Rifamycins LXI: In vivo Inhibition of RNA Synthesis by Rifamycins

The action of rifamycin SV and rifamide (diethylamide of rifamycin B[1]) on protein synthesis was first investigated by FRONTALI et al.[2] who found that in cell-free extracts of *Bacillus subtilis* these rifamycins produce a strong inhibition of amino acid incorporation into proteins. Further experiments indicating that the site of rifamide action may be at ribosomial level were successively reported[3,4]. Recently HARTMANN et al.[5] and WEHRLI et al.[6] found that several rifamycins inhibited the DNA directed RNA polymerase reaction in vitro and suggested a direct action on the polymerase enzyme. Similar results were independently obtained by UMEZAWA et al.[7] which reported evidence of inhibition of the initiation of RNA synthesis by rifamycins B and SV in a system including RNA polymerase extracted from *Escherichia coli*.

Since only partial data have so far been reported[4,5] on the in vivo mode of action it appeared interesting to assess whether the inhibition of RNA synthesis or the inhibition of protein synthesis at the ribosomial level was the primary lethal effect of rifamycins on growing microbial cells.

The effect of 80 μg/ml of rifampicin[8] on [C^{14}] uracil and [C^{14}] phenylalanine uptake by growing *E. coli* cells is shown in Figure 1 (on this strain the m.i.c. of rifampicin is about 2 μg/ml). An immediate block of uracil uptake is observed, while the uptake of the amino acid continues although at a reduced rate for about 8 min. This finding indicates that rifampicin penetrates very rapidly into the cells and that the primary effect of the antibiotic is an inhibition of the RNA synthesis. The time elapsing between the block of the uracil uptake and that of phenylalanine is in agreement with the accepted estimate of the half life of messanger RNA.

Similar results were observed (Figure 2) when rifampicin was added to cultures of *B. subtilis*, indicating that the action is not qualitatively different in gram-negative and gram-positive bacteria. The possibility of differences in the mechanism of action of rifampicin and rifamide was ruled out by repeating the experiment on *B. subtilis* using the latter (Figure 3) as the inhibiting agent.

The effect on the DNA synthesis was studied by determining the uptake of [C^{14}]thymine by *E. coli* and *B. subtilis* auxotrophs strains. As shown in Figures 4 and 5 the DNA synthesis continues after the complete inhibition of RNA synthesis at a progressively reduced rate, for about a generation time, indicating that the antibiotic does not interfere directly with DNA synthesis. This is in agreement with the in vitro finding[4] that the DNA directed RNA synthesis is not depressed by rifamycins. The possibility that rifampicin could prevent the utilization of [C^{14}]uracil by inhibiting the conversion of this compound to UTP was also examined. According to the technique proposed by GROS et al.[9], *E. coli* cells were plasmolized to allow the penetration of labelled UTP through the cell membrane barrier. The uptake of

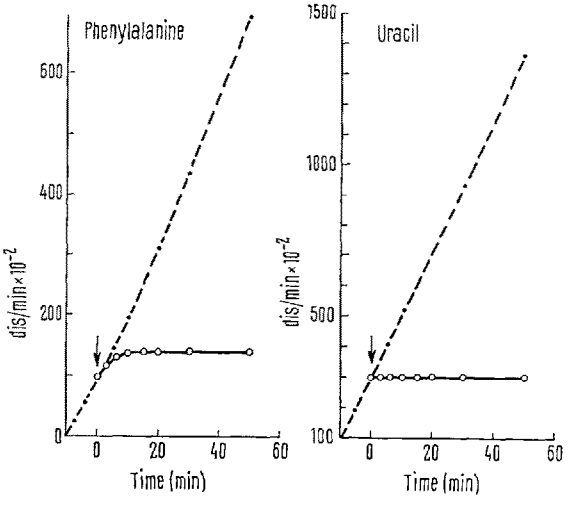

Fig. 1. Effect of rifampicin on phenylalanine and uracil uptake by *E. coli* cells. –●– control, –○–○– rifampicin (80 μg/ml). To 50 ml of *E. coli* cultures growing in Davis medium were added 5 μg/ml of 2-[C^{14}] uracil (sp. act. 1.2 μC/μ*M*) or 10 μg/ml of [C^{14}] phenylalanine (sp. act. 0.73 μC/μ*M*). Rifampicin was added after 10 min to 25 ml aliquots of the cultures. Samples (2 ml) were withdrawn at intervals, diluted with 2 ml of cold 10% TCA and the radioactivity of the insoluble fraction determined in a thin window counter.

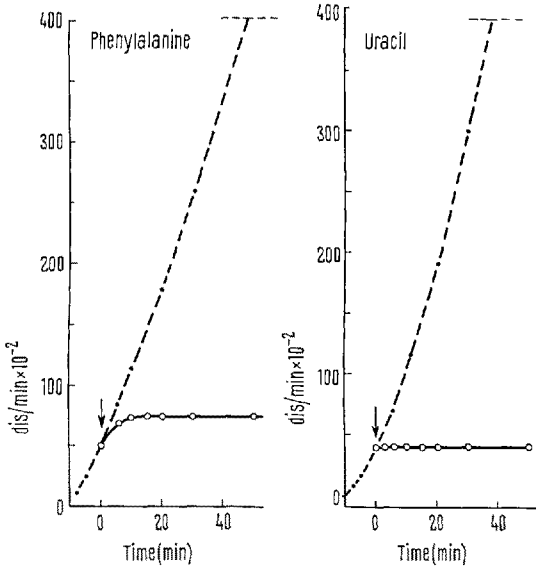

Fig. 2. Effect of rifampicin on phenylalanine and uracil uptake by *B. subtilis*. –●– control, –○–○– rifampicin (0.1 μg/ml). *B. subtilis* was grown in the Von Borstell M 40 medium. Additions and procedure were as described under Figure 1.

[1] N. MAGGI, G. G. GALLO and P. SENSI, Farmaco, Ed. pratica *20*, 147 (1965).
[2] L. FRONTALI, L. LEONI and G. TECCE, Nature *203*, 84 (1964).
[3] C. CALVORI, L. FRONTALI, L. LEONI and G. TECCE, Nature *207*, 417 (1965).
[4] L. FRONTALI and G. TECCE, *Antibiotics (I). Mechanism of Action* (Ed. D. GOTTLIEB and P. D. SHAW; Springer-Verlag, Berlin 1967), p. 415.
[5] G. HARTMANN, K. O. HONIKEL, F. KNÜSEL and J. NÜESCH, Biochim. biophys. Acta *145*, 843 (1967).
[6] W. WEHRLI, J. NÜESCH, F. KNÜSEL and M. STAEHELIN, Biochim. biophys. Acta *157*, 215 (1968).
[7] H. UMEZAWA, M. SATOSHI, H. YAMAZAKI and K. NITTA, J. Antibiot., Tokyo *21*, 234 (1968).
[8] Rifampicin is the common name of 3-(4-methyl-1-piperazinyl-iminomethyl)-rifamycin SV. N. MAGGI, R. PALLANZA and P. SENSI, Antimicrob. Ag. Chemother. 765 (1965).
[9] F. GROS, J. GALLANT, R. WEISBERGER and M. CASHEL, Molec. Biol. *25*, 555 (1967).

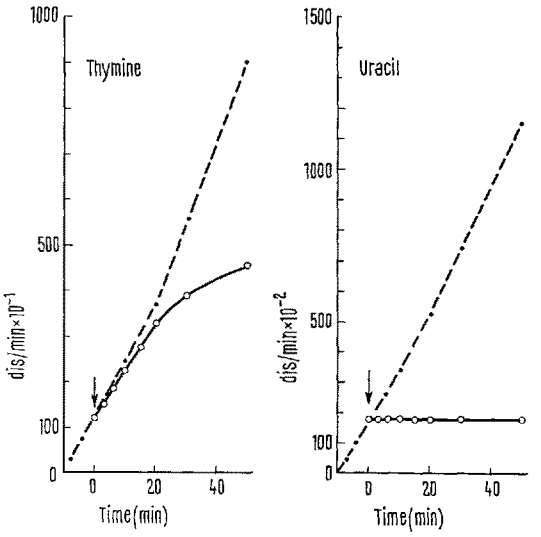

Fig. 3. Effect of rifamide on phenylalanine and uracil uptake by *B. subtilis* cells. –●– control, –○–○– rifamide (1 μg/ml). *B. subtilis* was grown in Von Borstell M40 medium. Additions and procedure were as described under Figure 1.

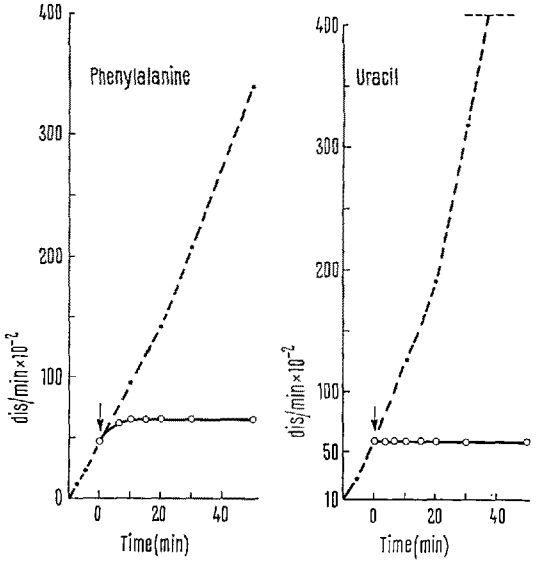

Fig. 4. Effect of rifampicin on thymine and uracil uptake by *E. coli* cells. –●– controls, –○–○– rifampicin 40 μg/ml. A Thy⁻ Me⁻ auxotroph strain of *E. coli* was grown in Davis medium plus 10 μg/ml of thymine and methionine. Procedure as described under Figure 1. Additions: 2[C^{14}] thymine 0.05 μC/ml (sp. act. 58.3 μC/μ*M*); 2[C^{14}] uracil 5 μg/ml (sp. act. 1.2 μC/μ*M*).

[C^{14}]uracil and [C^{14}]UTP into the plasmolized cells was rapidly inhibited by rifampicin (Figure 6).

It can thus be concluded that the experiments with growing *E. coli* and *B. subtilis* cells provide evidence that the primary in vivo effect of relatively high concentrations of rifamycins is the inhibition of the DNA directed RNA synthesis, probably through the inhibition of the RNA polymerase action, in agreement with the in vitro findings reported by other authors. The effect of lower concentration of rifampicin on *E. coli* cells is now under investigation and will be reported elsewhere.

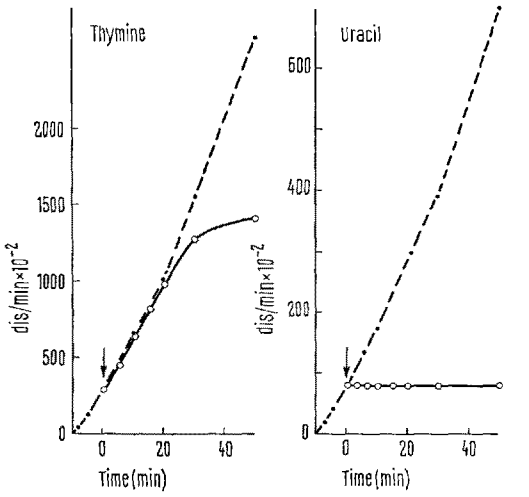

Fig. 5. Effect of rifampicin on thymine and uracil uptake by *B. subtilis* cells. –●– controls, –○–○– rifampicin (1 μg/ml). A Thy⁻ auxotroph strain of *B. subtilis* was grown in Von Borstell M40 medium plus 10 μg/ml of thymine. Procedure as described under Figure 1. Additions as reported under Figure 4.

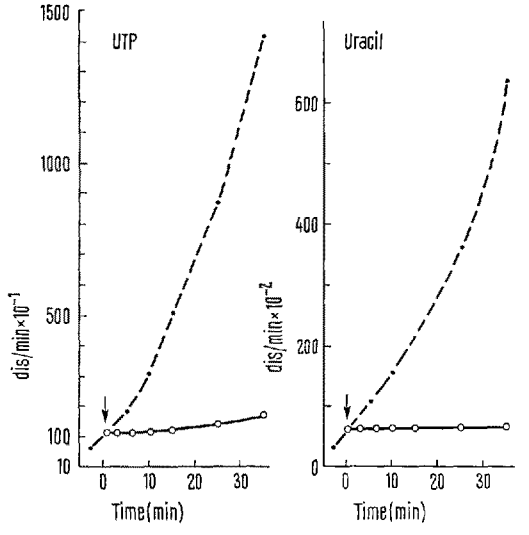

Fig. 6. Effect of rifampicin on UTP and uracil uptake by plasmolized *E. coli* cells. –●– controls, –○–○– rifampicin (80 μg/ml). *E. coli* cells were plasmolized in 2 *M* sucrose in 0.01 *M Tris* (pH 8) and then diluted into Davis medium buffered with 0.05 *M Tris* (pH 7.4) as described by GALLANT and CHASEL[10]. Additions: [C^{14}] UTP 0.05 μC/ml (sp. act. 30.4 μC/μ*M*), 2[C^{14}] uracil 0.05 μC/ml (sp. act. 1.2 μC/μ*M*). Procedure as described under Figure 1.

Riassunto. L'azione della rifampicina e della rifamide su batteri in fase logaritmica è stato studiato determinando l'effetto sull'incorporazione di uracile, timina, fenilalanina e uridina-trifostato marcati con C^{14} da parte di colture di *E. coli* e *B. subtilis*. I risultati indicano che il blocco della sintesi dell'RNA precede quello della sintesi delle proteine e del DNA.

G. C. LANCINI and G. SARTORI

Research Laboratories, Lepetit S.p.A., 20124 Milano (Italy), 14 June 1968.

[10] J. GALLANT and M. CHASEL, J. Biol. *25*, 545 (1967).