

## FREE NUCLEOTIDES IN RETINA

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### 1. Introduction

The various types of free nucleotides or nucleotide coenzymes of animal tissues play a fundamental role in several metabolic pathways [1, 2]. Thus, it is of interest to know the nature and the distribution of these compounds in retina. There are only few data in the literature concerning this subject. So far we know ATP [3] and NAD [4] are the sole nucleotides as yet determined in retina. In this paper the whole set of free nucleotides detectable in bovine and rat retina was studied.

### 2. Material and methods

The retina were taken either from young bovine eyes at the slaughter house or from Wistar rats at the laboratory within 5 min after death of the animals. For the bovine retina, the eyeballs were quickly removed and cut into anterior and posterior hemispheres. The retina were peeled off from the posterior hemisphere and frozen in liquid nitrogen. Concerning the rat retina, an incision of the eyeball *in situ* allowed the lens to be removed and the retina to be picked up with pincers which were plunged into liquid nitrogen. Acid-soluble extraction with perchloric acid and chromatographic fractionation on a Dowex 1X8 column of the nucleotides were performed as described earlier [5]. Nucleotide materials in column eluates were identified on the basis of different criteria: UV absorption spectra, coincident elution from the column with authentic samples added to tissue extracts, cochromatography with authentic

compounds by thin layer chromatography in at least 4 solvent systems depending on different pH. Following systems were employed:

Plates	Solvents
Silica gel F <sub>254</sub> Merck	Methanol-water-NH <sub>4</sub> OH, d 0.923 60:20:10
PEI cellulose F Merck	Acetic acid 1 N-CiLi 3 M, 90:10
PEI cellulose F Merck	LiCl gradient 0.3 M to 1.2 M
PEI cellulose F Merck	Formate gradient pH 3.4, 0.5 M to 4 M.

### 3. Results

The amount of free nucleotides in retina (table 1) is lower when compared to other nervous tissues such as brain; under optimal conditions, about 300  $\mu$ moles per 100 g wet weight were found [6]. A higher amount of nucleosides mono- and diphosphates can be noted. This may be due either to very active degradation enzymes or to a block of the phosphorylation of the nucleosides di- and tri-phosphates resulting from the interruption of the blood supply and therefore of the substrates immediately after killing the animals. In some experiments, not reported here, where the delay between killing the animals and extraction of the nucleotides was longer than usual (30 min), an equilibration of the three nucleotides took place which suggests the action of adenylate kinase activity. As in other tissues, very low levels of cytidylic nucleotides exist in retina. There are several specific features concerning the distribution of the

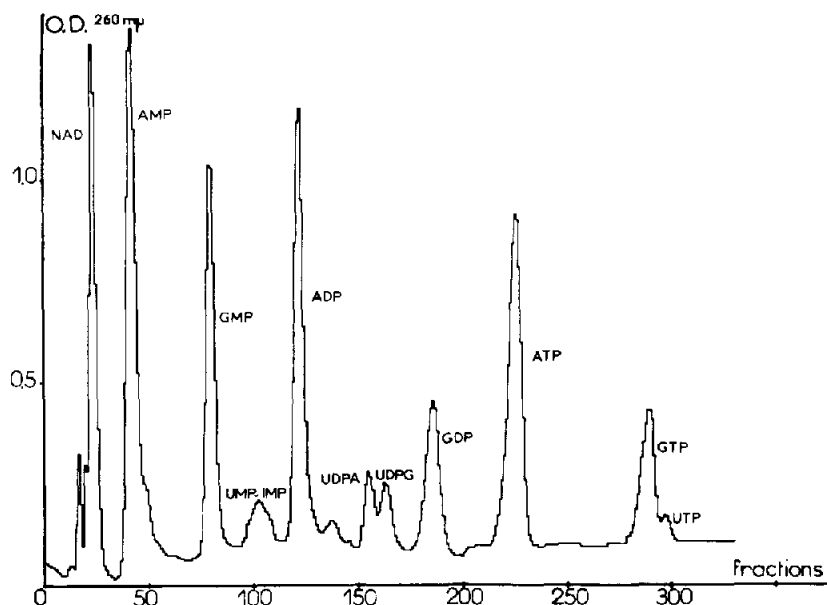


Fig. 1. Chromatography of acid-soluble extract of 16 bovine retina (5.3 g wet weight) on Dowex 1X8 200–400 mesh formate form. Column  $0.8 \times 28$  cm. Elution in 4 ml fractions. Discontinuous convex concentration gradients. Mixing chamber 250 ml contains water at the beginning. Reservoir: from fraction 1–31  $\text{HCOOH}$  1 N; 32–94  $\text{HCOOH}$  4 N; 95–189  $\text{HCOOH}$  4 N,  $\text{HCOONH}_4$  0.2 N; 190–265  $\text{HCOOH}$  4 N,  $\text{HCOONH}_4$  0.4 N; 266-end  $\text{HCOOH}$  4 N,  $\text{HCOONH}_4$  0.8 N.

Table 1  
Distribution of free nucleotides in bovine and rat retina.

	Bovines			Rats		
	$\mu\text{moles}/100$ g wet weight	percent of total Nt	percent Nt same base	$\mu\text{moles}/100$ g wet weight	percent of total Nt	percent Nt same base
AMP	48.7	17.7		52.7	21.5	
ADP	41.0	14.9	48.0	40.7	16.6	49.0
ATP	42.5	15.4		26.7	10.9	
GMP	30.2	11.0		58.4	23.8	
GDP	25.1	9.1	28.4	17.5	7.1	34.3
GTP	22.7	8.3		8.4	3.4	
UMP-IMP	10.0	3.6		11.1	4.5	
UDPA	11.6	4.2	12.3	6.0	2.4	11.0
UDPG	9.2	3.3		10.0	4.1	
UTP	3.4	1.2		traces		
CMP	8.4	3.1	3.1	traces		
NAD	22.1	8.0	8.0	13.5	5.5	5.5
Total	274.9			245.0		

different types of nucleotides. Whereas the total amount of free nucleotides of retina is lower than in brain, the level of NAD is at least twice that in brain, so are the UDP sugars. But the most striking values concern the distribution of the adenylic and guanylic nucleotides which account respectively for 49 and 34% of the total nucleotides compared to 71 and 9.3% in rat brain. The amount of guanine nucleotides is the highest noted until now in any tissue.

In summary, the distribution of free nucleotides in retina is characterized by a much lower amount of adenylic nucleotides and a much higher amount of guanylic nucleotides than in any other tissue. The significance of these findings is under investigation.

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#### References

- [1] P.Mandel, Metabolic turnover in the nervous system, in: Handbook of Neurochemistry, vol. 5, ed. A.Lajtha (Plenum Press, New York) in press.
- [2] J.L.Strominger, *Physiol. Rev.* 40 (1960) 55.
- [3] S.Williams, R.A.Paterson and H.Heath, *J. Neurochem.* 15 (1968) 227.
- [4] C.N.Graymore, in: *Biochemistry of the Retina*, ed. C.N. Graymore (Academic Press, 1965) p. 83.
- [5] J.Kleithi and P.Mandel, *Biochim. Biophys. Acta* 24 (1957) 642.
- [6] P.Mandel and S.Edel-Harth, *J. Neurochem.* 13 (1966) 591.