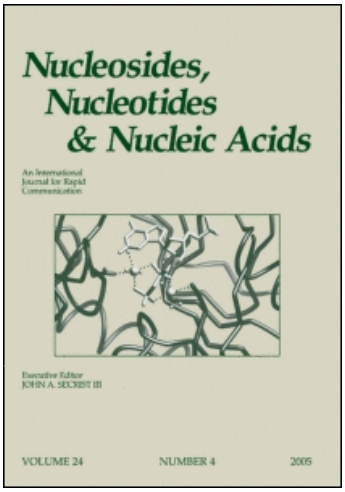

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QUEUINE CONTENT OF HEMATOPOIETIC AND NEURONAL TISSUES

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The transfer RNAs for asp, asn, his and tyr contain queuine in the first position of their anticodons (1). Queuine is 7-(((cis-4,5-dihydroxy-2-cyclopenten-1-yl)-amino)-methyl)-7-deazaguanine (2) and it cannot be synthesized by higher mammals. It must be obtained from the diet or the intestinal flora (3). The original transcripts of these tRNAs contain guanine in the wobble position. This guanine is enzymatically excised by cleavage of the N glycoside bond and is replaced by queuine (4,5). The function of queuine is not fully understood, but it is known that (q-) $tRNA^{tyr}$ occasionally reads through the amber stop codon to form an elongated run-through polypeptide. The (q+) $tRNA^{tyr}$ does not make this error (6). Also, there is recent evidence that queuine-containing tRNAs play a role in the regulation of oxidative metabolism in both prokaryotes (7) and eukaryotes (8). In fact, it has now been shown that molecular oxygen is required for the conversion of (q-) to (q+) tRNA (8). During a study of purine metabolism in diverse species we found canine bone marrow to be remarkably deficient in queuine-containing tRNA. We felt it to be of importance to determine if this was also true for other canine tissues and for other species. We also determined that the enzyme that inserts queuine into tRNA was present in canine bone marrow and we describe some of the properties of this enzyme.

METHODS

The tRNAs were extracted from the indicated tissues with phenol and charged with their cognate amino acids as previously described and analyzed by RPC-5 chromatography (9). The enzyme guanine-queuine tRNA transglycosylase was purified and assayed as described by Howes and Farkas (10).

The rate of transport of queuine by red cells was determined by washing the cells with phosphate-buffered saline pH 7.4 and suspending the cells in Tris buffer, pH 7.4, 1:10 V/V. ^{3}H queuine sp. act 1.0 mci/mmol was added to a final concentration of 0.5 uM. The temperature was 37^{o}. At the indicated times a 0.1 ml aliquot of the cell suspension was pipetted into 2 ml of ice cold isotonic saline. The cells were then pelleted by centrifugation at 1000 g at 0^{o}. A separate experiment showed that there was no uptake of queuine into or

Table I.

Percentage of Queuine-containing $tRNA^{His}$ in the Bone Marrow of Different Species

Species	$tRNA^{His}$ (% Q+)
dog	0
cat	0
lion	0
ferret	60
cow	67
rabbit	80
pig	53

release of queuine from the cells at these low temperatures. An aliquot of the supernatant was added to a scintillation counting cocktail to determine the concentration of extracellular queuine. In order to determine the amount of queuine inside the cells, the cells were first washed two more times with ice cold saline. The cell pellet was then treated with hydrogen perioxide to prevent color quenching by the heme and the ^3H queuine in the cells was determined with a liquid scintillation counter (11).

RESULTS AND DISCUSSION

We studied the isoacceptor tRNA patterns in normal dog bone marrow using RPC-5 chromatography. The first tRNA that we looked at was $tRNA^{His}$ and were surprised to find that it did not contain queuine. This observation was unexpected because normal tissues are usually (q+) and high levels of (q-) tRNA are associated with malignant cells (12). This was confirmed by showing that the canine bone marrow $tRNA^{His}$ did not react with BrCN (9) or periodate (13). We examined the other members of the queuosine family and found that $tRNA^{asn}$ was completely (q-) whereas $tRNA^{Asp}$ was 53% (q+). These results confirm our previous report that the queuine insertion enzyme has a greater affinity for $tRNA^{asp}$ than for $tRNA^{His}$ and $tRNA^{asn}$ (14). We looked at the bone marrow tRNAs of other species and found that $tRNA^{His}$ was largely (q+) in rabbit, cow and pig and (q-) in the cat and lion. At first glance one might notice that species that are predominantly (q-) are carnivores and do not have the luxuriant intestinal flora characteristic of herbivores. It is known that the microbes of the intestinal flora can provide queuine to their vertebrate host (3). We therefore examined the bone marrow tRNAs of the ferret which is a carnivore but is not a canine or feline. The carnivore hypothesis for absence of (q+) tRNA was dispelled when we found that the ferret bone marrow $tRNA^{His}$ was predominantly (q+) (see Table I). It was of

Table II:

Uptake of ^3H Reduced Queuine into Red Cells of Different Species.

	V cell/V outside	DPM ^3H cell / DPM ^3H outside
Dog	31	2
Human	31	38
Mouse	30	32
Rabbit	31	33

interest to determine if the undermodification with respect to the queuosine family of tRNAs was limited to bone marrow or was true for other canine tissues as well. Dog liver $tRNA^{His}$ was predominantly (q+) and brain was 100% (Q+), but the circulating leukocytes which are largely derived from bone marrow were completely (q-). It therefore, became of special importance to determine the status of queuine in another lymphoid organ e.g. the spleen which turned out to be 40% (q+).

We tested homogenates of bone marrow to determine if guanine, queuine tRNA transglycosylase was present. The enzymatic activity was found to be present at about the same levels as it is in other species but the properites of the canine enzyme were very different from the enzymes of other species. The enzyme purified from rat and rabbit tissues and from plants did not require a cofactor (10,15). However, the dog bone marrow enzyme lost activity upon dialysis. Activity could be restored by adding back Co^{2+} or Cu^{2+}. Furthermore, the Km for queuine and guanine were identical, 0.13 uM which may account for why canine bone marrow is so deficient in queuine. The other queuine insertion enzymes have a greater affinity for queuine than for gunine (15).

The ability of red cells from different species to take up queuine was determined. Rather than measuring the rate, we allowed the inward and outward transport of queuine to reach a steady state and then determined the amount of radioactivity inside the cell and in the extracellular fluid. These experiments demonstrated that in human, rabbit and mouse red cells at equilibrium, the queuine concentration in the cells and the extracellular fluid are equal. However, in the canine cells the amount of queuine in the extracellular fluid exceeded that in the cells, indicating that the canine red cell membrane does not readily allow the passage of queuine (Table II). A separate experiment showed that canine serum did not inhibit the uptake of queuine by human red cells (data not shown).

Queuine has two positive charges at physiological pH. Since the red cell membrane in canines discriminates against the passage of queuine we felt it of importance to determine if queuine crosses a much more discriminating obstacle, the blood brain barrier. We extracted the tRNA from dog brain and analyzed it for queuine. The brain tRNA was 100% (q+) for all four amino acids of the queuine family indicating that queuine is readily transported across the blood brain barrier.

Oncogenic retroviruses usually have a polycistronic message with the gene for the viral core protein at the 5' end and the message for reverse transcriptase at the 3' end. The two open reading frames are separated by a stop codon and a nonsense supressor is required for the translation of the reverse transcriptase (16). (q-) $tRNA^{Tyr}$ has been shown to read through the Amber codon (6). The availability of large amount of (q-) tRNA in some hematopoietic organs may indicate that polycistoronic messages occur in hematopoietic tissues. The fact that queuine insertion requires oxygen (8) indicates that this may be a mechanism for adjusting the translational apparatus of bone marrow to the availability of oxygen.

DEFINITION AND ACKNOLWEDGEMENTS

(q+) is tRNA that contains queuine in the first position of the anticodon. (q-) tRNA is the precursor of (q+) tRNA and contains guanine instead of queuine in this position. This work was supported by NIH grants ESO4079, HD 14062 and by a generous gift form the Lupus Foundation of America.

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