The Characterization of the Non-Histone Chromosomal Proteins of the Main Classes of Nuclei from Rat Brain Fractionated by Zonal Centrifugation

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(Received 29 September 1977)

1. Non-histone chromosomal proteins were isolated from the cell nuclei of whole rat brain and nuclei from different types of brain cells. 2. Brain nuclei were fractionated by zonal centrifugation into five zones deriving from five main categories of brain cells. These are the neuronals, astrocytes I, astrocytes II, oligodendrocytes I and oligodendrocytes II. 3. The non-histone chromosomal proteins were analysed by (a) sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, (b) electrofocusing electrophoresis and (c) two-dimensional electrophoresis. The results of this analysis showed a limited specific pattern of non-histone chromosomal proteins from the different classes of nuclei. Differences were found to exist between the proteins from neuronal and glial nuclei. In particular one polypeptide band with mol.wt. 10000 and pI 8.5 was found to be present in the non-histone protein fractions of neuronal nuclei, and absent from the corresponding fractions of nearly all the other classes of nuclei. 4. Two other classes of nuclear proteins, buffered-saline-soluble and 0.35M-NaCl-soluble, were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis along with the non-histone chromosomal. The similarities and differences among these groups of proteins are discussed. 5. The patterns of non-histone chromosomal proteins during development were investigated by studying them in two age groups of animals: in infant rats (10 days old) and adult rats. The polypeptide that was found to be specific for the proteins of neuronal nuclei of adult rats is present in all the classes of nuclei of infant rats.

The heterogeneity of the cells composing the brain is a major obstacle in the study of the biochemical processes in this organ. Recent development in the techniques of fractionation of cells and of cell nuclei have revealed that different cells have a wide range of biological activities. Differences were observed in the activities of nuclear enzymes like RNA polymerase (Austoker *et al.*, 1972) and DNA polymerase (Stambolova *et al.*, 1973) measured in the different types of brain cell nuclei.

Protein synthesis is a major aspect of brain metabolism. The characterization and the activities of brain cell nuclear proteins have been the subject of many studies (for example, see Dravid & Burdman, 1968; Burdman *et al.*, 1970; Burdman, 1972; Szijan & Burdman, 1973; Olpe *et al.*, 1973; Fujitani & Holoubek, 1974). Evidence is presented by several investigators that among the nuclear proteins, non-histone chromosomal proteins may be involved in the positive control of gene expression (see, for example, Allfrey, 1970; Baserga & Stein, 1971; Spelsberg *et al.*, 1972; McClure & Hnilica, 1972; Stein *et al.*, 1974; Cameron & Jeter, 1974; Olson &

Busch, 1974). In the present paper we report the characterization of non-histone chromosomal proteins extracted from nuclei of whole brain and nuclei derived from three main categories of brain cells; neurons, astrocytes and oligodendrocytes in two ages of animals.

Materials and Methods

Animals

Male albino rats, fed *ad libitum* on diet GR 3 EK with controlled lighting, were either 10 days old or weighed 90 g.

Isolation and fractionation of brain nuclei

The animals were killed by cervical dislocation and the brains were quickly removed. All subsequent operations were carried out at 0–4°C unless otherwise stated. The nuclei were prepared by the method of Løvtrup-Rein & McEwen (1966) as modified by Austoker *et al.* (1972). The crude nuclear pellet was purified by centrifugation in 2.4M-sucrose (final concentration of the sucrose in the suspension approx. 1.85M) containing 1mM-MgCl₂ and 1mM-sodium cacodylate buffer, pH 6.4, for 60 min at 50000g. The yield of nuclei was approx. 130×10^6

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nuclei/g of brain tissue. For experiments on unfractionated nuclei six to ten animals were used.

The nuclei were separated into zones by zonal centrifugation in a discontinuous gradient of sucrose from 2.0 to 2.8M as described by Austoker *et al.* (1972). Centrifugation lasted for 2h at 8–10°C and 45000 rev./min in an MSE B XIV titanium rotor. The contents of the rotor were displaced with a 45% (w/v) solution of sodium metrizoate (Nyegaard & Co., A/S Oslo, Norway) in 1M-sucrose, pH7.0. The effluent was passed through a flow cell of 1cm path-length in a Gilford spectrophotometer and monitored at 500nm. The effluent was collected manually into fractions corresponding to the individual peaks. The nuclear fractions were diluted by adding a suitable volume of 0.32M-sucrose, pH6.4, containing 1mM-MgCl₂ and 1mM-sodium cacodylate, to a final concentration of sucrose of approx. 2.0M. Samples from the diluted fractions were taken for microscopy, nuclear counts, and determination of protein and DNA content. The nuclei were recovered by centrifugation at 102000g for 30min at 5°C. The nuclear pellets were used for the extraction of nuclear proteins.

The integrity and purity of nuclear preparations was examined under oil immersion by using a phase-contrast microscope (type M20; Wild Photomicroscope, Heerbrugg, Switzerland).

The criteria of identification of the different types of nuclei were (a) size of the nuclei, (b) distribution of chromatin and (c) localization and number of the nucleoli (Glees & Meller, 1968; Rappoport *et al.*, 1969; Burdman, 1972).

Nuclear counts

The nuclei immediately after their preparation (unfractionated or nuclei prepared after the zonal centrifugation and diluted as described above) were counted after a suitable dilution (1:250 or 1:500) was made with 0.9% (w/v) NaCl (Baxter Laboratories, Thetford, Norfolk, U.K.) containing 5mM-MgCl₂ in a model F Coulter counter with a 100µm orifice tube (aperture setting 16; attenuation 0.500; threshold 6.5).

The nuclear counts were also determined indirectly by DNA analysis.

Analytical methods

DNA was measured by the method of Burton (1956) as described by Widnell & Tata (1964) and with the modification introduced by Croft & Lubran (1965).

Protein

Protein was determined by the method of Lowry *et al.* (1951). Bovine serum albumin was used as standard for all nuclear proteins except histones, when calf thymus histone was used.

Extraction of nuclear proteins

Non-histone proteins from brain nuclei were extracted by the procedure suggested by Gronow & Griffiths (1971). The nuclear pellet was treated with deionized 8M-urea and 50mM-sodium phosphate buffer, pH7.6. The urea solution was deionized with AnalaR Amberlite M.B.3 (British Drug House, Poole, Dorset, U.K.). The suspension was homogenized in a glass/glass homogenizer with 20 strokes. After 30min at 10°C, it was centrifuged at 2500g for 25min at 4°C. The supernatant was collected and the pellet treated once more with the above medium. The combined supernatants of this extraction procedure were called total non-histone proteins.

Extraction of non-histone chromosomal proteins required the preliminary removal of nuclear proteins other than chromosomal proteins and subsequent precipitation of chromatin. For this reason the method of extraction with 8M-urea was modified as follows: the nuclei immediately after their preparation were washed twice with 0.14M-NaCl, 10mM-Tris/HCl, 5mM-EDTA, pH8.0, for 20min each time at 4°C. Chromatin was pelleted from the suspension by centrifugation at 2500g for 30min at 4°C. The supernatants from both washes were combined and constituted the 'saline-soluble' protein fraction. In some experiments the pellet of chromatin was further purified by resuspension in 1.7M-sucrose in 0.14M-NaCl, 10mM-Tris/HCl, 5mM-EDTA, pH8.0, and centrifugation at 50000g for 2.5–3h (Elgin & Bonner, 1970). The chromatin pellet was then extracted with 8M-urea, 50mM-sodium phosphate buffer, pH7.6, as described above. The combined supernatants from this extraction constituted the non-histone-chromosomal-proteins fraction. The insoluble material left after the extraction of non-histone chromosomal proteins was extracted twice with ice-cold 0.4M-H₂SO₄ for 30min at 4°C followed by centrifugation at 2500g for 20min. The material soluble in 0.4M-H₂SO₄ constituted the histone fraction. The residue left after the extraction of histones was dissolved in 0.05M-Tris buffer, pH8.0, containing 1% sodium dodecyl sulphate at 37°C overnight and is termed 'residual proteins'.

Treatment of chromatin with 0.35M-NaCl

In some experiments the chromatin pellet after washing with the 0.14M-NaCl, 10mM-Tris/HCl, 5mM-EDTA, pH8.0, was treated twice with 0.35M-NaCl, 10mM-Tris/HCl, 5mM-EDTA, pH8.0, each time for 20min at 4°C (Johns & Forrester, 1969; Goodwin & Johns, 1972). After this treatment the extraction of non-histone chromosomal proteins was carried out as described previously. The 0.35M-NaCl extracts were analysed on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis along with the non-histone chromosomal proteins.

In some experiments 1 mM-phenylmethanesulphonyl fluoride (Sigma Chemical Co., Poole, Dorset, U.K.) was present in the solutions for the extraction of proteins.

A schematic representation of the extraction procedure is shown in Fig. 1.

Gel electrophoresis

Non-histone chromosomal proteins were analysed on three types of acrylamide gels.

(1) *Sodium dodecyl sulphate/polyacrylamide gel electrophoresis*. Polyacrylamide gels (7.5%) containing 0.1% sodium dodecyl sulphate were prepared in 0.1 M-sodium phosphate buffer, pH 7.1, as described by Shapiro *et al.* (1967) and Weber & Osborn (1969). In some experiments 13 and 15% acrylamide gels have been used.

The following proteins were used as standards for the determination of molecular weights: bovine serum albumin (68000; Armour Pharmaceutical Laboratories, Eastbourne, Sussex, U.K.), glutamate dehydrogenase (53000), aldolase (40000) (both from Boehringer, Mannheim, Germany), myoglobin (17200), pancreatic ribonuclease (13700) and ribonuclease T1 (11200) (all from Sigma). All of them were dissolved in 0.01 M-sodium phosphate buffer, pH 7.1, containing 1% sodium dodecyl sulphate, 1% 2-mercaptoethanol and 10% glycerol.

(2) *Electrofocusing electrophoresis*. Electrophoresis was carried out by the method of Gronow & Griffiths (1971) in 5% (w/v) acrylamide gels, containing 8 M-urea (AnalaR grade, deionized) with 2% Ampholine carrier ampholytes (LKB-Producter A.B., Stockholm, Sweden) in a Shandon disc-electrophoresis

Fig. 1. Procedure for the fractionation of nuclear proteins from brain nuclei

apparatus (Shandon Southern Products, Runcorn, Cheshire, U.K.) for 5 h at room temperature and at 150 V (constant voltage). The anode solution was 5% (w/v) orthophosphoric acid and the cathode 5% (v/v) ethane-1,2-diamine.

(3) *Two-dimensional polyacrylamide-gel electrophoresis*. This was carried out by the method described by Martini & Gould (1971) with some modifications. Non-histone chromosomal proteins were subjected to electrofocusing electrophoresis as described above, the only difference being the size of the gel rods and the load. The gel rods were 7 cm long with a diameter of 0.3 cm and the load 120 μ g of protein.

The gel rods after electrofocusing electrophoresis and after having been washed with 12.5% (w/v) trichloroacetic acid were incubated in buffers containing sodium dodecyl sulphate and 2-mercaptoethanol, pH 7.1 (Shapiro *et al.*, 1967; Martini & Gould, 1971). Electrophoresis was carried out on gradient acrylamide slabs [Gradipore gels; Universal Scientific (Uniscil), London, U.K.]. The gradient of acrylamide in the slabs was from 4 to 28%. Electrophoresis was carried out in a Uniscil vertical slab electrophoresis apparatus at 35 mA/gel slab under constant voltage at room temperature for approx. 9 h, with electrode buffer 0.1 M-sodium phosphate/0.1% sodium dodecyl sulphate, pH 7.1. The slabs were stained overnight in 0.1% Coomassie Brilliant Blue in methanol/acetic acid/water (5:1:4, by vol.) and destained by bathing in methanol/acetic acid/water (1:1:8, by vol.). After destaining they were photographed on a plastic grid (10 cm $\times$ 10 cm) and stored in 7.5% acetic acid.

Results

Adult rats

Fractionation of nuclei. Brain nuclei were fractionated by isopycnic centrifugation into five distinct zones. Zone I included mainly neuronal nuclei, some astrocyte nuclei and a small percentage of oligodendrocyte nuclei. Zones II and III were composed of astrocyte nuclei with about 15% contamination by neuronal and oligodendrocyte nuclei. In zone IV the majority of nuclei were oligodendrocyte and zone V contained only oligodendrocyte nuclei. The percentage distribution of nuclei in each zone was similar to the one described by Austoker *et al.* (1972). Although this method does not give total purity in all fractions the degree of resolution is high enough to enable the properties of each class of nuclei, both morphological and chemical, to be studied.

Chemical composition of nuclei. The protein and DNA contents of both unfractionated and fractionated nuclei were determined. The protein value found for unfractionated nuclei was around 32.6 μ g and the DNA value around 7.0 μ g/nucleus. Table 1 gives

the protein and DNA values of the nuclei of the five zones. The amount of DNA in all the types of nuclei varies between 6.8 and 7.4 μ g/nucleus. Large differences are found in the protein content of various types of nuclei. The highest value is found in zone I where the majority of nuclei are neuronal. The lowest value is in zone V (oligodendrocyte nuclei) and is less than half that of zone I.

Extraction of nuclear proteins. Total non-histone proteins, extracted by the one-step procedure suggested by Gronow & Griffiths (1971), from unfractionated nuclei, gave a yield of $59.2 \pm 2.1\%$ of the total nucleoprotein. The modification of this procedure gave about 27% of total nucleoprotein recovered in the 8 M-urea extract, whereas about 30% was recovered in the buffered 0.14 M-NaCl extract (ribosomal and globulin-like proteins according to the classification of Busch & Steele, 1964; Hnilica, 1967). The recoveries of each class of nuclear proteins are given in Table 2. It is obvious from this Table that both classes of nuclear proteins make up the total non-histone proteins extracted by the one step procedure of Gronow & Griffiths (1971). In the experiments where the chromatin pellet was further purified by resuspension and centrifugation in 1.7 M-sucrose containing 0.14 M-NaCl, as suggested by Elgin & Bonner (1970), the recoveries of non-histone chromosomal proteins and their patterns after sodium

Table 1. *Content of DNA and protein of adult rat brain nuclei separated into zones by centrifugation*
Values are means for seven determinations.

Zone	DNA (μ g/nucleus)	Protein (μ g/nucleus)	Protein/ DNA ratio
I	7.2 ± 0.1	39.2 ± 2.4	5.4 ± 0.2
II	6.8 ± 0.3	35.6 ± 4.2	5.2 ± 0.3
III	7.3 ± 0.07	30.7 ± 1.6	4.1 ± 0.2
IV	7.06 ± 0.3	19.6 ± 2.1	2.4 ± 0.3
V	7.4 ± 0.08	17.9 ± 2.1	2.4 ± 0.2

Table 2. *Amounts of protein extractable from unfractionated brain nuclei of adult rats*

Nuclear proteins were extracted from brain nuclei of male albino rats (90 g) by procedure (B) of Fig. 1. Results are expressed as means for eight experiments.

	Content (%)	Content (μ g/nucleus)
Total nuclear proteins	100	32.6 ± 0.8
Soluble proteins	29.2 ± 1.6	11.5 ± 0.9
Non-histone chromosomal proteins	27.4 ± 0.7	8.9 ± 0.4
Histones	17.2 ± 1.5	4.9 ± 0.5
Residual proteins	5.6 ± 0.8	1.5 ± 0.06

dodecyl sulphate/polyacrylamide-gel electrophoresis were identical with those where this additional stage of purification was not applied. Consequently, this step was omitted in the subsequent experiments.

Treatment of chromatin with 0.35M-NaCl. Johns & Forrester (1969) have shown in experiments with calf thymus deoxyribonucleoprotein that many cytoplasmic and nuclear sap proteins can be adsorbed non-specifically to chromatin during exposure to 0.14M-NaCl and that many of these adsorbed proteins could be removed from the chromatin with 0.35M-NaCl. In several experiments the chromatin preparations were washed once or twice with 0.35M-NaCl. It was found that the recoveries of non-histone chromosomal proteins were less by the amount of protein extracted in the 0.35M-NaCl extract and that the recovery of the histone fraction was lower than when this step was omitted. The recoveries of nuclear proteins are shown in Table 3. Of the total nucleoprotein 9–12% was recovered in the 0.35M-NaCl extract and only about 14% in the non-histone chromosomal fraction. Johns & Forrester (1969) reported that the experiments with rat liver have indicated that washing with 0.30M-NaCl solubilized

the chromatin and that this method could not be used. Our experiments indicate that with brain chromatin, only part was solubilized during the 0.35M-NaCl wash. Similar results on brain chromatin were observed by Olpe *et al.* (1973). The lower percentage of histone extracted in experiments in which a 0.35M-NaCl wash was included can be explained by the fact that at this NaCl concentration, and above, the lysine-rich histones are removed (Ohlenbusch *et al.*, 1967).

Nuclear proteins from nuclei fractionated by zonal centrifugation were extracted by procedure (B) of Fig. 1. The recoveries of nuclear proteins in every zone are shown in Table 4. Neuronal nuclei contain more non-histone chromosomal and soluble proteins than oligodendrocyte nuclei. Astrocyte nuclei maintain an intermediate position between the other two classes with respect to the amount of soluble and non-histone chromosomal proteins. The preparations of non-histone chromosomal proteins contained no DNA or RNA detectable by chemical analysis. The amount of histone remains constant within the limits of the experimental error in all the classes of nuclei. Therefore the differences in total nucleoprotein noted previously in Table 1 seem to be due mainly to the soluble and non-histone chromosomal proteins. As can be seen from Tables 2–4, the sum of the recoveries of each class of nuclear proteins, given as percentages of the total nuclear protein, amount to 70–80%. The two main reasons for the discrepancy are the difficulty of handling small volumes of solutions containing 8M-urea that adhere to the walls of containers, and the variable amounts of the proteins tightly bound to DNA that are not extractable in the last step using Tris/HCl/sodium dodecyl sulphate.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of non-histone chromosomal proteins. Unfractionated brain nuclei. The non-histone chromosomal proteins were resolved on the basis of molecular weight into 25 major bands and 5–6 minor ones that are partially masked between the most heavily stained bands. Their molecular weights were

Table 3. *Effect of washing nuclei with 0.35M-NaCl on the recovery of non-histone chromosomal proteins*

Nuclear proteins were extracted from brain nuclei of male albino rats (90g) by procedure (D) of Fig. 1. Results are expressed as means $\pm$ S.E.M. for four experiments of the percentage of protein recovered in each fraction taking the total protein of the unwashed nuclei as 100%.

	Protein recovered (%)
Proteins soluble in 0.14M-NaCl	29.5 $\pm$ 2.1
Proteins soluble in 0.35M-NaCl (1st wash)	8.9 $\pm$ 0.5
Proteins soluble in 0.35M-NaCl (2nd wash)	3.0 $\pm$ 0.1
Non-histone chromosomal proteins	13.9 $\pm$ 0.8
Histones	9.4 $\pm$ 0.8
Residual proteins	5.2 $\pm$ 0.6

Table 4. *Recoveries of nuclear proteins from different classes of brain nuclei*

Nuclear proteins were extracted from fractionated brain nuclei of 90g animals by procedure (B) of Fig. 1. Results are expressed as percentage of the total protein extracted in each class and pg/nucleus. These values represent the means $\pm$ S.E.M. for seven experiments.

Zone	Soluble proteins		Non-histone chromosomal protein		Histones		Residual proteins	
	(%)	(pg/nucleus)	(%)	(pg/nucleus)	(%)	(pg/nucleus)	(%)	(pg/nucleus)
I	22.2 $\pm$ 3.2	8.1 $\pm$ 1.5	30.8 $\pm$ 2.6	11.30 $\pm$ 0.5	6.74 $\pm$ 1.8	2.0 $\pm$ 0.2	8.3 $\pm$ 0.2	1.7 $\pm$ 0.09
II	22.9 $\pm$ 3.7	8.3 $\pm$ 0.6	23.1 $\pm$ 1.2	8.62 $\pm$ 0.4	7.12 $\pm$ 1.7	2.3 $\pm$ 0.08	7.4 $\pm$ 0.6	1.8 $\pm$ 0.1
III	22.8 $\pm$ 3.3	4.8 $\pm$ 0.2	26.4 $\pm$ 2.4	8.38 $\pm$ 0.5	10.3 $\pm$ 0.8	2.6 $\pm$ 0.3	5.7 $\pm$ 0.5	1.1 $\pm$ 0.06
IV	12.9 $\pm$ 1.5	2.3 $\pm$ 0.5	23.9 $\pm$ 2.0	5.31 $\pm$ 0.6	17.9 $\pm$ 1.3	2.9 $\pm$ 0.4	6.3 $\pm$ 0.1	1.2 $\pm$ 0.07
V	10.7 $\pm$ 1.6	1.3 $\pm$ 0.04	22.8 $\pm$ 2.6	4.17 $\pm$ 0.1	19.5 $\pm$ 2.5	3.1 $\pm$ 0.2	6.4 $\pm$ 1.4	1.2 $\pm$ 0.03

Fig. 2. Densitometric tracings of non-histone proteins from unfractionated brain nuclei

Non-histone proteins were extracted from: (a) intact rat brain nuclei by procedure (A) of Fig. 1; (b) purified chromatin by procedure (C) of Fig. 1; (c) chromatin by procedure (B) of Fig. 1. Sodium dodecyl sulphate complexes of 8M-urea-soluble proteins were separated in 7.5% acrylamide gels at pH 7.1 as described in the Materials and Methods section.

found to be between 10000 and 165000. The number of bands of non-histone chromosomal proteins characterized by various authors varies, because it depends not only on the method of preparation of chromatin and non-histone chromosomal proteins, but also on the resolving power of the gel system used. Olpe *et al.* (1973), with an extraction similar to the one used in the present work, found 14 main bands and 5–6 minor ones for total brain nuclei. Platz *et al.* (1970) have separated bovine brain

chromatin phosphoprotein into 23 bands. Davis *et al.* (1972) reported about 31 bands of acidic deoxyribonucleoprotein in rat brain nuclei by using a high-resolution acrylamide-gel system. Wu *et al.* (1973) in a comparative study of several rat tissues found 12–18 major molecular-weight bands that make up the majority of the protein mass in most of the tissues studied. In most of the works quoted, either the preparation of proteins or the analytical system used

Fig. 3. Densitometric tracings of the non-histone chromosomal proteins

(a) Non-histone chromosomal proteins extracted before the 0.35M-NaCl wash (procedure B of Fig. 1); (b) non-histone chromosomal proteins extracted after the 0.35M-NaCl wash (the arrow indicates the position of the band missing); (c) 0.35M-NaCl-soluble proteins (1st extract; procedure D of Fig. 1). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described in the text.

differs from those used in the present work, so no direct comparisons can be made. In Fig. 2 are shown the densitometric tracings of three gels of non-histone chromosomal proteins corresponding to three isolation procedures (extractions from nuclei, from chromatin and from purified chromatin). The patterns closely resemble one another. The similarity of the patterns of total non-histone proteins and non-histone chromosomal proteins will be discussed below in connection with the sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of soluble proteins. Since the patterns of purified chromatin and chromatin not subjected to purification are very similar and the purification did not extract any bands of specific molecular weight, this step was omitted in the subsequent experiments. No differences in the electrophoretic patterns were observed when phenylmethanesulphonyl fluoride was present in the extraction media. In most experiments phenylmethanesulphonyl fluoride was not included.

Treatment of chromatin with 0.35M-NaCl before the extraction of non-histone chromosomal proteins solubilized part of it, as judged by the recoveries of proteins. Comparison of the electrophoretic patterns of non-histone chromosomal proteins from chromatin treated with 0.35M-NaCl with those that had not been treated showed that there is a partial removal of non-histone chromosomal protein bands in the region of molecular weights of about 80000–30000 and one band missing (mol.wt. 80000) in the fractions prepared after extraction with 0.35M-NaCl (Fig. 3). Proteins extracted in the first 0.35M-NaCl wash, which included the bulk of proteins extracted by this treatment, were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis under the same conditions as the other two fractions (treated and non-treated). The electrophoretic patterns of the 0.35M-NaCl-soluble proteins and those of non-histone chromosomal proteins exhibited similarity in the region of molecular weights mentioned above, but not identity, as was reported by Fujitani & Holoubek (1973, 1974). Olpe *et al.* (1973) found in their study of brain chromosomal proteins that with 0.35M-NaCl, 12 of the main bands could be partially solubilized, but only one band was extracted completely. The present results are in agreement with those of Olpe *et al.* (1973). The partial solubilization of chromatin and the similarity of the patterns of proteins is explained by the fact that 0.35M-NaCl extracts non-histone proteins loosely bound to chromatin, as was shown by Kostraba *et al.* (1975) and Umansky *et al.* (1976).

The electrophoretic patterns of non-histone chromosomal proteins from different classes of brain nuclei exhibited a striking similarity. One difference of a qualitative nature was found in the non-histone chromosomal proteins. A band of approx. mol.wt 10000 was present in the non-histone chromosomal

Fig. 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of non-histone chromosomal proteins

Brain nuclei from 14 male albino Wistar rats (90g body wt.) were isolated and fractionated into five zones as described in the text. Non-histone chromosomal proteins were prepared by procedure (B) of Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described in the Materials and Methods section. (a) Non-histone chromosomal proteins from neuronal nuclei (zone I). The arrow indicates the band that was found to be specific for this class of nuclei. (b) Non-histone chromosomal proteins from oligodendrocyte II nuclei (zone V).

proteins from neuronal nuclei (zone I) and absent from oligodendrocyte and astrocyte II (zone III) nuclei. It seems to be present as a very faint band, not easily detected, in preparations from zone II (astrocytes I) where the relative contribution of neuronal nuclei is high. About 15% of nuclei in this zone are neuronal and this contamination is sufficient to explain the presence of this faint band in the proteins from zone II. This protein band appears to be specific for neuronal proteins. The electrophoretograms are presented in Figs. 4 and 5. Other differences found are of a quantitative nature. The preparations of non-histone chromosomal proteins from oligodendrocyte nuclei (zones IV and V) contain two low-molecular-weight bands that seem more strongly

Fig. 5. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of non-histone chromosomal proteins from: (a) astrocyte I nuclei (zone II); (b) astrocyte II nuclei (zone III).

Electrophoretic patterns of non-histone chromosomal proteins from: (a) astrocyte I nuclei (zone II); (b) astrocyte II nuclei (zone III).

stained than the corresponding ones from other zones. It seems that the protein species corresponding to these polypeptide bands may be present in higher amounts in these zones than in the others; but it must be remembered that the dye-binding response with Coomassie Brilliant Blue is linear only in the range of 1–20 μ g of protein/band (Wu *et al.*, 1973). Olpe *et al.* (1973) fractionated total brain nuclei into two classes, large and small, which may correspond to neuronal and oligodendrocyte nuclei of our preparations. They found one specific protein band for large (neuronal) nuclei and one specific for small (oligodendrocyte) nuclei. Although the number of bands present in their preparations is much less than the number of bands found in ours, the specific protein band found in their preparation of large nuclei seems to correspond to the band of neuronal nuclei of the present experiments. On the other hand Dravid & Burdman (1968) fractionated nuclei with the technique of Løvtrup-Rein & McEwen (1966) and they could not show any electrophoretic differences between soluble acidic proteins isolated from the

nuclei of neurons, astrocytes and oligodendrocytes. Tashiro *et al.* (1974) fractionated nuclei from cerebral cortex into two fractions: neuron-rich and oligodendroglial nuclei. They claim the existence of a group of high-molecular-weight non-histone proteins in neuronal chromatin that is apparently absent from oligodendroglial chromatin. However, they found that all components present in neuron-rich chromatin are detected in oligodendroglial chromatin, but in much smaller amounts, and they suggest that this may be due to a small proportion of neuronal nuclei (less than 5%) present in the oligodendroglial nuclear preparation. It is questionable whether such a small percentage of neuronal nuclei in the preparation would account for the similarity of these two fractions.

The present results indicate a limited specific protein pattern for the different classes of nuclei. The only major difference found as reported above is the presence of a protein band in the non-histone chromosomal proteins of neuronal nuclei and its absence from nearly all the other classes. This protein of mol.wt. 10000, which has been shown in experiments reported below to have an iso-electric point of 8.5, has a size that is sufficiently near that of histone H4 (mol.wt. 11300) for it to be argued that it was this histone and not a non-histone chromosomal protein. This is inherently unlikely because if the neuronal protein were histone H4, there seems no reason why it should not appear in proteins extracted with 8M-urea from the other types of brain nuclei. However, to eliminate this possibility, gel electrophoresis has been carried out in 13 and 15% gels as well as in 7.5% acrylamide. The neuronal protein was always resolved from histone H4 and had mol.wt. 10000. A sample of the protein was recovered from a 15% slab gel by staining the two strips cut from each side of the sample slot with Coomassie Blue and using these as markers to guide the cutting out of the mol.wt. 10000 band. The gel was macerated and extracted with 6M-HCl and the extract freeze-dried. The residue was taken up in 6M-HCl and heated at 105°C for 18 h in a sealed tube in an atmosphere of N₂. Amino acid analysis, by ion-exchange chromatography, indicated an amino acid composition that was quite different from that of histone H4. For example, whereas in histone H4 the ratio of aspartic and glutamic acid to arginine plus lysine is 1:2.3, in the protein of mol.wt. 10000, it is 1:0.76. Similarly, in the histone H4 the serine/threonine ratio is 1:3.5, and in the brain protein 2.97:1. The alanine/valine ratio is 1:2.8 in histone H4, and 1.9:1 in the brain protein. It is noteworthy that the amino acid analysis of the mol.wt. 10000 protein is distinct from that of the brain protein S-100, which has a molecular weight of the order of 30000, and has a high content of acidic amino acids, is found in glial as well as in neuronal cells and may, probably as an artefact of

Fig. 6. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of soluble proteins

Proteins soluble in 0.14M-NaCl, 0.01M-Tris/HCl, 5mM-EDTA, pH8.0, were extracted from: (a) neuronal nuclei (zone I). The arrow on the densitometric tracing indicates the position of the band missing; (b) oligodendrocyte nuclei (zone V). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described in the text.

homogenization, appear in the nuclear fraction (Donato, 1976).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of soluble proteins. The patterns of non-histone chromosomal proteins from unfractionated brain nuclei extracted either from nuclei or from chromatin were similar (Fig. 2). Similar results were reported by Barrett & Gould (1973) in experiments with rat liver. MacGillivray & Rickwood (1974), however, showed that the two-dimensional patterns of proteins extracted from nuclei in NaCl/Tris were markedly different from those extracted from chromatin.

To examine the relationship between the soluble proteins and non-histone chromosomal proteins, the buffered-saline-soluble proteins from fractionated nuclei were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis under the same conditions as those for non-histone chromosomal

proteins. The densitometric tracings of the gels (and their photographs) are shown in Figs. 6 and 7. The proteins were resolved into 17 major molecular weight bands and three or four minor faint bands on the upper part of the gel. The bulk of the protein components from all the classes of nuclei seem to be concentrated in the region of mol.wts. 55000–20000. Bands with higher molecular weights (about nine bands) are considerably less stained compared with all others and there are two rather strongly stained bands with apparent mol.wts. 13700 and 12000. The profiles of these proteins within the different classes were similar. Some differences were observed between the proteins from zone I (neuronal nuclei) compared with those of zones III and V (astrocyte and oligodendrocyte nuclei respectively). One band seems to be missing in the protein extracts of neuronal nuclei (approx. mol.wt. 45000; its position is indicated by an arrow on the densitometric tracing). The other difference concerns the shape of the two low-molecular-

Fig. 7. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of soluble proteins

Soluble proteins were extracted from: (a) astrocyte II nuclei (zone III); (b) oligodendrocyte I nuclei (zone IV). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described in the Materials and Methods section.

weight bands. These are well separated in the fractions from astrocyte and oligodendrocyte nuclei, whereas in neuronal nuclei they appear (in the photographs and under a magnifying lamp) as one broad double band. Differences of qualitative character were not observed between the preparations from astrocyte and oligodendrocyte nuclei. Comparison of the electrophoretic profiles of soluble proteins with non-histone chromosomal proteins revealed some similarities. They have some bands in common especially in the region of molecular weight where for the soluble proteins, most of the material is concentrated. Soluble protein extracts seem to lack some of the bands with higher molecular weights, characteristic of the profile of non-histone chromosomal proteins. Extracts from neuronal nuclei lack the band that was found to be specific for the non-histone chromosomal proteins of these nuclei. Hnilica (1967) had indicated that nuclear-sap proteins extracted with NaCl and Tris have many common features, such as similar amino acid composition, with the non-histone chromosomal proteins. MacGillivray & Rickwood (1974) analysed the soluble proteins on two-dimensional electrophoresis. They found that soluble proteins from brain nuclei, although largely acidic in nature with many of them possessing a pI value of approx. 4.5, have different molecular weights.

The results of our experiments on one electrophoretic system, sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, showed that removal of nuclear-sap proteins is necessary before the extraction of non-histone chromosomal proteins.

Electrofocusing electrophoresis. Analysis of non-histone chromosomal proteins by electrofocusing electrophoresis carried out as described by Gronow & Griffiths (1971) revealed 18 major and 3–4 minor components of total brain non-histone proteins. The protein components focus at a pH region from 5.0–7.0. Similar pI values to those found in our experiments were reported for mouse liver, kidney and brain covering a pH region from 4.5 to 9.0 (MacGillivray & Rickwood, 1974) and for the liver non-histone chromosomal proteins the majority of the components focuses at pH 6.7–8.9 with few focusing at a more acidic pH (Gonzalez-Mujica & Mathias, 1973).

The patterns of non-histone chromosomal proteins from different classes of nuclei are similar. The proteins from neuronal nuclei seem to possess a component focusing at pH 8.5, which is absent from the preparations of all the other classes of nuclei. Figs. 8 and 9 show the electrophoretograms (and the photographs) of gels. The component present in the neuronal nuclei only is indicated by an arrow. Differences of quantitative character can be readily detected by comparing the relative height of the peaks in the electrophoretograms and the intensity of the

Fig. 8. *Electrophoretic profiles of non-histone chromosomal proteins after their separation by electrofocusing electrophoresis*

The direction of the pH gradient is shown in the Figure. (a) Non-histone chromosomal proteins from neuronal nuclei (zone I); (b) non-histone chromosomal proteins from oligodendrocyte II nuclei (zone V). The conditions of the electrophoresis are described in the text.

bands in the photographs among the proteins from different classes of nuclei.

Non-histone chromosomal proteins are often referred to as acidic proteins because of the excess of glutamic and aspartic amino acid residues. Separation of these proteins on a pH gradient showed that within this group are present components of a slightly basic nature and that the term 'acidic chromosomal proteins' does not fully characterize them.

Two-dimensional electrophoresis. Non-histone chromosomal proteins from different classes of brain nuclei were subjected to two-dimensional electrophoresis in a number of experiments. The patterns were compared by direct visualization of the slabs, mapping and photography. Photographs and maps of two-dimensional slabs with non-histone chromosomal proteins from three representative classes of nuclei are presented in Figs. 10–12. The slabs were

Fig. 9. *Electrofocusing electrophoresis of non-histone chromosomal proteins from astrocyte nuclei*
 (a) Non-histone chromosomal proteins from astrocyte I nuclei (zone II); (b) non-histone chromosomal proteins from astrocyte II nuclei (zone III).

photographed on a sheet of Perspex marked with a grid, so that the position of each spot can be defined precisely by two co-ordinates. On the maps of the slabs only the major, more strongly stained, components are shown. Some of the fainter ones are better shown on the photographs. The protein components were resolved into both spots and bands. One characteristic feature of the patterns of non-histone chromosomal proteins on the second dimension seems to be the existence of bands extending over a pH range in some cases of about 2pH units that have apparently the same molecular weight. A typical example of this is the low-molecular-weight double broad band present in all classes of nuclei with co-ordinates: horizontal 2.7–4.0 and vertical (downwards) 6.6. The use of sodium dodecyl sulphate/polyacrylamide-gel and electrofocusing electrophoresis alone had shown that non-histone chromosomal proteins represent a heterogeneous group of proteins with a wide range of molecular weights and isoelectric points. The combination of the two techniques in a single analysis revealed a more complex

Fig. 10. *Two-dimensional electrophoresis of non-histone chromosomal proteins from neuronal nuclei (zone I)*

Photograph and map of the components of non-histone chromosomal proteins subjected to two-dimensional electrophoresis. The horizontal axis represents isoelectric focusing in the first dimension. The direction of the pH gradient is shown at the bottom of the map. The vertical axis represents sodium dodecyl sulphate/polyacrylamide-gel electrophoresis in the second dimension. Molecular weight markers are bovine serum albumin (68000; co-ordinates: horizontal 0.5, vertical 4.0) and ovalbumin (43000; co-ordinates: horizontal 0.5; vertical 4.8).

pattern and at least 50 components may be observed. Although many of them are faint, nevertheless they can be distinguished from one another. Better resolution is achieved in the region of mol.wt. 70000 to 30000. Most of the components are concentrated in two pH regions. The bulk of protein is found in a pH range 4.5–7.0. The second region is a narrower one corresponding to pH 8.0–8.5, where there is a set of bands separated on the second dimension, one after the other, indicating that there is a population of polypeptides with the same or quite close isoelectric

Fig. 11. Two-dimensional electrophoresis of non-histone chromosomal proteins from oligodendrocyte II nuclei (zone V)

For details see the legend to Fig. 10.

points, but with different molecular weights. These observations apply to protein preparations from all classes of brain nuclei. The fraction of the proteins from neuronal nuclei, focusing between pH 8.0 and 8.5 in the first dimension, is resolved into 12–14 polypeptides in the second. A fast-moving component belonging to this population of polypeptides was not observed in the proteins from other classes of nuclei. Electrofocusing electrophoresis showed that proteins from neuronal nuclei contain one additional component, focusing at pH 8.5, which appears to coincide in pI value with the polypeptide found in the two-dimensional gels of neuronal nuclei (Fig. 10). This component has mol.wt. approx. 10000 and a pI value of 8.5. Its co-ordinates on the grid are 1.6 and 7.2. Amongst the proteins from astrocyte and

Fig. 12. Two-dimensional electrophoresis of non-histone chromosomal proteins from astrocyte II nuclei (zone III)

For details see the legend to Fig. 10.

oligodendrocyte nuclei, differences that can be detected are of a quantitative character only.

The original observation by Gronow & Griffiths (1971) that under the conditions used for the separation in the first dimension, by electrofocusing electrophoresis, histones run off the alkaline end of the gel was confirmed, using both a commercial preparation of histones, and a sample prepared by extraction of chromatin with 0.4M-HCl. This is not surprising, because of the high isoelectric points of these proteins (Malamud & Drysdale, 1978). RNA runs off the gel at the other end. Hence if the preparations of non-histone chromosomal proteins were contaminated by RNA or histone, both contaminants would be lost in the separation in the first dimension. This affords additional proof to the argument that the protein of mol.wt. 10000 and pI 8.5 is not histone H4. To check on the possibility that a small amount of DNA, below the concentrations detectable by

chemical analysis, might adhere specifically to certain groups of proteins in such a way as to influence their mobility in the second direction, two-dimensional gels of the non-histone chromosomal proteins were stained with ethidium bromide solutions. No fluorescence was observed. Hence it was concluded that DNA was absent from our preparations or lost in the electrofocusing electrophoresis.

A number of authors have reported the use of two-dimensional electrophoresis for the separation of non-histone chromosomal proteins. Barrett & Gould (1973) compared the patterns of non-histone chromosomal proteins from different tissues and different species. MacGillivray & Rickwood (1974) separated non-histone chromosomal proteins from mouse liver, kidney and brain. Peterson & McConkey (1976) reported the separation of non-histone chromosomal proteins from HeLa cells into a very large number of components. It is difficult to compare their patterns, particularly those of brain proteins, with ours, since the whole procedure of fractionation is quite different to the one we followed.

Infant rats (10-day-old)

The protein and DNA contents of both unfractionated and fractionated nuclei have been deter-

Table 5. *Contents of nuclear proteins from 10-day-old male rats*

Nuclear proteins were extracted from brain nuclei of 10-day-old male rats by procedure (B) of Fig. 1. The contents are expressed as percentages of the total nucleoprotein and as pg/nucleus. The values are means $\pm$ S.E.M. for five experiments.

	Proteins	
	(% of total)	(pg/nucleus)
Total nucleoprotein	100	40.3 $\pm$ 1.8
Soluble proteins	29.2 $\pm$ 1.5	11.1 $\pm$ 0.7
Non-histone chromosomal proteins	30.3 $\pm$ 0.5	11.5 $\pm$ 0.2
Histones	15.7 $\pm$ 0.4	4.8 $\pm$ 0.2
Residual	4.8 $\pm$ 0.2	1.4 $\pm$ 0.07

Table 6. *Content of DNA and protein of infant rat brain nuclei separated into zones by centrifugation*
Values are means for five determinations.

Zone	DNA (pg/nucleus)	Protein (pg/nucleus)	Protein/ DNA ratio
I	7.1 $\pm$ 0.2	50.1 $\pm$ 0.8	7.0 $\pm$ 0.3
II	7.0 $\pm$ 0.7	38.3 $\pm$ 0.1	5.4 $\pm$ 0.5
III	7.7 $\pm$ 0.3	33.4 $\pm$ 0.9	4.3 $\pm$ 0.3
IV	7.4 $\pm$ 0.1	31.4 $\pm$ 1.3	4.2 $\pm$ 0.2
V	7.5 $\pm$ 0.2	25.2 $\pm$ 0.4	3.3 $\pm$ 0.2

mined. Brain nuclei from 10-day-old rats contain more protein per nucleus: around 40pg (Table 5) compared with 32pg found in the adults (Table 2). Results of protein and DNA determinations in the five zones of nuclei after zonal centrifugation are presented in Table 6. The amounts of protein vary significantly within the different classes of nuclei. Zone I contains twice as much protein compared with zone V (oligodendrocyte nuclei). This is in agreement with the findings for fractionated nuclei of adult rats.

Extraction of nuclear proteins. The percentage recoveries of nuclear proteins, extracted by procedure (B) of Fig. 1, as well as the amount of protein expressed as pg/nucleus, are presented in Table 5. These results indicate a greater amount of non-histone chromosomal proteins extracted from nuclei and a greater content of protein per nucleus compared with those for adult rats (Table 2). Szijan & Burdman (1973) observed a higher relative content of chromatin acidic proteins in 7-day-old rats than in the adult rat, suggesting that at this age there is considerably more synthesis and content of these proteins concomitant with an enhanced DNA replication observed in isolated purified nuclei of young animals (Burdman, 1972). Recovery of 30% of the total nucleoprotein in the fraction of non-histone chromosomal proteins was found in 11-day-old chick embryo brain (Graziano & Huang, 1971). The histone and residual protein contents remain the same, within the limits of the experimental error, in both ages of animals.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of non-histone chromosomal proteins. The patterns of non-histone chromosomal proteins from unfractionated nuclei after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis were almost identical with those of adult rats. Differences of quantitative character were observed between the two profiles, the most noteworthy of them being a low-molecular-weight band, the last band with mol.wt. approx. 10000, that seems more strongly stained in the preparations from young animals. Szijan & Burdman (1973) reported differences in the electrophoretic profile between the acidic proteins of chromatin from 7-day-old rats and from adult rat. In our preparations of non-histone chromosomal proteins from unfractionated nuclei, we could not detect any differences of a qualitative nature between the two ages of the animals.

The profiles of non-histone chromosomal proteins from different classes of nuclei after zonal centrifugation were very similar if not identical. The densitometric tracings of proteins from neuronal and oligodendrocyte nuclei are shown in Fig. 13. Among the protein preparations from the different classes, differences of a quantitative character were detected from those reported earlier for the adult animals. The most

important observation was the presence of the low-molecular-weight band (mol.wt. 10000; indicated by an arrow) in the protein preparations from all the classes of nuclei. This protein band was found to be specific for the non-histone chromosomal-protein fraction of neuronal nuclei (zone I) of adult animals. As mentioned above, this particular band is much more heavily stained in the proteins of unfractionated nuclei of 10-day-old rats, compared with the same preparation from the adults. Its presence in the

unfractionated nuclei of the adult rats is probably due to the contribution of neuronal nuclei.

Two-dimensional electrophoresis. A fairly close resemblance is obvious when comparison is made between the two-dimensional patterns of the major classes of brain nuclei from adult rats with the corresponding classes from infant animals. However, as has already been pointed out, there is present in the neuronal nuclei from adult rats a component, pI value 8.5, that is absent from the gels of the astrocyte and oligodendrocyte nuclei, but that is found in all classes of nuclei from infant rats. Thus the analysis in two dimensions reinforces the conclusions from the experiments in one dimension only.

Discussion

Non-histone chromosomal proteins were extracted from unfractionated and fractionated nuclei of adult and infant rats by a procedure based on the method suggested by Gronow & Griffiths (1971) with modifications to ensure the efficient extraction of non-histone chromosomal proteins and to provide sufficient quantities of material so that further characterization was possible in the various electrophoretic systems used. It is unlikely that the various groups of proteins, extracted by the methods we have used, are contaminated to any significant extent by proteins of a specifically cytoplasmic nature for two reasons. First, the nuclei after the conventional method of purification have been fractionated by zonal centrifugation. During the separation itself and during the recovery of the nuclei from the separated fractions, any residual contamination would be leached out. Secondly, if there were cytoplasmic contamination then one would expect to observe neither the differences in the amounts of the different classes of protein in the various types of nuclei, nor the specific differences in the individual proteins within these groups, and in particular the possession by the neuronal nuclei of a protein of mol.wt. 10000 and pI 8.5. The present experiments do not give a definitive answer to the problem of whether non-histone chromosomal proteins extracted from chromatin are contaminated by cytoplasmic proteins, as was suggested by Johns & Forrester (1969), although they argue against it. They provide an indication that treatment of chromatin with 0.35M-NaCl may remove the loosely bound non-histone proteins as was shown by Kostraba *et al.* (1975) and Umansky *et al.* (1976).

It is not possible to fractionate nuclei that have been treated with detergents such as Triton X-100 to remove the nuclear membrane because nuclei treated in this way tend to clump together and this vitiates the zonal fractionation. Hence it might be argued that specific differences in the patterns of proteins from the various classes of brain nuclei derive from

Fig. 13. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of non-histone chromosomal proteins from neuronal and oligodendrocyte nuclei

Brain nuclei from 25 male albino Wistar rats (10-day-old) were isolated and fractionated into five zones as described in the text. Non-histone chromosomal proteins were extracted by procedure (B) of Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described in the Materials and Methods section. (a) Non-histone chromosomal proteins from neuronal nuclei (zone I); (b) non-histone chromosomal proteins from oligodendrocyte nuclei (zone V).

the membrane rather than the chromatin alone. However, in two experiments in which the nuclei were washed after fractionation with 0.5% (w/v) Triton X-100, only minor changes in the patterns of the non-histone proteins were observed and the neuronal protein of mol.wt. 10000 was scarcely affected. This, coupled with the substantial amount of this protein, makes it unlikely that proteins of the nuclear membrane exert a dominant effect on the patterns of non-histone chromosomal proteins revealed in the electrophoretic system.

The three electrophoretic systems used for the analysis of non-histone chromosomal proteins showed that this group of proteins is complex and heterogeneous. A limited specific-protein pattern was observed among the non-histone chromosomal proteins from the various classes of brain nuclei after their separation by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The use of electrofocusing electrophoresis confirmed the findings of sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and showed that many of the components are not acidic. Because, in general, we have no indication of the content of glutamic and aspartic amino acids in the individual protein species the term 'non-histone chromosomal proteins' seems more appropriate than 'acidic nucleoproteins' for this group of proteins.

The combination of the two electrophoretic systems in the two-dimensional electrophoresis, apart from giving a clearer picture of the complexity and heterogeneity of non-histone chromosomal proteins, underlined the limited specificity of patterns obtained by the other two systems. Limited specificity of brain non-histone chromosomal proteins was reported by other authors (Olpe *et al.*, 1973; Tashiro *et al.*, 1974), whereas Dravid & Burdman (1968) could not detect any differences in the proteins from neurons, astrocytes and oligodendrocytes and Panchenko *et al.* (1974) could detect no differences in the proteins derived from four brain regions. Limited tissue and species specificity of non-histone chromosomal proteins was shown in a number of different species and tissues (Elgin & Bonner, 1970; MacGillivray *et al.*, 1971, 1972; Wu *et al.*, 1973).

The exact role of non-histone chromosomal proteins remains still unknown. This work was centred on the characterization of this group of proteins in the different classes of brain nuclei, but some of the findings may be related to the evidence presented by several authors that they may play a role in gene regulation. The content of non-histone chromosomal proteins in both ages of animals was higher in nuclei characterized by light disperse chromatin (euchromatin) (neuronal and astrocytes I nuclei) than in the nuclei with condensed chromatin-heterochromatin (oligodendrocyte nuclei; Table 1). Data on the incorporation of amino acids into the non-histone

chromosomal proteins correlated with a higher RNA polymerase activity of neuronal and astrocyte I nuclei (Austoker *et al.*, 1972) suggest a possible involvement of non-histone chromosomal proteins in gene regulation. The striking similarity of the patterns of non-histone chromosomal proteins among the different classes of nuclei may be explained by the fact that this group of proteins should contain structural proteins of chromatin and enzymes of nucleic acid and histone metabolism. Regulatory proteins, if indeed they are present among the non-histone chromosomal proteins, should be in small quantities, which would not have been detected by these methods that permit a qualitative rather than a quantitative characterization.

S. G. T. acknowledges with gratitude a research training scholarship from the Wellcome Foundation.

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