tor strength for this electronic transition as for normal hydrogen and assuming that the line is optically thin, we apply standard procedures (*11*) to derive an upper limit to the column density of X atoms of 2.0×10^{12} cm^{-2}. Since the column density of H is 1.0×10^{20} cm^{-2} (*9*), this implies that $n(X)/n(H) < 2 \times 10^{-8}$. This limit obtains for all hypothetical singly charged superheavies with isotope shifts greater than 120 km sec^{-1} or to particles with masses greater than about four times that of the proton.

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12. Partly supported by the National Aeronautics and Space Administration and the National Science Foundation. M.J. is an Alfred P. Sloan Foundation fellow.

23 November 1981

Queuine, a Modified Base Incorporated Posttranscriptionally into Eukaryotic Transfer RNA: Wide Distribution in Nature

Abstract. *Queuine, a modified base found in transfer RNA, appears to be a new dietary factor because (i) previous studies have shown that mice require it for the expression of queuine-containing transfer RNA's, but apparently do not synthesize it, and (ii) significant amounts of free queuine are present in common plant and animal food products.*

Unlike other transfer RNA (tRNA) modifications, queuine is synthesized first as a base which then is incorporated irreversibly (in an exchange reaction in which guanine is removed) into mature tRNA by the enzyme guanine: queuine tRNA transglycosylase (*1–3*). Queuine is found exclusively in the first position of the anticodon in tyrosine tRNA, histidine tRNA, asparagine tRNA, and aspartic acid tRNA (*4*). Queuine appears to be the immediate precursor of queuine-containing tRNA ([Q+]tRNA) in mammals (*1, 2, 5–7*) and has been identified as the free base in animal serum, amniotic fluid, and extracts of *Drosophila melanogaster* (*8, 9*). However, animals apparently do not synthesize queuine de novo, since germ-free mice on a defined diet do not synthesize [Q+]tRNA unless enabled to do so by any one of the following: loss of germ-free state, consumption of a normal Laboratory Chow diet, parenteral injection of queuine, or addition of queuine or [Q+]tRNA to the defined diet (*6*). While both gut flora and diet enable mice to synthesize [Q+]tRNA, it is not known whether this results from the feeding of queuine or its precursor. We report here that free queuine is widely distributed among eukaryotes, with significant levels present in plant and animal products common to the human diet (Table 1).

The data in Table 1 were obtained by means of a whole cell assay; when cultured in serum-free medium, the synthesis of [Q+]tRNA by L-M cells depends on queuine addition to the medium (*1, 9, 10*). Only queuine has been demonstrated to give this response (*9, 10*); the nucleoside of queuine, queuosine, is not active in the assay (*11*). The L-M cell assay, however, is tedious, unsuitable for more than a few samples at a time, and subject to nonspecific inhibition (*9*). Therefore, an additional chemically based assay was developed, on the basis of gas chromatography–mass spectrometry with selected ion monitoring (*12*). The abundant ions of *m/z* 379, 380, which are highly characteristic of the 7-deazaguanine nucleus (*8*) were monitored (Fig. 1). This method provides rigorous chemical evidence for the presence of queuine in human amniotic fluid, extracts of *D. melanogaster*, and coconut water. Previously, queuine had been positively identified only in bovine amniotic fluid (*8*). The isolates from *D. melanogaster* and coconut water were sufficiently pure to permit acquisition of full-scan mass spectra (M = *m/z* 709; M-CH_3 = 694) (*8*). Seven amniotic fluids from normal human pregnancies (16 to 28 weeks gestation) were estimated to contain queuine concentrations ranging from 2 to 84 ng/ml (mean = 29 ng/ml), on the basis of selected ion recording peak areas, referenced to standard queuine samples. There was no apparent relation of queuine concentration to gestation time (*13*).

Our data appear to be sufficient to explain the contribution of diet to [Q+]tRNA formation. However, diet must provide queuine both directly and indirectly (after salvage of free base from [Q+]tRNA), because the ability of germ-free mice to use dietary [Q+]tRNA for endogenous [Q+]tRNA synthesis implies a salvage mechanism. Salvage also would explain the contribution of gut flora to [Q+]tRNA formation in mice,

Table 1. Queuine content of natural products. Queuine was estimated from the appearance of [Q+]tRNA^{Asp} in the L-M cell line, with authentic queuine as a standard (*1, 9, 10*), and a millimolar extinction coefficient for queuine of 10.5 at 260 nm in H_2O (*22*). Most solid materials were blended as a 10 percent (weight to volume) aqueous slurry (tomato was blended whole) and centrifuged and then the supernatant was assayed. *Drosophila melanogaster* was extracted as described in (*9*). Milk was centrifuged and the skim portion was assayed. For multiple samples (number in parentheses) the range of values is given. Many products were negative for queuine by the L-M cell assay (*23*).

Source	Amount
Bovine amniotic fluid (third trimester) (3)	2300 to 3600 ng/ml
*Drosophila melanogaster** (wild type and mutants) (15)	0 to 1100 ng/g
Coconut water (ripe) (5)	87 to 530 ng/ml
Bovine pineal body	300 ng/g
Wheat germ	190 ng/g
Bovine seminal vesicle (adult)	110 ng/g
Bovine testicle (adult)	58 ng/g
Bovine serum (fetal) (2)	33 to 54 ng/ml
Tomato (fresh, ripe)	21 ng/g
Bovine milk (whole and skim) (2)	16 to 17 ng/ml
Bovine serum (calf)	14 ng/ml
Bovine milk (evaporated skim, canned)	12 ng/ml
Yogurt (commercial and homemade) (2)	4 to 6 mg/g
Goat milk (fresh)	3 ng/ml
Goat milk (evaporated, canned)	1 ng/ml
Human milk	1 ng/ml

*The values for *D. melanogaster* are derived from previously published data [figure 1 in (*9*)].

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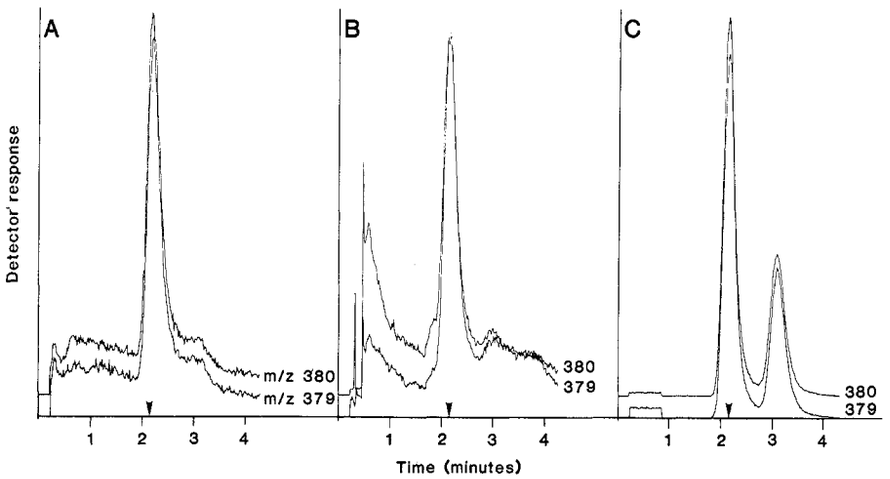

Fig. 1. Identification of queuine by gas chromatography–mass spectrometry and selected ion monitoring of the trimethylsilyl derivative. (A) Human amniotic fluid. Queuine was partially purified (*19*), then converted to the volatile trimethylsilyl derivative (*8*), prior to mass spectrometry (*12*). (B) Queuine was partially purified (*20*), and a portion of this material was subjected to mass spectrometry. (C) Human amniotic fluid. Queuine was purified to homogeneity (*21*), and a portion was subjected to mass spectrometry. (A to C) Arrows mark the elution time of the hexasilyl derivative of authentic queuine (M^+ = 709). The second peak, most notable in (C), is due to the pentasilyl derivative (M^+ = 637).

because rodents practice coprophagy and the stomach of rodents contains some microbial flora (*14*). Salvage may account for the high concentration of queuine in bovine sources (Table 1) as well, since salvaged material from the degradation of microbial RNA (from rumen bacteria) contributes significantly to tissue nucleic acid synthesis in ruminants (*15*). Finally, the possibilities remain that diet or gut flora may contribute a component of queuine, for example, the cyclopentenediol moiety, or that gut flora may contribute fully modified queuine.

Direct queuine synthesis has not yet been observed in any organism, although the high concentration of queuine in some plant sources (Table 1) suggests that plants may do so. *Escherichia coli*, the best studied organism in this regard, does not synthesize queuine directly; instead, a precursor of queuine is incorporated into tRNA (queuine is not a substrate for this reaction), with further modification occurring only at the polynucleotide level (*3*). No free queuine has been detected in *Escherichia coli* extracts (*3*).

The function of queuine remains unknown. Since long-term queuine-deficient mice have no apparent abnormalities (*6*), queuine must be of marginal significance under the protective conditions of gnotobiotic animal care. However, queuine metabolism is altered in neoplasia. Many tumors exhibit queuine-deficient tRNA (*5, 7, 16*); and queuine addition to Ehrlich ascites tumor–bearing mice can restore queuine to tumor tRNA and may inhibit tumor growth (*5*). Therefore, our findings may relate to the nutritional needs of the cancer patient and may suggest improved formulations for hyperalimentation and total parenteral nutrition (*17*).

The high concentration of queuine in the bovine pineal body (Table 1) is of interest because the pineal body contains the highest known concentration of biopterin in the body (*18*). This result supports a previously suggested (*9*) relation between queuine and pteridine metabolism.

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12. Isolated material was converted to volatile trimethylsilyl derivatives by heating the dried sample with *N,O*-bis(trimethylsilyl)acetamide, trimethylchlorosilane, and pyridine (90:1:10) for 1 hour at 100°C. Data were recorded with an LKB 9000S gas chromatograph–mass spectrometer: 0.9-m, 1 percent SP-2250 chromatographic column, 210°C, 30 cm^3 of He per minute; 70 eV of ionizing energy; Digital Equipment Corp. PDP-11/40 data system.
13. First- and second-trimester bovine amniotic fluids contain less than 1 percent of the third-trimester material. However, a large late gestation increase in human amniotic fluid queuine is unlikely to occur because early studies using the L-M assay (in a form not comparable quantitatively with the present assay) indicated no significant difference in the queuine contents of human amniotic fluids from 29, 32, 40, and 42 weeks gestation (data not shown).
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19. Amniotic fluid (4 ml) was applied to a column (0.5 ml) of Dowex-50 (H^+), then eluted successively with 3 ml of H_2O, 3 ml of 1.5*N* HCl, 3 ml of H_2O, and 2 ml of 3*N* NH_4OH. The NH_4OH eluate contained the queuine and was evaporated to dryness.
20. Flies (340 g; strain Oregon R) were blended with 4.4 liters of propanol, 3.5 percent NH_4OH, and 2-mercaptoethanol, 100:50:0.5 (by volume) and then centrifuged; the supernatant was lyophilized [see (*9*)]. The dry residue was dissolved in 500 ml of H_2O and applied to a Sephadex G-10 column (5 by 88 cm) in H_2O, then washed with 5 liters of H_2O and finally with 2 liters of 0.02*M* HCl. The fraction containing queuine (eluting between 620 and 930 ml after the addition of 0.02*M* HCl to the column) was identified, after lyophilization, by the L-M cell assay (yield 5 μg, by activity).
21. Coconut water (2500 ml from 14 coconuts purchased locally) was absorbed to Dowex-50 (H^+) (454 g in a scintered glass funnel), then eluted successively with 3 liters of H_2O, 3 liters of 1.5*N* HCl, 3 liters of H_2O, and 2 liters of 3*N* NH_4OH. The dark-colored NH_4OH eluate was collected, the NH_3 was removed by rotary evaporation, and the product was lyophilized. The residue (dissolved in 45 ml of H_2O) was applied to a cellulose column (2.5 by 93 cm, Whatman CF11) in H_2O and washed with H_2O (850 ml); the queuine was then eluted with 0.02*N* HCl. Queuine-containing fractions were identified [by evaporation of 0.5-ml portions, with subsequent thin-layer chromatography on cellulose with 3 percent ammonium formate as solvent (*1*)], pooled, and lyophilized. Final purification was by preparative thin-layer chromatography, with the use of the same system (yield, 66 μg).
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23. Natural products negative for queuine (1 < ng/g) by the L-M cell assay: apricot kernel, banana (ripe, peeled), cotton seed, bovine rumen fluid, date seed, date embryo tissue culture [J. F. Reynolds and T. Murashige, *In Vitro* **15**, 383 (1979)], green pea (fresh), mung bean, tobacco callus tissue culture, and water from immature coconuts (about 6 months and younger).
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6 October 1981; revised 11 January 1982