Matthews, H. R. (1968), *J. Chromatog. 36*, 302.
Midgley, J. E. M., and McIlreavy, D. J. (1966), *Biochem. J. 101*, 32P.
RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 751.
Robinson, W. E., and Gilham, P. T. (1967), *7th Intern. Congr. Biochem.*, p 660.
Sanger, F., Brownlee, G. G., and Barrell, B. G. (1965), *J. Mol. Biol. 13*, 373.
Sinha, N. K., Enger, M. D., and Kaesberg, P. (1965), *J. Mol. Biol. 12*, 299.
Sugiura, M., and Takanami, M. (1967), *Proc. Natl. Acad. Sci. U. S. 58*, 1595.
Takanami, M. (1967), *J. Mol. Biol. 29*, 323.
Thirion, J.-P., and Kaesberg, P. (1968), *J. Mol. Biol. 33*, 379.
Wagner, E. K., Penman, S., and Ingram, V. M. (1967), *J. Mol. Biol. 29*, 371.
Warner, J. R., Soiero, R., Birnboim, H. C., Girard, M., and Darnell, J. E. (1966), *J. Mol. Biol. 19*, 349.
Weith, H. L., Asteriadis, G. T., and Gilham, P. T. (1968), *Science 160*, 1459.
Young, R. J. (1968), *Biochemistry 7*, 2263.
Zachau, H. G., Dutting, D., and Feldmann, H. (1966), *Z. Physiol. Chem. 347*, 212.

Incorporation into Polypeptide and Charging on Transfer Ribonucleic Acid of the Amino Acid Analog 5′,5′,5′-Trifluoroleucine by Leucine Auxotrophs of *Escherichia coli**

Eric D. Fenster† and H. S. Anker

ABSTRACT: Some leucine auxotrophs of *Escherichia coli* were previously shown to be adaptable to grow on 5′,5′,5′-trifluoroleucine in a chemostat. In these experiments bacteria *naive* to trifluoroleucine were tested for their ability to carry out various reactions when confronted with this analog in the absence of leucine. A crude extract of aminoacyl synthetases charged about two to three times as much trifluoroleucine as leucine to transfer ribonucleic acid. The trifluoroleucine charging was inhibited, in decreasing order of effectiveness, by leucine, isoleucine, phenylalanine, and cysteine.

Intact cells incorporated trifluoroleucine into polypeptide for about an hour, whereupon incorporation ceased. The initial rate was somewhat greater than the initial rate of leucine incorporation. *In vivo* incorporation of trifluoroleucine was inhibited by leucine, isoleucine, and phenylalanine. When histidine was used as an indicator of protein synthesis *in vivo* the ratio of trifluoroleucine to histidine incorporation was about twice the leucine to histidine ratio. The trifluoroleucine to histidine ratio was reduced in the presence of isoleucine or phenylalanine; the leucine to histidine ratio was unaffected. A combination of isoleucine and phenylalanine decreased the trifluoroleucine to histidine ratio below the leucine to histidine ratio. Ribonucleic acid synthesis, as measured by uracil incorporation, was markedly reduced by incubation in trifluoroleucine. Synthesis was fully restored by 1 μg/ml of chloramphenicol, an amount too low to relax ribonucleic acid synthesis in the absence of amino acids.

The adaptation of some leucine auxotrophs of *Escherichia coli* to growth in 5′,5′,5′-trifluoroleucine was reported by Rennert and Anker (1963). The organisms were grown in a chemostat in which the leucine of the medium was gradually replaced by trifluoroleucine. Amino acid analysis of the total protein of adapted bacteria showed that trifluoroleucine was present in nearly the same relative concentration as leucine was in unadapted bacteria. No leucine was found in the fully adapted bacteria. In the experiments described below some responses of unadapted bacteria to trifluoroleucine were studied. Trifluoroleucine was found to be recognized as leucine, isoleucine, and phenylalanine both in the tRNA-charging reaction *in vitro* and in polypeptide synthesis *in vivo*.

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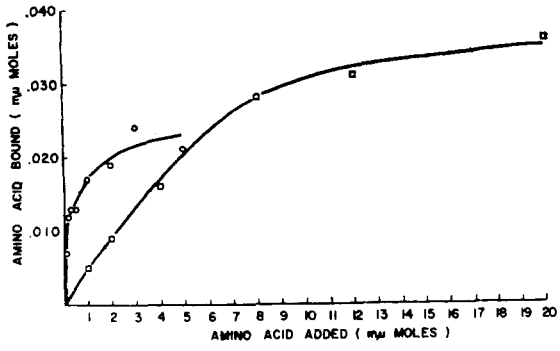

FIGURE 1: Amino acid charged to tRNA as a function of concentration during 1.5-min incubation. (○) Leucine; (□) trifluoroleucine.

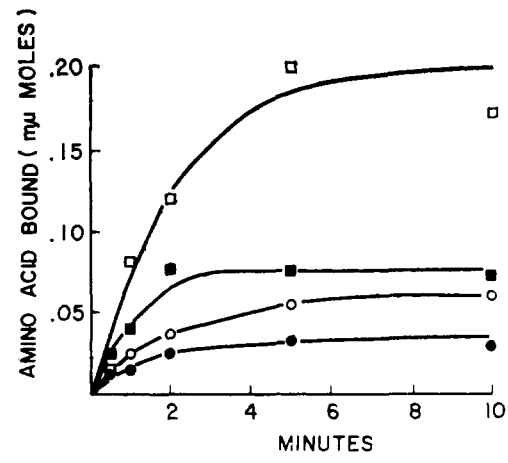

FIGURE 2: Rate of charging of tRNA by 2×10^{-6} M leucine or 4×10^{-6} M trifluoroleucine. (○) Leucine; (□) trifluoroleucine; (●) leucine in the presence of 5×10^{-4} M of 18 amino acids; (■) trifluoroleucine in the presence of 5×10^{-4} M of 18 amino acids.

Materials and Methods

Tritiated Trifluoroleucine. DL-Trifluoroleucine (50 mg) was tritiated according to the Wilzbach procedure (Dorfman and Wilzbach, 1959; Volk Radiochemical Co., Lot No. LB4270). The crude reaction product, about 35 mg (30 mCi), dissolved in 5 ml of water was passed through a Sephadex G-10 column (1 × 20 cm) and 3-drop fractions were collected. One microliter from each fraction was spotted on Whatman No. 1 paper and tested for reaction to ninhydrin. The column fractions which reacted were pooled, dried, and redissolved in water. Only half the radioactivity was recovered. The ninhydrin-positive eluate was streaked on Whatman No. 3MM paper (18 × 22 in.) and chromatographed in a solvent consisting of 1-butanol–glacial acetic acid–water (4:1:2). There were three radioactive areas, but only one was ninhydrin positive. The ninhydrin-positive region was cut out, eluted with 0.1 N NH_4OH, dried, redissolved in water, and rechromatographed. This time there was found only a single peak of radioactivity which coincided with the ninhydrin-positive area. This area was cut out, eluted with 0.1 N NH_4OH, lyophilized, and dissolved in 10 ml of water. The amino acid concentration of this solution was found by the quantitative ninhydrin method (Cocking and Yemm, 1954) to be 5.3 μmoles/ml. The radioactivity determination gave 7×10^6 cpm/μmole. It is probable that only the L isomer of trifluoroleucine is incorporated by bacteria (Rennert and Anker, 1963) and in all calculations of the amounts and activity of trifluoroleucine, values were used relative to the L isomer.

Other Radiochemicals. L-Leucine-^{14}C (specific activity 25 Ci/mole), histidine-^{14}C (specific activity 231 Ci/mole), and uracil-5-^{3}H (specific activity 6×10^3 Ci/mole) were commercial preparations (New England Nuclear Corp.)

Transfer Ribonucleic Acid. tRNA from *E. coli* B (General Biochemicals) was stripped of amino acids by incubation in 0.5 M Tris-HCl (pH 8.8) for 45 min at 37°. The solution was made 1 M in NaCl and the tRNA was precipitated by two volumes of ethanol, dialyzed against water, and lyophilized.

Bacterial Cultures. Strains K12-C2F9 (leu$^-$thr$^-$B$_1^-$ alkaline phosphatase constitutive, from F. Rothman) or B33 (leu$^-$ from S. Gots) were grown, except where indicated, in minimal medium containing per liter of distilled water: 10.5 g of Na_2HPO_4, 4.0 g of KH_2PO_4, 12.0 g of NH_4Cl, 1.2 ml of a 6% solution of $MgSO_4$, 0.6 ml of a 4.44% solution of $CaCl_2$, 24 ml of glycerol, 125 mg of L-Thr, 2 mg of thiamine, and amino acid(s) as indicated. In later experiments deionized water was used, 0.5 mg of $Fe_2(SO_4)_3$ was added, and the $CaCl_2$ was omitted. The modified medium had less tendency to form precipitates on autoclaving.

Bacteria for most experiments were taken from a chemostat in which the reservoir contained minimal medium plus 10^{-4} M L-leucine and the generation time was approximately 3 hr. In some experiments batch cultures in the exponential phase of growth were used.

Aminoacyl Synthetases. Strain C2F9 was grown in 3 l. of minimal medium containing 3×10^{-4} M leucine to an optical density of 0.3–0.4 at 650 mμ and centrifuged. The resulting paste was crushed in a Hughes Press at −30°. The disrupted preparation was resuspended in 3 ml of 0.01 M Tris-HCl buffer (pH 7.2) 0.01 M in $MgCl_2$ and centrifuged for 3 hr at 100,000*g*. The upper four-fifths of the supernatant was passed through a Sephadex G-25 column and fractions of about 0.5 ml were collected. Ten microliters of each fraction was tested for enzyme activity as described below, and the more active fractions were pooled, divided into 1-ml aliquots, and stored at −15°. Upon thawing, 1 μl of β-mercaptoethanol was added as a preservative and the enzyme solution was then kept at 4°.

Charging of tRNA. Reaction mixtures containing 3.3 μmoles of Tris buffer, 3.3 μmoles of Mg^{2+}, 50 μg of tRNA, 0.5 μmole of neutralized ATP, 1 μmole of β-mercaptoethanol, the appropriate amino acid(s), and 0.01 ml of the enzyme extract in 0.3-ml total volume (pH 7.2) were incubated at room temperature. The reaction was stopped with 0.3 ml of cold 10% trichloroacetic acid. After standing for 10 min in an ice bath, the samples were filtered through Millipore filters and washed with 5% trichloroacetic acid. The filters were dissolved in 10 ml of Bray's solution and counted in a liquid scintillation counter.

In Vivo Incorporation of Labeled Compounds. The general method for measuring acid or uracil incorporation was to filter bacteria onto Millipore filters of 0.45-μ pore size and resuspend in minimal medium. After appropriate additions the cells were incubated at 37°; portions were removed at intervals, mixed with an equal volume of 10% trichloroacetic acid, and allowed to stand for 10 min. The samples were filtered onto Millipore filters and the test tube and filter were thoroughly rinsed with 5% trichloroacetic acid. In zero-time controls the radioactivity due to free amino acids remaining on the filter was insignificant. Radioactivity due to free amino acids remaining in the cells after trichloroacetic acid treatment was negligible, since almost no radioactivity was found in experiments carried out in the presence of chloramphenicol. Radioactivity due to amino acids charged to tRNA was also insignificant since the intracellular tRNA content is relatively small.

Results

The Charging of tRNA with Trifluoroleucine. A crude enzyme extract was prepared and the charging reaction carried out as described in Methods. It was found that trifluoroleucine could be charged to tRNA, and the dependence upon substrate concentrations for leucine and trifluoroleucine was determined (Figure 1). The reaction was essentially complete within 5 min with either leucine or trifluoroleucine, but the amount of tRNA charged at the end of this time was about three times greater for trifluoroleucine than for leucine (Figure 2). In this figure is also shown the rate of leucine and trifluoroleucine charging in the presence of a manifold excess of 18 of the other amino acids found in proteins (cysteine was not included). Both leucine and trifluoroleucine were charged at lower levels than in the absence of the other 18 amino acids. Microbiological assay showed that the mixture was free of leucine.

The 20 standard amino acids were then tested individually for their ability to inhibit the charging of tRNA by trifluoroleucine, with the results given in Table I. The charging of trifluoroleucine was inhibited by leucine, isoleucine, phenylalanine, and cysteine, while the charging of leucine was inhibited by trifluoroleucine, isoleucine, and phenylalanine.[1] Cysteine was the only amino acid which inhibited charging of trifluoroleucine without affecting charging of leucine. Inhibition by phenylalanine and cysteine were nearly additive in the trifluoroleucine-charging reaction. None of the other amino acids inhibited the charging reaction of leucine or trifluoroleucine to a measurable extent. The charging of isoleucine and phenylalanine was not inhibited by trifluoroleucine under the experimental conditions used.

Uptake of Trifluoroleucine into Trichloroacetic Acid Insoluble Material in Vivo. A chemostat culture of strain B33 was filtered and resuspended in minimal medium. The suspension was divided in half, made 5×10^{-5} M in leucine-^{14}C and trifluoroleucine-^{3}H, respectively, and incubated at 37°. Samples (1 ml) were removed at intervals and added to 1 ml of 10% trichloroacetic acid. The precipitable counts as a function of time for the two amino acids are shown in Figure 3. There was no lag in trifluoroleucine uptake, and during the first 15 min it was incorporated at a greater rate than leucine (Figure 3, right). Trifluoroleucine incorporation stopped completely within 1 hr while leucine incorporation continued exponentially (Figure 3). Trifluoroleucine uptake was nearly completely inhibited by 100 μg/ml of chloramphenicol. Formation of active β-galactosidase corresponding to the incorporation of trifluoroleucine could not be detected when the culture was simultaneously induced by isopropyl β-D-thiogalactopyranoside.

The extent of incorporation of leucine or trifluoroleucine *in vivo* was then determined in the presence of those amino acids which inhibited the tRNA-charging reaction (Figure 4). Isoleucine and phenylalanine inhibited trifluoroleucine incorporation, but cysteine did not. None of the three amino acids inhibited leucine uptake. The incorporation of leucine-^{14}C was slightly stimulated by cysteine and phenylalanine, perhaps because an increased growth rate was possible when the biosynthesis of these amino acids was spared.

Several experiments were done in which leucine and trifluoroleucine incorporation were compared with the incorporation of histidine. Histidine was chosen because it had no effect on the charging of leucine or trifluoroleucine and since its synthesis is repressed when it is present in the growth medium. The extent of incorporation

TABLE I: Effect of Amino Acids on the Charging of tRNA by Leucine and Trifluoroleucine.[a]

Amino Acid Charged	Amino Acid Added	nmole Bound	% of Control
Leucine	None	0.055	100
	Phenylalanine	0.037	67
	Cysteine	0.054	98
	Phenylalanine plus cysteine	0.035	64
	Isoleucine	0.020	36
	Trifluoroleucine	0.025	45
Trifluoroleucine	None	0.218	100
	Phenylalanine	0.101	46
	Cysteine	0.173	79
	Phenylalanine plus cysteine	0.084	38
	Isoleucine	0.024	11
	Leucine	0.019	9

[a] Reaction mixture: 10^{-9} mole of leucine-^{14}C or 2×10^{-8} mole of trifluoroleucine-^{3}H; other amino acids: 2×10^{-7} mole; incubated for 5 min; other details given in Methods.

[1] Isoleucine and phenylalanine were assayed chromatographically and found to be free of leucine.

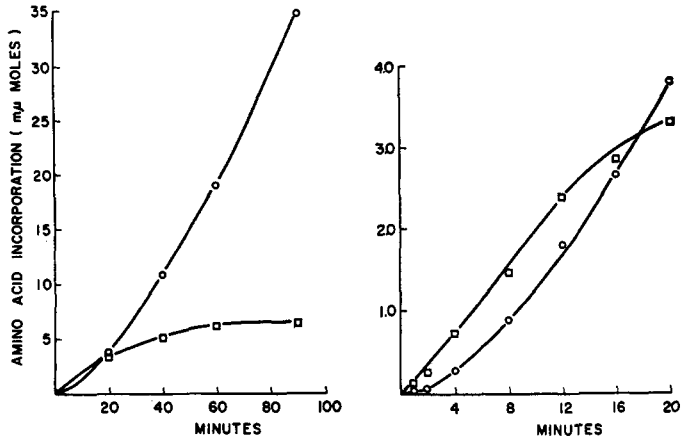

FIGURE 3: *In vivo* rate of incorporation. (O) Leucine; (□) trifluoroleucine (enlarged representation of initial rates on the right).

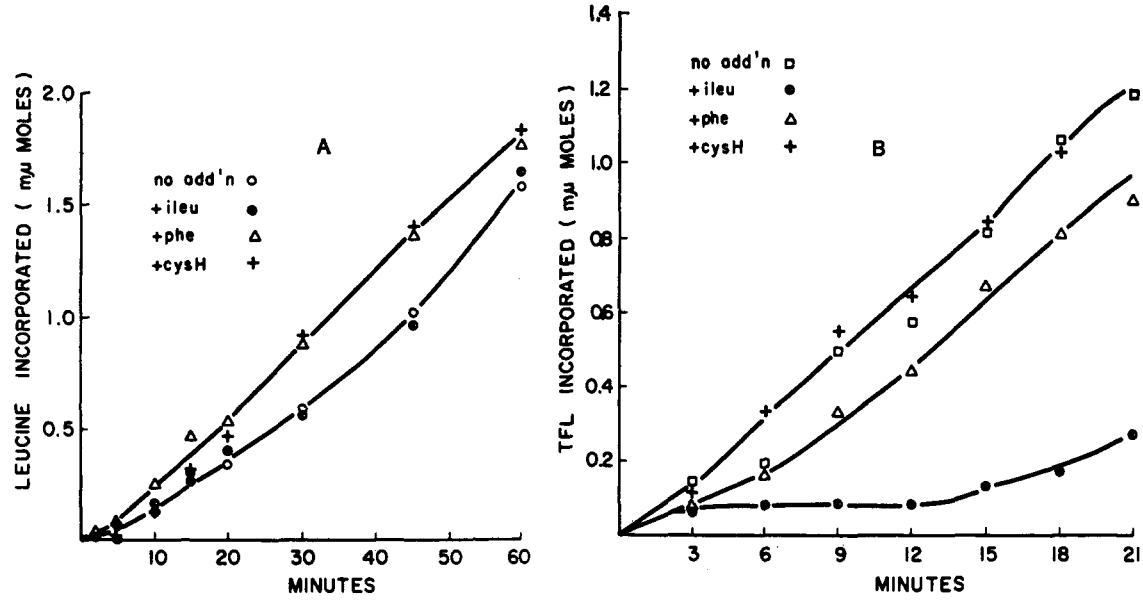

FIGURE 4: *In vivo* rate of incorporation in the presence of a tenfold excess of isoleucine, phenylalanine, or cysteine: leucine on left (A); trifluoroleucine on right (B).

of the labeled histidine added could thus be regarded as a measure of all the histidine used in protein synthesis.

Strain C2F9 was grown in the chemostat with histidine present at a concentration of 10^{-4} M. The culture was filtered, resuspended in minimal medium, and incubated for 10 min. The following mixtures were prepared and incubated at 37°: leucine-^{14}C–histidine; leucine–histidine-^{14}C; trifluoroleucine-^{3}H–histidine; and trifluoroleucine–histidine-^{14}C. All amino acid concentrations were 10^{-4} M. A second set of mixtures was prepared similar to the above except that isoleucine (10^{-4} M), phenylalanine (10^{-3} M), or cysteine (10^{-3} M) was added. The trifluoroleucine to His ratio was always about twice that of Leu to His when no additions were made. Phenylalanine and isoleucine had no effect on the Leu to His ratio, but the trifluoroleucine to His ratio was reduced; cysteine had no effect on either ratio. Figure 5 shows the data obtained in the presence of isoleucine (A) and phenylalanine (B). If both phenylalanine and isoleucine were added the trifluoroleucine to His ratio fell below the Leu to His ratio (Figure 6).

RNA Synthesis in the Presence of Trifluoroleucine. A chemostat culture of strain C2F9 was filtered, resuspended in minimal medium, and incubated for 5 min. To 1-ml portions containing neither leucine nor trifluoroleucine, or 10^{-4} M leucine, or 10^{-4} M trifluoroleucine were added 0.1 μg of uracil-5-^{3}H and 0–5 μg of chloramphenicol. After incubation for 20 min, the reaction was stopped with 10% trichloroacetic acid and radioactivity incorporation was measured (Figure 7). The results show that in the absence of chloramphenicol the uptake of uracil was greatly reduced when trifluoroleucine was substituted for leucine, though it was higher than in the absence of both amino acids. However the uptake of uracil was essentially identical in the presence of trifluoroleucine or leucine if 1–5 μg/ml of chloramphenicol was added to the reaction mixture. This concentration of chloramphenicol was far below the level of 50 μg/ml required to relax RNA synthesis in the absence of amino acids.

The effect of low levels of chloramphenicol on amino acid incorporation is also shown in Figure 7. The ex-

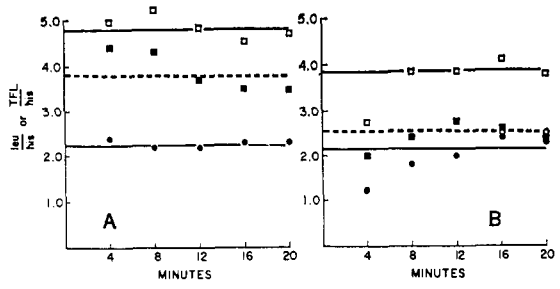

FIGURE 5: *In vivo* Leu to His and trifluoroleucine to His ratios as a function of time. (○) Leu to His; (□) trifluoroleucine to His; (●) Leu to His in the presence of 10^{-4} M isoleucine (A: left panel) or 10^{-3} M phenylalanine (B: right panel); (■) trifluoroleucine to His in the presence of 10^{-4} M isoleucine (A) or 10^{-3} M phenylalanine (B). The lines give the averages of the ratios (◑ indicates that ○ and ● coincide).

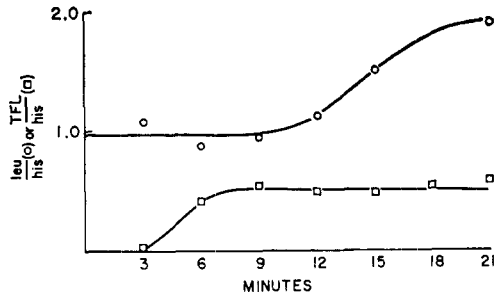

FIGURE 6: *In vivo* Leu to His and trifluoroleucine to His ratios as a function of time in the presence of 5×10^{-4} M isoleucine plus 10^{-3} M phenylalanine. (○) Leu to His; (□) trifluoroleucine to His.

periment was carried out as described above except that radioactive amino acids were used, uracil was omitted, and the incubation was allowed to proceed for 30 min. It can be seen that protein synthesis was only moderately inhibited at a concentration of 2 μg/ml of chloramphenicol which gave maximum stimulation of RNA synthesis in the presence of either leucine or trifluoroleucine.

Discussion

The sorting of amino acids for correct placement in polypeptide chains takes place at the level of activation to AMP–amino acids and charging to tRNA. A single enzyme catalyzes both steps of the reaction and there is specificity at each step, which reduces the chances of error (Bergmann, 1962). Isoleucine aminoacyl synthetase is a known exception in which a mistake can be made at the first step: it activates valine, but does not normally transfer valine to a tRNA (Bergmann, 1962). Valine, on the other hand, partly inhibits the transfer of isoleucine to isoleucine tRNA. Inhibition does not require, therefore, that the inhibitor is actually charged to the tRNA whose charging it inhibits.

The excessive charging of trifluoroleucine relative to leucine suggested that trifluoroleucine could charge to other species of tRNA in addition to leucine tRNA and the inhibition of trifluoroleucine charging by leucine, isoleucine, phenylalanine, and cysteine indicated that trifluoroleucine was charged to the tRNAs corresponding to these amino acids. Leucine charging was inhibited to a lesser extent by isoleucine or phenylalanine (Table I). It has been reported that leucine charging was not affected by other amino acids if present at equal concentrations (Bergmann, 1962). It is likely, therefore, that the inhibition of leucine charging in the experiments reported here was due to the presence of the inhibitory amino acid at 200 times the concentration of leucine; however, it is also possible that the leucine assay used was not sufficiently sensitive to detect the degree of contamination of isoleucine or phenylalanine implied by the observed inhibition. Leucine incorporation *in vivo* (Figures 4 and 5) was not decreased by the presence of any other amino acid(s).

It seemed necessary to show that the results obtained by the charging experiments with trifluoroleucine *in vitro* could also be demonstrated in intact organisms. Trifluoroleucine was incorporated into bacterial polypeptides in the absence of leucine and without any prior adaptation. The probability that the product was of polypeptide nature was supported by its insolubility in 5% trichloroacetic acid, its retention on Millipore filters, the nearly complete inhibition of incorporation by chloramphenicol, and the relatively constant ratio of trifluoroleucine to His incorporated as a function of time. The absence of a lag in the incorporation of trifluoroleucine suggested that there was no difficulty in transporting trifluoroleucine into the cell.

When leucine or trifluoroleucine incorporation was

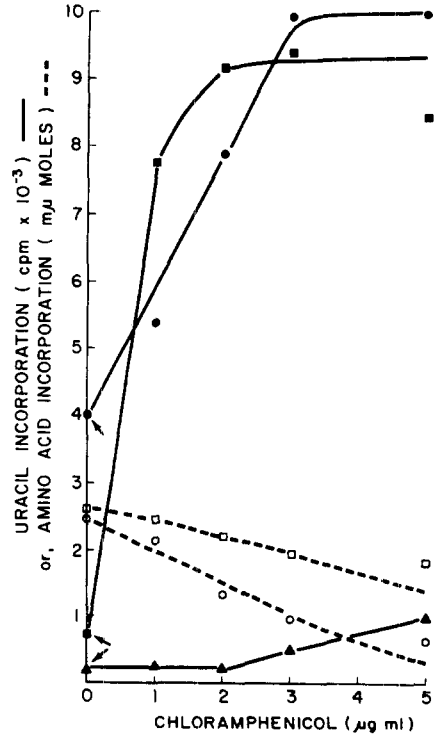

FIGURE 7: Uracil-5-^{3}H uptake as a function of the chloramphenicol concentration *in vivo*. (▲) No leucine or trifluoroleucine; (●) 10^{-4} M leucine; (■) 10^{-4} M trifluoroleucine. Inhibition of amino acid incorporation as a function of the chloramphenicol concentration *in vivo:* (○) leucine-^{14}C; (□) trifluoroleucine-^{3}H.

compared with histidine uptake, the trifluoroleucine to His ratio was always found to be greater than the Leu to His ratio, a result which was in accord with the hypothesis that trifluoroleucine was inserted into other than the leucine positions of polypeptides. The presence of isoleucine and phenylalanine each reduced the trifluoroleucine to His ratio without affecting the Leu to His ratio. Cysteine had no effect but since it is present in relatively small amounts in proteins the experimental procedure may not have been sensitive enough to reveal a change of ratio. When isoleucine and phenylalanine were both added (Figure 6), the trifluoroleucine to His ratio was less than the Leu to His ratio. Under these circumstances the isoleucine and phenylalanine sites were presumably blocked to incorporation of trifluoroleucine. Yu and Rappaport (1966) had reported that there is more than one leucine aminoacyl synthetase in *E. coli*. One of their enzyme fractions could charge leucine to all the leucine tRNAs while another fraction charged only half of them (Yu and Rappaport, 1966). It is not known by which of the charging enzyme fractions trifluoroleucine is recognized. Multiple leucine tRNA synthetases have been found in rabbits and these attach leucine to different species of leucine tRNA (Strehler *et al.*, 1967). It has also been shown that charging enzymes prepared from mouse embryos of less than 12 days of gestation can charge trifluoroleucine to some of the embryonic leucine tRNAs, while enzymes prepared from adult mouse liver charge only traces of trifluoroleucine to leucine tRNA from mouse liver. The difference is due both to the enzymes and to the tRNAs (O. M. Rennert, personal communication).

Although polypeptide synthesis after the transfer of bacteria to trifluoroleucine proceeded initially at a normal rate a large reduction in the rate of RNA synthesis was immediately seen as determined by uracil incorporation. The addition of isoleucine, cysteine, and phenylalanine to trifluoroleucine did not change the rate of uracil incorporation (Fenster, 1967).

Nearly complete cessation of RNA synthesis can result from a nutritional shiftdown (Neidhardt, 1964). After transfer to trifluoroleucine the increase in viable cell count (Fenster, 1967) and the DNA content of the cells do not proceed beyond the values expected for the completion of the division cycle in progress and the rate of polypeptide synthesis decreases (Figure 3). In the usual shiftdown experiment the synthetic activities eventually resume at a new rate while in the presence of trifluoroleucine growth is never recovered. In these experiments the leucine and trifluoroleucine concentrations used were much higher than the actual leucine concentration in the chemostat from which the organisms were obtained and the rate of incorporation of histidine-C^{14} was the same in the presence of trifluoroleucine as in the presence of leucine. It follows therefore that trifluoroleucine does not produce a shiftdown in the rate of polypeptide synthesis equivalent to a simple nutritional shiftdown.

Cessation of RNA synthesis can also occur after removal of an amino acid for which a strain of bacteria is auxotrophic (Gros and Gros, 1956); while the mechanism of this effect is not known there is good evidence that amino acid activation is a prerequisite for normal RNA synthesis (Fangman and Neidhardt, 1964). In the absence of either trifluoroleucine or leucine a low level of chloramphenicol does not relax. If trifluoroleucine were to fail in charging to one of the (rarer) leucine tRNA species it would be expected to be an inadequate substitute for leucine in the control of RNA synthesis. This inadequacy was apparent in the absence of chloramphenicol (Figure 7), but when small amounts (1–2 μg/ml) of chloramphenicol were added RNA synthesis in trifluoroleucine was relaxed to about the same degree as RNA synthesis in leucine (Figure 7). These results could be due to one of several processes: the addition of trifluoroleucine could initiate protein breakdown with release of sufficient quantities of leucine to charge the (rarer) leucine tRNAs not charged by trifluoroleucine. Since leucine at 1 nmole/ml stimulated RNA synthesis to a measurable extent (E. D. Fenster, unpublished data) protein degradation is unlikely to be due to the addition of 2 μg/ml of chloramphenicol alone (Figure 7). The initiation of protein breakdown by trifluoroleucine would require an as yet unknown mechanism. Or, alternatively, trifluoroleucine could interfere with the charging of, without itself charging, some other tRNA, thus reducing the concentration of this charged tRNA and thereby decreasing RNA synthesis. However, RNA synthesis in trifluoroleucine was not increased by the presence of the other 18 amino acids. Under these circumstances addition of small amounts of chloramphenicol could perhaps relax RNA synthesis (Kurland and Maaløe, 1962).

The mechanism of adaptation of leucine auxotrophs to growth on trifluoroleucine remains obscure. Since the relative amount of trifluoroleucine in adapted organisms is close to the amount of leucine present before adaptation, it seems probable that the adapted bacteria had acquired the ability to charge all leucine tRNAs with trifluoroleucine, and to exclude trifluoroleucine from all nonleucine tRNAs.

References

Bergmann, F. H. (1962), *Methods Enzymol. 5*, 708.

Cocking, E. C., and Yemm, E. W. (1954), *Biochem. J. 58*, xii.

Dorfman, L. M., and Wilzbach, K. E. (1959), *J. Phys. Chem. 63*, 799.

Fangman, W. L., and Neidhardt, F. C. (1964), *J. Biol. Chem. 239*, 1844.

Fenster, E. D. (1967), Ph.D. Thesis, University of Chicago, Chicago, Ill.

Gros, F., and Gros, F. (1956), *Biochim. Biophys. Acta 22*, 200.

Kurland, C. G., and Maaløe, O. (1962), *J. Mol. Biol. 4*, 193.

Neidhardt, F. C. (1964), *Progr. Nucleic Acid Res. Mol. Biol. 3*, 144.

Rennert, O. M., and Anker, H. S. (1963), *Biochemistry 2*, 471.

Strehler, B. L., Hendley, D. D., and Hirsch, G. P. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 1751.

Yu, C.-T., and Rappaport, H. P. (1966), *Biochim. Biophys. Acta 123*, 134.