CYTOPHOTOMETRIC MEASUREMENT OF THE CONTENT OF DNA IN NUCLEI OF LYMPHOCYTES FROM THE CORTEX AND MEDULLA OF THE THYMUS

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In textbooks and in reviews of the histology of the thymus [2, 5-7] the variation in the staining properties of the cortex and medulla is attributed entirely to differences in the number of lymphocytes in the two layers of the thymic lobules. In fact, many more lymphocytes are found in the cortex than in the medulla, although it has been observed [3, 4] that less chromatin is present in the nuclei of the lymphocytes in the medulla than in the cortex. This is evidently associated with the lower content of desoxyribonucleic acid (DNA) in the cell nuclei of the medullary zone of the thymus. On this basis it has been suggested that the thymus is an organ for supplying the body with DNA in the juvenile period, by providing it with actively developing cells in which DNA is reutilized [3, 4].

In this investigation the content of DNA in the nuclei of the lymphocytes of the cortical and medullary layers of the thymus was determined cytophotometrically.

EXPERIMENTAL METHOD AND RESULTS

The cytophotometric determination of the DNA content was carried out by a two-wave method [1]. An apparatus assembled in the laboratory of microscopy (Head, Professor E. M. Kheisin), Institute of Cytology, Academy of Sciences of the USSR, was used in the investigation.

As a first step the intensity of absorption of the chosen object—the nuclei of the lymphocytes of the thymus—was determined over the whole of the visible part of the spectrum (Fig. 1). Working wavelengths were selected from the absorption values given by these structures in the various parts of the spectrum—480 and 510 mμ. This choice of range of wavelengths was determined by the size of the test object (nucleus of the lymphocytes) and also by the fact that in this zone absorption is strictly proportional to changes in wavelength. As Fig. 1 shows, the curve of intensity of absorption against wavelength is a straight line between wavelengths of 480 and 510 mμ.

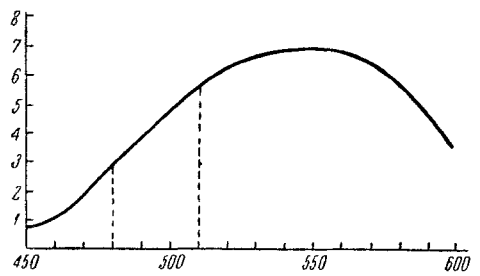

Fig. 1. Intensity of absorption of the visible part of the spectrum by nuclei of the lymphocytes. Along the axis of ordinates—intensity of absorption of light (in relative cytophotometer readings); along the axis of abscissas—wavelength given by monochromator (in mμ).

The sections used in the investigation were cut from a pig fetus to a thickness of 5 μ and stained for DNA by Feulgen's method. By means of the mechanical stage of the microscope the nucleus of the lymphocyte was moved into the path of a monochromatic beam of light, and the amount of unabsorbed light determined the photocurrent of the signal. The amount of light of another wavelength passing through the same nucleus was also measured. The total luminous flux of the probe (the amount of light passing through a probe outside the limits of the test tissue) was measured. The total luminous flux was also measured in a bundle of light of two wavelengths.

In this way four indices were obtained for each measured nucleus: I_1—the luminous flux with a wavelength of 480 mμ passing through the part of the area of the probe occupied by the nucleus; I_2—the luminous

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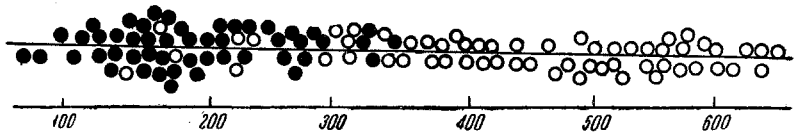

Fig. 2. DNA content in nuclei of the lymphocytes of the medulla (black circles) and cortex (white circles) of the thymus. The numbers indicate the relative DNA content in the nuclei of the lymphocytes.

DNA Content in Nuclei of Lymphocytes of the Thymus

Statistical index	Nuclei of lymphocytes in cortex of thymus	Nuclei of lymphocytes of medulla of thymus
Limits	127–702	97–411
Mode	445.6	206.4
Median	450.1	211.7
Standard deviation (δ)	144	74

flux with a wavelength of 480 mμ outside the section; I_0^1–the luminous flux with a wavelength of 510 mμ passing through the same nucleus; and I_0^2–the luminous flux with a wavelength of 510 mμ outside the section. The ratios I_1/I_2 and I_0^1/I_0^2 were calculated, and from Michaelis's tables the value of the optical constant A, proportional to the amount of matter in the measured nucleus, was found.

The results of the measurements are given in Fig. 2. The scatter of the black circles (the DNA content in the nuclei of the medulla) in this figure occurs in one region of values, and the scatter of the white circles (the DNA content in the nuclei of the cortex) in another region of values.

Altogether 110 measurements were made (60 nuclei in the cortical zone and 50 nuclei of lymphocytes in the medulla). The results of a statistical analysis of the data are given in the table.

The cytophotometric results thus confirmed the earlier observations, made by a visual method, concerning the difference in the DNA content of the nuclei in the cortex and medulla of the thymus.

Is this decrease in the DNA content in the nuclei of the lymphocytes in the thymus after migration from the cortex into the medulla [8] dependent on a change in their ploidy? Differences in the ploidy of nuclei of lymphocytes in the thymus have been demonstrated [9], but in homogenates. It is not yet certain, therefore, whether this difference in ploidy relates to the lymphocytes of the different zones of the thymic lobules. Further investigations are needed to answer the question.

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