ASYMMETRICAL HISTOGRAM OF THE NUCLEOLAR NUCLEIC ACID CONTENT AS A POSSIBLE INDICATOR OF RIBOSOMAL RNA AMPLIFICATION IN PURKINJE CELLS OF THE RAT CEREBELLUM

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In most Purkinje cells of the cerebellum the DNA content in the nuclei corresponds to its diploid level (2c) in rats, cats, chickens, and man [5]. In some of these neurons however, the DNA content is unusual for nonproliferating cells, namely intermediate between the 2c and 4c levels [1-3]. The number of these H2c Purkinje cells in rats is small, on average 3-5% [4]. It was important to determine the excess of nucleolar DNA. If, during photometry of the nucleus, the zone of the nucleolus (nucleolus and perinucleolar chromatin) was screened, the DNA-Feulgen content was reduced to the 2c level [5]. On the basis of this observation it was suggested that ribosomal DNA undergoes amplification in Purkinje cells. If this hypothesis is correct, H2c cells ought to differ from 2c cells in the possession of an increased RNA content, especially in the nucleoli.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 1 month and weighing 40-50 g.

Pieces of cerebellum were fixed in a mixture of formalin, alcohol, and acetic acid (3:1:0.3) for 1 h and embedded in paraffin wax by the usual technique. After dewaxing sections of known thickness the Purkinje cells were photographed on the MUF-6 microscope (objective 50, λ = 265 nm) before and after extraction of the nucleic acids (5% $HCLO_4$ at 90°C for 6 min). Photometry of the negatives was carried out on MF-4 or IFO-451 microphotometers. With the first instrument the mean optical density of the nucleolus was determined from measurements at three points within the organoid. Next, by means of an enlarger, the contour of the nucleolus was traced. To determine the nucleic acid content the product of optical density and size of the nucleolus was calculated. The IFO-451 microphotometer, which is equipped with an integrator, enabled the integral optical density, i.e., the nucleic acid content, to be determined automatically. In the case of measurements of the DNA content in the perinucleolar chromatin, the nucleolus and the zone of the nucleus with the nucleolus and the perinucleolar chromatin were subjected to photometry separately; the first value was subtracted from the second.

EXPERIMENTAL RESULTS

Radition in the UV region of the spectrum is known to be absorbed by DNA and RNA in the same zone of the spectrum, and to an equal degree. Nevertheless, the nucleoli differed from the chromatin granules (Fig. 1). The nucleoli were spherical in shape and much larger than the chromatin granules. After extraction of nucleic acids the nucleoli were clearly distinguishable as a ring formed by proteins of the perinucleolar chromatin.

Both nucleic acids are contained in the nucleolus. However, nearly all absorption of UV radiation was due to ribosomal RNA, a component of the subunits of ribosomes. In the perinucleolar chromatin practically all absorption is due to DNA.

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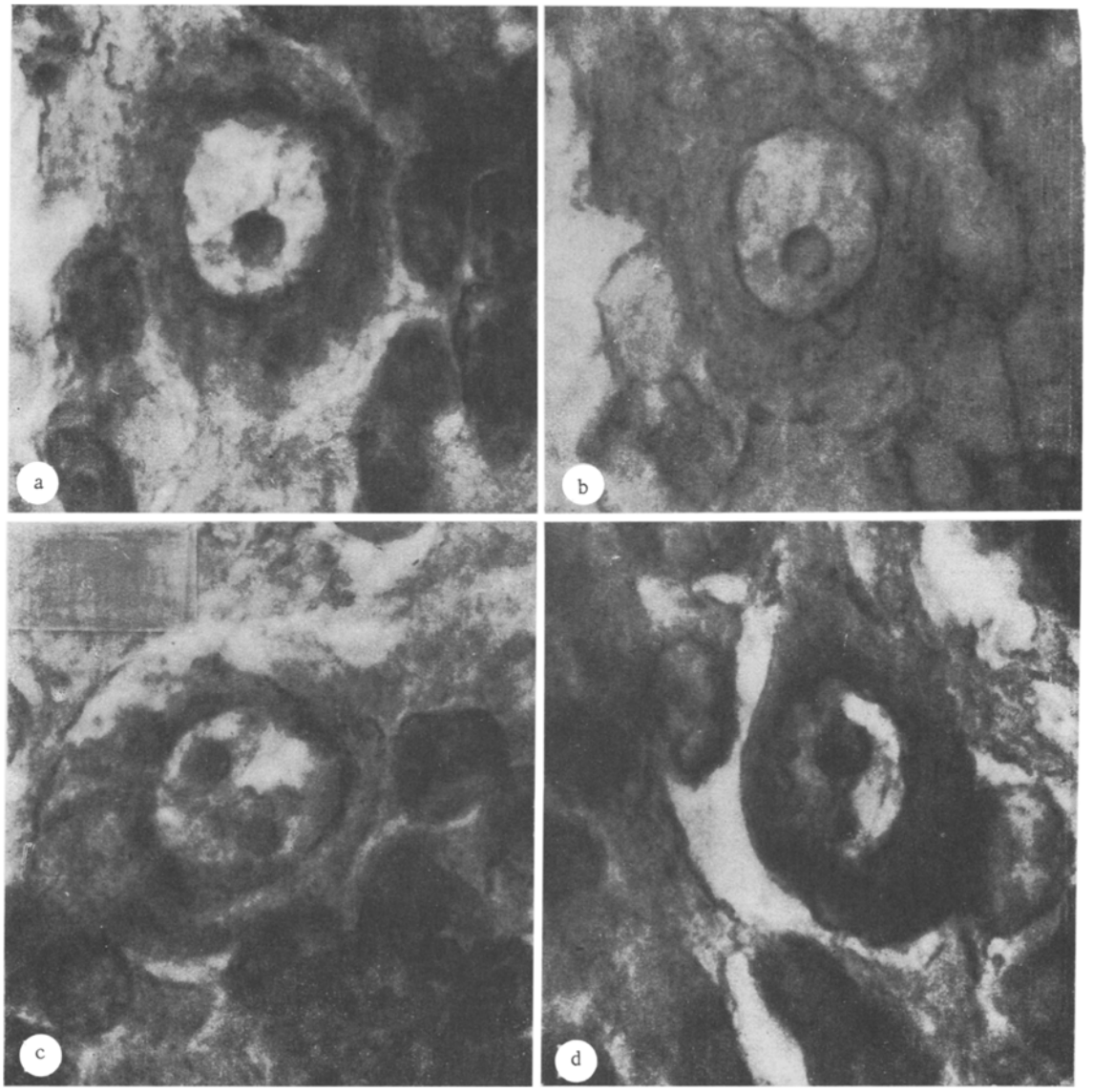

Fig. 1. Purkinje cell with typical content of nucleic acids in nucleolus (see maximum of Gaussian curve in Fig. 4) before (a) and after (b) extraction of nucleic acids. Mononucleolar (c) and binucleolar (d) Purkinje cells with increased nucleic acid content in nucleoli. MUS-6 microscope, objective 58 × 08, ocular 8.

The result of functional changes in RNA in the nucleoli ought to be a normal distribution of the measured amounts. The shape of the variation curve could be due to sections through the nucleolus (but this is unlikely, considering that the diameter of the nucleolus is only 1/4-1/5 the thickness of the section). The same distribution could be produced by random errors of cytophotometry. The normal, Gaussian distribution was in fact discovered when nucleoli of glial cells were studied (Fig. 2). The histogram of the nucleic acid content was strictly symmetrical. The histogram of the nucleoli in the Purkinje cells differed in its appearance (Fig. 3). Both histograms of nucleic acid content, for practical purposes limited to RNA, were asymmetrical. Most values were grouped in the normal distribution, but a few, about 5%, lay outside the Gaussian curve.

Some Purkinje cells have two nuclei, a few have three. It will be seen in Figs. 2 and 3 that most of the aggregated values of nucleic acids in two nucleoli fall on the right of the Gaussian curve. The percentage of binucleolar neurons in that part of the histogram lying outside the limits of the normal distribution was particularly high. However, cells with only one nucleolus also were present here.

Both in this and in the previous investigation [5] DNA of the nucleolus was not specially studied. Possible changes in it cannot be ruled out. The DNA content was definitely changed

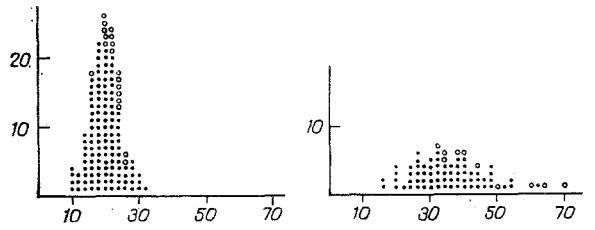

Fig. 2

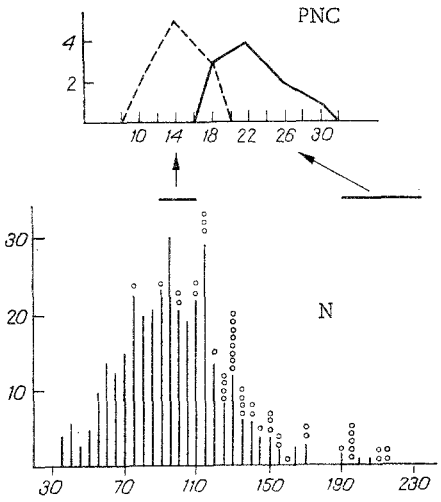

Fig. 3

Fig. 2. Distribution of nucleic acid content in nucleoli of Purkinje cells (right) and glial cells (left) according to results of photometry on the IFO-451 instrument. Each point represents one mononucleolar cell, each circle one binucleolar cell. Abscissa — nucleic acid content in nucleolus (in conventional units); ordinate — number of cells.

Fig. 3. Distribution of nucleic acids in nucleoli (N) and perinucleolar chromatin (PNC) of Purkinje cells according to results of photometry of negative. Vertical lines — mononucleolar cells, circles — one binucleolar cell; five animals, 389 cells. Abscissa — nucleic acid content (in conventional units); ordinate — number of cells.

in the perinucleolar chromatin (Fig. 3). Comparison of the DNA content in the perinucleolar chromatin of Purkinje cells with the typical RNA content in their nucleoli (the center of the Gaussian curve) with cells in which the nucleolar nucleic acids lay outside the limits of the normal distribution revealed an excess of DNA in the perinucleolar chromatin of the latter cells. It was impossible to determine the total DNA content in the nuclei in an investigation conducted on sections, i.e., to correlate changes discovered in nucleic acids in the nucleolus and perinucleolar chromatin with the H2c effect. Such an association is likely. The changes themselves are in good agreement with the hypothesis of DNA amplification in some Purkinje cells.

It would be interesting in the future to study nucleoli of Purkinje cells at the maximum and minimum of functional changes in the RNA content in the cytoplasm of the neurons. It would be important to study RNA synthesis, and not only changes in its content. We know that uridine is incorporated more actively in kidney cells *in vitro* into nuclei with two nucleoli, than into those with only one [6]. It these cells, however, nucleolar DNA was not amplified. Preliminary data on hybridization of ribosomal RNA with DNA and on incorporation of uridine into Purkinje 2c and H2c cells have confirmed the hypothesis of amplification of ribosomal genes in some Purkinje cells.

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