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PAPER

NUCLEAR MORPHOLOGY AND RNA CONTENT AS MARKERS OF FUNCTIONAL INTEGRITY OF NERVE CELLS, WITH APPLICATION TO THE STUDY OF DEMENTIA

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ABSTRACT

Under steady, or normal, conditions, cytophotometric and morphometric measurement of the amount of RNA and nuclear and nucleolar volume shows that for nerve cells of any particular type, all 3 are interrelated and set at genetically pre-determined levels that allow the production of precisely the correct amount of protein needed by cells of that size to maintain their metabolic economy. However, in abnormal situations, especially ones of neurological disease, such as the dementia of middle age and later life (Alzheimer's disease) these features are reduced in amount or size, to match altered requirements for protein by these cells. Furthermore, decreases in protein synthesis capacity correlate with degree of mental impairment. These findings indicate that measurements of RNA content, nuclear and nucleolar volume can act as markers of functional integrity of nerve cells.

INTRODUCTION

In certain chronic neurological disorders such as motor neurone disease, the loss and atrophy of specific nerve cell types such as anterior horn cells of the spinal cord and Betz cells of the precentral gyrus of the cerebral cortex can be readily discerned under the light microscope by eye alone. However, in many other situations the question of precisely which nerve cells are affected and to what extent is not so easily resolved by this simplistic approach. This limitation has for many years restricted our understanding of both the aetiology and pathogenesis of many disorders of the nervous

system; a way of distinguishing objectively those cells affected by the disease process, from others which are spared, is required.

Although the production of large quantities of protein is usually associated with the liver or pancreas, the level of such within the brain is similarly high, with most of this being synthesized by the nerve cells themselves. A measurement of protein production might therefore be one way of assessing nerve cell function. However, in humans, kinetic studies of protein synthesis cannot be easily performed since tissues obtained at autopsy are usually the only ones available for study. What is needed, therefore, is a method whereby it is possible to measure reliably, even in tissues obtained many hours after death, certain cell components which act as indices of protein synthetic capacity.

Biochemistry has shown that the RNA within the cytoplasm is located within the ribosomes; hence the amount of this may indicate the number of ribosomal sites upon which protein synthesis can occur. The nucleolus is the site of production of this cytoplasmic RNA; its volume is related to the rate of synthesis of ribosomal RNA (Watson 1968) and may therefore indicate ribosome turnover. Since the amount of DNA within nerve cells is uniformly diploid (Mann and Yates 1974), the volume of the nucleus indicates the degree of dispersion of the chromatin, and thus indicates proportion of transcriptively active DNA. Measurements of RNA content, nuclear and nucleolar volume therefore provide potentially valuable indices of nerve cell protein synthesis especially since they are all stable for long intervals after death (Mann et al. 1978) and, being readily identifiable in tissue sections, can be easily quantified by morphometric methods.

MATERIALS AND METHODS

RNA is demonstrated in paraffin sections of 16μm thickness using the metachromatic dye, Azure B (Shea 1970). In such preparations RNA is specifically stained dark blue, DNA a weak green. The nucleolus is prominent and the nuclear membrane well defined. Sections of this thickness include the maximum diameter of nucleus and nucleolus and permit a sufficiently large "slice" of neurone perikaryon to be measured for RNA content while adhering to Beer-Lambert law of light absorption (Mann 1972). RNA content is measured in such sections using a Leitz MPV microphotometer at a

wavelength of 580 nm (Mann et al. 1978) which ensures that light absorption by the Azure B-DNA complex is negligible. Correction for non-specific light loss is made using a cytoplasmic blank. Nucleolar and nuclear diameters are measured by micrometry in the same RNA stained sections using a Leitz ocular micrometer at a magnification of x1250. Nucleolar volume was calculated from formula, $V=\frac{\pi}{6}D^3$ where D=diameter; nuclear volume from $V=\frac{\pi}{6}ab^2$ where a=major diameter and b=minor diameter. Cytoplasmic RNA content, nuclear volume and nucleolar volume were measured in 60 nerve cells of each of those types shown in Table 1, in a 56 year old patient dying free from neurological or psychiatric disease. Relationships between each were examined by regression analysis.

RESULTS AND DISCUSSION

TABLE 1. Correlations between cytoplasmic RNA content (R), nuclear volume (V1) and nucleolar volume (V2) for nerve cells of types shown and for mean values of all types. All are significant, $p<0.001$, except those marked *, $p<0.01$.

CELL TYPE	R x V1	R x V2	V1 x V2
BETZ CELL	0.78	0.77	0.80
ANTERIOR HORN CELL	0.62	0.64	0.58
TRIGEMINAL MOTOR CELL	0.63	0.74	0.57
TROCHLEAR MOTOR CELL	0.67	0.75	0.53
PURKINJE CELL	0.56	0.81	0.34*
NEURONE OF DENTATE NUCLEUS	0.62	0.79	0.38*
PYRAMIDAL CELL OF HIPPOCAMPUS	0.65	0.83	0.60
MEAN VALUES FOR ALL CELL TYPES	0.972	0.981	0.993

Measurement of these features in individual nerve cells of any one type in normal individuals shows close linear relationships between each of them (Table 1). Furthermore, these relationships hold even more strongly when overall mean values from different cell types are correlated (Table 1).

TABLE 2. Mean total nuclear volume (N_1), nucleolar volume (N_2), cytoplasmic RNA content (R) and perikaryon surface area (P) for cells of the superior mesenteric ganglion, containing one to five nuclei, together with ratios of values of nuclear volume, nucleolar volume and cytoplasmic RNA content, to surface area, in such cells.

	NUMBER OF NUCLEI PRESENT IN THE CELL				
	1	2	3	4	5
NUMBER OF CELLS	30	10	5	2	2
TOTAL N_1	1267.2	1857.8	3069.1	3327.0	3926.1
TOTAL N_2	40.6	57.7	91.5	102.3	125.0
TOTAL R	21.3	28.8	47.1	56.4	69.6
P (μm^2)	2767	3860	6310	7201	8962
N_1/P	0.46	0.48	0.49	0.46	0.44
N_2/P	0.0143	0.0149	0.0145	0.0142	0.0139
R/P	0.0077	0.0075	0.0076	0.0078	0.0078

Examination of single and multinucleated nerve cells of sympathetic ganglia (Table 2) shows that the amount of RNA (R) within the cell is proportional to the total volume of the nuclei (N_1) and the nucleoli (N_2) with the summated values of each being related to the size of the cell as indicated by perikaryon surface area (P). Therefore, in normal individuals the RNA content, nuclear and nucleolar volumes of nerve cells are all interrelated and set at genetically predetermined levels that allow the production of precisely that amount of protein needed by cells of that size to maintain their metabolic economy. In neurological disease, however, the metabolism of nerve cells is often altered and usually towards a failure of function. This change may not be apparent in conventional preparations examined under the microscope by eye alone, but RNA staining combined with morphometry detects such alterations. This point can be illustrated by reference to a study of Alzheimer's disease.

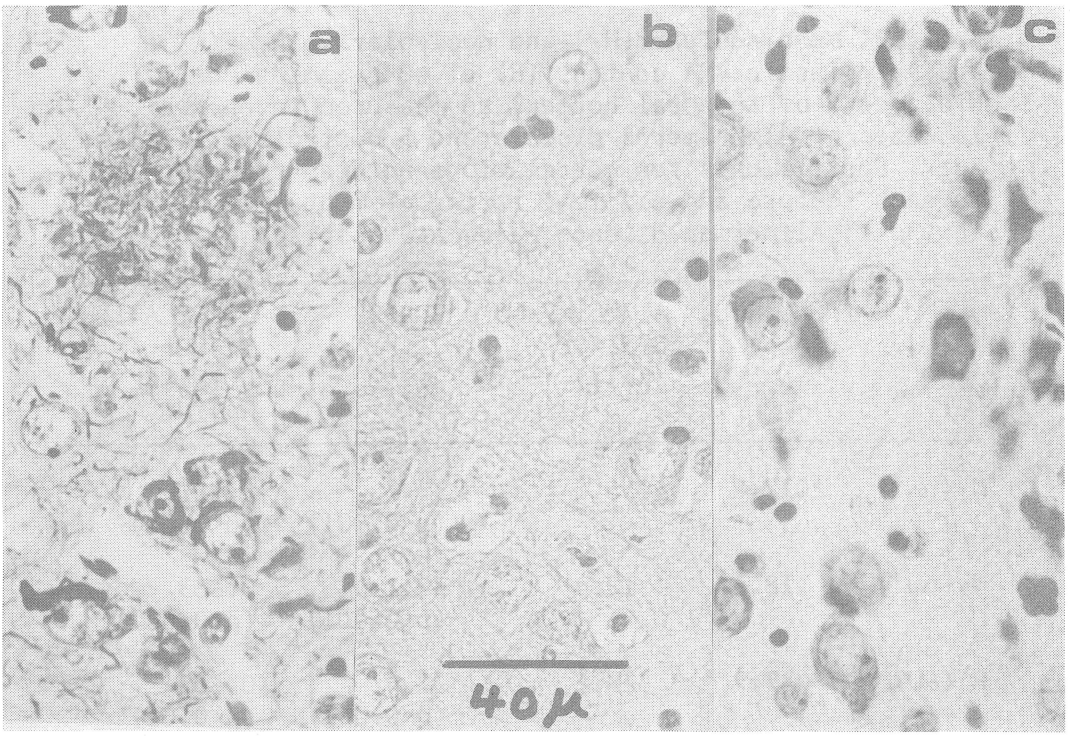

Fig.1. Temporal cortex in a case of Alzheimer's disease stained by Gros-Bielchowsky (a), haematoxylin (b) and Azure B (c) methods. The magnification is the same in each instance.

In sections of cerebral cortex stained by silver impregnation techniques (here Gros-Bielchowsky) the characteristic pathological features of Alzheimer's disease, namely senile plaques and neurofibrillary tangles, are obvious (Fig.1a). In the same view in haematoxylin and eosin prepared sections (Fig.1b) it is difficult to discern these features, nor can we detect with any precision which nerve cells are involved in these processes and to what extent. However, in Azure B stained sections (Fig.1c), RNA loss is obvious and different degrees of change within neurones apparent.

Nuclear and nucleolar volume and cytoplasmic RNA are all significantly reduced in Alzheimer's disease (Table 3) and the magnitude of the reduction in each correlates significantly with the degree of mental impairment in that patient, as measured in tests of cognitive function (Neary et al. - unpublished observations). Both sets of measurements thus reflect the failure of neocortical neurones that occur in this disorder.

TABLE 3. Mean nuclear (N_1) and nucleolar volume (N_2) and cytoplasmic RNA content (R) of 40 pyramidal cells of layer 5 of temporal cortex, as measured in each of 13 cases of Alzheimer's disease and 5 controls of similar age. Coefficients for correlations between reduction in each feature and clinical rating of mental impairment in the 13 Alzheimer disease cases are also shown.

	CONTROL (n=5)	ALZHEIMER'S DISEASE (n=13)	% LOSS	CORRELATION WITH CLINICAL RATING
$N_1(\mu m^3)$	2017.8	1258.2	37.7 ($p<0.001$)	0.528 ($p<0.01$)
$N_2(\mu m^3)$	18.1	12.3	32.1 ($p<0.001$)	0.683 ($p<0.001$)
R (AU)	37.9	29.2	22.9 ($p<0.001$)	0.602 ($p<0.01$)

In summary, therefore, details are presented of a morphometric technique which allows the assessment of protein synthesis within nerve cells, and in doing so, indicates alterations in their capacity for function. These kinds of measurements are valuable since they clarify the extent of nerve cell involvement in diseases of the human nervous system using tissues which, despite being readily available, are usually unsuitable for most biochemical studies of nerve cell function and provide a useful adjunct to histopathological diagnosis.

REFERENCES

Mann DMA. Nucleic acid content of cells of the human nervous system. Ph.D. Thesis. University of Manchester 1972.

Mann DMA. Barton CM, Davies JS. Postmortem changes in human central nervous tissue and the effects on quantitation of nucleic acids and enzymes. Histochem J 1978;10:127-35.

Mann DMA, Yates PO. Polyploidy in the human nervous system. J Neurol Sci 1974;18:183-96.
Shea JR. A method for the in situ estimation of absolute amount of RNA using Azure B. J Histochem Cytochem 1970;18:143-52.
Watson WE. Observations on the nucleolar and total cell body nucleic acid of injured nerve cells. J Physiol 1968;196:655-76.