Presence of queuine in Drosophila melanogaster: correlation of free pool with queuosine content of tRNA and effect of mutations in pteridine metabolism

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ABSTRACT

Queuine, a modified form of 7-deazaguanine present in certain transfer RNAs, is shown to occur in Drosophila melanogaster adults in a free form and its concentration varies as a function of age, nutrition and genotype. In several, but not all mutant strains, the concentrations of queuine and the Q(+) (queuine-containing) form of $tRNA^{Tyr}$ are correlated. The bioassay employs L-M cells which respond to the presence of queuine by an increase in their Q(+)$tRNA^{Asp}$ that is accompanied by a decrease in the Q(-)$tRNA^{Asp}$ isoacceptors. The increase in Q(+)$tRNA^{Tyr}$ in Drosophila that occurs on a yeast diet is accompanied by an increase in queuine. Similarly the increase of Q(+)tRNAs with age also is accompanied by an increase in free queuine. In two mutants, brown and sepia, these correlations were either diminished or failed to occur. Indeed, the extract of both mutants inhibited the response of the L-M cells to authentic queuine. When the pteridines that occur at abnormally high levels in sepia were used at 1×10^{-6}M, the inhibition of the L-M cell assay occurred in the order biopterin > pterin > sepiapterin. These pteridines were also inhibitory for the purified guanine:tRNA transglycosylase from rabbit but the relative effectiveness then was pterin > biopterin > sepiapterin. Pterin was competitive with guanine in the enzyme reaction with $K_i = 0.9 \times 10^{-7}$M. Also when an extract of sepia was chromatographed on Sephadex G-50, the pteridine-containing fractions only were inhibitory toward the L-M cell assay or the enzyme assay. These results indicate that free queuine occurs in Drosophila but also that certain pteridines may interfere with the incorporation of queuine into RNA.

INTRODUCTION

Either queuine (1) or guanine can occur in the wobble position of the anticodons of $tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{His}$, and $tRNA^{Tyr}$ (2) without a demonstrable effect on the ability of the tRNAs to participate in translation (3-7). These tRNAs can exist predominantly as either Q(+) or Q(-) forms; some cells contain a mixture of the two. The ratio of Q(+) to Q(-) forms is a function of age, nutrition, metamorphic stage, and genotype of Drosophila (7-9), and of the nutritional status (10) and the presence of

tumor or organ-regenerating properties (3, 5, 11-17) in mice.

The biosynthesis of both pteridines and queuine is linked to the metabolism of guanine. The conversion of guanosine triphosphate to dihydroneopterin triphosphate by GTP cyclohydrolase I occurs in both bacteria and eukaryotes (18-20). The enzymology of queuine biosynthesis has not been defined, but it has been shown that Salmonella typhimurium will convert labeled guanine to labeled queuine with the loss of C-8, as occurs in pteridine biosynthesis (21). In addition pteridines and queuine share common chemical properties in that they are fluorescent and adsorb to Sephadex at neutral pH (22). In this report the relationship between queuine and certain pteridines is extended.

A bioassay for queuine has been developed (23) that utilizes the L-M strain of mouse fibroblasts in tissue culture. An activity designated as Q-factor exists in serum, amniotic fluid, and other sources that can be detected by this bioassay: in a serum-free medium the $tRNA^{Asp}$ of L-M cells is predominantly in the Q(-) forms, but a few hours after the addition of Q-factor, the Q(+) forms of $tRNA^{Asp}$ increase at the expense of the Q(-) forms. The amount of Q(+)$tRNA^{Asp}$ is proportional to the amount of Q-factor added to the culture at concentrations less than 0.02 A_{260} unit/50 ml of culture medium and Q(+)$tRNA^{Asp}$ ranges from 3 to 98% of the total depending on the amount of Q-factor added (22). Recently, the Q-factor from amniotic fluid was identified as queuine (24). It is presumed that queuine in the medium is transported into the L-M cells and becomes a substrate for guanine:tRNA transglycosylase, the enzyme that removes guanine from the anticodon of the Q-family of tRNAs and replaces it with either guanine or queuine (10, 12, 25).

In this study we have identified queuine in Drosophila melanogaster and have found that growth conditions which affect queuosine levels in tRNA, as well as mutations which affect pteridine metabolism, also affect the levels of free queuine. In addition we have found that the L-M cell assay for queuine is subject to inhibition by substances that are present in pteridine mutants. Furthermore, the guanine:tRNA transglycosylase of rabbit erythrocytes has been found to be inhibited by three pteridines that commonly accumulate in one of these mutants.

METHODS AND MATERIALS

Drosophila melanogaster mutants are described in Lindsley and Grell (26), except for pr^{c4} and pr^{m2b} which were isolated recently (Grell and

Jacobson, unpublished). Mutants were selected for their abnormal levels of various pteridines: purple (pr and pr^{bw}) are leaky mutants that produce a number of pteridines including drosopterins at ~ 20% of the normal level; sepia (se) produces no drosopterins but does produce elevated levels of several simpler pteridines (sepiapterin, biopterin and pterin); white (w) and brown (bw) are devoid of all pteridines (27, 28). Vermilion (v) and cinnabar (cn) are mutants that prevent the synthesis of brown eye pigments; they are used on occasion in combination with the pr mutants so that the red eye pigments (drosopterins) can be observed more directly.

The extraction of queuine from the adult fly followed the procedure described earlier for pteridine extraction with ammoniacal isopropanol (29). The extract was lyophilized to dryness and dissolved in water. In the standard assay, an amount equivalent to 0.2 g of flies (usually 3 ml) was added to culture medium to yield a total volume of 50 ml, which was then filter-sterilized and distributed between two tissue culture flasks (150 cm^2 growing surface each) of confluent or nearly confluent L-M cells; the cells were harvested after 24 hr. The isolation of L-M cell tRNA, aminoacylation with radioactive aspartic acid, and chromatography on an RPC-5 column were described earlier (23). The L-M cell assay yields the proportion of Q(+) to total $tRNA^{Asp}$; i.e., the sum of the radioactivity recovered in the $[Q+]tRNA^{Asp}$ peaks (1 and 3) divided by the sum of the radioactivity recovered in $tRNA^{Asp}$ (peaks 1, 2, 3, and 4). In some instances, peaks 1 and 2 were not resolved completely after RPC-5 chromatography and the proportion of $[Q+]tRNA^{Asp}$ was determined by the radioactivity in peak 3 divided by the sum of the radioactivity in peaks 3 and 4. Both methods of determining the proportion of $[Q+]tRNA^{Asp}$ yield comparable results (see ref. 23). Control values (no addition to the medium) of 0.02 ± 0.01 have been subtracted and the data are expressed as the proportion of $[Q+]tRNA^{Asp}$/0.2 gram of flies. In the standard L-M cell assay, 0.01 A_{260} units of pure queuine routinely yields Q(+)/total $tRNA^{Asp}$ values of 0.35 ± 0.05 (see Table III and ref. 22). Only queuine has been demonstrated to give this response (22-24 and J. R. Katze, unpublished observations), and queuine has been identified by mass spectroscopy after isolation from D. melanogaster strain Oregon-R (B. Basile, J. R. Katze, and J. A. McCloskey, unpublished observations); however, the possibility has not been eliminated that other agents may mimic queuine. The existence of substances in the extracts of D. melanogaster that inhibit or interfere with this bioassay must be

recognized when results from this type of assay are evaluated.

Queuine was obtained from bovine amniotic fluid as described previously (22, 24). Pterin was purchased from Sigma Chemical Company; biopterin was from Smith, Kline, and French; and sepiapterin was isolated from the sepia mutant. Each was chromatographically homogeneous by thin-layer chromatography (29). The millimolar extinction coefficients at pH 7 for sepiapterin (10.3 at 423 nm), biopterin (6.3 at 354 nm), and pterin (21.4 at 251 nm) were used to determine concentrations of these substances (30). [U-^{14}C]pterin had a specific activity of 59 mCi/mmol and [^{3}H]guanine of 7 Ci/mmol.

The guanine:tRNA transglycosylase was prepared from rabbit erythrocytes and fractionated by the published procedure through the step that employs Sephadex G-200 (31). The assay conditions were described previously (22).

Chromatography of Drosophila Tyr-tRNA and measurement of the relative abundance of peak 1 [Q(+)] and peak 2 [(Q(-)] were described earlier (32).

Sephadex chromatography. Young adult flies of the sepia strain (5 g) were extracted with 2:1 mixture of isopropanol-3.5% NH_4OH in 0.5% 2-mercaptoethanol (200 ml) and the 8000xg supernatant was rotary evaporated to remove the alcohol. An amount equivalent to 1.5 g of flies was chromatographed on a column of Sephadex-G-50 (1.5 x 115 cm) equilibrated with 0.1 M NH_4HCO_3. In a preliminary chromatogram fractions 10-12 (8 ml each) correspond to excluded molecules (Blue Dextran), 26-30 to magnesium acetate and 30-35 to retarded compounds such as biopterin. The Blue Dextran was observed visually, the magnesium acetate by its refractive index and biopterin by its blue fluorescence. The NH_4HCO_3 was displaced by 380 ml of water and then the column was eluted with 400 ml of 0.02 N HCl. The pools of these fractions were lyophilized to dryness three times to remove the NH_4HCO_3.

RESULTS

Presence of free queuine in Drosophila. An earlier study showed that D. melanogaster produced much more Q(+)tRNA when raised on a diet of dried brewers' yeast and that this increase in Q(+)tRNA was prevented when the diet was supplemented with glucose (33). To determine whether a correlation existed between the concentrations of Q(+)tRNAs and of queuine in the D. melanogaster we employed the L-M cell bioassay. The v; bw and bw strains were raised on diets of yeast or yeast plus glucose; their extracts

were prepared and administered to L-M cells. The results in Table I show that the Q(+) form of $tRNA^{Asp}$ of the L-M cells is markedly higher when the extract of flies fed the yeast diet without added glucose is compared to flies raised on yeast and glucose. Thus, there is a direct correlation between the level of Q(+)tRNA in D. melanogaster and the response of the L-M cells to extracts from the flies.

Certain pteridine mutants were also assayed to determine the correlation between the amount of $Q(+)tRNA^{Tyr}$ in the fly and the response of L-M cells to extracts. The flies were raised on standard medium and harvested daily. Some were frozen at -80° immediately, and others were transferred to fresh medium and maintained for 14 days. Aliquots of each age were analyzed for $tRNA^{Tyr}$ isoacceptors by RPC-5 chromatography and for apparent queuine by the L-M cell assay (Table II). In three mutants, pr, pr^{bw}, and pr; cn, the correlation was positive; higher levels of $Q(+)tRNA^{Tyr}$ were correlated with higher levels of queuine. However, pr; se showed an inverse relationship when 1- and 14-day-old flies were compared.

Table I. Variation in apparent queuine content of D. melanogaster raised on different diets

	L-M Cell $tRNA^{Asp}$ [Q(+)/total]	
Genotype	Yeast	Yeast + Glucose
Experiment 1		
v; bw	0.07	< 0.01
bw	0.07	0.04
Experiment 2		
v; bw	0.14	0.01
bw	0.08	0.01

The D. melanogaster mutant strains were raised on a diet of 4% dried brewers' yeast and 1.5% agar ± 5% glucose (33), collected at 1 day of age, and stored at -80°. Extracts equivalent to 0.2 g of adult flies caused changes in $tRNA^{Asp}$ of L-M cells that are represented by the ratio of the Q(+) forms to the total as described in Methods and Materials. The flies in experiments 1 and 2 were raised at different times on identical diets.

Table II. Measurement of apparent queuine levels and Q(+)tRNA^{Tyr} levels at different ages in various pteridine mutants of Drosophila

Genotype	Age (days)	L-M Cell tRNA^{Asp} [Q(+)/total]	Drosophila tRNA^{Tyr} [Q(+)/total]
pr	1	0.12	0.81
pr; cn	1	0.01	0.45
	14	0.10	0.95
pr^{bw}	1	0.01	0.34
	14	0.04	0.85
pr; se	1	0.04	0.22
	14	<0.01	0.48
se	1	<0.01	0.24
pr^{c4}; cn/pr^{m2b}; cn	1	<0.01	0.33
	14	0.01	0.28

Apparent queuine concentrations were determined as described in Table I; Drosophila tRNA was isolated, charged with [^{14}C]tyrosine, and chromatographed on RPC-5; then the ratio of Q(+) isoacceptor to total tRNA^{Tyr} was calculated.

Another exception is seen in se, which had no queuine yet was not abnormally low in the Q(+) forms of tRNA^{Tyr}. In the case of pr^{c4}; cn/pr^{m2b}; cn the queuine pool remained low and the Q(+)tRNA^{Tyr} failed to increase with age. A previous study on four strains of D. melanogaster (7) and the present study of the strains shown in Table II showed an increase in Q(+)tRNAs in older flies compared with young ones. Thus, the failure to find an increase in

either queuine or Q(+)tRNA^{Tyr} in the case of $\underline{pr^{c4}; cn/pr^{m2b}; cn}$ is noteworthy.

The data from Tables I and II and additional analyses are plotted in Fig. 1 for estimation of the correlation between the levels in $\underline{D.\ melanogaster}$ of queuine (L-M cell assay) and Q(+)tRNA^{Tyr}. The calculated linear regression is shown by the line; the correlation coefficient is 0.71. This relatively high correlation coefficient indicates that the levels of apparent queuine and Q(+)tRNA^{Tyr} are related, as would be expected if the queuine pool in $\underline{D.\ melanogaster}$ were a limiting factor for Q(+)tRNA formation by the transglycosylase. The absence of a complete correlation indicates that other factors are also important.

$\underline{\text{Inhibitory effect of pteridines}}$. The genotypes that contain $\underline{se}$ stand out as exceptions in Table II in that $\underline{se}$ itself caused no stimulation of Q(+)tRNA^{Asp} formation by the L-M cells and $\underline{pr; se}$ caused a moderate stimulation at 1 day of age but not at 14 days. Accordingly, these extracts were examined to determine if they interfered with the assay for authentic queuine (Table III). In experiment 1, the sepia extract inhibited by 59%. Several pteridines are known to be elevated in the $\underline{se}$ mutant, namely, sepiapterin, biopterin, and pterin at 21, 4, and 6 times,

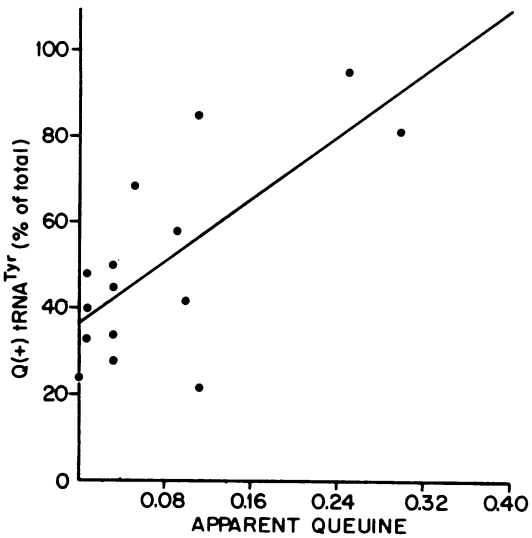

Fig. 1. Correlation between concentrations of apparent queuine and Q(+)tRNA^{Tyr} in $\underline{Drosophila}$. Apparent queuine concentrations were determined as described in Table I. The abscissa values are the proportion of L-M cell Q(+)tRNA^{Asp} to the total.

Table III. Inhibition of L-M cell assay for queuine by pteridines and Drosophila extracts

Addition	L-M Cell $tRNA^{Asp}$ [Q(+)/Total]	Inhibition (%)
Experiment 1		
None	0.01	-
Queuine	0.38	-
Sepia extract	0.01	-
Sepia extract + queuine	0.16	59
Experiment 2		
None	0.01	-
Pterin	0.02	-
Biopterin	0.01	-
Sepiapterin	0.01	-
Queuine	0.29	-
Queuine + pterin	0.27	7
Queuine + biopterin	0.23	21
Queuine + sepiapterin	0.33	0
Experiment 3		
None	0.03	-
Oregon-R extract	0.02	-
White extract	0.02	-
Brown extract	0.02	-
Sepia extract	0.02	-
Queuine	0.31	-
Queuine + Oregon-R extract	0.26	17
Queuine + white extract	0.30	3
Queuine + brown extract	0.19	41
Queuine + sepia extract	0.18	45

The extract from 0.2 g Drosophila (Oregon-R is wild type; sepia, white, and brown are mutants) was assayed with L-M cells as described in Table I. Where noted queuine was present at 0.01 A_{260} unit per 50 ml culture medium and pteridines (biopterin, sepiapterin, and pterin) were present at 10^{-6} M. The low value obtained for bw is consistent with the value obtained in Table I for flies raised on yeast + glucose; all flies in this table were raised on standard medium (yeast, glucose, sucrose, and corn meal).

respectively, the amounts in wild type (28). In experiment 2, these pteridines were added to the L-M cells at 1 x 10^{-6}M; biopterin and possibly pterin were inhibitory. In experiment 3, other strains are compared. The

white and brown mutants are completely deficient in the red eye pigments (drosopterins) and have no other detectable pteridines either (27). Again, the sepia extract inhibited the queuine assay but white did not. Interestingly, the brown extract was also strongly inhibitory and the wild type (Oregon-R) moderately so. These experiments demonstrate that certain mutants contain more inhibitory activity than others and that biopterin, and possibly pterin, are inhibitory.

Guanine: tRNA transglycosylase. Guanine:tRNA transglycosylase from rabbit erythrocytes was assayed by measuring the exchange between guanine in the anticodon of yeast tRNA and free guanine. Yeast tRNA has been shown to be completely Q(-) (34). Each of the pteridines that se contains in elevated amounts was shown to inhibit the enzyme to some degree: sepiapterin < biopterin < pterin (Fig. 2). The amounts to produce approximately 30% inhibition were 1×10^{-4} M, 9×10^{-5} M, and 7×10^{-8} M, respectively. The inhibition was studied further, and, as shown in Fig. 3, the inhibition was competitive with guanine with a K_i of 9×10^{-8} M.

Since pterin was a competitive inhibitor of the transglycosylase and had a K_i that was comparable to that of queuine, it was necessary to determine if pterin was incorporated into tRNA. Two tubes were incubated as described by Howes and Farkas (31) except that one tube contained [^{14}C]pterin (59 mCi/mmol) and the other contained [^{14}C]guanine diluted with unlabeled guanine to the same specific activity as that of pterin. The incubation mixtures were sterilized by filtration through a Millipore

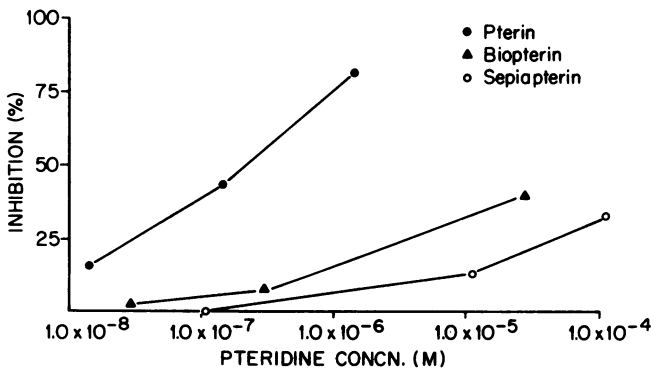

Fig. 2. Inhibition of guanine:tRNA transglycosylase by some pteridines. Inhibition of the enzyme was measured under standard assay conditions except that sepiapterin, biopterin, or pterin was present at the concentrations indicated.

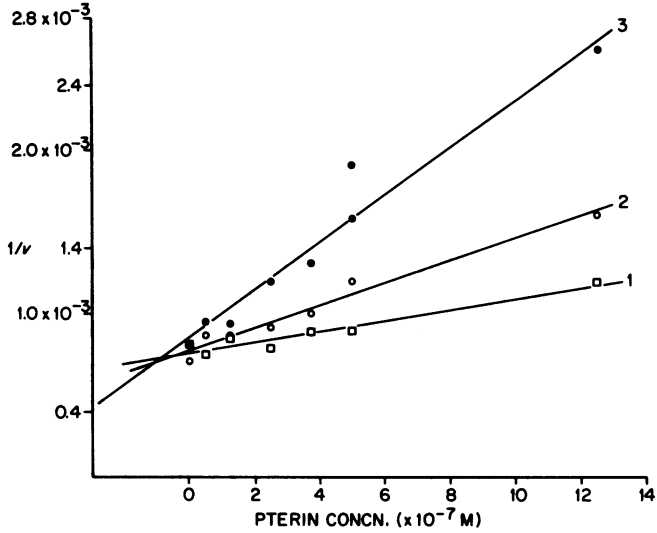

Fig. 3. Inhibition of guanine:tRNA transglycosylase by pterin. Dixon plot. The points shown were obtained by use of the standard assay at guanine concentration of 1.67, 1.12, and 0.56 μM for 1, 2, and 3, respectively. The lines are calculated by the method of least squares.

filter (0.45 μm) into sterile tubes, which then were incubated for 8 hr at 37°. At 0 and 8 hr, aliquots were removed, precipitated with HCl, and prepared for counting (31). The experiment, which was run in duplicate, showed that no pterin was incorporated into tRNA, whereas 7.6 pmol of [^{14}C]guanine were incorporated. The lower limit of [^{14}C]pterin that would have been detected in this experiment was 0.5 pmol.

An indication that certain pteridines in the extract of se are responsible for the inhibitory effect on the L-M cells was obtained. Pteridines such as sepiapterin, biopterin and pterin behave abnormally when chromatographed on Sephadex in that they are adsorbed and eluted after the included small molecules and ions. When an extract of se was chromatographed on Sephadex G-50 only the retarded fraction (30-48) exhibited inhibition of the appearance of Q(+)tRNA^{Asp} caused by queuine in the L-M cells (Table IV). Similarly when regions of the Sephadex chromatograh were added to the transglycosylase assay the sole inhibitory fraction was the retarded one (Table IV). This is an indication that it is indeed the pteridines in the extract of sepia that caused the inhibition of

Table IV. Inhibitory properties of fractions obtained from Sephadex chromatography of sepia extract

Fraction Added[1]	L-M Cell Assay[2]	Transglycosylase Assay[3]
	Inhibition (%)	(Inhibition Units)
None	0	0
Excluded (9–15)	3	33
Included (16–29)	10	152
Adsorbed (30–48)	38	8979
Water Wash (49–96)	0	80
HCl (97–147)	0	0

1. The fractions (8 ml) were pooled as indicated by the fraction numbers in parenthesis so as to include all eluted material.

2. Inhibition of the L-M cell assay was determined as in Table III and represents the mean of two determinations. The amount of each Sephadex fraction added to the L-M cell assay was equivalent to 0.2 g flies and the amount of queuine was 0.01 A_{260} unit in all cases.

3. The uninhibited enzyme catalyzed the incorporation of 2634 cpm of [^{3}H]guanine into yeast tRNA. One unit of inhibitor is defined as that amount which caused a decrease in incorporation of 100 cpm. The inhibition was linear between 30% and 60% inhibition with amount of inhibitor added to the reaction. The total number of inhibitory units in each pool is shown.

the L-M cell assay and that the mechanism of this inhibition is due to their effect on the transglycosylase.

DISCUSSION

Queuine, previously identified only in bovine amniotic fluid (22-24) is present in adult Drosophila. The amount appears to vary with age, nutrition and genotype. The data in Fig. 1 indicate an apparent range between 0 and 6 μmoles/kg for the queuine content of adult Drosophila, assuming a mM extinction coefficient for queuine of 7.5 at 260 nm (P. F. Crane, J. R. Katze and J. A. McCloskey, unpublished). These values for queuine may be correct for some genotypes but for others are no more than a minimum. A positive correlation between the apparent queuine measurements

and the Q(+)tRNA^{Tyr} was obtained in 15 experiments but since the correlation coefficient was less than 1.0 the influence of other factors must be considered. One factor is the activity of the enzyme that inserts queuine into the anticodon of tRNA^{Tyr}, guanine:tRNA transglycosylase; the variation of the activity of the enzyme during metamorphosis indicates one or more regulatory mechanisms for its control (37, 38).

The present data suggest that another factor is the level of certain pteridines in the organism. This evidence is of interest since the involvement of pteridines in queuine metabolism and utilization has not been observed heretofore. Since both pteridines and queuine are derived from guanine (18-21), and folic acid is required for purine biosynthesis, a mechanism that regulates the intracellular levels of these compounds may exist.

Pteridine biosynthesis is most active in the last day of pupal and the first day of adult life as judged by the developmental profiles of GTP cyclohydrolase (37, 38) and sepiapterin synthase (39, 40). The rate of appearance of Q(+)tRNAs also increases in the late larval period and continues for several days after the adult emerges (8, 9). Among mutants that alter the rate of pteridine biosynthesis it is of note that the level of GTP cyclohydrolase in the young adult fly is lowered in $\underline{pn}^2$ and $\underline{ras}^2$ mutants (38) and that the sepiapterin synthase is 20% of normal in $\underline{pr}$ and $\underline{pr}^{bw}$ mutants; the $\underline{pr}$ locus appears to contain the structural gene for sepiapterin synthase (20). In $\underline{pr}$ no pteridines are increased but several, including sepipterin, are decreased to low concentrations. Strain-dependent differences in the rate of Q(+)tRNA appearance are known; the appearance of Q(+)tRNA is rapid in Samarkand but only 1/3 as fast in Oregon-R (7). These processes may all be examined to establish possible correlations between changes in pteridine and queuine metabolism.

When the L-M cells are used to quantitatively measure queuine concentrations, it is assumed that external queuine is transported into the cytoplasm and then incorporated into tRNA by guanine:tRNA transglycosylase. Any substance that interfered with the transport of queuine or the level of activity of the transglycosylase would compromise this bioassay. In $\underline{v; bw}$, $\underline{pr}$, $\underline{pr}^{bw}$, and $\underline{pr; cn}$, and possibly $\underline{bw}$, there is a positive correlation in the flies between the amount of apparent queuine and the amount of Q(+)tRNA^{Tyr}. This may indicate that the L-M cell assay gives an accurate measure of the queuine concentration in these extracts. In the case of $\underline{pr}^{c4}\underline{; cn}/\underline{pr}^{m2b}\underline{; cn}$ the correlation still is positive in that

neither component changed with age; in all other strains and mutants examined, an age-dependent increase in the proportion of Q(+)tRNA^{Tyr} has been observed (Table II and ref. 7), so the absence of change in this mutant is significant and may indicate that another parameter is important. Indeed, in the cases of se and pr; se it is likely that other parameters do exist; the bioassay fails to detect queuine in se, but the level of Q(+)tRNA^{Tyr} is not abnormally low; in pr; se the bioassay fails to detect queuine at 14 days, a time when Q(+)tRNA^{Tyr} has increased (Table II). Since sepiapterin, biopterin, and pterin are all increased in se, these pteridines were tested as inhibitors of the bioassay; at 10^{-6} M, biopterin and, to a lesser extent, pterin did inhibit (Table III). The inhibition of the L-M cell assay by the extracts of se may be caused by these pteridines since the Sephadex chromatogram contained inhibitory material only in the position that pteridines occupy. The inhibition by the brown extract is indicative that another substance(s) must also be considered, since this mutant contains none of these pteridines (27). The inhibitor(s) may actually increase with age according to the results from pr; se.

The enzyme guanine:tRNA transglycosylase catalyzes the exchange of guanine in the wobble position of the anticodon of the Q-family of tRNAs with free guanine. The enzyme's true function is to replace the wobble guanine with queuine (12, 25, 41). Inhibition of the rabbit guanine:tRNA

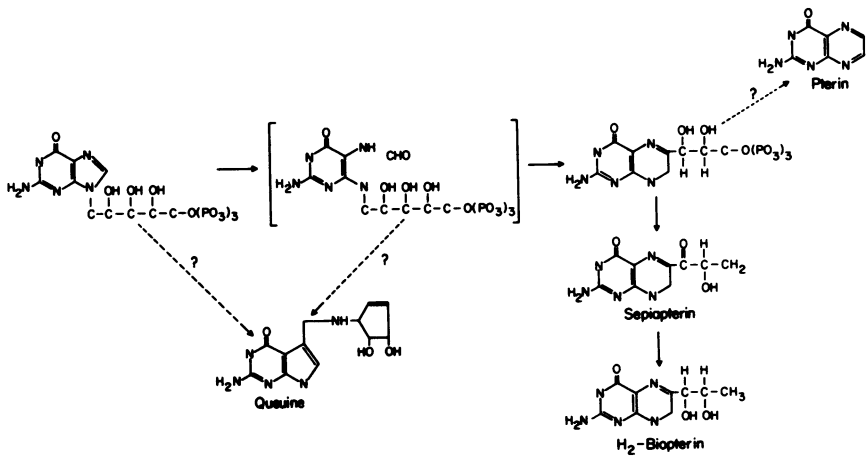

Fig. 4. Metabolic conversion of GTP to pteridines and queuine. The solid arrows represent reactions that have been demonstrated in Drosophila; reactions shown by dotted arrows have not been demonstrated.

transglycosylase by pterin > biopterin > sepiapterin indicates that these pteridines may be a factor in the inhibition of the L-M cell bioassay. The inhibition of the rabbit transglycosylase by pterin is competitive with guanine (K_i = 9 x 10^{-8} M); the structures of these two ligands are closely related. The binding constants of this enzyme for guanine and queuine are 1.5 x 10^{-7} M and 4.5 x 10^{-8} M respectively (22, 31). The interrelationship of pteridines and queuine warrants further examination.

In the pathway for pteridine biosynthesis in Drosophila (Fig. 4) the lesion for pr occurs early in the pathway (20). The biochemical defect for se is not known, only that the drosopterin eye pigments are not accumulated and that certain simpler pteridines (pterin, biopterin, and sepiapterin) accumulate (28). If queuine is a product of GTP metabolism, the various pteridine mutants might act by "sparing" GTP and making more available for queuine synthesis. On the other hand, if queuine is derived from some pteridine, its level may be decreased by the same lesion that causes decreased levels of certain pteridines. The pr^{c4}; cn/pr^{m2b}; cn mutant is a more extreme form of the purple mutation than either pr or pr^{bw}, and it is of interest that both the queuine pool and the Q(+)tRNA pool remain low.

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1. Queuine is 7-[(cis-4,5-dihydroxy-2-cyclopenten-1-yl)-amino]-methyl-7-deazaguanine. Q(+) and Q(-) refer to the tRNA isoacceptors that do and do not contain queuine, respectively, in the wobble position.
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