REACTION OF *N*-METHYL-*N*-NITROSOUREA WITH DNA OF NEURONAL AND GLIAL CELLS *IN VIVO*

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1. Introduction

On the basis of observations on carcinogenesis *in vitro* by tumour viruses, chemicals and irradiation Sachs [1] has suggested that the development of a visible tumour *in vivo* requires (a) a change in the control mechanisms for cell replication (b) fixation of this change in the cell so that it can be transmitted as a hereditary property and (c) growth of a single tumour cell into a visible tumour. He has further suggested that chemical carcinogens may induce changes in cellular control mechanisms by interacting with DNA while admitting that the primary interaction might be with some other intracellular constituent or directly with the cell membrane. Fixation of the transformed state is postulated to be dependent on cell replication occurring soon after the initial interaction between the carcinogen and the cell [2–5]. Some evidence consistent with such a mechanism has recently been presented by Stewart and Magee [6] who have shown that single doses of dimethylnitrosamine, sufficient to induce kidney tumours in rats, are followed within a week by stimulation of DNA synthesis and cell replication. The methylation of DNA and/or other cellular components induced in the kidney by dimethylnitrosamine [7] could be responsible for the initial change in cellular control mechanisms postulated by Sachs.

The related carcinogen, *N*-nitrosomethylurea, induces tumours in a variety of organs in the rat and, under appropriate conditions, can induce a high incidence of brain tumours. The induced tumours appear always to arise from glial cells and never from neuronal cells [8]. Rat neuronal cells do not divide while glial cells retain this capacity throughout life. It is therefore possible that the failure of tumour induction in neuronal cells may be related to their inability to respond to a stimulus to divide. An alternative explanation might be that the interaction of the carcinogen with the neuronal cells might be less than that with the glial cells and thus insufficient to induce tumours. This possibility has been examined by measurement of the extent of methylation of DNA in fractionated nuclei from brains of rats treated with *N*-nitrosomethylurea. In this way it has been possible to compare the extent of interaction between the carcinogen with neuronal cell nuclei with that of various classes of glial cell nuclei.

2. Materials and methods

Twelve female Wistar rats (90 g) received an intravenous injection of *N*[^{14}C]methyl-*N*-nitrosourea (253 μCi/mmole, 1 mmole/kg body wt.) and were killed 2 hr later. Nuclei were isolated from the pooled brains by a modification of the method of Løvtrup-Rein and McEwen [9] and separated in the MSE

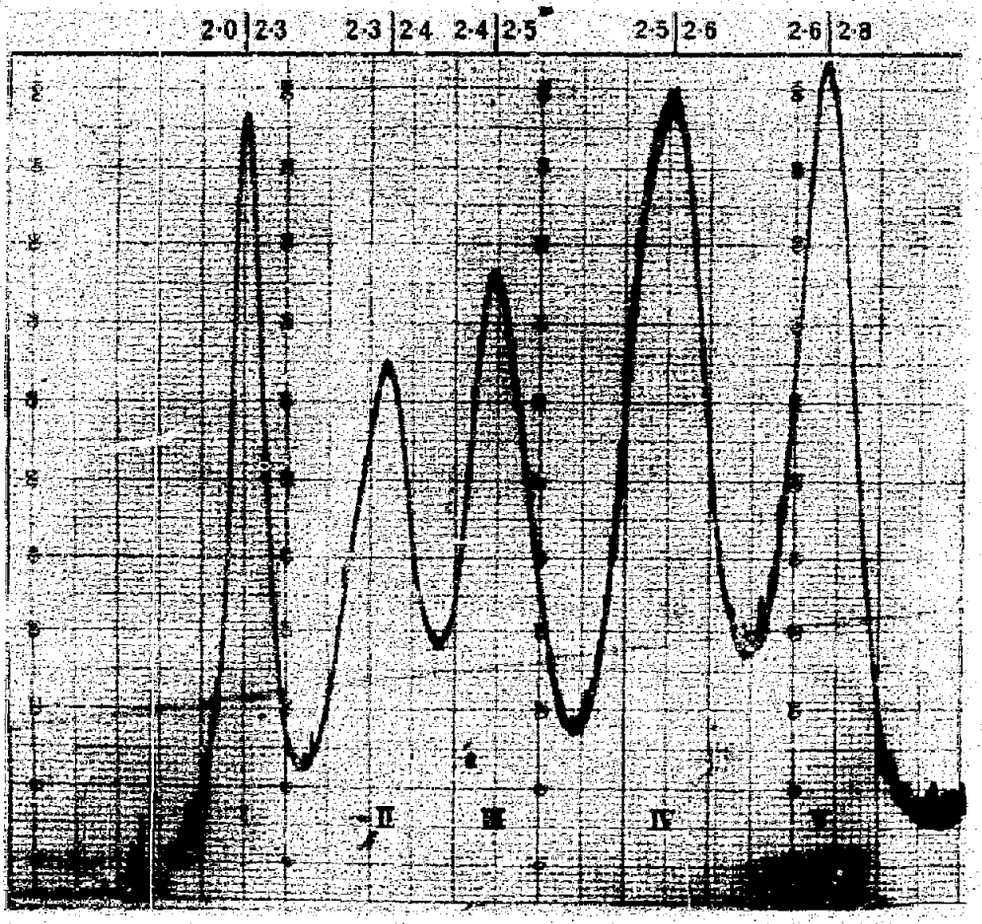

Fig. 1. Separation of nuclei from whole rat brain by zonal centrifugation in a B-XIV Zonal Rotor. The diagram shows the light scattering profile of the effluent monitored at 600 nm. The gradient consists of discrete zones of sucrose in 1 mM sodium cacodylate buffer pH 6.4 containing 1 mM $MgCl_2$. The concentrations of sucrose are indicated along the top of the figure. Centrifugation was at 45 000 rpm for 2 hr at 10°. The effluent is collected in fractions. Appropriate fractions are bulked for the recovery of the nuclei from the five main zones.

B-XIV zonal rotor using a discontinuous sucrose gradient [10]. After centrifugation for 2 hr at 8–10° and 45,000 rpm, the contents of the rotor were passed through a flow cell and light scattering of the effluent was monitored at 600 nm. Five distinct nuclear fractions were obtained (fig. 1) and classified according to their morphological features [10]. DNA was isolated from each fraction by phenol extraction [7] and hydrolyzed in N HCl (100°, 1 hr). The amount of the main alkylation product, 7-methylguanine, was determined after ion exchange chromatography on Dowex 50, H^+ form [7, 11].

3. Results

As shown in table 1 NMU, although inducing almost exclusively gliomas, reacted more extensively with DNA in the nuclei of the neuronal fraction than with that of astrocytes and oligodendrocytes. The specific radioactivity of total nuclear proteins was also higher in the neuronal fraction but some of the [^{14}C]radioactivity is known to enter the C_1-pool and may be incorporated into amino acids [7]. The differences in the specific radioactivities of the nuclear proteins are, therefore, no true indication of the level of interaction of NMU with proteins and may also reflect differences in the incorporation of amino acids into proteins of the various classes of brain nuclei.

Table 1
Alkylation *in vivo* of rat brain nuclear DNA by *N*-methyl-*N*-nitrosourea.

Fraction no.	Type of nucleus and proportion present in each fraction*		7-Methylguanine in nuclear DNA**	Specific activity of nuclear proteins (dpm/mg)
I	Neuronal Astrocytic	(70%) (23%)	0.188	229
II	Astrocytic Neuronal	(81%) (15%)	***	163
III	Astrocytic Oligodendrocytic	(84%) (15%)	0.170	170
IV	Oligodendrocytic	(92%)	0.153	110
V	Oligodendrocytic	(100%)	0.159	105

* Average data from several experiments. For details see [10]. The nuclei of the granule cells which are difficult to differentiate from those of the oligodendrocytes are probably to be found in Fractions III and IV.
** The amount of 7-methylguanine is expressed in mol% of guanine (see [7]).
*** The amount of DNA isolated from this fraction was too small for quantitative estimation.

4. Discussion

The results clearly show that methylation of neuronal cell nuclear DNA was no less extensive than that of the DNA of the various glial nuclear fractions. The same conclusion may also apply to the proteins of neuronal and glial nuclei. The use of nuclear rather than whole cell fractions was preferred because of the better separations that can be obtained with brain by the former method. There is no reason to suppose that there are marked quantitative differences between the degree of methylation of nuclear and cytoplasmic components of neuronal and glial cells and the extent of methylation of nuclear DNA is probably a valid measure of the degree of initial interaction of the carcinogen with the cell. Autoradiographic studies have indicated that nitrosomethylurea is evenly distributed in the brain within minutes after intravenous injection. It is concluded, therefore, that there is no difference between the extent of the initial interaction of the carcinogen with the neuronal and the glial cells and that the failure of neuronal tumour induction may be determined by the failure of these cells to divide. Several authors have pointed out that brain tumours induced by NMU are preferentially located in the periventricular zones of the lateral ventricles [8, 12, 13]. These are the only regions in which a significant glial cell proliferation seems to persist in the adult mammalian brain [14].

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