# THE UTILIZATION OF L-ADENOSINE BY MAMMALIAN TISSUES

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## 1. Introduction

Recently, we have been interested in a systematic study of the behaviour of L-nucleosides and nucleotides. These compounds, enantiomers of the naturally occurring nucleic acid components, are now easily accessible by unequivocal synthetic procedures [1–3] which eliminate any possibility of contamination by natural derivatives. On investigation of numerous enzymes and enzymatic systems *in vitro* it has been demonstrated [3, 4] that the nucleotide derivatives of the L-ribo series are resistant towards many nucleolytic enzymes as determined by the mode of binding of the nucleoside residue to the enzyme molecule. This observation led us to investigate the biochemical properties of L-nucleosides *in vivo*. The experiments with bacterial cells showed that there is essentially no transport of L-ribonucleosides through the cell wall of the bacteria investigated [5]. Thereafter, we examined the possibility of L-nucleoside utilization in mammalian tissue systems *in vivo*. In this paper we report the results obtained with L-adenosine applied intraperitoneally to mice.

## 2. Material and methods

8-$^3$H-L-adenosine was prepared by an alkali-catalyzed tritium-exchange reaction [6] of L-adenosine [1] in tritium oxide. The product thus obtained (specific activity 37.5 mCi/mmole) was essentially free of adenine (as shown by borate buffer electrophoresis) and chromatographically homogeneous. This compound was administered intraperitoneally to a mouse ("H"-strain) in one dose of 36.6 $\mu$Ci.

Simultaneously, the same experiment was performed with a leukemic animal (5 days after leukemic inoculation).

## 3. Results

The excretion of radioactivity in urine of experimental animals (fig. 1) shows a remarkable time delay in L-nucleoside excretion. Moreover, the difference between healthy and leukemic animals favours the accumulation of L-nucleoside in the latter. The paper chromatographic analysis of urine constituents showed that about 97% of the radioactive material consisted of L-adenosine, the residual activity being formed by L-inosine. There were no traces of adenine within the range of experimental error.

The distribution of labelled L-adenosine in samples from various tissues was analyzed by direct counting in liquid scintillation apparatus. The data summarized in table 1 show the distribution of L-nucleoside in different tissues. It might be pointed out that the content of L-nucleoside is significantly higher in leukemic tissues.

On analyzing the tissue homogenates, the main part of the radioactivity was found in a 70% ethanol soluble fraction, the content being again remarkably higher with leukemic tissues. The pool was analyzed for nucleotide content by paper chromatography in 2-propanol–concentrated aqueous ammonia–water (7:1:2) system. In most tissues examined (table 2), the material contained small but detectable amounts of L-AMP. The only exception was found with liver

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Table 1
Distribution of L-adenosine in mice tissues (cpm)[a].

| Time after administration (hr) | Liver | | Spleen | | Thymus | |
|---|---|---|---|---|---|---|
| | N | L | N | L | N | L |
| 0.5 | 17,400 | 22,500 | 31,800 | 43.100 | 29,400 | 43,500 |
| 1.0 | 8,100 | 15,000 | 7,800 | 16,200 | 7,200 | 20,400 |
| 2.0 | 2,000 | 8,600 | 2,400 | 9,000 | 2,700 | 10,500 |
| 3.0 | 1,500 | 6,900 | 1,450 | 6,900 | 1,800 | 8,400 |
| 6.0 | 1,150 | 6,050 | 1,290 | 3,900 | 900 | 5,400 |
| 11.0 | 930 | 1,700 | 1,050 | 1,800 | 900 | 2.550 |
| 24.0 | 900 | 997 | 750 | 1,350 | 750 | 1,050 |

[a] Values for 10 mg of wet tissues; N = normal, L = leukemic.

homogenate the pool fraction of which contained up to 40% L-AMP. The enantiomeric purity of this material was checked by treatment with the whole snake venom (*V. russelli*) which did not afford any significant amount of nucleoside material (L-nucleoside 5′-phosphates are not dephosphorylated by snake venom 5′-nucleotidase [3]). Both this enantiomeric purity of labelled nucleotide and the analysis of L-adenosine fraction of the same sample which did not reveal any trace of labelled adenine eliminate the possibility of L-adenosine utilization for the synthesis of natural nucleosides *de novo* under the experimental conditions.

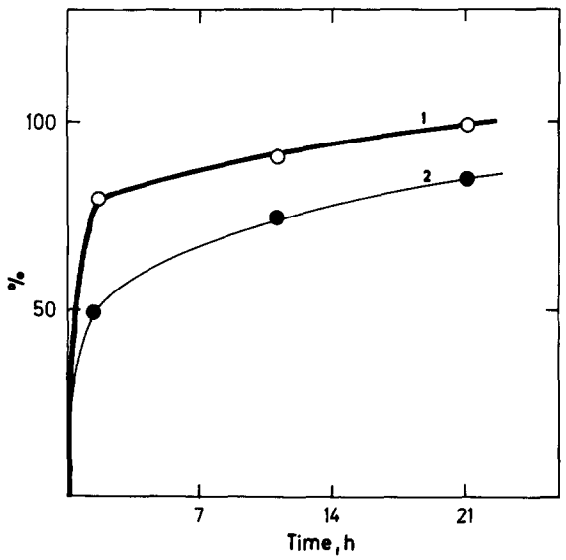


Fig. 1. Excretion of L-adenosine-$^3$H in mouse urine. Values refer to percentage of radioactivity excreted as function of time (administered dose = 100%). (1) Normal, (2) leukemic.

On detailed analysis by citrate buffer (pH 3.5) electrophoresis, the nucleotidic fraction of liver homogenate was shown to contain 2% L-ADP in addition to the bulk of L-AMP discussed above. On the other hand, the presence of L-ATP could not be proven within the limits of experimental error. L-AMP seems to be a substrate for nucleotide kinases of liver cells. In this connection, L-adenosine incorporation into liver RNA was to be examined. The TCA insoluble fraction of liver homogenate contains a significant amount of L-adenosine, dependent upon the quantity of the material applied. From the estimation of the radioactivity of native liver RNA [7] it follows that there is 0.015 mol. % L-adenosine residue incorporated

Table 2
Phosphorylation of L-adenosine in mice tissues.

| Tissues | Total radioactivity[a] (cpm) | | L-AMP (%) | |
|---|---|---|---|---|
| | N | L | N | L |
| Thymus | 19,800 | 24,200 | 3.5 | 3.5 |
| Spleen | 18,200 | 16,600 | 5.0 | 6.5 |
| Liver | 36,800 | 28,000 | 45.0 | 43.0 |
| Brain | 4,950 | 6,450 | traces | traces |
| Kidney | 49,500 | 70,050 | none | none |
| Muscle | 1,950 | 3,200 | none | none |
| Heart | 1,050 | 1,100 | none | none |
| Blood | 30,200 | 36,000 | 8.0 | 7.0 |

Time after administration, 1.30 hr.
[a] For 25 mg of wet tissues, N = normal, L = leukemic.

into liver RNA. The character of its linkages as well as the detailed analysis of the incorporarion into RNA are under study.

## References

[1] A. Holý and F. Šorm, Coll. Czechoslovak Chem. Commun. 34 (1969) 3383.
[2] A. Holý, Tetrahedron Letters (1971) 189.
[3] A. Holý and F. Šorm, Coll. Czechoslovak. Chem. Commun. 36 (1971) in press.
[4] Mechanism and Control Properties of Phosphotransferases, Joint Biochemical Symposium USSR-GDR, May 3–7, 1971, Schloss Reinhardsbrunn (Akademie-Verlag, Berlin) (1971).
[5] I. Votruba, A. Holý and F. Šorm, in preparation.
[6] J. Filip and A. Holý, unpublished results.
[7] K. Scherrer and J.E. Darnell, Biochem. Biophys. Res. Commun. 7 (1962) 486.