Presence of High Adenosine Triphosphatase Activity in Lenses and Erythrocytes of Embryonic and Young Chicks

Previous ion transport studies have shown that embryonic chick lenses are capable of a very high uptake of rubidium-86 (Rb^{86})[1]. Cation transport in lenses and erythrocytes, and active transport in general, is believed to be mediated by the enzyme system, adenosine triphosphatase (ATPase), which provides the required energy by the hydrolysis of ATP[2–4]. We have, therefore, determined the activity of both ouabain-sensitive and ouabain-insensitive ATPase in the lens and erythrocytes of 12-13-day-old embryonic chicks and 4–7-day-old chicks, and have then made comparisons with the activities of these enzymes in mature domestic fowls and members of other species.

Lenses were dissected from 12–13-day-old embryonic chicks as described before[5]. Blood was collected, before the dissection of lenses, by allowing the chicks to bleed freely in a petri dish. Blood, with other adherhing fluids, was immediately collected in heparin with a pasteur pipette. Pooled blood from 5 chicks was centrifuged, washed once with normal saline and then haemolysate was prepared for ATPase assay. Rabbit blood was collected from ear veins and blood from rats and young chicks by heart puncture under pentobarbital anaesthesia. Blood from pigeons was collected from wing veins. Lenses and erythrocytes from mature domestic fowls were collected from commercial slaughter houses. Lenses were dissected from rats, rabbits and young chicks immediately after sacrificing with an overdose of pentobarbital.

Haemolysates from erythrocytes were prepared and the ATPase assay was done according to the procedure of BREWER et al.[6]. In the case of avian erythrocytes 0.1 ml of haemolysate was used for the assay, whereas 0.3–0.5 ml was used for humans and other animal species. The red cell enzyme activity was expressed as μmoles phosphorous liberated/g haemoglobin/h at 44 °C. The conditions of assay and the validity of the expression of results have been described by BREWER et al.[6]. Lenses were homogenized in 0.1 *M* histidine-0.1 *M* imidazole buffer at pH 8.0. Generally 10 embryonic chick lenses or 5 young chick lenses were homogenized in 1 ml of buffer whereas approximately 10% (w/v) homogenate in the same buffer was made from fowl, rat, rabbit and human lenses dissected at autopsy. Enzyme assay in homogenate was made using appropriate aliquot in place of haemolysate as in the red cell ATPase assay. Protein in the homogenate was determined according to the method of LOWRY et al.[7]. Results were expressed as μmoles phosphorous liberated/g protein/h at 44 °C. Rb^{86} uptake study was done with embryonic chick and other lenses as described before[1].

Comparative Rb^{86} uptake by lenses is shown in Table I. Rb^{86} uptake by the much smaller chick lens was several times higher than that of rabbit and rat lenses. Correcting for protein content of the lenses, the relative uptake is even higher with embryonic chick lenses, followed by that of young chick lenses and the lenses of fowls.

ATPase activity in the lenses of the various groups is set out in Table II. As expected, chick lenses had a much higher activity of both ouabain-sensitive and insensitive ATPase. Young chick lenses had similar activity to those from embryonic chicks. In all 3 species studied there was a considerable proportion of ouabain-sensitive, Na^+-K^+-dependent ATPase in the lens and the Rb^{86} uptake paralleled the Na^+-K^+-ATPase activity in lenses. Human lenses had comparatively low ATPase activity.

We then investigated whether this high activity in lens was also reflected in the erythrocytes; the results are shown in Table III. Erythrocytic ATPase activity was

Table I. Comparative Rb^{86} uptake by lenses from various species

Species	Age	No.	Rb^{86} uptake	
			cpm/lens	cpm/mg lens protein
Domestic fowl	Embryonic	12	6213	13,219
Domestic fowl	4–7 day old	10	12125	8,083
Domestic fowl	Mature	12	10621	602
Rat	Adult	6	1018	68
Rabbit	Adult	6	2121	20

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Table II. Adenosine triphosphatase activity of lenses from various species

Species	Age or other distinction	No.	ATPase activity[a] (Mean ± S.D.)		
			Total	Ouabain-insensitive	Ouabain-sensitive
Domestic fowl	Embryonic	8P	379 ± 87	194 ± 44	185 ± 65
Domestic fowl	4-7-day-old	8P	347 ± 56	134 ± 21	213 ± 30
Domestic fowl	Mature	8P	170 ± 21	73 ± 13	97 ± 10
Rat	Adult	8P	115 ± 20	63 ± 12	52 ± 11
Rabbit	Adult	8	34 ± 11	14 ± 5	20 ± 12
Human	Post-mortem	10	36 ± 11	18 ± 6	18 ± 5

P, indicates pooled samples. [a] Expressed as μmoles phosphorous liberated/g protein/h at 44 °C.

Table III. Erythrocytic adenosine triphosphatase activity of various species

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

P, indicates pooled samples. [a] Results are expressed as μmoles 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 humans[9]. SPERELAKIS[10] 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 Rb^{86} uptake by chick lenses from a medium devoid of K^+ may thus represent compensation for increased K^+ permeability[11].

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 Rb^{86} dans leur cristallin.

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The Non-Histone Protein Pattern of Rat Brain During Ontogenesis[1]

There are not many investigations of the ontogenetic changes in the non-histone protein (NHP) pattern[2–4], 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. myelinization, 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 previously[5]. For better purification of the nuclei, two CHAUVEAU[6] 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 GRIFFITHS[7] (0.05 sodium phosphate pH 7.6 containing: 8 *M* urea, 10%

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