	3	4	5	6	7	8	9	10
1	0,30	0,22	0,26	0,30	0,16	0,14	0,18	0,20	0,16	0,20
2	0,28	0,30	0,32	0,30	0,14	0,16	0,14	0,14	0,20	0,14
3	0,22	0,26	0,30	0,24	0,15	0,20	0,16	0,15	0,17	0,17
4	0,30	0,30	0,28	0,30	0,14	0,18	0,15	0,17	0,15	0,19
5	0,26	0,28	0,25	0,30	0,16	0,15	0,18	0,14	0,16	0,16
6	0,25	0,26	0,26	0,26	0,17	0,16	0,17	0,20	0,14	0,20
7	0,32	0,22	0,22	0,22	0,16	0,14	0,14	0,18	0,18	0,15
8	0,30	0,30	0,22	0,22	0,18	0,20	0,20	0,16	0,19	0,18
9	0,26	0,24	0,24	0,24	0,14	0,14	0,16	0,16	0,16	0,20
10	0,28	0,26	0,26	0,22	0,14	0,16	0,16	0,15	0,15	0,20
M	0,28	0,26	0,26	0,26	0,15	0,16	0,16	0,17	0,16	0,17
±m	0,01	0,01	0,01	0,01	0,007	0,008	0,01	0,008	0,01	0,007
1	0,28	0,30	0,23	0,32	0,14	0,15	0,17	0,19	0,20	0,19
2	0,30	0,25	0,23	0,26	0,15	0,15	0,18	0,18	0,18	0,20
3	0,30	0,25	0,30	0,24	0,14	0,15	0,14	0,14	0,15	0,17
4	0,26	0,27	0,25	0,23	0,16	0,18	0,15	0,16	0,17	0,14
5	0,27	0,30	0,22	0,25	0,20	0,17	0,18	0,14	0,14	0,14
6	0,23	0,24	0,26	0,26	0,18	0,16	0,17	0,18	0,14	0,15
7	0,25	0,25	0,30	0,22	0,14	0,14	0,17	0,15	0,17	0,17
8	0,25	0,22	0,32	0,30	0,18	0,14	0,16	0,17	0,16	0,16
9	0,28	0,26	0,25	0,26	0,17	0,18	0,14	0,16	0,19	0,14
10	0,26	0,25	0,24	0,22	0,16	0,20	0,18	0,14	0,20	0,15
M	0,27	0,26	0,26	0,26	0,16	0,16	0,17	0,16	0,17	0,16
±m	0,01	0,01	0,01	0,01	0,008	0,009	0,007	0,01	0,01	0,008

EXPERIMENTAL RESULTS

The study of single cells showed that the DNA content in the lepra cell is relatively constant and correlates clearly with its structural state. For instance, the mean indices of extinction of young, poorly vacuolated lepra cells (in patients tested before treatment) varied from 0.23 to 0.28. In lepra cells under the influence of treatment the intensity of the Feulgen reaction was constantly much lower (extinction index 0.16-0.18). The decrease in extinction during destruction of the lepra cells took place mainly on account of DNA-fuchsin granules with the highest optical density. For instance, in the young lepra cell an extinction of between 0.46 and 0.82 was found at 23% of the points of measurement, whereas in the disintegrative cells it was found in only 0.5% (Fig. 1a, b). Compared with the results of cytophotometry of other cells in the lepromatous infiltration, the DNA content (intensity of the Feulgen reaction) in the lepra cells was constantly lower than in lymphocytes and fibroblasts, whose mean extinction was 0.39 and 0.38, respectively. Analysis of the histograms (Fig. 1c, d) showed the virtual absence of granules of DNA-fuchsin of low optical density, with an extinction of under 0.26, in the lymphocytes and fibroblasts, although they were comparatively numerous in the young (31.7% of points of measurement) and, in particular, in the fragmentary lepra cells (64.2% of points of measurement).

Since it was impossible to determine the intensity of the reaction in serial sections at different levels of the same cell, a photometric investigation was made of fragments of the focus of infiltration, which was clearly discernible throughout the extent of both series of sections at different depths. An area of infiltration consisting mainly of lepra cells with a minimal number of other cells was chosen. The area was divided into 8-12 rectangular (because of the rectangular shape of the scanning grating) fields, and these were measured successively. The results of photometry of the fragments of the focus of infiltration for each section were aggregated by the Wang-720C computer. The results of photometry of series of sections with respect to mean extinction values are given in Table 1. The cell fields of lepra cells had mean extinction values close to those obtained during the investigation of single cells of similar structure. Some increase in extinction could be explained by a sprinkling of single lymphocytes and fibroblasts among the mass of lepra cells. Marked fluctuations in the mean extinction index were constantly discovered in individual sections within the series. During the investigation of fields of young lepra cells they accounted for up to 30% (0.22-0.30) of the total, but up to 35% of disintegrated lepra cells (0.14-0.20). Meanwhile, the results of photometry aggregated for series of sections were relatively close within the limits of each clinical group. In the untreated patients the extinction index varied from 0.25 to 0.26, and during regression from 0.15 to 0.17. The differences for the groups of patients were statistically significant.

The results thus show that if the DNA content is determined in histological sections stained by the Feulgen method, investigation of single sections may show considerable fluctuations; reliable results can therefore be obtained only by the study of serial sections.

LITERATURE CITED

1. L. S. Agroskin, V. Ya. Brodskii, and G. V. Papayan, *Tsitologiya*, No. 4, 513 (1976).
2. F. E. Vishnevetskii, "Intravital morphological investigations of the liver and spleen of patients with leprosy," Author's Abstract of Doctoral Dissertation, Moscow (1969).
3. N. A. Vdovina, *Uchen. Zapiski Inst. Isuchen. Lepry (Astrakhan')*, No. 4 (9), 168 (1964).