

## KINETIC STUDIES ON AMINO ACID UPTAKE AND PROTEIN SYNTHESIS IN LIVER OF TEMPERATURE ACCLIMATED TOADFISH

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Previous studies on the compensation of liver protein synthesis that occurs with temperature acclimation of toadfish have indicated that protein synthetic rate is controlled at the steps of polypeptide chain elongation and release (Haschemeyer, 1969a). Rate increases in cold-acclimated fish have been correlated with elevated levels of elongation factor I, the enzyme that promotes binding of aminoacyl-transfer RNA at the codon recognition site (Haschemeyer, 1969b). Chain elongation rate in liver is determined from the distribution of radioactivity between soluble completed proteins and total protein (including ribosome-bound growing chains) at short times ( $< 5$  minutes) after injection of labelled amino acids. Use of the method, however, requires that uptake of the precursor and activation to aminoacyl tRNA be rapid compared with polypeptide chain assembly. We present here a new method for simultaneous measurement of L-amino acid uptake, activation, and incorporation into growing polypeptide chains by liver *in vivo*. Preliminary results have been reported (Haschemeyer and Persell, 1971).

### MATERIALS AND METHODS

#### *Animals*

Adult toadfish, body weight  $250 \pm 30$  g, about 90% male, were collected by the Supply Department at the Marine Biological Laboratory in June (1971 and 1972) and utilized in the following months of July and August. Fish were maintained in running sea water aquaria at  $22^\circ$  (normal laboratory sea water supply) or at  $11^\circ \pm 1^\circ$  using a Neslab seawater heat exchanger. The toadfish were fed killifish (*Fundulus heteroclitus*) to appetite until two days before experiment. When acclimation and experimental temperatures differed, 1 hour was allowed for adjustment to the new temperature before experiment.

#### *Measurement of uptake and protein synthetic rates*

The procedures for anesthesia and hepatic portal vein injection were described previously (Haschemeyer, 1969a). The injection solution contained 2  $\mu\text{Ci}$  of L-[U- $^{14}\text{C}$ ] amino acid mixture (0.01  $\mu\text{mole}$  total) with 4  $\mu\text{Ci}$  of D-[1- $^3\text{H}(\text{N})$ ] mannitol or [G- $^3\text{H}$ ]inulin in 1% NaCl, pH 7.2. Another series utilized 3  $\mu\text{Ci}$  of L-[U- $^3\text{H}$ ]leucine with 1-2  $\mu\text{Ci}$  of D-[1- $^{14}\text{C}$ ]leucine or [Carboxyl- $^{14}\text{C}$ ]-1-amino-cyclopentane-1-carboxylic acid (cycloleucine). All isotopes were obtained from New England Nuclear Corp. In most experiments the air bladder of the fish was



deflated by puncturing in order to relieve pressure against the hepatic portal vein that interfered with blood flow. The labelled compounds were injected into the portal vein in 0.1 ml saline carrier over a 3-second period. After an additional elapsed time of 3 seconds to 8 minutes the liver was rapidly excised and transferred to a Sorval Omnimixer can for homogenization as previously described (Haschemeyer, 1969a). Excision time (about 5 seconds) and time to homogenization (about 20 seconds) were noted for each animal. Immediately after liver excision, blood was collected in a heparinized syringe from the cut end of the hepatic veins leading to the heart. External pressure was applied to the heart region to facilitate drainage. After centrifugation the plasma was treated with cold 10% trichloroacetic acid (TCA). An aliquot of the supernatant fluid was analyzed to obtain the plasma free  $^{14}\text{C}/^3\text{H}$  ratio. Radioactivity in the *S* (soluble protein) and *T* (total protein) fractions of the liver homogenate was determined on filter paper discs after fractionation according to Mathews, Oronsky and Haschemeyer (1973); washing of the discs included hot TCA for elimination of aminoacyl-tRNA (Haschemeyer 1969a). Assay of the combined incorporation into protein and aminoacyl-tRNA in these fractions was made by omitting the hot acid step, and the latter was obtained by difference. No radioactivity associated with the labelled marker substances (mannitol, inulin, D-leucine, cycloleucine) was found in the acid-precipitable fractions. The cold TCA supernatant fluid of an homogenate aliquot was analyzed to determine total recovery of  $^{14}\text{C}$  and  $^3\text{H}$  as free radioactivity in the liver. Counting was done in Aquasol (New England Nuclear) with a Packard scintillation spectrometer. Efficiency and overlap determination for the double label system was made with toluene internal standards. Results were normalized to an injection dose of 1  $\mu\text{Ci}$  for each component.

#### *Determination of liver spaces*

Water content was determined by drying either whole livers or blotted tips of lobes to constant weight in an oven at  $90^\circ$ . Determination of the time dependence of distribution of various substances in plasma and liver was done by injecting through a gill artery a saline solution containing 1–3  $\mu\text{Ci}$  of two of the substances listed in the previous section in  $^{14}\text{C}$ - $^3\text{H}$  pairs. After a given time for equilibration at  $22^\circ$  (5 minutes to 5 hours) blood samples were taken from the gills and from an internal vessel in heparinized syringes and the liver was rapidly excised and homogenized. Free radioactivities were determined for the cold TCA supernatant solution of plasma and liver homogenate aliquots in Aquasol. After correction for dilutions the recovery of each substance in liver (per g) was divided by its concentration in plasma (per ml) to obtain the volume in ml/g occupied by that substance in liver at the time of sampling.

## RESULTS

At any time  $t$  after portal injection of a radioactive L-amino acid (*A*) the recovery in the excised liver will be the sum of amounts present in intracellular (*i*) and extracellular (*e*) spaces:

$$A_{\text{Liver}} = A_i + A_e \quad (1)$$



The intracellular contribution may be further subdivided as:

$$A_i = A_i^{\text{Free}} + A_i^{\text{Protein}} + A_i^{\text{aa-tRNA}} \quad (2)$$

The quantities  $A_i^{\text{Protein}}$ ,  $A_i^{\text{aa-tRNA}}$  and the sum  $[A_i^{\text{Free}} + A_e]$  which represents total free radioactivity in liver are determinable by use of the hot and cold acid extraction procedures described in materials and methods.  $A_i^{\text{aa-tRNA}}$  refers to amino acid residues in aminoacyl transfer ribonucleic acid. These quantities thus yield  $A_{\text{Liver}}$ . The value of  $A_e$  must be determined by use of the marker data in order to obtain the desired  $A_i$ .

The recovery of the marker substance (B) in the liver will represent that amount present in extracellular space ( $B_e$ ) plus any of the marker that has reached intracellular space by diffusion or other non-specific uptake processes (*e.g.*, endocytosis):

$$B_{\text{Liver}} = B_e + B_i^{\text{diff}} \cong B_e \quad (3)$$

In the present study markers were used which showed negligible accumulation in liver during the short time course of the experiments. The term  $B_i^{\text{diff}}$  thus may be neglected without significantly affecting the final equations for the active transport of A.

Comparing equations (1) and (3) it is apparent that under circumstances of active accumulation of A by liver the total recovery  $A_{\text{Liver}}$  will exceed  $B_{\text{Liver}}$  by an amount proportional to the extent of specific or active transport (above that associated with non-specific processes). In order to eliminate the unknown  $A_e$  from equation (1) we use the measured value  $(A/B)_{\text{Plasma}}$  obtained for plasma draining from the liver to approximate the ratio  $A_e/B_e$  in the extracellular space of liver. In this way  $A_e$  may be calculated directly from the experimental data, as follows:

$$A_e = (B_{\text{Liver}}) (A/B)_{\text{Plasma}} \quad (4)$$

$A_i$  for each experimental time is then obtained from the measured total recovery of A in liver ( $A_{\text{Liver}}$ ) by means of equation (1).

The time course for intracellular accumulation of radioactivity of the L-amino acid mixture at two experimental temperatures is presented in Figure 1. Fish of different acclimation groups were not distinguishable by this measurement, and data normalized to the average maximum uptake achieved for each experimental group are averaged together. The results clearly reveal a mechanism in liver for rapid intracellular uptake of L-amino acids injected into the hepatic portal vein. The system is essentially equilibrated within 1 minute at 24° and in about 2 minutes at 11°. After this time isotope ratios in plasma  $[(A/B)_{\text{Plasma}}]$  were in the range 0.2 to 0.5 compared with an injected ratio of 1.0, whereas uncorrected distribution ratios for liver  $[A_{\text{Liver}}/B_{\text{Liver}}]$  were as high as 6. At  $t > 1$  minute (24°) the extracellular component  $A_e$  in liver calculated from equation (4) averaged  $4 \pm 2\%$  of the injected dose. The distribution ratio for the free intracellular and extracellular pools  $[A_i^{\text{Free}}/A_e]$  reached values of about 15.

Recovery of D-mannitol ( $B_e$ ) in liver showed an approximately exponential decline with time ( $t_{1/2} \sim 20$  seconds at 24° and 35 seconds at 11°). By extrapolation of  $B_e$  to the level of the injected dose, a measure of flow time between the portal vein and the hepatic veins draining the liver (for the supine anesthetized fish) was obtained: 5–10 seconds at 24°; 10–15 seconds at 11°. Average recovery of



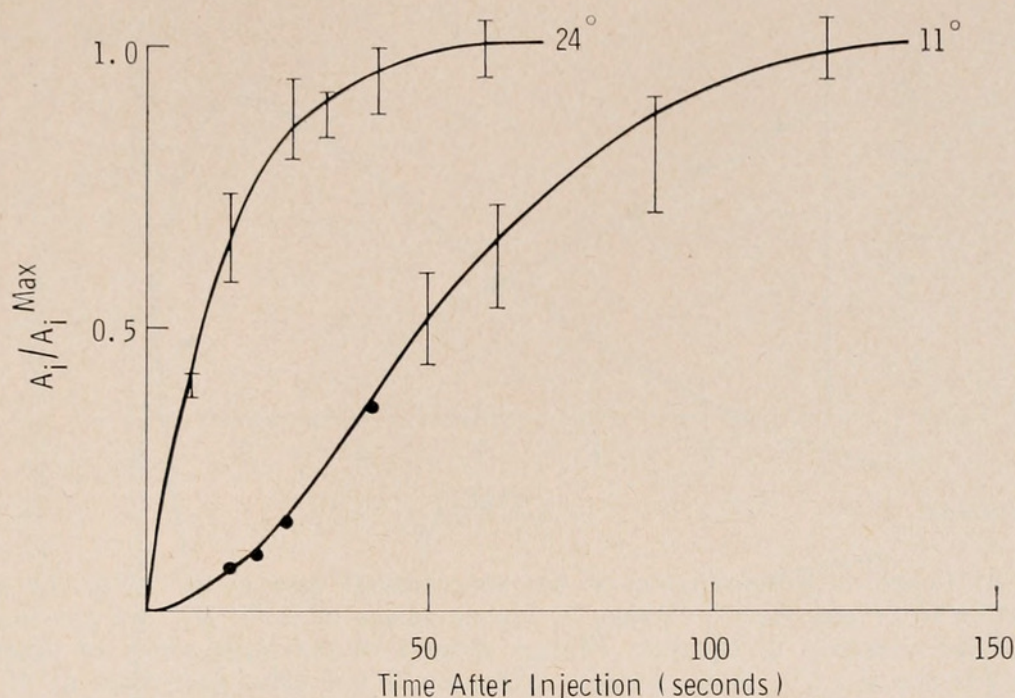


FIGURE 1. Kinetics of uptake of L-amino acids into intracellular space of toadfish liver following intraportal injection. Averaged data with standard error bars [based on  $A_i(t)$ ] are shown for the ratio of uptake  $A_i$  at short incubation times to the maximum or equilibrium value  $A_i^{Max}$  obtained at  $t > 1$  minute at 24° and  $t > 2$  minutes at 11°.

D-mannitol (normalized to a 1  $\mu$ Ci injection) in liver in the time range of 1–2 minutes after injection at 24° was  $0.18 \pm .06$ . Other markers tested were not significantly different: D-leucine,  $0.24 \pm 0.04$ ; inulin, 0.24; cycloleucine,  $0.26 \pm 0.04$ . None of these substances showed evidence of accumulation in liver during the short experimental periods used.

The accumulation process for  $A_i$  may be described by the simple differential equation:

$$dA_i/dt = k_a A_e - k_b A_i^{Free} \quad (5)$$

where the rate constant  $k_a$  for uptake into intracellular space will depend upon transport characteristics and, in this experimental system, on flow rate as well. The second term refers to efflux processes that remove  $A$  from the intracellular space. If protein turnover is neglected, efflux may be approximated by a first-order term in  $A_i^{Free}$ , the free radioactive pool. The rate constant  $k_b$  is dependent upon characteristics of the efflux system (*e.g.*, diffusion) that leads to loss of  $A_i$  from the intracellular space of liver as well as on flow rate.

Figure 2 presents a numerical solution of equation (5), *i.e.*,  $(\Delta A_i/\Delta t)/A_e$  vs.  $A_i^{Free}/A_e$ , based upon the 24° data of Figure 1 and the corresponding averaged data for  $A_e$  and  $A_i^{Free}$  obtained in the same experimental series. The intercept and slope of this plot yield the following values for the parameters of equation (5):  $k_a = 2.7 \text{ minutes}^{-1}$ ;  $k_b = 0.5 \text{ minutes}^{-1}$  (based on the earlier time points). From the value of  $k_a$  the half-time for uptake is found to be 15 seconds. At 11° an uptake half-time of 30 seconds may be calculated from the maximum rate of accumulation (Fig. 1) and the corresponding levels of  $A_e$  in this time range. The shape of the experimental curve at 11° (Fig. 1) clearly deviates from that pre-



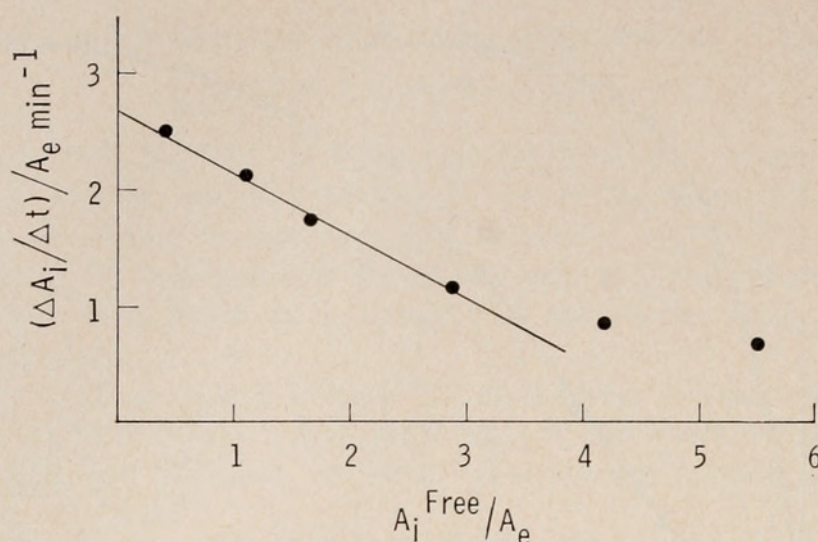


FIGURE 2. Graphical differentiation of the experimental data at 24° (Fig. 1) combined with  $A_i^{\text{Max}}$  (Table I) and  $A_e(t)$ . Points at higher values of  $A_i^{\text{Free}}/A_e$  are not included in evaluation of the constants of equation (5) on account of the large effect of experimental variation in this region. The linear portion yields uptake and efflux half-times of 15 seconds and 80 seconds, respectively.

dicted by equation (5). This is likely to be due to flow rate dependency and to the inadequacy of equation (5) for describing the system with the experimental data obtainable at short times after injection.

#### *Amino acid uptake at equilibrium and protein synthetic parameters*

At  $t > 1$  minute at 24° and  $t > 2$  minutes at 11° intracellular recovery of the L-amino acid radioactivity [ $A_i$  of equation (2)] appeared to have reached equilibrium within experimental error. Averaged results for  $A_i^{\text{Max}}$ , normalized to an injection dose of 1  $\mu\text{Ci}$ , are presented in Table I for fish of two temperature acclimation groups matched for body weight and nutrition. Total uptake was slightly higher for the 22°-acclimated group measured at 24°, but this may have been associated with differences in endogenous amino acid pools. Although both groups were starved for two days before experiment, the effect of starvation is likely to differ for fish at the two acclimation temperatures. In an earlier series 22°-accli-

TABLE I

*Amino acid uptake levels and protein synthetic parameters for liver of toadfish in vivo at 24° and 11°. Results are normalized to 1  $\mu\text{Ci}$  injection of the 15 L-amino acid mixture and presented with standard error and number of animals; body weight =  $250 \pm 30$  g; liver weight =  $6.8 \pm 1.5$  g*

Experimental temperature	Acclimation temperature	Experimental time range	$A_i^{\text{Max}}$	$t_e$ (min)
24°	22°	1–5 min	$0.76 \pm 0.10$ (6)	$4.0 \pm 0.4$
	11°		$0.63 \pm 0.05$ (6)	$2.5 \pm 0.3$
11°	22°	2–8 min	$0.56 \pm 0.05$ (6)	$17 \pm 2$
	11°		$0.61 \pm 0.06$ (6)	$11.5 \pm 1.5$



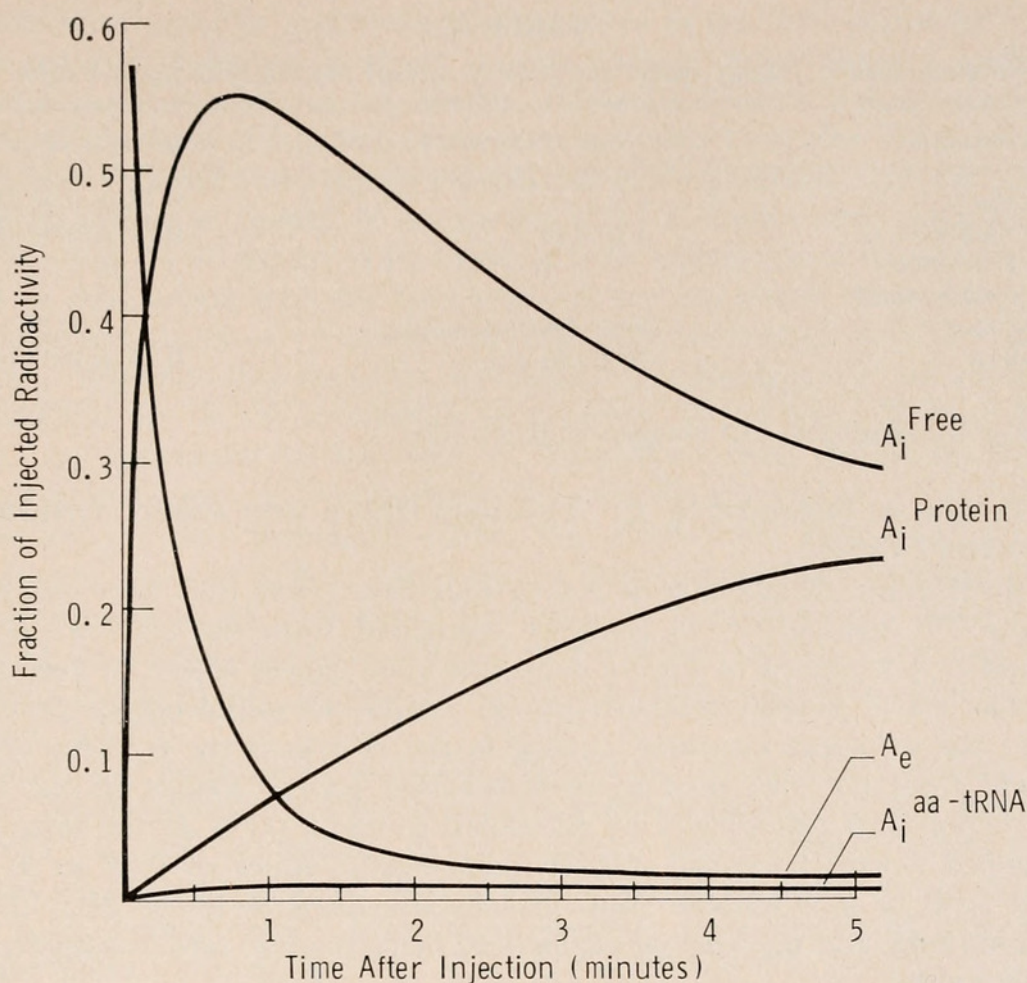


FIGURE 3. Time course in the distribution of radioactive L-amino acids among various liver pools following hepatic portal vein injection *in vivo* at 24° in toadfish acclimated for two weeks at 11°.  $A_i^{\text{Free}}$ —intracellular free (cold trichloroacetic acid soluble) amino acid pool;  $A_i^{\text{Protein}}$ —protein (hot acid insoluble) fraction;  $A_e$ —free amino acid pool attributed to extracellular space [from equation (4)];  $A_i^{\text{aa-tRNA}}$ —aminoacyl transfer RNA.

mated fish that had been fed continuously showed a reduced average  $A_i^{\text{Max}}$  of  $0.52 \pm 0.08$  at 24°.

Average polypeptide chain assembly time  $t_c$  (or the average time for one round of protein synthesis on all active ribosomes) was determined for each group from the distribution of radioactivity between growing (ribosome-bound) and completed chains as a function of incubation time up to  $t_c$  (Haschemeyer, 1969a). Values of  $t_c$  obtained from incorporation of the L-amino acid mixture and of L-leucine alone are given in the last column of Table I. The results at 24° indicate a slightly higher synthetic rate than previously reported at 22°. Part of the difference is due to temperature; other contributing factors are a shorter period of starvation before experiment and a change in methodology that reduced loss during centrifugation of membrane-bound protein. Cold acclimated fish showed more rapid protein synthesis (smaller  $t_c$ ) at both experimental temperatures. Within each acclimation group  $Q_{10}$  for the process measured by  $t_c$  (polypeptide chain elongation and release) was about 3. Net incorporation into protein ( $A_i^{\text{Protein}}$ ), though subject to more individual variation, roughly paralleled the



TABLE II

*Determination of tissue spaces accessible to various substances in toadfish liver*

Substance	Time after injection	Space* ml/g wet weight
Water	—	$0.78 \pm 0.02$ (8)
D-mannitol, D-leucine, cycloleucine	1 to 3 hr	$0.72 \pm 0.03$ (4)
D-mannitol	Zero (extrapolated)	0.31
Inulin	10 to 30 min	$0.30 \pm 0.03$ (5)

\* Including standard error and number of observations.

elongation rates as measured by  $t_c$ . This is in accord with results obtained at 22° (Haschemeyer, 1969a).

The distribution of labelling of the various liver pools that were measured directly ( $A_i^{\text{Protein}}$  and  $A_i^{\text{aa-tRNA}}$ ) or were calculated from the marker distribution ( $A_i^{\text{Free}}$  and  $A_e$ ) is shown in Figure 3 by smoothed curves based on averaged data for the most active protein synthetic group. After the initial uptake period (0 to 1 minute) the principal measured change in the system is the transfer of labelled amino acid from the free pool to protein-bound form. Levels in the hot acid soluble fraction (aminoacyl-tRNA) amounted to about 0.5% of the injected dose, and remained essentially constant over experimental times of 0.25 to 5 minutes. The final level of incorporation into protein amounted to about 25% of the injected amino acid, and the average normalized incorporation rate was approximately 0.05  $\mu\text{Ci}/\text{minute}$ .

#### *Space determinations in liver*

Table II presents data for liver volume occupied by various substances, in relation to plasma concentration, at various times after arterial injection. Water content amounted to 78% of wet weight. After equilibration times of 1 to 3 hours diffusible substances such as mannitol occupied a liver space of 0.72 ml/g wet weight. A time series in the range of 10 to 60 minutes for mannitol distribution between plasma and liver was plotted in the form:  $\log [1\text{-mannitol space}/\text{H}_2\text{O space}]$  vs. time to yield an extrapolated value at zero time of 0.31 ml/g, representing extracellular space occupied before permeation into intracellular spaces occurred. The half-time for equilibration obtained from the slope of the line was 20 minutes. The recovery of mannitol in liver, extrapolated to zero time, was 8.2% of the injected amount. Recovery after 30–60 minutes was 5%. Inulin space at equilibrium (10 to 30 minutes) was 0.30 ml/g; recovery, extrapolated to zero time, was 5.4% of the original dose. In contrast, recovery of  $^3\text{H}$ -L-leucine injected by the same route averaged 19% at times from 5 to 60 minutes after injection.

#### DISCUSSION

A method is introduced here for determination of the time course and equilibrium level of uptake by liver of radioactively labeled L-amino acids supplied as a pulse via the hepatic portal vein. Analysis of the distribution of amino



acids between intracellular and extracellular spaces in the time period of 5 seconds to 8 minutes after injection is based on concomitant measurements in liver and plasma of a marker substance of similar diffusivity that is not concentrated by liver. The time course of labelling of the liver aminoacyl transfer RNA and protein pools was also followed and average polypeptide chain assembly time determined.

The time course of uptake of a mixture of 15 L-amino acids supplied by the portal vein (Fig. 1) is shown to be rapid at both experimental temperatures ( $24^{\circ}$  and  $11^{\circ}$ ) in comparison with the time required for one round of protein synthesis ( $t_c$ ) at these temperatures (4 minutes and 17 minutes, respectively, for  $22^{\circ}$ -acclimated fish). Equilibration times for the uptake process depended on experimental temperature but not on the acclimation temperature of the fish. Because the measurements are influenced by the rate of blood flow from the portal to hepatic veins, uptake half-times (15 seconds at  $24^{\circ}$  and 30 seconds at  $11^{\circ}$ ) determined from equation (5) may principally reflect flow rate rather than transport parameters. Minimal values for flow time between injection site and drainage site were about 5–10 seconds at  $24^{\circ}$  and 10–15 seconds at  $11^{\circ}$ . The latter corresponds to a flow rate of 20 ml/min/kg body weight assuming the volume swept out is about half the liver inulin space. [Hepatic blood flow in dog is 30–35 ml/min/kg (Spurr and Dwyer, 1972)].

It is likely that most uptake occurs in the first pass of the injection fluid through the liver. Thereafter the system equilibrates by elimination of the extracellular components ( $A_e$  and the marker  $B_e$ ) due to the influx of fresh blood from the portal vein and hepatic artery. The half-time for efflux of  $A_i$  from the liver is found to be 80 seconds at  $24^{\circ}$  [from  $k_b$  in equation (5)], compared with about 20 seconds for efflux of the marker  $B_e$ . This is not unexpected, since  $A_i$  is subject to continuous influx-efflux processes along the portal to hepatic vein pathway and to possible recycling of isotope re-entering the liver through the hepatic artery.

For the mixture of 15 L-amino acids injected at a total concentration of 0.1 mM (in 0.1 ml), intracellular uptake amounted to 60–70% of the injected quantity (Table I) at  $t \geq 1$  minute at  $24^{\circ}$  or  $t \geq 2$  minutes at  $11^{\circ}$ . Accumulation for L-leucine alone injected at 0.05 mM was about one-half as much. Following the initial uptake period, transfer of radioactivity from the free amino acid pool to protein-bound form is observed (Fig. 3). The distribution ratio between intracellular and extracellular spaces [ $A_i^{\text{Free}}/A_e$ ] averaged about 15, based on recoveries in whole livers. When corrected for relative volumes of the two spaces [ $v_i = 0.41$  ml/g,  $v_e = 0.31$  ml/g (Table II)], the intracellular to extracellular concentration ratio is about 11.

These results are consistent with the operation of an active transport system in toadfish liver serving to accumulate L-amino acids against a concentration gradient. Comparison of the concentrations of the injection solutions with tissue amino acid levels [about 20  $\mu\text{mole/g}$  for the amino acid mixture (Haschemeyer, 1968) and 0.2  $\mu\text{mole/g}$  for leucine in similarly fed fish (Haschemeyer, unpublished)] indicates that transport probably was uphill throughout the measurement period. Selective accumulation of amino acids in liver relative to plasma has previously been observed (in mammals) after long periods of equilibration (see, *e.g.*, Harrison and Christensen, 1971). Data indicating active transport for several amino acids have been obtained in rat liver slices (Tews and Harper, 1969; Crawhall and Davis, 1971). The uptake of nonmetabolizable amino acids has been



studied in perfused livers (Chambers, Georg and Bass, 1965; Mallette, Exton and Park, 1969).

The distribution ratios reported above do not include that portion of accumulated amino acid that has been transferred to aminoacyl-tRNA or protein at the time of measurement. Inclusion of this component would lead to higher uptake ratios. Also, no account has been taken of protein turnover in liver in the present study. Because of the short experimental times used it is doubtful that degradation processes would contribute significant radioactivity to the measured free amino acid pool. This factor complicates the interpretation of longer time experiments. The quantitative results for total  $A_i$  and the transfer of radioactivity from the free amino acid pool into protein (Fig. 3) indicate negligible loss of amino acids through chemical conversion. Such processes, *e.g.*, amino acid oxidation, may affect uptake results in long-term experiments (Fisher and Kerly, 1964).

Recovery of radioactivity in the aminoacyl tRNA pool amounted to about 0.5% of the injected amount for the amino acid mixture. The rapidity of equilibration of the pool (within 15–30 seconds at 24°) indicates that precursor activation kinetics will not cause serious error in the determination of polypeptide chain assembly times from incorporation data at  $t > 1$  minute. Quantitative evaluation of the aminoacyl tRNA pool is possible from the data of Figure 3 and Table I for cold-acclimated fish measured at 24°. For this group the normalized incorporation rate into protein (0.05  $\mu\text{Ci}/\text{min}/\text{liver}$ ) may be combined with the polypeptide chain synthesis rate [5.7 nmole/min/liver based on  $t_c = 2.5$  min and ribosome concentration of 2.1 nmole/g (Haschemeyer, 1969a)] to obtain the average specific activity of the intracellular precursor pool. If the average polypeptide chain synthesized has 435 amino acid residues, this result turns out to be 0.020  $\mu\text{Ci}/\mu\text{mole}$  aminoacyl-tRNA. The normalized recovery of radioactivity in aminoacyl-tRNA then corresponds to an active pool of 37 nmole/g liver, representing an average of 18 molecules of aminoacyl tRNA per ribosome.

Overall protein synthesis in this system based on  $t_c$  for cold-acclimated fish at 24° is 2.5 mg/g liver/hour, the highest value obtained in toadfish liver to date. The synthesis rate for 22°-acclimated fish measured at 24° ( $t_c = 4.0$  min) is 1.6 mg/g liver/hour, about  $\frac{1}{5}$  of the mammalian rate calculated from  $t_c$  (1.16 min) and liver ribosome concentration in rat (Mathews, Oronsky and Haschemeyer, 1973). When temperature and ribosome concentration differences are taken into account, it would appear that the protein synthetic system of toadfish liver is comparable to the mammalian liver with respect to levels of substances involved in control of elongation rate.

The results herein provide further evidence of the value of poikilothermic vertebrates for the study of rate processes involved in liver protein metabolism *in vivo*. Body temperature may be varied both for examination of acute temperature effects and for study of adaptive changes associated with acclimation. Extension of the new methodology to determine transport parameters and hormonal influences on these kinetic systems is in progress.

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#### SUMMARY

1. A new method is described for measurement of intracellular uptake of amino acids by liver in toadfish (*Opsanus tau*) *in vivo* with simultaneous determination of incorporation into aminoacyl transfer RNA and of polypeptide chain assembly time, a kinetic parameter for protein synthesis.

2. Mixed L-amino acids or L-leucine supplied by a pulse injection into the hepatic portal vein are strongly concentrated by liver (up to 75% of dose) compared with D-amino acid or mannitol markers. Uptake occurs against an apparent concentration gradient with halftimes of 15 seconds at 24° and 30 seconds at 11°.

3. The uptake of pulse-injected L-amino acids into intracellular space of liver and the equilibration of the aminoacyl-tRNA pool are rapid compared with the kinetics of polypeptide chain assembly and thus do not interfere with assembly time determination.

4. Polypeptide chain assembly times were 4.0 and 2.5 minutes for warm- and cold-acclimated fish, respectively, at 24° and were 17 and 11.5 minutes for the same groups measured at 11°. The  $Q_{10}$  (for acute temperature change) was about 3 for both acclimation groups.

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