CHANGES IN LEUCYL-tRNA DURING SPONTANEOUS AND INDUCED METAMORPHOSIS OF BULLFROG TADPOLES[1]

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Triiodothyronine (T_3) altered the chromatographic profiles of ^{3}H- and ^{14}C- leucyl-tRNA charged *in vivo* in the liver, kidney, tail, and gills of bullfrog tadpoles. Though the chromatographic profiles differed from tissue to tissue, two main leucyl-tRNA components were observed for each tissue. Twenty-four hours after T_3 injection the proportion of the rapidly eluted tRNA was reduced and the more slowly eluted tRNA component was increased. A similar shift in leucyl-tRNA was observed in liver and kidney slices from spontaneously metamorphosing tadpoles.

Recent studies of developing or differentiating organisms ranging from bacteria to mammals (4-6, 8, 9) have indicated that alterations in certain tRNAs may play an important role in regulating protein synthesis at the translational level. In the adaptor-modification and codon-restriction hypotheses (14, 15), it has been proposed that some effectors can induce specific changes in protein synthesis through changes in tRNA. The recognition of the fine structure of tRNA codewords has been shown to be general for a broad spectrum of organisms extending from bacteria to amphibia and mammalia (10).

Since thyroid hormones can initiate the drastic cell differentiation associated with anuran metamorphosis (3), we have studied the leucyl-tRNA profiles in T_3 treated and spontaneously metamorphosing bullfrog tadpoles. The alteration of co-chromatographic profiles

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Fig. 1

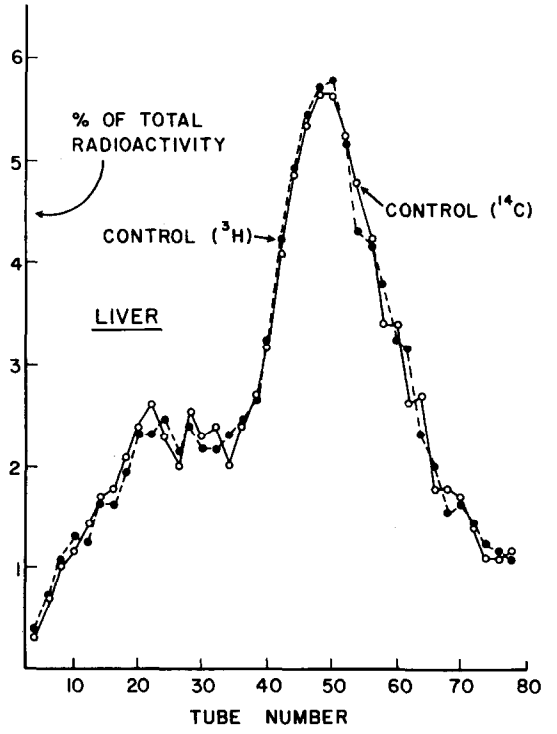
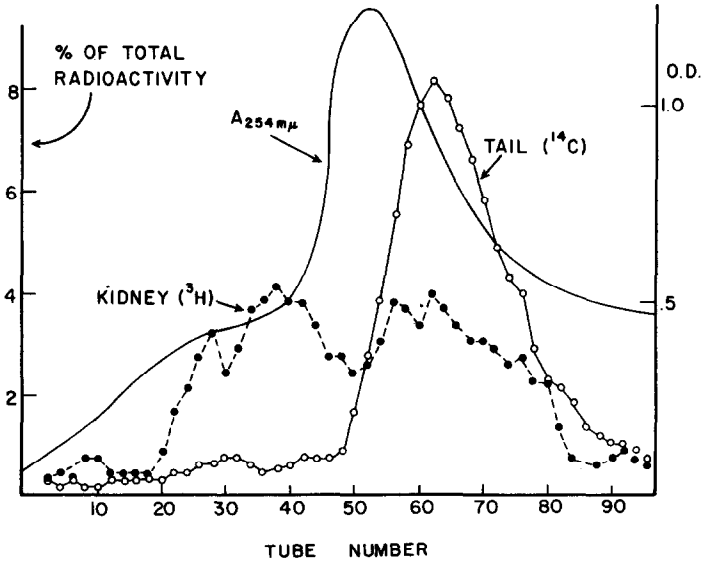


Fig. 2



Figs. 1 and 2.

Methylated silicic acid column chromatographic profiles of leucyl-tRNA from control tadpoles, stage IX. Fig. 1, liver leucyl-tRNA, labeled with ^{3}H- and ^{14}C- leucine. Fig. 2, kidney ^{3}H-leucyl-tRNA and tail ^{14}C-leucyl-tRNA (^{14}C). See text for experimental details. Each point represents the average of two 2.0 ml fractions from that column. The A_{254} value is also included to confirm the location of the nucleic acid fraction.

of leucyl-tRNA has been first examined *in vivo* because any alteration *in vivo* is more likely to have real physiological significance and the artifactual results of *in vitro* charging can be avoided (19). Leucyl-tRNA was chosen because of its extensive degeneracy in codons (12) and the effect of T_3 on the leucine incorporation into the proteins of various tissues in the tadpole has been investigated (17).

Materials and Methods

Rana catesbeiana tadpoles, stage IX to XI were used at 23-25°. T_3 was injected at a dose of 0.30 nanomoles/g body weight 24 hours before the injection of labeled leucine. To a group of 4 to 5 animals L-leucine-4, 5-^{3}H (100µc/10 g of tadpole weight) or L-leucine ^{14}C (U) (15 µc/10 g) (New England Nuclear) were injected. After 20 min. the organs were excised and frozen on dry ice. The tissues from 4-6 control animals (^{14}C labeled) and 4-6 T_3-treated animals (^{3}H labeled) were pooled and the nucleic acids were extracted by the method of Kruh *et al.* (1964). Extracts were co-chromatographed using 0.050 M phosphate buffer at pH 5.9 using a slight modification of the method of Axel *et al.* (1967). Methylated albumin silicic acid (13) was used instead of methylated albumin kieselguhr. The RNA was eluted with 200 ml of a linear gradient of 0.20 M - 1.2 M NaCl at a flow rate of 2 ml/min. Aliquots (2.0 ml) were mixed with 15 ml of a water-miscible scintillator mixture (1.0 ml methanol, 14 ml dioxane, 6% napthalene and 0.8% PPO) and counted in a Beckman scintillation counter. The results have been corrected for background, converted into DPM and the percent of total radioactivity recovered was determined by a computer program (2). The range of DPM recovered in tRNA was 20,000 to 40,000 for ^{3}H and 5,000 to 10,000 for ^{14}C.

Results

Fig. 1 illustrates the close similarity of the profiles of ^{3}H- and ^{14}C-labeled leucyl-tRNA from the livers of control animals. Superimposable profiles were also obtained in other tadpole tissues studied *in vivo* or when kidney or liver slices were used. Any significant differences in profiles are believed to reflect the effect of T_3 treatment. Profile differences might be larger at later times, although the 24 hour period after T_3 injection was sufficient to produce significant differences in leucine uptake into protein (17). That the radioactivity was only due to leucyl-tRNA was checked by the incubation of eluates at pH 10.0 at 35° for 30 min. to

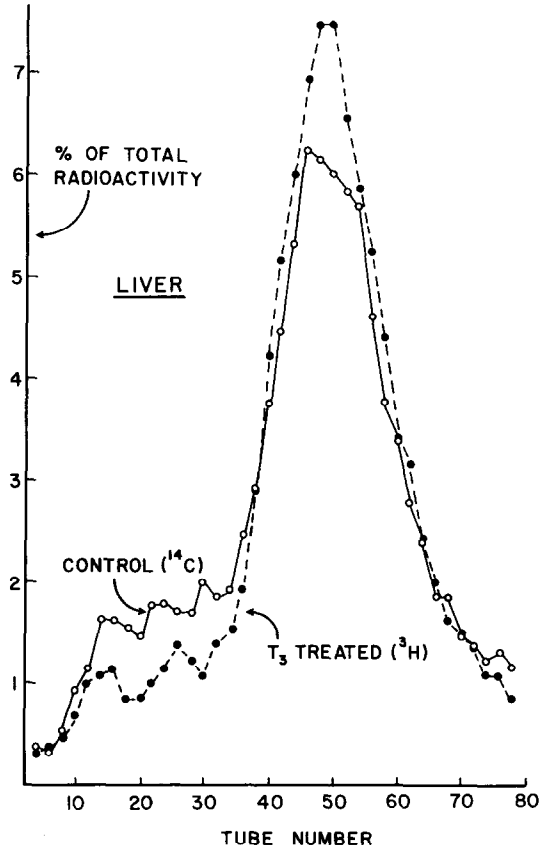

% OF TOTAL
RADIOACTIVITY
LIVER
CONTROL (^{14}C)
T_3 TREATED (^{3}H)
TUBE NUMBER

Fig. 3

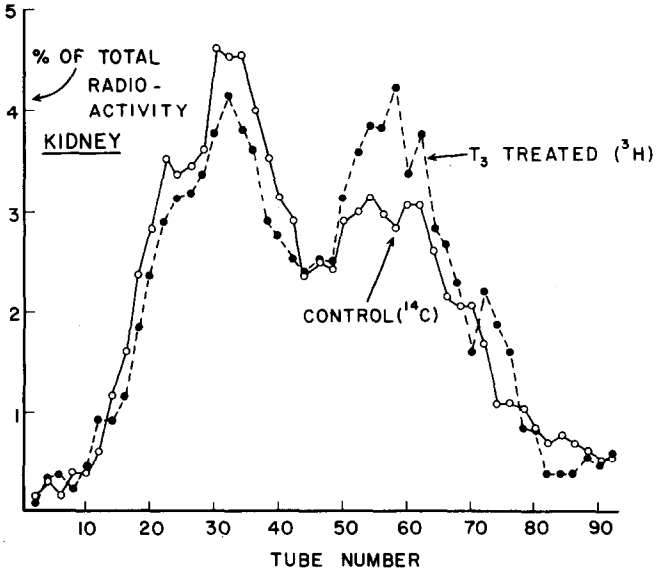
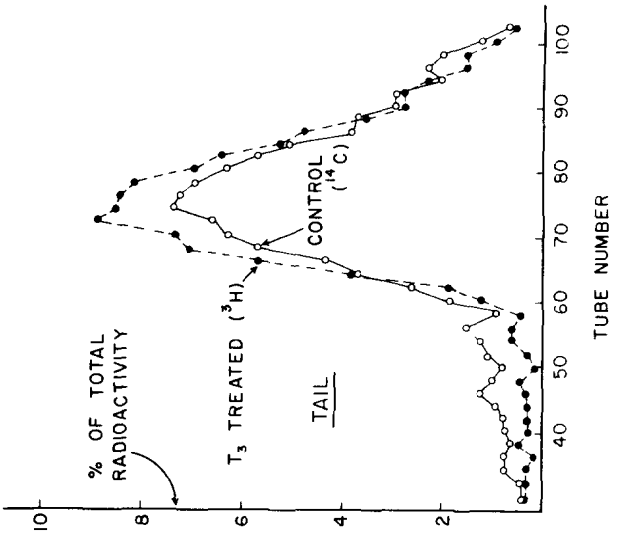

% OF TOTAL
RADIO-
ACTIVITY
KIDNEY
T_3 TREATED (^{3}H)
CONTROL(^{14}C)
TUBE NUMBER

Fig. 4

Fig. 6



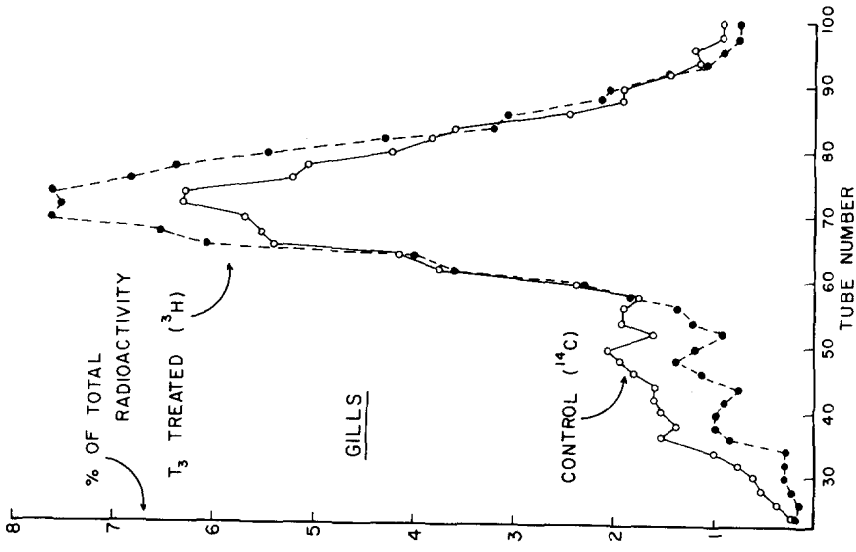


Fig. 5

Figs. 3 to 6.

A comparison of the chromatographic profiles of labeled leucyl-tRNA from liver, kidney, gills and tail of control and T_3 injected tadpoles. Experimental details are defined in the text and in the legend to Fig. 1.

Fig. 7

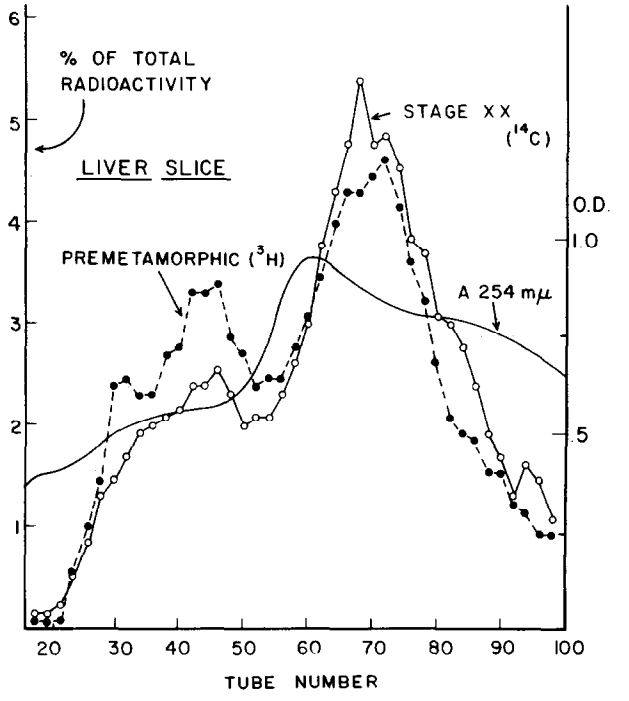
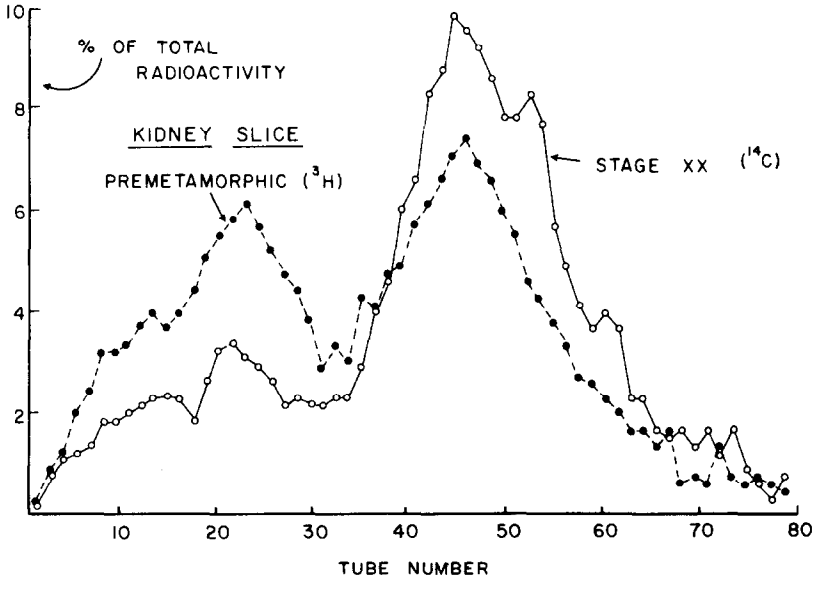


Fig. 8



Figs. 7 and 8.

The chromatographic profiles of leucyl-tRNA from liver and kidney slices incubated with ^{3}H-leucine (premetamorphic tadpoles) and ^{14}C-leucine (metamorphic tadpoles). Incubation mixture; frog Ringer's buffered with $NaHCO_3$ (0.025 M), pH 7.5, Gas phase CO_2, O_2 (5:95), at 25°. Other details are presented in legend to Fig. 2.

strip the charged leucine (18) followed by cold TCA precipitation. The washed TCA precipitate showed no radioactivity.

Fig. 2 shows representative profiles of co-chromatography of leucyl-tRNA from kidney and tail obtained from control animals. When compared with the leucyl-tRNA profiles for the liver and gills, some important differences become clear. Kidney had similar amounts of two major peaks representing two main components appearing around tubes no. 30 ± 5 (Component I) and tubes no. 60 ± 5 (Component II) (Figs. 2, 4, 8). In liver, component II is even more prominent (Figs. 1, 3, 7). Component I becomes considerably less noticeable in gill tissue (Fig. 5) and is barely detectable in the tail (Figs. 2, 6).

The effect of T_3 injection on the profiles of leucyl-tRNA extracted from tadpole liver, kidney, gills and tail are illustrated in Figs. 3-6. The observed differences varied in size, but are consistent for every pair of tubes representing that particular tRNA fraction. In all the tissues the relative proportion of component I was decreased and that of component II increased by T_3 treatment. To determine if the T_3 induced alteration in profiles is also found during spontaneous metamorphosis, the leucyl-tRNAs of liver and kidney from premetamorphic (stage IX) and metamorphic climax (stage XX) tadpoles were compared. In these experiments slices of each tissue were incubated with ^{3}H- or ^{14}C- leucine *in vitro* and the nucleic acids were extracted. A similar alteration was observed in liver and kidney slices from normal metamorphosing animals (Figs. 7, 8), with an even greater shift from component I to II in the kidney.

Discussion

Though in the tadpole there are no available studies on components of synonymous tRNA and corresponding codons, it is generally accepted that the components of leucyl-tRNA are related to codon degeneracy (12, 16). The alteration of profiles of leucyl-tRNA in the tadpole strongly suggests that T_3 has an effect on leucine incorporation into proteins through a codon-related mechanism. Since the tRNA was charged *in vivo*, we cannot identify the particular reaction affected by T_3. Profiles may be altered by change(s) of either tRNA, component-specific synthetases (14, 20) of the binding of amino acylated tRNA to polysomes. However, the present findings of a proportional change in components of leucyl-tRNA produced *in vivo*, suggests further studies on the detailed mechanism with some hope of their physiological relevance.

The multiplicity of the effects of T_3 on leucine incorporation into the proteins of various tissues of the tadpole has been demonstrated (17). While T_3 stimulated the incorporation of leucine into liver protein, it reduced leucine incorporation in the kidney, gills, and tail. However in these four tissues, T_3 had a uniform effect on profiles of leucyl-tRNA, i.e. the proportional increase in component II and a decrease in component I. This suggests the possibility that T_3 may affect specific codon(s) of leucine uniformly in these tissues. This, in turn, may result in multiple effects depending on the combinations of the codons which are active in these different tissues (14).

The differences in the profiles in different tadpole tissues reported here contrast with the absence of major differences in tRNA profiles in non-differentiating tissues (11). Major differences in tRNAs have been reported only when tumor cells were compared to normal cells (1, 11).

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EVIDENCE THAT 3-PHOSPHOSERINE MAY BE A PRECURSOR OF VITAMIN B_6 IN ESCHERICHIA COLI

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Summary: The steps in the biosynthesis of vitamin B_6 are unknown. 3-phosphoserine is shown here to be the likely primary precursor since, during serine starvation, a mutant lacking 3-phosphoserine: oxoglutarate transaminase is unable to synthesize vitamin B_6 whereas a mutant lacking 3-phosphoserine phosphatase synthesizes vitamin B_6 at two times the normal rate.

The sequence of biosynthetic reactions which lead to the formation of vitamin B_6 have not been described, but some years ago Morris (1) reported a puzzling and still unexplained relationship existed between serine and certain pyridoxine mutants of E. coli. We have been reinvestigating this problem to see if its resolution might give a clue to the compounds involved in vitamin B_6 biosynthesis. During this study we found the interesting data reported below, which show serine is not a precursor of pyridoxine but that 3-phosphoserine may be.

MATERIALS AND METHODS

Organisms and conditions of growth. WG1100, WG1143, and WG1145 were derived from WG1, a wild type strain of Escherichia coli B after N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis and growth at 45°C in a manner similar to that described for pyridoxine mutants (Dempsey, J. Bacteriol., in press). Based upon frequency

of occurrence of these mutant phenotypes, and upon their rates of reversion to prototrophy, each of these mutants is a result of a single different mutation (Dempsey, submitted for publication). For the experiments reported here, cells from 50 ml overnight, aerated, 39°C cultures of the mutants in glucose minimal medium (2) plus 500 mg/liter L-serine and 0.1 mg/liter pyridoxine were washed in saline and used to inoculate 1 liter of fresh identical medium at 39°C. The cultures were grown with vigorous aeration to a density of 0.7 mg dry weight/ml. The cells were harvested by centrifugation at 35°C for 15 minutes at 4000 x g, washed once with saline, returned to fresh medium lacking only serine and pyridoxine and aerated vigorously for 3 hours at 39°C, during which time samples were withdrawn every 30 minutes for analysis. The cells were then harvested, washed with saline, and frozen at -70°C.

Cell mass and pyridoxine content of culture. Cell mass was measured as previously described (2). For pyridoxine analysis two 5 ml samples were removed from each culture every thirty minutes, mixed immediately with 5 ml 0.11 N H_2SO_4 and refrigerated until all samples were taken. The total pyridoxine content was then determined by bioassay as described previously (2).

Assays. Enzyme assays for 3-phosphoserine:oxoglutarate transaminase and 3-phosphoserine phosphatase were those described by Pizer (3). The assay for 3-phosphoglycerate dehydrogenase was that of Umbarger, et al. (4). For enzyme measurements the cells were thawed, disrupted by sonic oscillation, and centrifuged 30 minutes at 40,000 x g. The supernatant fluid was carried through the streptomycin and ammonium sulfate steps described by Pizer (3) before assay.

RESULTS

The data in Table I show that each of the three mutants lacks

TABLE I

Activities of Serine Biosynthetic Enzymes in Three Escherichia Coli Mutants

Strain No.	growth requirements	3-phospho-glycerate dehydrogenase	3-phosphoserine oxoglutarate transaminase	3-phosphoserine phosphatase
		μ moles/min/mg protein		
WG1100	Serine	.024	0.40	.0003
WG1143	Serine	<.002	0.37	.03
WG1145	Serine and Pyridoxine	.024	<.004	.03

a different enzyme of serine biosynthesis. WG1145, a member of a group of mutants which show an absolute requirement for both serine and pyridoxine, lacks 3-phosphoserine:oxoglutarate transaminase. WG1100 and WG1143 lack 3-phosphoserine phosphatase and 3-phosphoglycerate dehydrogenase respectively.

Figure I shows that during starvation for their required nutrients, each of three mutants synthesizes vitamin B_6 at a different rate. WG1143 synthesizes vitamin B_6 at the rate usually seen

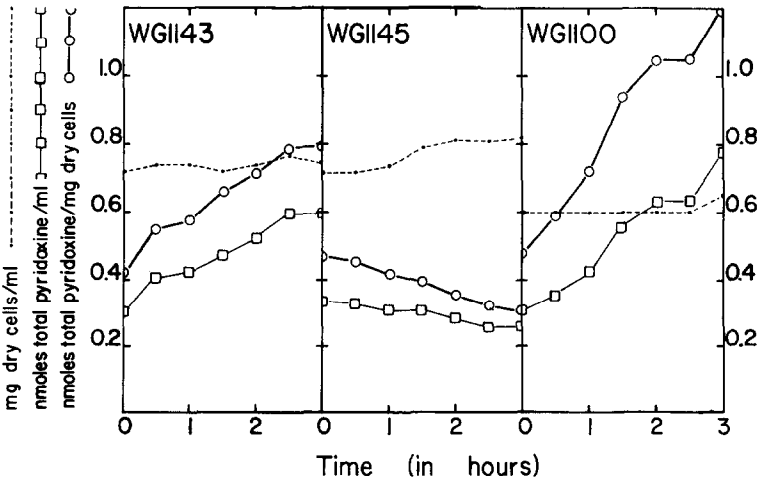

Figure I. Cell mass and vitamin B_6 changes during serine starvation of serineless mutants.

in amino acid starvations, namely 1.3 X 10^{-10} moles/mg/hr. (2). WG1145 appears to totally stop vitamin B_6 synthesis. WG1100, on the other hand, synthesizes vitamin B_6 at twice the normal rate during serine starvation. (2.4 X 10^{-10} moles/mg/hr.)

DISCUSSION

The findings are consistent with the metabolic scheme shown in Figure II. It would appear that 3-phosphoserine is an immediate precursor of vitamin B_6 because (i) mutants lacking 3-phosphoserine: oxoglutarate transaminase cannot synthesize pyridoxine, instead they require pyridoxine as well as serine for growth, (ii) mutants able to make phosphoserine but not serine do not require pyridoxine; they instead synthesize it at twice the normal rate. The increased rate of pyridoxine biosynthesis during serine starvation of the mutant lacking 3-phosphoserine phosphatase is tentatively presumed to result from the larger pool of 3-phosphoserine which would be expected during serine starvation of this type of mutant.

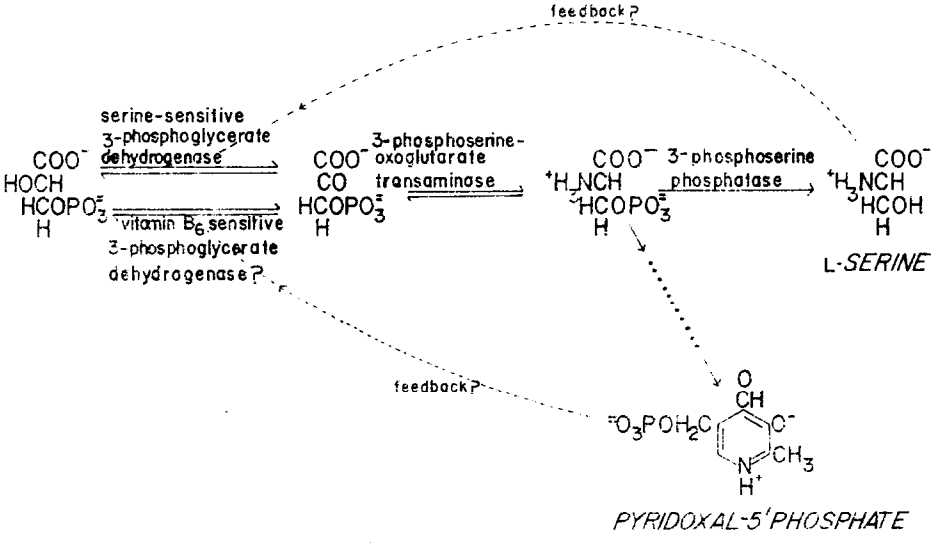

Figure II. Proposed metabolic scheme interrelating vitamin B_6 and serine biosyntheses.

The normal rate of pyridoxine synthesis seen during serine starvation of the mutant lacking 3-phosphoglycerate dehydrogenase may be explained by postulating the existence of a second 3-phosphoglycerate dehydrogenase. In keeping with the precedent set for this kind of arrangement by the existence of several different aspartokinases, one might expect, in turn, such an isozyme has its activity regulated by vitamin B_6. Both of these speculations are shown in Fig. II.

If the scheme proposed in Figure II is true it can also account for the failure, to date, for any pyridoxine mutants of E. coli to accumulate detectable amounts of pyridoxine precursors. There are two obvious reasons for this, firstly, 3-phosphoserine is phosphorylated and thus it and any phosphorylated derivatives of it would not readily pass the cell membrane; secondly, the reaction which generates phosphoserine is catalyzed by a transaminase. Transaminases usually use pyridoxal-5'-phosphate as a coenzyme and if this transaminase is not exceptional then the unusual situation is had of a coenzyme being required for its own synthesis. Experiments are in progress to verify the existence of such a unique control system.

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