

Table III. Erythrocytic adenosine triphosphatase activity of various species

Species	Age	No.	ATPase activity* (Mean \pm S.D.)		
			Total	Ouabain-insensitive	Ouabain-sensitive
Domestic fowl	Embryonic	12P	429 \pm 49	186 \pm 36	243 \pm 56
Domestic fowl	4-7-day-old	6P	421 \pm 48	258 \pm 36	163 \pm 24
Domestic fowl	Mature	8	273 \pm 36	146 \pm 26	127 \pm 13
Pigeon	Adult	5	132 \pm 13	84 \pm 9	48 \pm 13
Rat	Adult	10	106 \pm 13	69 \pm 16	37 \pm 9
Rabbit	Adult	10	72 \pm 23	65 \pm 24	7 \pm 4
Human	Adult	50	21 \pm 5	13 \pm 4	8 \pm 2

P, indicates pooled samples. *Results are expressed as μ moles phosphorous liberated/g haemoglobin/h at 44 °C.

much higher in embryonic and young chicks than in mature fowls; the activity in fowls was higher than in pigeons, which in turn was higher than rats, rabbits and humans. Comparisons with ATPase activity of erythrocytes from various other mammalian species showed that chick erythrocytes had many times higher activity of both ouabain-sensitive and insensitive ATPase (unpublished observation). It thus appears likely that the high levels in embryonic and young chicks reflect both the early stage of development and some factor common to avian erythrocytes, perhaps the nucleated state of mature red cells. One inexplicable observation was the very low level of ouabain-sensitive ATPase in rabbit erythrocytes in our test system.

Although in many species of animals it has been demonstrated that the Na⁺-K⁺-dependent ATPase activity is related to the erythrocytic K⁺ concentration^{6,8}, the erythrocytic K⁺ concentration of fowls is comparable to that of rats, rabbits and humans⁹. SPERELAKIS¹⁰ observed that in chick heart and skeletal muscles, the intracellular K⁺ concentration reaches a steady state level at a very early embryonic stage, but due to an increase in K⁺ permeability with embryonic age, the cation pump and hence the membrane Na⁺-K⁺-ATPase activity is greatly increased to compensate for the cation leak. It is possible that this increased potassium leak is also operative in embryonic chick erythrocytes and lenses, gradually diminishing with age, but still remaining greater than in

mammalian species, and thus contributing to the high Na⁺-K⁺-dependent adenosine triphosphatase activity. The very rapid and high Rb⁸⁶ uptake by chick lenses from a medium devoid of K⁺ may thus represent compensation for increased K⁺ permeability¹¹.

Résumé. Dans le cristallin et les erythrocytes de poussins à l'état embryonnaire et quelques jours après l'éclosion, l'activité de l'ATPase est beaucoup plus intense que chez d'autres animaux. Ce fait correspond bien à la forte incorporation de Rb⁸⁶ dans leur cristallin.

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The Non-Histone Protein Pattern of Rat Brain During Ontogenesis¹

There are not many investigations of the ontogenetic changes in the non-histone protein (NHP) pattern²⁻⁴, although such a study might provide support for the proposed involvement of NHP in transcriptional mechanisms. Since many important biochemical and morphogenetic processes, e.g. myelination, cell proliferation and synaptic connections, occur in the rat brain during the first 25 postnatal days, qualitative or quantitative changes in the NHP pattern could be expected in young animals, if this pattern reflects the differentiation and metabolic activity of a cell. The NHP pattern of rat brain up to the age of 20 days has therefore been compared with that of adult animals. Quantitative changes of the NHP content in brain and liver nuclei have been investigated by measuring the NHP/DNA ratios during ontogenesis.

Methods. Total brain and liver nuclei of 5-, 10- and 20-day-old and of adult (350-400 g) rats (Ivanovas SIV 50)

were prepared as described previously⁵. For better purification of the nuclei, two CHAUVEAU⁶ procedures were performed. Three successive extractions of NHP from the brain and liver nuclei were then carried out with the modified medium of GRONOW and GRIFFITHS⁷ (0.05 sodium phosphate pH 7.6 containing: 8 M urea, 10%

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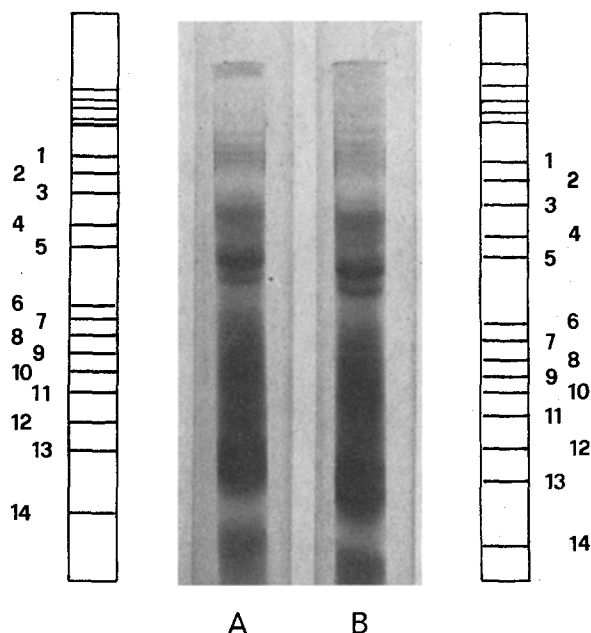
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glycerol, 10^{-3} M N-ethyl-maleimide), using a magnetic stirrer at 10°C for 20 min (1 part extraction volume to 4 parts of original tissue weight). DNA was extracted from the insoluble residue with 5% HClO_4 at 70°C for 15 min and determined by the method of BURTON⁸. Total NHP amounts were determined in the GRONOW and GRIFFITHS' extract by the method of LOWRY⁹. Protein samples were separated by disc-electrophoresis on 3×60 mm gels with the system of SHAPIRO et al.¹⁰, as

NHP/DNA ratios in the developing and adult liver and brain of the rat

Organ	Age	Experiments			Mean
		1	2	3	
Brain	5 d	2.4	2.4	1.8	2.2
	10 d	3.2	2.7	2.5	2.8
	20 d	3.1	2.6	2.4	2.7
	adult	2.6	3.4	2.4	2.8
Liver	5 d	2.1	2.8	1.7	2.2
	10 d	2.4	2.6	2.2	2.4
	20 d	3.3	2.7	3.0	3.0
	adult	3.2	2.8	2.7	2.9



Disc-electrophoresis of GRONOW and GRIFFITHS' extract of total brain from a 5-day-old (A) and adult (B) rat. 20–50 μg proteins were separated for $1\frac{1}{2}$ h with $1\frac{1}{2}$ mA per gel. Gels were then stained with amido black.

modified by ELGIN and BONNER¹¹. Methodological details have been published elsewhere¹².

Results and discussion. A reproducible pattern of 14 main bands for brain and 12 for liver nuclei was obtained from adult animals. Comparison of these patterns shows that the GRONOW and GRIFFITHS soluble proteins have a limited organ specificity, which we have described in another publication¹². The electrophoresis of protein samples extracted from 5-, 10- and 20-day-old rats and adult animals all gave the same general pattern and number of bands (Figure). There is a slight increase of the NHP/DNA ratio for both liver and brain nuclei during ontogenesis, as can be seen from the Table. At the age of 5 days liver and brain nuclei both have a ratio of 2.2, whereas in adult rats the ratios for brain and liver nuclei were found to be 2.8 and 2.9 respectively.

This unchanged NHP pattern during ontogenesis is not compatible with the supposed function of NHP in transcription processes in brain nuclei. Although NHP are synthesized during the first 10 postnatal days, no new bands could be demonstrated by electrophoresis in that period and we could not find any quantitative changes in the densitometric profile of the gels. Our findings are consistent with the observations of BURDMAN² who did not find any differences between the NHP patterns of 1-day- and 8-day-old rat brains, but noticed an increase of total nuclear protein during that period. In rat liver, however, electrophoretic differences of NHP during ontogenesis³ and during liver regeneration have been observed¹³. It is therefore possible that the changes in the nuclear proteins of brain nuclei during development are too small to be detected by present techniques.

Zusammenfassung. Die «non-histone» - Proteine (NHP) aus Hirn- und Leberzellkernen der Ratte wurden bei 5, 10 und 20 Tage alten und adulten Tieren extrahiert und das NHP/DNA-Verhältnis bestimmt. Die NHP aus den Gehirnzellkernen wurden disk-elektrophoretisch aufgetrennt. Leber- und Gehirnkernzeigten eine geringe Zunahme des NHP/DNA - Quotienten während der ersten 20 postnatalen Tage. Es konnten jedoch für diese Altersstadien keine Veränderungen im Proteinmuster des Hirns gefunden werden.

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A Bipolar Electrode for Localized Directional Stimulation

During a recent study of the cerebral cortical consequences of stimulation of the pyramidal tract, it has been found that unless great care is taken, interference occurs due to inadvertent stimulation of the adjacent medial lemniscus¹. This problem is especially difficult in experiments on smaller mammals such as rats, and a special

bipolar electrode has therefore been devised to overcome as far as possible problems of stimulus spread.

Materials and methods. A diagram of the tip of the electrode to be described is shown in Figure 1. The method of construction was as follows: a hollow dental needle of external diameter 400 μm was used and through this was