# The action of thienylalanine on protein and ribonucleic-acid synthesis in liver slices

While studying the relationship of ribonucleic-acid (RNA) metabolism to protein synthesis in the liver, we have made some observations which indicate that the action of β-(2-thienyl)-DL-alanine on mammalian cellular metabolism cannot be adequately explained by the hypothesis that it interferes with utilization of phenylalanine during protein synthesis[1].

Tissue slices were prepared from the pooled livers of several male rats and 400 mg samples were suspended in 4 ml Krebs-Ringer bicarbonate solution in flasks. Into different flasks were put either labelled amino acids (1 μc 2-$^{14}$C-glycine or in some experiments 2 μc $^{35}$S-methionine/flask), $^{32}$P-orthophosphate (25 μc/flask) or in a few experiments 8-$^{14}$C-adenine (2 μc/flask). Where required, DL-thienylalanine and L-phenylalanine were added to the medium to give a final concentration of 7 m*M*. After gassing with 95% $O_2$–5% $CO_2$, the flasks were incubated at 37° under aseptic conditions. The reactions were terminated by adding 0.5 vol 30% trichloroacetic acid. For counting of $^{14}$C or $^{35}$S in protein, the precipitate was washed twice with 5% trichloroacetic acid followed by lipid extraction[2] and performic-acid treatment to remove labelled amino acids not bound in peptide linkage[3]. The dried protein specimens were counted at infinite thickness. Flasks containing $^{32}$P were assayed for the specific activity of RNA[4]. In the case of flasks containing $^{14}$C-adenine, adenylic acid was prepared from the tissue RNA by ionophoresis[4], eluted and counted; after determining the P content of the eluate, the specific activity of RNA adenine was computed. Control flasks in which the tissue was inactivated with trichloroacetic acid at the start of incubation showed negligible uptake of all isotopes.

Our first series of experiments demonstrated an inhibitory action of thienylalanine on uptake of isotopes into RNA as well as protein. All flasks were incubated for 3 h and, in a typical experiment, those containing the inhibitor showed a reduction of uptake into protein of 63% for $^{14}$C-glycine and 69% for $^{35}$S-methionine, compared with a reduction of incorporation into RNA of 72% and 67% respectively for $^{32}$P and $^{14}$C-adenine.

In the second series of experiments, incorporation of $^{14}$C-glycine into protein and $^{32}$P into RNA were examined after incubation periods of 1, 2 and 3 h (Table I). Inclusion of thienylalanine

TABLE I

INHIBITORY ACTION OF THIENYLALANINE ON INCORPORATION OF LABELLED PRECURSORS BY THE PROTEIN AND RNA OF LIVER SLICES, AND REVERSAL OF INHIBITION BY PHENYLALANINE*

| *Labelled precursor* | *Time of incubation (h)* | *Uptake by untreated slices* | *Uptake in presence of thienylalanine* | | |
|---|---|---|---|---|---|
| | | | *Alone* | *+ phenylalanine simultaneously* | *+ phenylalanine after 1 h* |
| $^{14}$C-glycine into protein** | 1 | 107 | 84 | 72 | — |
| | 2 | 220 | 92 | 138 | — |
| | 3 | 405 | 76 | 190 | 77 |
| $^{32}$P into RNA*** | 1 | 132 | 96 | 126 | — |
| | 2 | 200 | 89 | 186 | — |
| | 3 | 299 | 89 | 156 | 103 |

* The data are the means of two replications of the experiment.
** The uptake of $^{14}$C-glycine into protein is given in counts/min/planchet.
*** The uptake of $^{32}$P by RNA is given in counts/min/100 μg P.

in the medium had only a slight inhibitory effect on incorporation of labelled precursors into protein and RNA during the first hour of incubation, but thereafter further uptake was completely inhibited. This suggests either that thienylalanine takes some considerable time to attain an inhibitory concentration within the cell, or else that the true inhibitor is a metabolite of thienylalanine. In this experiment, the effect of adding phenylalanine to flasks containing thienylalanine was also examined. When phenylalanine was added at the start of incubation, there was partial reversal of the inhibitory action of thienylalanine. However, when it was added after 1 h of incubation, that is, when inhibition was already complete, there was no reversal of the action of the inhibitor. This suggests that phenylalanine acts competitively by preventing penetration of thienylalanine into the cell. Once the antagonist has entered, phenylalanine can no longer reverse its action on protein and RNA synthesis, presumably because the mode of action of

thienylalanine on cellular metabolism does not involve intracellular competition with phenylalanine.

These findings indicate that the inhibitory effects of thienylalanine may be of a rather general and non-specific character, and we accordingly studied its action on an independent synthetic system not involving phenylalanine, namely the formation of *p*-aminohippuric acid from *p*-aminobenzoic acid and glycine. The liver slices were used in the proportion of 100 mg wet weight to 4 ml Krebs-Ringer bicarbonate medium containing 0.001 *M* *p*-aminobenzoic acid and 0.01 *M* glycine (COHEN AND MCGILVERY[5]). The concentrations of thienylalanine and phenylalanine added to the medium were those used previously. Formation of *p*-aminohippuric acid was measured by the method of COHEN AND MCGILVERY[5].

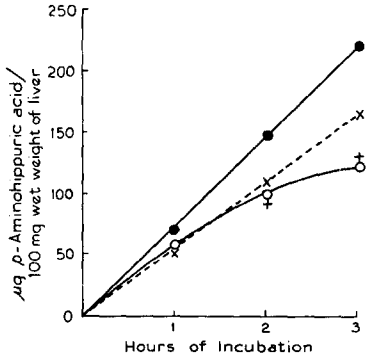


Fig. 1. The inhibitory action of thienylalanine on *p*-aminohippuric acid synthesis by liver slices and the reversal of inhibition by phenylalanine. The data are the means of five replications. ●——● untreated slices; ○——○ thienylalanine alone; ×---× thienylalanine with phenylalanine added simultaneously; + thienylalanine with phenylalanine added after 1 h.

Fig. 1 shows that the pattern of action of thienylalanine on this synthetic reaction is strictly similar to its effect on isotope incorporation into protein and RNA. Less *p*-aminohippuric acid is formed in the presence of thienylalanine, and this effect can be partly reversed by adding phenylalanine to the medium at the start of incubation, but not when added 1 h after incubation has begun. In these experiments it was also established that, unlike phenylalanine, L-α-alanine in 7 m*M* concentration does not reverse the inhibition (data not recorded in Fig. 1).

The findings with *p*-aminohippuric acid synthesis can be adequately accounted for by the explanation already offered for the isotopic data, namely that competition between thienylalanine and phenylalanine is limited to the cell surface and that, once the inhibitor has penetrated the cell, it exerts a more general toxic action. The inhibitory action of thienylalanine on RNA synthesis as well as on protein synthesis is thus likely to be part of a general effect on synthetic processes and does not necessarily mean that there is a direct relationship between the rates of formation of protein and RNA.

These conclusions apply to experiments carried out under our conditions and do not exclude the possibility of a more specific action of thienylalanine on protein synthesis, perhaps at much lower concentrations of the inhibitor (*cf.* RABINOVITZ, OLSEN AND GREENBERG[6]).

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