Effect of Thyroxine Treatment on the Transfer of Amino Acids from Aminoacyl Transfer Ribonucleic Acid into Protein by Cell-Free Extracts from Tadpole Liver*

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ABSTRACT: Characteristics of the system catalyzing the transfer of amino acids from aminoacyl transfer ribonucleic acid to polypeptides have been studied using cell-free extracts from tadpole liver. Optimal conditions for the transfer reaction have been found to be pH 7.6 and 25°, with an optimal magnesium concentration of about 60 mM. Microsomes and ribosomes exhibit a significant level of endogenous incorporation in the absence of added supernatant. The requirement for guanosine 5′-triphosphate is not absolute and there is no requirement for monovalent cations or a sulfhydryl compound. Sparsomycin, a specific inhibitor of the transfer reaction, gives a 90% inhibition at a concentration of 2.6×10^{-4} M. Microsomes from liver of thyroxine-treated tadpoles showed a level of incorporation about 114% greater than microsomes prepared from untreated animals.

The stimulation of the transfer reaction was greater in microsomal preparations than in ribosomal preparations from thyroxine-treated animals. Tadpole liver ribosomal preparations revealed the presence of a factor (probably ribonuclease) capable of breaking down rat liver polysomal aggregates when the two preparations were mixed.

Accelerated metamorphosis of tadpoles by thyroxine is accompanied by a number of biochemical changes in the liver, *viz.*, induction of the enzymes of the ornithine–urea cycle with concomitant *de novo* net synthesis of carbamyl phosphate synthetase (Metzenberg *et al.*, 1961; Tatibana and Cohen, 1964, 1965) and increased rate of synthesis of RNA (Nakagawa and Cohen, 1967; Nakagawa *et al.*, 1967). Current studies in this laboratory have been focused on elucidating the mechanism of action of thyroxine during thyroxine-induced tadpole metamorphosis. Thyroxine has been shown to exert an effect at the level of transcription (Kim and Cohen, 1966) as seen in an increase in the efficiency of chromatin, isolated from liver of thyroxine-treated tadpoles. The present studies were undertaken in order to determine whether there was any influence at the level of translation. Since the amino acid activation step in protein synthesis had been previously shown not to be affected by thyroxine treatment (Degroot and Cohen, 1962), we examined the transfer step by measuring the rate of transfer of [^{14}C]aminoacyl-tRNA to polypeptide on microsomes and ribosomes isolated from thyroxine-treated tadpoles. Some preliminary aspects of this study have been reported (Unsworth and Cohen, 1967).

Materials and Methods

Animals. Tadpoles, *Rana catesbeiana*, weighing between 7 and 10 g, were purchased from Lemberger Co., Oshkosh, Wis. Tadpoles were maintained in 15° water and fed chopped spinach once a week. Thyroxine treatment was carried out at 25° for various periods of time, as described by Paik and Cohen (1960).

Biochemicals. Stripped *Escherichia coli* B tRNA (General Biochemicals) was charged with reconstituted [^{14}C]protein hydrolysate (Schwarz BioResearch Inc.), essentially as described by Nathans and Lipmann (1961). The final product contained about 100,000 cpm/mg of tRNA, and was resistant to acid hydrolysis. However, 96% of the total radioactivity was released from the tRNA after alkaline hydrolysis, indicating that the radioactive amino acids are not free but present in the form of aminoacyl-tRNA complexes. Sparsomycin was a gift from Dr. C. G. Smith, Upjohn Co., Kalamazoo, Mich. All the nucleotides, phosphoenolpyruvate, and RNase were purchased from Sigma Chemical Co. Uniformyl labeled L-[^{14}C]phenylalanine tRNA (*E. coli* B) was purchased from New England Nuclear (5.7 mg/μCi). Poly U was obtained from Miles Chemical Co. and pyruvate kinase from Calbiochem. Sodium deoxycholate was obtained from Fisher and Co.

Preparation of Microsomes and Ribosomes. The livers were removed and washed with 0.15 M KCl solution. The pooled livers were then homogenized in three volumes of 0.25 M sucrose solution in standard buffer A (50 mM Tris (pH 7.4), 5 mM $MgCl_2$, and 25 mM KCl) using six strokes of an all-glass hand homogenizer. The homogenates were centrifuged at 6000*g* for 15 min to provide a postmitochondrial supernatant.

MICROSOMES. Suitable volumes of postmitochondrial

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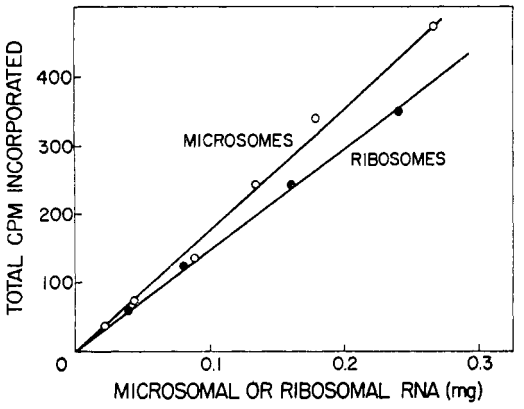

FIGURE 1: Effect of increasing amounts of tadpole liver microsomes or ribosomes on labeling of TCA-insoluble protein. Conditions were as described under Methods, except that the amount of microsomes or ribosomes per tube was varied. Tubes containing microsomes were incubated at 25° for 30 min. Tubes containing ribosomes were incubated at 25° for 1 hr.

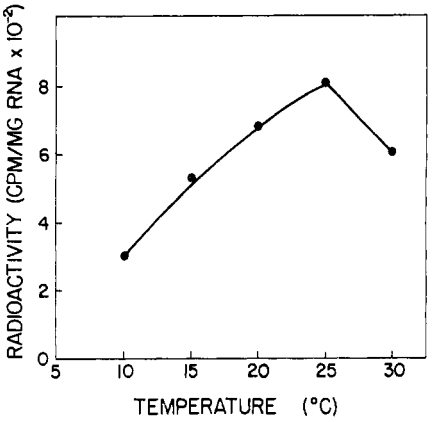

FIGURE 3: Effect of temperatue on transfer of amino acids from [^{14}C]aminoacyl-tRNA into TCA-insoluble protein of tadpole liver microsomes. Incubation was carried out for 30 min under condtions described in Methods, except that the temperature was varied as shown.

supernatant were centrifuged at 130,000g for 2 hr. The pellet was rinsed several times with standard buffer A, gently homogenized in this buffer, and centrifuged at 5000g for 10 min to remove insoluble material. The light brown microsomal suspension was then utilized in aminoacyl-tRNA transfer experiments.

RIBOSOMES. Preparation of ribosomes by direct extraction from microsomes with deoxycholate (as described by Korner, 1961) resulted in an 80% loss in OD_{260}-absorbing material. This was probably due to the action of endogenous ribonuclease, since good yields of ribosomes were obtained by addition of deoxycholate to the postmitochondrial supernatant. The presence of a ribonuclease inhibitor in rat liver supernatant has been described by several workers (Blobel and Potter, 1966; Lawford *et al.*, 1966), and a similar inhibitor may well be present to some extent in tadpole liver supernatant. Ribosomes were prepared by the addition of 0.5 ml of 10% (w/v) deoxycholate to 4.5 ml of postmitochondrial supernatant. This was then layered over 5 ml of 0.5 M sucrose solution in standard buffer A, and the ribosomes sedimented by centrifugation for 2 hr at 130,000g. The pellets, which were stored at −20°, retained their activity for several weeks.

Ribosomal Profiles. Ribosomal pellets were taken up in a suitable volume of standard buffer. Portions of 25 μl (having an OD_{260} of about 10) were layered over 15–30% (w/v) sucrose gradients (4.6-ml volume) which had been cooled to 0°. The gradient solutions contained standard buffer salts. The gradients were centrifuged for 1 hr at 37,000 rpm in a Spinco SW 39 rotor and allowed to stop without braking. The bottom of the tube was punctured and the optical density of the effluent was continuously monitored at 260 mμ by passage through a flow-through cell in a Gilford Model 2000 recording spectrophotometer. The flow rate was kept constant by means of a Buchler pump.

Preparation of Postmicrosomal Supernatant. When required, the postmicrosomal liver supernatant was either passed through Sephadex G-25 or dialyzed against standard buffer A containing 0.25 M sucrose, in order to remove inhibitors from the cell sap (Mansbridge and Korner, 1963).

Analytical Methods. RNA was determined with a modified Schmidt–Tannhauser procedure (Fleck and Munro, 1962). One optical density unit (at 260 mμ) of the hydrolysate was found to correspond to 32.5 μg of RNA (Nakagawa and Cohen, 1967). Protein was determined according to Lowry *et al.* (1951).

Composition of the Reaction Mixture. The following reaction mixture (0.5 ml) was used to assay the transfer activity: 50 mM Tris-HCl buffer (pH 7.6), 60 mM magnesium chloride, 0.25 mM GTP, 10 mM PEP,[1] 20 μg of pyruvate kinase, 50 μg of [^{14}C]aminoacyl-tRNA, and 0.2 mg of ribosomal or microsomal RNA. Incubation was at 25°. The reaction was stopped with 1 ml of cold

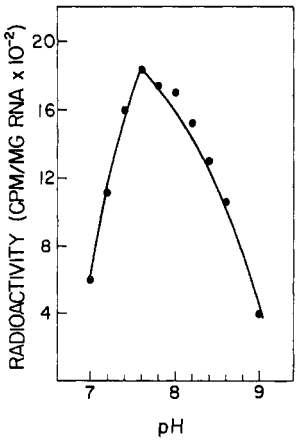

FIGURE 2: Effect of pH on the transfer of amino acids into TCA-insoluble protein of tadpole liver ribosomes. Incubation was carried out for 1 hr under conditions described in Methods, except that the pH of 50 mM Tris-HCl buffer was varied from 7 to 9.

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PEP, phosphoenolpyruvate; TCA, trichloroacetic acid; RNase, bovine pancreas ribonuclease A (protease free); PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene.

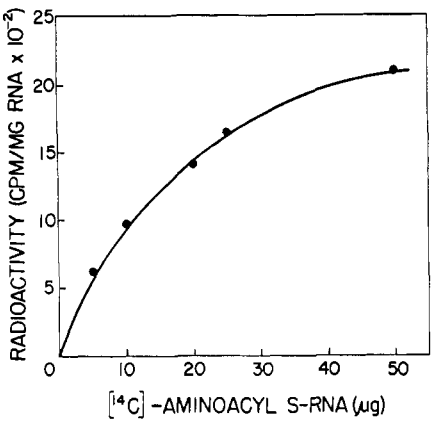

FIGURE 4: Effect of [^{14}C]aminoacyl-tRNA concentration on labeling of TCA-insoluble protein of tadpole liver ribosomes. The ribosomes were incubated for 1 hr as described under Methods, except that the concentration of the labeled precursor was varied.

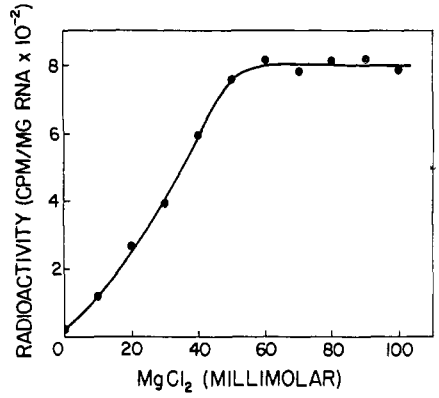

FIGURE 5: Effect of magnesium on the transfer of amino acids to TCA-insoluble protein of tadpole liver microsomes. The microscomes were incubated for 30 min as described under Methods, except that the magnesium concentation was varied from 0 to 100 mM.

10% TCA. The precipitate was spun down and extracted for 20 min with hot (90°) 5% TCA, followed by two washes with 5% TCA containing 2% Casamino Acids, one ethanol wash, one ethanol–ether wash, and finally two washes in ether. The dry precipitate was dissolved in a small volume of formic acid and counted in a Tri-Carb liquid scintillation counter with 15 ml of dioxane–PPO–POPOP solution (Bray, 1960).

Results

Characterization of the Incorporation System. Transfer of ^{14}C-labeled amino acids into TCA-insoluble protein by cell-free extracts of tadpole liver exhibits complete dependence upon the presence of microsomes or ribosomes in the incubation medium (Figure 1). In the present studies about 0.2 mg of microsomal or ribosomal RNA was added to each incubation. The reaction had a pH optimum of 7.6 (Figure 2) and the transfer reaction reached a maximum at a temperature of 25° (Figure 3). The system was saturated with 50 μg of [^{14}C]aminoacyl-tRNA/0.5 ml of reaction mixture (Figure 4) and was found to be dependent upon added magnesium for activity (Table I). However, upon increasing the Mg^{2+} concentration from 0 to 100 mM the activity steadily increased to reach a maximum and plateau between 50 and 100 mM (Figure 5). The optimum magnesium concentration varied slightly from one batch of tadpoles to another; however, 60 mM $MgCl_2$ was usually a suitable concentration for good activity. No requirement for a sulfhydryl compound was noted; in fact the addition of reduced glutathione had an inhibitory effect (Table I). Addition of monovalent cations to the incubation medium was also inhibitory, 100 mM NH_4Cl inhibiting 20% and 100 mM KCl 25% (Figure 6). The time course of incorporation of the ribosomal and microsomal systems is shown in Figure 7. A slightly higher amount of incorporation which reached a maximum after 30 min was observed in

TABLE I: Effect of Various Components of the Reaction Mixture on the Transfer of Amino Acids.[a]

Conditions	[^{14}C]Amino Acids Incorp (cpm)
Complete	330
−Ribosomes	0
−Supernatant	337
−$MgCl_2$	7
−PEP, phosphoenolpyruvate kinase	346
−GTP	287
−PEP, GTP, phosphoenolpyruvate kinase	312
+KCl (100 mM)	245
+NH_4Cl (100 mM)	270
+Glutathione (5 mM)	250
+Glutathione (20 mM)	8
+100 μg of RNase	16

[a] The complete reaction mixture is described under Methods. Supernatant protein (1 mg) was used per tube and the incubation time was 1 hr.

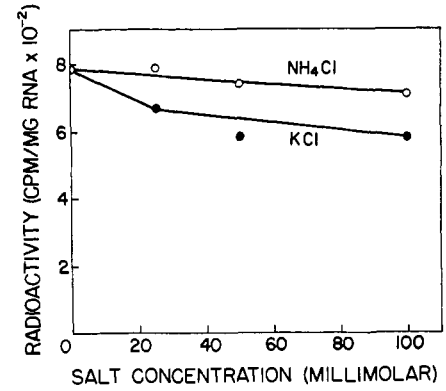

FIGURE 6: Effect of monovalent cations on the transfer of [^{14}C]amino acids to tadpole liver microsomes. The microsomes were incubated for 30 min as described under Methods, except that increasing amounts of either NH_4Cl or KCl were added to the medium prior to incubation at 25°.

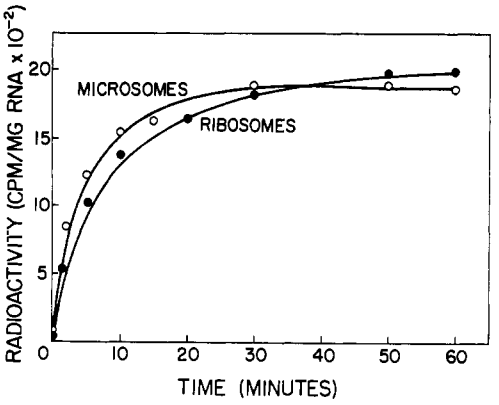

FIGURE 7: Time course of amino acid transfer from [^{14}C]-aminoacyl-tRNA to tadpole liver ribosomes or microsomes. Incubation was carried out at 25° as decribed under Methods, except that the reaction was allowed to proceed for times varying from 0 to 60 min.

the case of microsomes whereas the radioactivity incorporated reached a plateau after 1 hr in the case of ribosomes. In the present studies all microsomal systems were incubated at 25° for 30 min and all ribosomal systems for 1 hr.

There was a high endogenous incorporation of labeled amino acids into protein, by both microsomes and ribosomes, in the absence of added postmicrosomal supernatant. It can be seen from Table I that addition of supernatant to ribosomes did not produce a stimulation in the extent of incorporation. In fact, it has proved possible to remove only 40% of the transfer activity by repeated washing with standard buffer. Therefore, supernatant was not routinely added to the microsomal incubation mixtures. Variable effects were noted when pH 5 supernatant preparations were substituted for postmicrosomal supernatant, and therefore, pH 5 supernatant preparations were not routinely employed. There is only a small requirement for an added energy source or GTP, possibly because there are sufficient endogenous cofactors present (Table I). The system is also sensitive to added RNase at 100-μg concentration.

Addition of an Inhibitor of Aminoacyl Transfer to Polypeptide. Sparsomycin has been reported to be a highly effective inhibitor of protein synthesis by extracts of *E. coli* (Goldberg and Mitsugi, 1966) and rabbit reticulocytes (Colombo *et al.*, 1966). Furthermore, inhibition is exerted specifically on aminoacyl transfer to polypeptide The effect of addition of sparsomycin on the tadpole ribosomal transfer system was therefore examined. It can be seen in Figure 8 that the extent of inhibition is dependent upon the concentration of the antibiotic. We obtained a 90% inhibition at 2.6 × 10^{-4} M sparsomycin concentration, which corresponds with the values reported for *E. coli* extracts by Goldberg and Mitsugi (1967).

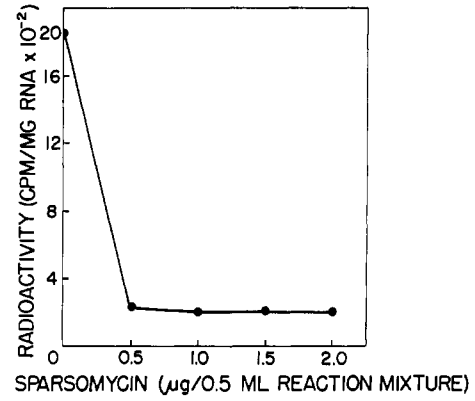

FIGURE 8: Effect of sparsomycin on the transfer of amino acids to tadpole liver ribosomes. Incubation was carried out for 1 hr as described under Methods, except that the concentration of sparsomycin was varied from 0 to 2 μg in the reaction mixture.

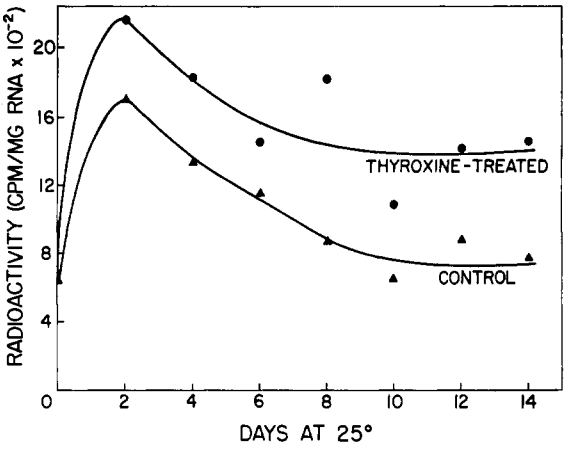

FIGURE 9: Effect of exposure of tadpoles to thyroxine on the extent of transfer of labeled amino acids into TCA-insoluble protein of tadpole liver microsomes. On day 0, tadpoles were taken from 15° water and either placed in 25° water (control animals) or placed in 25° water containing 2.6 × 10^{-8} M thyroxine. Tadpoles were sacrificed at varying intervals of time and microsomes prepared from the livers. The microsomes were incubated as described under Methods.

Effect of Thyroxine Treatment on Aminoacyl Transfer into protein. Tadpoles were kept at 15° and on day 0 were placed in either 25° water (control animals) or 25° water containing 2.6 × 10^{-8} M thyroxine. An increase of two- to threefold in the extent of [^{14}C]amino acid transfer to microsomes prepared from the livers of both groups of animals was observed by the second day (Figure 9). However, by day 10, the values for the control animals returned to their zero-day level and the values for the thyroxine-treated animals reached a plateau with incorporation values about 600 cpm/mg of microsomal RNA higher than the controls. This protein radioactivity was found to be bound to the microsomes and was not released into the supernatant upon incubation at 25°. It seems possible that the increase in incorporation, observed with the control animals, could be in response to the temperature change to which the animals were subjected. This possibility was tested by keeping the tadpoles at 25° for 20 days prior to thyroxine treatment. On day 0 the animals were divided into two batches, one batch serving as controls and the other placed in 2.6 × 10^{-8} M thyroxine solution. It can be seen from Figure 10 that microsomes isolated from the control group show no significant variation in incorporation during the 14-day period of the experiment. However,

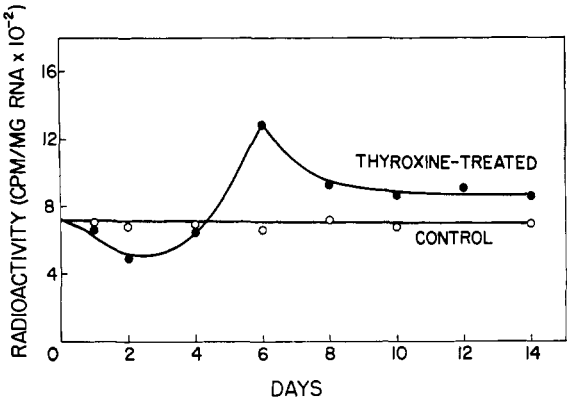

FIGURE 10: Transfer of labeled amino acids to tadpole liver microsomes isolated from tadpoles kept at 25° for 20 days prior to thyroxine treatment. Microsomes were prepared and incubated for 30 min as described under Methods.

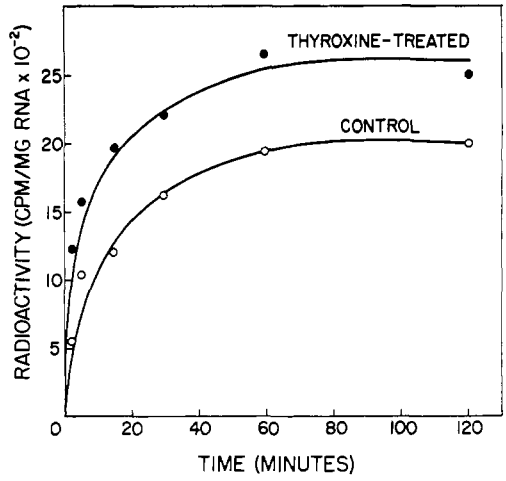

FIGURE 11: Time course of incorporation of amino acids from [^{14}C]aminoacyl-tRNA into ribosomes prepared from the livers of both thyroxine-treated and control tadpoles. Tadpoles were kept at 25° for 20 days, then placed in 2.6×10^{-8} M thyroxine solution. Tadpoles were sacrificed after 6 days and ribosomes were prepared and incubated for 1 hr as described under Methods.

after a lag period of some 4 days, microsomes from thyroxine-treated animals start to show an increased extent of aminoacyl transfer into protein, reaching a maximum level of about 114% greater than the control animals on day 6. The decrease, observed during the first 4 days, is of unknown significance, but may be associated with the similar lag period observed in RNA synthesis during metamorphosis induced by thyroxine (Nakagawa *et al.*, 1967).

The preceding experiments, describing the effect of exposure of tadpoles to thyroxine solution and its influence on the rate of aminoacyl transfer into protein, were performed on a cell-free microsomal system. Ribosomes prepared from tadpoles exposed to thyroxine for 6 days, demonstrated an increased rate of transfer activity when compared with ribosomes isolated from control animals (Figure 11). However, the ribosomes from thyroxine-treated tadpoles showed only a 25% higher level of incorporation than the ribosomes from control animals. It seemed possible that this could be explained by the variation in the time taken for various batches of tadpoles to respond to thyroxine. However, ribosomes prepared every other day for 10 days, from thyroxine-treated and control animals (as in Figure 10 for microsomes), never showed greater than a 25% increase in incorporation, compared with a 114% increase observed with the microsomal system.

We have attempted to determine whether this increased transfer activity, observed after thyroxine treatment, is associated with the transfer factor, quality of the ribosomes, or the nature of the messenger. The first approach was to verify the observation of Grossi and Moldave (1959) that the transfer activity is present in the neutralized supernatant of the fraction precipitated at pH 5; and then to perform crossover experiments with microsomes prepared from thyroxine-treated and control animals placed in supernatant fractions prepared from either control or thyroxine-treated animals. The first experiment was unsatisfactory because fresh preparations of pH 5 supernatant frequently showed little or no activity. This phenomenon has been described by Fox *et al.* (1965) in the case of cell-free preparations from *Drosophila melanogaster*. The second experiment proved to be unfeasable because repeated washing with standard buffer removed only 40% of the endogenous transfer activity from tadpole microsomes. Since crossover experiments were rendered unfeasable by the nature of the system, an attempt was made to remove endogenous messenger from microsomes, followed by assay of their protein-synthesizing capacity after addition of poly U and [^{14}C]phenylalanine following the procedure of Weksler and Gelboin (1967). However, preliminary experiments with tadpole microsomes indicated that high specific activity [^{14}C]phenylalanine and poly U produced a labeling pattern which was dependent neither upon magnesium concentration, nor upon the presence

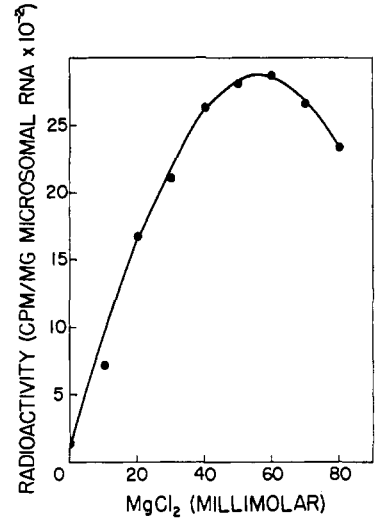

FIGURE 12: Effect of magnesium on the transfer of phenylalanine. Reaction was carried out using 57 μg of [^{14}C]-phenylalanine tRNA and 100 μg of poly U in 0.5 ml of reaction mixture. Other conditions as described in Methods except that the magnesium concentration was varied from 0 to 80 mM. Incubation was at 25° for 30 min.

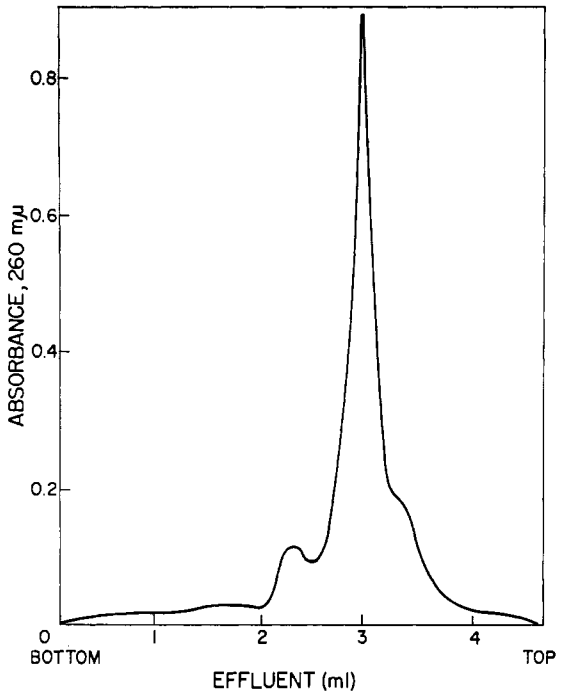

FIGURE 13: Distribution of tadpole postmitochondrial ribosomes on a linear sucrose density gradient. Tadpoles were kept at 15° prior to ribosomal preparation. A linear 15–30% (w/w) sucrose gradient (4.6-ml volume) which contained, in addition to sucrose, 50 mM Tris (pH 7.4), 5 mM $MgCl_2$ (or in some cases 60 mM $MgCl_2$), and 25 mM KCl, was prepared using standard procedures. Ribosomal pellet suspension (25 μl) was layered over the gradient, which was centrifuged for 1 hr at 37,000 rpm in a Spinco SW 39 rotor and allowed to stop without braking. The bottom of the tube was punctured and the optical density of the effluent was continuously monitored at 260 mμ by passage through a flow cell of a Gilford Model 2000 recording spectrophotometer. Direction of sedimentation is toward the left.

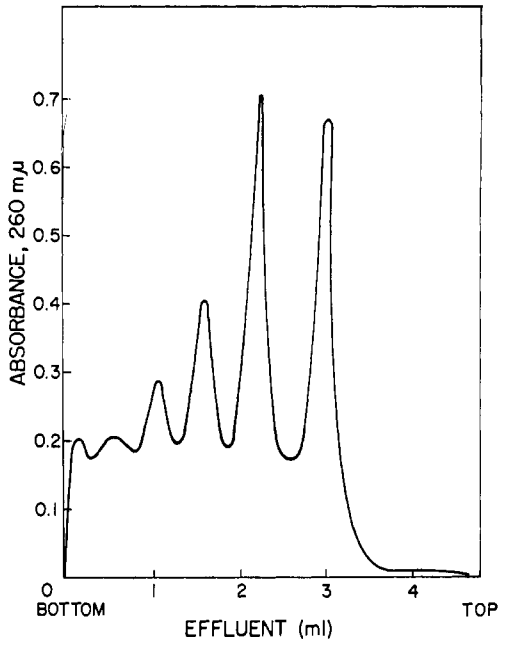

FIGURE 14: Sucrose density gradient profile of ribosomes isolated from the liver of a 24-hr-starved rat. Isolation procedures were exactly as used for tadpole liver ribosomal preparations (Figure 13).

of poly U. Since the transfer system, starting with an aminoacyl compound, gave reproducible results, it was concluded that the preparation lacked an adequate phenylalanine-activating enzyme system. We, therefore, repeated the above experiments but used [^{14}C]phenylalanine tRNA and poly U instead of labeled amino acid and poly U. It can be seen from Figure 12 that the poly U stimulated incorporation is dependent upon magnesium concentration. This is reported with the reservations inherent in a single determination. However, it may be relevant to note that the shape of the curve and the magnesium concentration at which maximal incorporation occurred correspond well with the findings shown in Figure 5.

Experiments utilizing [^{14}C]phenylalanine tRNA and poly U tended to give rather erratic results thus making the usefulness of this technique uncertain. The experiments were further complicated by our inability to remove endogenous messenger from tadpole microsomes or ribosomes. Standard procedures, involving preincubation (Weksler and Gelboin, 1967) or RNase treatment (Munro *et al.*, 1964) did not adversely affect the incorporating ability of either microsomes or ribosomes. Tadpole microsomes or ribosomes were insensitive to preincubation at 25° for 30 min or incubation at 25° for 20 min with 20 μg/ml of RNase, prior to aminoacyl incorporation.

These observations suggest that either the ribosomes are present predominantly in the monomeric form, or as polysomes which are in some way protected from breakdown of their mRNA by added ribonuclease. Analysis of tadpole liver ribosomal profiles showed a predominantly monosomal pattern (Figure 13). However, rat ribosomes prepared by an identical procedure showed essentially a polysomal profile (Figure 14). Mixing rat and tadpole liver ribosomal preparations resulted in the degradation of the rat polysomal peaks (Figure 15), suggesting the presence of a nuclease in the tadpole preparations. In spite of a variety of efforts, we were unable to find a technique which would reduce significantly the activity of the tadpole liver nuclease. Thus the significance of the failure to demonstrate any quantitative changes in the sucrose density gradient profiles of liver ribosomes from premetamorphic and thyroxine-treated tadpoles remains to be determined.

Discussion

The requirements, as reported by many workers, of the amino acid transfer system include ribosomes, soluble enzymes, GTP, mRNA, Mg^{2+}, K^+, or NH_4^+ and a sulfhydryl compound (Nathans *et al.*, 1962; Fessenden and Moldave, 1961; Takanami, 1961; Lamfrom and Squires, 1962; Heredia and Halvorson, 1966). However, it would appear from the present work, that the transfer system in *R. catesbeiana* tadpole liver differs from these reports in several respects.

(1) Repeated washing in standard buffer only reduced the incorporating activity of tadpole microsomes by

40%. Lack of requirement of supernatant has been reported for *D. melanogaster* microsomes by Fox *et al.* (1965) and for *Saccharomyces fragilis* ribosomes by Downey *et al.* (1965). This would suggest that transfer enzymes are tightly bound to tadpole microsomes. (2) There is no absolute requirement for GTP, a finding which also agrees with the situation in *Drosophila* cell-free systems (Fox *et al.*, 1965). (3) There is a lack of requirement for a monovalent cation such as KCl or NH_4Cl, as well as a sulfhydryl compound. A similar observation has been reported for a protein incorporating system utilizing microsomes prepared from pigeon pancreas (Redman *et al.*, 1966). (4) There is an unusually high requirement for magnesium (about 60 mM) in the tadpole transfer system. A more usual concentration in other systems for maximum activity is of the order of 10 mM. Ribosomes tend to aggregate above a magnesium concentration of about 20 mM (Petermann, 1964). However, tadpole ribosomes were found not to aggregate at the very high magnesium levels routinely used during this work (Unsworth and Cohen, 1967).

The experiments demonstrating inhibition of incorporation by low concentrations of sparsomycin leave no doubt that we are dealing with a transfer system.

The results of the experiments reported, using thyroxine-treated tadpoles, indicate that an increase in the extent of the transfer reaction is a relatively early response to thyroxine treatment. However, the magnitude and timing of the response is affected by the temperature at which the tadpoles are kept prior to thyroxine treatment. Animals acclimatized at 25° for 20 days, prior to thyroxine treatment, show a level of incorporation 114% greater than the control animals. This demonstrates that thyroxine may exert an effect at the level of translation and would thus be consistent with the report of Sokoloff *et al.* (1963) that the stimulatory effect of thyroxine is located at the transfer step in protein synthesis. Of interest is the observation that ribosomes isolated from tadpoles exposed to thyroxine for 7 days show only a 25% higher incorporating ability than ribosomes prepared from control animals. The greater sensitivity of microsomes to thyroxine treatment seems related to factors which are removed by deoxycholate treatment. A similar situation has been reported by Kato *et al.* (1965) who observed that microsomes isolated from phenobarbital-treated rats had 114% greater L-[^{14}C]phenylalanine incorporating activity than did microsomes from control animals. However, phenobarbital treatment caused only a 37% increase in the incorporating activity of ribosomes. Kato *et al.* (1965) emphasized the importance of newly synthesized membrane components in their system, and the significance of membrane-attached particles in protein synthesis has been indicated by the work of Campbell *et al.* (1964), Sargent and Campbell (1965), Moore and Umbreit (1965), and Lim and Adams (1967). Also of possible relevance is the suggestion by Pitot (1964) that certain rat liver mRNAs may be stabilized by an intimate association with the endoplasmic reticulum. Tata (1967) has reported the appearance of newly synthesized membrane-bound RNA in liver during induction of metamorphosis in *R. catesbeiana* tadpoles. Thyroxine treatment of tadpoles has been shown to cause a marked increase in the amount of hepatic endoplasmic reticular membranes (Cohen, 1967). It has also been shown that a high *in vivo* incorporation of injected [^{14}C]phenylalanine is associated with a microsomal membrane fraction in tadpole liver (unpublished work). Thus, an increase in both membrane synthesis and aminoacyl transfer to polypeptides occurs as a result of exposure of tadpoles to thyroxine.

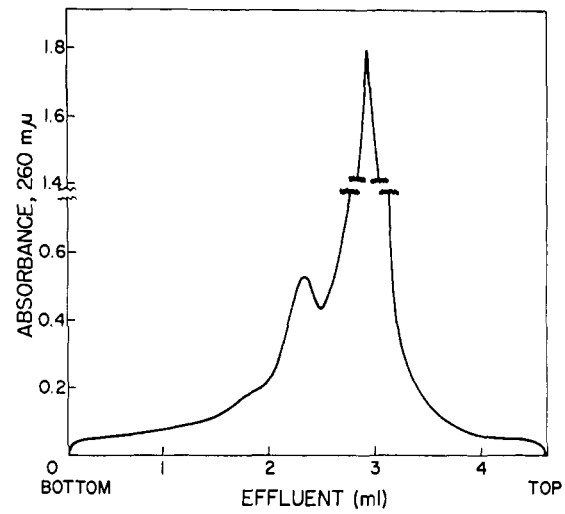

FIGURE 15: Effect of mixing tadpole ribosomal preparations with rat liver polysomal preparations. Rat and tadpole ribosomes were prepared as described under Methods. Equal volumes of each ribosomal pellet suspension were mixed together and allowed to remain at 0° for 1 hr. After this time 25 μl of the mixture was layered over a 15-30% sucrose gradient and analyzed as described in the legend to Figure 13.

The fact that tadpole microsomes and ribosomes resist attack by RNase is of interest. It may be that tadpole liver contains an RNase-stable polysomal fraction as is the case in eggs of *Ascaris lumbricoides* (Kaulenas and Fairbairn, 1966) and in developing chick down feathers (Humphreys *et al.*, 1964). However, in the examples cited above, the stability of these polysomes is associated with inactivity, which is released by removal of a protective protein coat by trypsin treatment. We did not find the activity of *R. catesbeiana* ribosomes to be significantly affected by preincubation with trypsin. The resistance of microsomes and ribosomes to RNase may be correlated with the high resistance to enzymatic hydrolysis reported for amphibian egg RNAs (Burr and Finamore, 1963). The experiment which we report in Figure 13 indicates that the majority of the ribosomes in our *in vitro* preparations was mainly in the monomeric form. This may explain our inability to remove endogenous mRNA from tadpole ribosomes, since it is known that monosomes are resistant to RNase (Munro *et al.*, 1964) and also, mRNA may be partly shielded by the ribosome (Takanami and Zubay, 1964). Reports of incorporation of [^{14}C]amino acids by young rat brain preparations (Lim and Adams, 1967) are of particular interest, since the active fraction was both resistant to RNase and predominantly monosomal in nature.

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