RIFAMYCINS: A GENERAL VIEW 1594

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INTRODUCTION

It is probably too early for a conclusive review on the rifamycins. In fact, while the various aspects of their antibacterial activity are adequately understood, the same is not true for their activity on viruses and eukaryotic cells. Therefore, this review will necessarily be unbalanced. Some rifamycin derivatives like rifamycin SV, rifamide, and rifampicin have already been employed in the therapy of bacterial infections; many derivatives, however, are still being used only as tools in studying the molecular biology of bacteria, viruses, and cells.

Two properties make rifamycins particularly interesting for the molecular biologist: (*a*) the high specificity of action against the enzymes involved in the synthesis of polynucleotides; (*b*) the great number of possible chemical modifications of the fundamental molecule, a fermentation product of *Streptomyces mediterranei*, which give rise to derivatives with different specificities against the DNA-dependent RNA polymerase of bacteria and/or of eukaryotic cells, as well as against the RNA-dependent DNA polymerase from tumor viruses and other sources. Up to date, 750 derivatives have been

synthesized at the Lepetit Research Laboratories and several hundred others have been synthesized by Ciba-Geigy. The complete evaluation of the biological activities of all these derivatives will require many years' work.

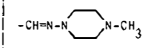
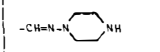
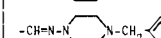
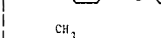
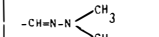
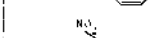
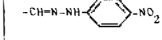
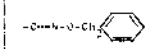
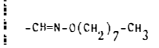
In the last few years, several reviews and papers dealing specifically with rifamycins or with the enzymes against which rifamycins are active have been published. The reviews by Sensi and colleagues (1, 2) belong to the first group. Many reviews on RNA polymerase and transcription are available (3–14, 63, 74); in addition, two collections of papers on transcription have been published (13, 14). Finally, there are more general reviews specifically dealing with rifamycins and other antibiotics active on the synthesis of polynucleotides (15–18, 235, 236).

Papers on the activity of rifamycins against the RNA-dependent DNA polymerase (reverse transcriptase) of tumor viruses can be found in the Proceedings of the 2nd Lepetit Colloquium (19), in two papers still in press (20, 21) and in a recent review on reverse transcriptase published by Gallo (22).

CHEMISTRY

Rifamycins are a family of antibiotics originally isolated in Italy in 1959 (23) from the fermentation broths of *S. mediterranei* (24). Under special fermentation conditions, i.e. in the presence of diethylbarbituric acid, the main product is rifamycin B (XI, Table 1) whose chemical structure was determined in 1964 (25). The steric configuration of the molecule has also been determined by X-ray spectrography (26, 27). The most up-to-date three-dimensional model is shown in Figure 1. Structurally, the rifamycins consist of a naphthoquinonic chromophore which is spanned by an aliphatic ansa chain between the nitrogen on C-2 and the oxygen on C-12 of the chromophoric moiety. Rifamycin SV (I, Figure 2 and XIII, Table 1) is derived from rifamycin B (the natural fermentation product) by removal of the glycolic group bound to C-4 of the naphthoquinone. Rifamycin SV has been used since 1962 under the trademark Rifocin in the parenteral treatment of tuberculosis. Other derivatives with antibacterial activity have been obtained by chemical modification of the natural molecule (rifamycin B) and of its derivatives. In 1961, even before the complete structure of the rifamycins was elucidated, several derivatives with antibacterial activity were obtained (starting from rifamycin O, II, Figure 2) by condensation at C-4 of the chromophoric group with aromatic amines, hydrazides, aminohydrazones, and aminoguanidines (28, 29). The diethylamide of rifamycin B (commonly known as rifamide, XII, Table 1) was synthesized in 1963 and used in therapeutic treatment under the trademark Rifocin M (30). In 1966, the phenazynic derivatives such as rifazine (IV, Figure 2) (31) and phenoxazynic derivatives were obtained (32). In 1966, an interesting intermediate was also obtained: the 3-formylrifamycin SV (XIV) from which several derivatives with remarkable biological activity were in turn obtained (33, 34). From a therapeutic point of view the most important of these products is a 3-(4-methylpiperazinoiminomethyl) derivative of rifamycin SV commonly known as rifampicin (in the

TABLE 1 : RIFAMYCIN DERIVATIVES (I)

ROMAN NUMBER	NAME OR LEPETIT CODE NAME		R'	R''
XI	rifamycin B		-H	$-OCH_2COOH$
XII	rifamide		-H	$-OCH_2CON(C_2H_5)_2$
XIII	rifamycin SV		-H	-OH
XIV	3-formyl rifamycin SV		-CHO	-OH
XV	3-piperazino-	(Ciba-Geigy)		-OH
XVI	3-morpholino-	(Ciba-Geigy)		-OH
XVII	rifampicin	AF/AMP		-OH
XVIII	N-demethyl rifampicin (3-piperazinoiminomethyl)	AF/AP		-OH
XIX	3-(4-benzylpiperazinoimino-methyl) of 3-formyl rifamycin SV	AF/ABP		-OH
XX	3-(4-benzyl-2,6-dimethyl piperazinoiminomethyl) (cis)- of 3-formyl rifamycin SV	AF/ABDP		-OH
XXI	dimethylhydrazone of 3-formyl rifamycin SV	AF/DMI		-OH
XXII	diphenylhydrazone of 3-formyl rifamycin SV	AF/DFI		-OH
XXIII	2-4-dinitrophenylhydrazone of 3-formyl rifamycin SV	AF/DNFI		-OH
XXIV	O-benzyloxime of 3-formyl rifamycin SV	AF/BO		-OH
XXV	O-diphenylmethyloxime of 3-formyl rifamycin SV	AF/05		-OH
XXVI	O-butyloxime of 3-formyl rifamycin SV	AF/010		-OH
XXVII	O-n-pentyloxime of 3-formyl rifamycin SV	AF/012	$-CH=N-O(CH_2)_4-CH_3$	-OH
XXVIII	O-n-octyloxime of 3-formyl rifamycin SV	AF/013	$-CH=N-O(CH_2)_7-CH_3$	-OH
XXIX	O-3-Phenylpropyloxime of 3-formyl rifamycin SV	AF/015		-OH

United States as rifampin (XVII, Table 1), and under the trademarks Rifadin and Rimactane (35, 36). Because of its activity against *Mycobacterium tuberculosis* rifampicin is regarded as a major antituberculosis drug. It is also used in the treatment of infections caused by Gram-positive and Gram-negative bacteria. The range of chemical modifications of rifamycins has not yet been fully explored. Recently, a series of O-alkyl and arylalkyl oximes of the 3-formyl rifamycin SV (XXIV, XXV, XXVI, XXVII, XXVIII, XIX) has been synthesized which prove particularly active on the RNA-dependent DNA polymerase of RNA tumor viruses (20, 21, 37).

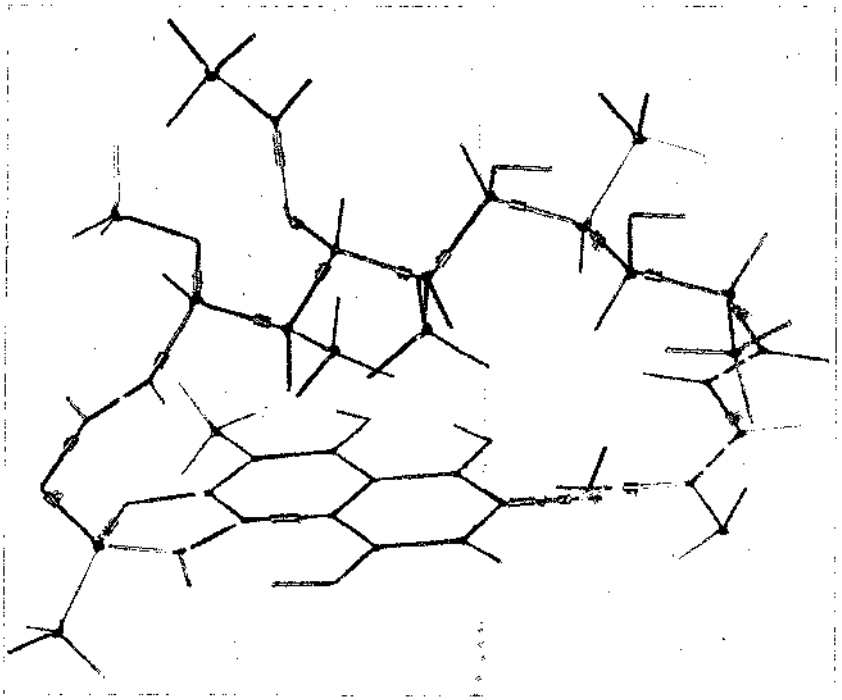


FIGURE 1. Dreiding stereomodel of rifamycin SV based on X-ray data (26). (By courtesy of G. G. Gallo.)

Additional studies on the chemical structure of rifamycins produced by *S. mediterranei* when grown in the absence of diethylbarbituric acid are still under way.

Other antibiotics which, like rifamycins, are composed of a chromophoric nucleus spanned by an aliphatic ansa are known, and they are grouped under the common name of ansamycins. Streptovaricins (X, Figure 2) (38–41) and tolypomycins belong to this group (42–44). Streptovaricins have antibiotic activities closely resembling those of rifamycins, they have the same mechanism of action (45, 46), and they show cross-resistance with rifamycins (47–50). Some streptovaricins also exhibit an activity on poxviruses and on RNA-dependent DNA polymerase (51, 52).

RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND ANTIBACTERIAL ACTIVITY

Even before knowing the mechanism of action of rifamycins, Sensi and colleagues (1) faced the problem of establishing which functions of the molecule were necessary for the antibacterial activity, in order to synthesize derivatives with improved biological properties as compared to those of the nat-

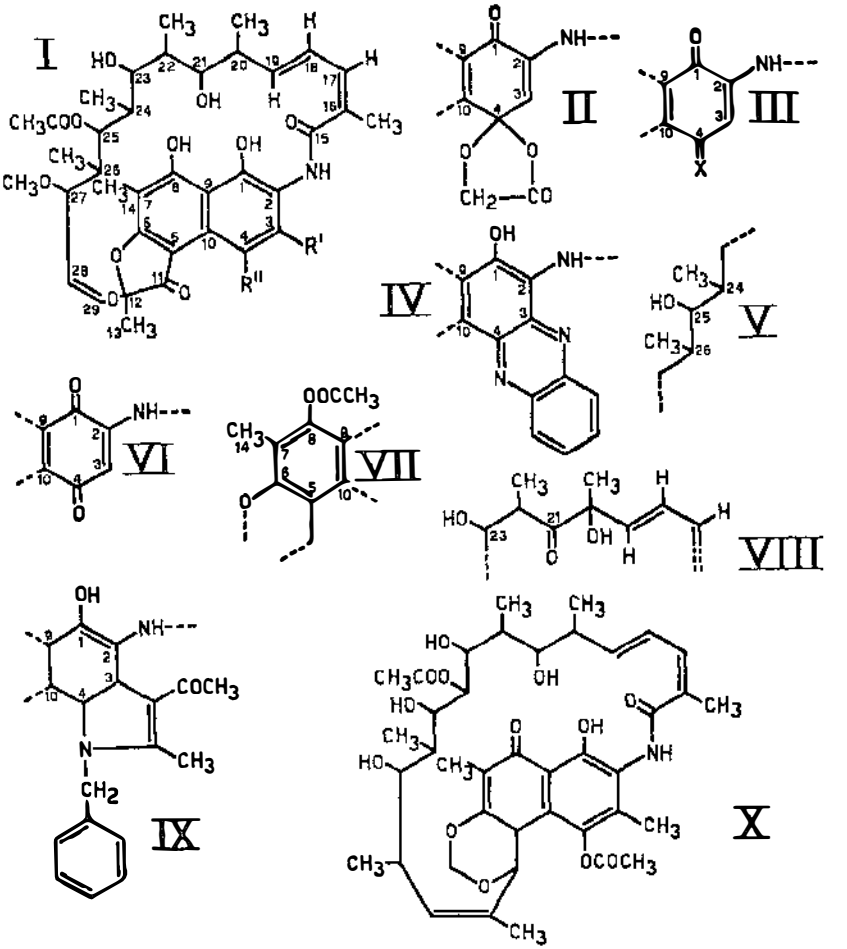


FIGURE 2. Chemical structures of rifamycins: I: Structure of rifamycin SV derivatives (for R′ and R″ see Table 1). II: Partial structure of rifamycin O. III: Partial structure of quinone rifamycins. IV: Partial structure of rifazine. V: Partial structure of 25-O-deacetylrifamycins. VI: Partial structure of rifamycin S. VII: Partial structure of 8-O-acetylrifamycins. VIII: Partial structure of rifamycin Y. IX: General structure of pyrrolorifamycins. X: Structure of streptovaricin D.

ural product of fermentation (rifamycin B). They found that modifications of the ansa chain are generally accompanied by loss of activity; in particular, it was found that the presence of both hydroxyl groups at the C-21 and C-23 positions is required for biological activity. Rifamycin Y (VIII, Figure 2) that has a keto group at the C-21 position is inactive. Hydrogenation of the

double bonds in the ansa chain reduces the antibacterial activity of the molecule presumably by affecting the rigidity of the ansa chain. On the contrary, it was found that the desacetylation of the group bound at the C-25 position, while reducing the activity against Gram-positive bacteria, does not affect the activity against *M. tuberculosis*. This modification affects the ability of the molecule to penetrate into bacteria, as was confirmed in 1969 (52) when the 25-O-desacetylrifamycin (V, Figure 2) was found to be active against isolated RNA polymerase. The chromophoric moiety alone has no activity. The presence of a free carboxyl group in the glycolic moiety of rifamycin B reduces the ability of this derivative to penetrate the cells as proved by the fact that the dialkylamide derivatives of rifamycin B, in which the carboxyl group is blocked, are active on whole bacteria (1, 53) and by the fact that rifamycin B is active on the bacterial enzyme (52, 54, 55). The quinone or the hydroquinone form of the chromophoric group are equally active (1, 53). The presence of the hydroxyl group at the C-4 position is not required for biological activity as shown in 4-deoxyrifamycin SV and in several other derivatives with a C-4 substitution (1). The presence of the hydroxyl group in the C-8 position is necessary, as proved by the fact that 8-O-acetylrifamycin S is inactive on RNA polymerase. It should be pointed out that the hydroxyl group in C-21, C-23, and at C-1 or C-8 positions are present in streptovaricin, tolypomycin and in all rifamycins active on RNA polymerase. These four hydroxyl groups, however, are also present in the 16–17–18–19–28–29 hexahydrorifamycin SV (where all the double bonds of the ansa are hydrogenated) which is poorly active on RNA polymerase (52), indicating that not only their presence is required but also that their steric position must not be changed. The condensation of side chains at the C-3 and/or C-4 positions allows one to obtain the majority of derivatives that retain antibacterial activity. Some of these derivatives are also active on RNA polymerase purified from rifampicin-resistant mutants (unpublished data). The condensation at the C-3 position of rifamycin SV of an N-bound cyclic amine confers activity against rifampicin-resistant staphylococcal mutants; this activity increases with the size of the nitrogenated ring.

According to Knüsel and co-workers (56), these derivatives are not active against rifampicin-resistant RNA polymerase but exhibit a different mechanism of action which, however, has not been identified. Also, the condensation at the 3-formylrifamycin SV of O-alkylhydroxylamines generates a series of derivatives active against rifampicin-resistant bacterial mutants, and also active against the RNA polymerase of such mutants (R. J. White, personal communication). Other products active on rifampicin-resistant mutants have been obtained by reacting the 3-formylrifamycin SV with substituted 1-aminopiperazines. Furthermore, derivatives active on rifampicin-resistant mutants have been obtained by condensation on rifamycin S derivatives of substituted β-aminoacrylamides, esters and ketones (pyrrolorifamycins, IX, Figure 2). Apparently, a side chain linked to C-3 and/or to C-4 positions increases the affinity of the molecule for the rifampicin-resistant RNA polymerase.

Mechanism of Antibacterial Action

Activity on whole cells.—The derivatives that have been most widely used in these studies are in the order: rifampicin, rifamycin SV, rifamycin B and rifamide.

Rifampicin exhibits bactericidal activity on Gram-positive and Gram-negative bacteria and on mycobacteria (57, 58). Also, Chlamydozoaceae (trachoma agent), which are considered unusually small bacterial cells, are reported to be inhibited by rifampicin and by some rifamycin SV derivatives (233). The primary target of rifampicin on whole bacteria is the synthesis of RNA (57, 59). Following the addition of rifampicin, the rate of RNA synthesis decreases exponentially with time after a short lag period. Both the lag and the slope of the decrease in rate are concentration-dependent (60, 62, 72). Ribosomal, transfer, and messenger RNA synthesis are equally affected by rifampicin (61). Protein synthesis stops later, after a time interval corresponding to the life of messenger RNA. The synthesis of DNA goes on until a round of replication has been completed.

Activity on DNA-dependent RNA polymerase.—The isolation and the characterization of the enzyme which transcribes DNA have been described elsewhere (3–14, 63, 74). This enzyme has been found to be the target of rifamycins (54, 55, 64). The synthesis of RNA in vitro requires the presence of the enzyme, of the DNA template, of bivalent ions (Mg^{2+}, Mn^{2+}), and of the 4 nucleoside-triphosphates. The reaction takes place in several steps: binding of the enzyme to template, initiation of polymerization, RNA chain elongation, chain termination, and enzyme release.

Rifamycins bind to and inactivate free enzyme but do not interfere with complex formation (64). One mole of rifampicin bound to one mole of RNA polymerase is sufficient to inhibit the RNA synthesis. The binding is rapid and the stability of the complex (enzyme+antibiotic) is temperature-dependent (76, 77). Rifamycins are also inhibitory if added to the enzyme-template complex before the addition of purine riboside-triphosphate. Therefore, complex formation per se does not protect the enzyme against rifamycins. Rifamycins are not inhibitory if added after chain elongation has started (64, 65), or when the enzyme-template complex is preincubated with the purine riboside-triphosphates, which are found at the 5′ terminus of RNA molecules (64). If the enzyme-template complex is preincubated at temperatures above 17°C it becomes partially resistant to rifamycins (66–68); this effect has been attributed to DNA denaturation (69, 70) and to enzyme conformational changes (71). So & Downey (73), however, have suggested that the partial protection of the enzyme-template complex above 17°C could be due to residual traces of triphosphates contaminating the reaction mixture.

The resistance of the complex is lost if triphosphates are not added at the same time as rifampicin (68). These findings induced Travers to hypothesize the existence of two forms of complexes which are in equilibrium, rifampicin blocking the transition from form I to form II; form II possibly being the one

which is able to bind the first purinetriphosphate (74, 75) and is rifampicin-resistant. Sippel & Hartmann have shown that there are two classes of enzyme-DNA complexes, one when the enzyme is specifically bound to promoters (in the presence of σ factor) and another when the enzyme is bound unspecifically to DNA. Of these two complexes the first one is the most rifampicin-resistant (68). This fact has provided an opportunity for estimating the number of promoter sites in various phage DNAs (66).

Mutation to Rifampicin Resistance

Spontaneous mutants can be quite easily isolated by plating about 10^8 bacteria on agar dishes containing bactericidal concentrations of rifampicin. Such mutants have been found in all the bacterial species investigated. The mutation rates to rifampicin resistance have been evaluated in *Staphylococcus aureus* and *Bacillus subtilis* (56) and also in *M. tuberculosis* (115, 127). Single-step mutants with different levels of resistance can be isolated varying from slightly to highly resistant (>2000 μg/ml). All resistant mutants (whatever the level of resistance) from which an RNA polymerase has been purified, possess a modified enzyme that is more resistant to the drug when tested in vitro (48, 49, 78–90). The majority of these data were obtained with *Escherichia coli,* but similar results have also been obtained with other bacterial species such as *S. aureus* (81), *B. subtilis* (91–95), and *Mycobacterium smegmatis* (96, 114). The level of resistance of a strain depends on two factors: (*a*) the intrinsic level of resistance of the RNA polymerase, (*b*) the permeability of the cell to the drug. This is clearly shown in *E. coli* where, in the majority of cases, cells are a hundred times more resistant than the purified enzyme. On the contrary, *S. aureus* cells and their RNA polymerase are almost equally sensitive. Finally, strains of *E. coli,* highly sensitive to rifampicin, such as RS522 (derived from PA607) have been obtained by mutagenic treatment and have an altered cell wall (97). In fact, they also turn out to be more sensitive than the parental strain to streptolydigin and other antibiotics. In addition, they may have lost the cell wall receptors for T7 and λ phages (our unpublished data). The introduction of certain R factors carrying resistance to other antibiotics can decrease the level of resistance to rifampicin in both *rif-r* mutants and sensitive strains without affecting the RNA polymerase, and this effect is due to a permeability change (98).

As yet, no *rif-r* mutants have been isolated that owe their resistance to a decreased permeability, but this might only be due to a rather low frequency of occurrence in comparison with that of the RNA polymerase mutants.

All the rifampicin-resistant mutants that have been mapped in *E. coli* either by conjugation (78–80, 82–87) or by transduction (48, 49, 101), are located near the *arg* H locus. Temperature-sensitive mutants have also been found but, although they also map near the *arg* H locus, it is not completely clear whether they are in the same cistron (49, 84, 99, 100, 181). A temperature-sensitive mutation has been isolated that seems to be located in a subunit

other than β (99). A new class of mutants has been recently isolated in *E. coli* (180). These mutations have been called *groN* since they block phage λ development by interference with the action of the product of phage gene *N*. This mutation is closely linked to the rifampicin resistance locus; purified RNA polymerase from *groN* mutants is more sensitive to rifampicin than the enzyme from *gro*$^+$. It is suggested that the *groN* mutation produces a structural change in the bacterial RNA polymerase such that it can no longer interact properly with the phage *N* product. The fact that different *rif-r* mutants (with different levels of resistance) can be isolated indicates that the binding site for rifampicin involves many amino acids.

The binding site for rifampicin is located on the so-called core enzyme (103). Certain evidence has been gathered indicating that the binding site for the drug is located on the β subunit. Zillig and collaborators (89, 102) have isolated the RNA polymerase from five independent *rif-r* mutants, finding that the β subunit of one of such enzymes had a different electrophoretic mobility. Furthermore, they have shown that rifampicin binds and cosediments with the β subunit of a wild-type enzyme. In addition, the appropriate reconstitution experiments have been carried out with the separated subunits of resistant and sensitive enzymes (102).

Rifampicin-resistant mutations have also been mapped in *B. subtilis* (230). Some *B. subtilis rif-r* mutants form spores with altered morphology (231). There is certain evidence that the β subunit in *B. subtilis* also is involved in rifampicin resistance (91–93). Some *rif-r* mutants of *B. subtilis* are unable to sporulate. Since during sporulation one of the β subunits undergoes a proteolytic cleavage, it has been suggested that the *rif-r* mutation modifies this subunit in such a way as to make it inaccessible to the cleaving enzyme (92). The *rif-r* enzymes have a lower binding affinity for rifampicin and the affinity is directly related to the level of resistance (15). There is no clear-cut information about the exact location of the genes for the other subunits, although it has been suggested that in *E. coli* they could map near the *rif* gene and be regulated as a single operon (104, and Zillig, personal communication).

In *E. coli* merodiploids, the question of the dominance of the *rif-r* mutation has been investigated. Cases both of dominance and of recessiveness have been reported (48, 49, 99, 105, 106, 179, 181).

According to Babinet, the phenotypic effect of the *rif-r* mutation in the heterozygous strains varies greatly from one *rif-r* mutation to another (179). The *rif-r* allele can be in fact either dominant to the wild-type *rif-s* allele or partially dominant or recessive, but in all cases the enzyme extracted from the heterozygotes has a higher resistance to the drug than the enzyme extracted from the wild type. According to this author, in *rif-s/rif-r* merodiploid strains there is no evidence of two populations of RNA polymerases, and he interprets his results by hypothesizing some kind of intramolecular complementation between sensitive and resistant β subunits. Other authors claim that the *rif-r* mutation is, as a rule, recessive to the *rif-s* and propose an

explanation that is in conflict with the one given by Babinet (105, 106, 181). All these problems require further consideration since, in some cases, the techniques employed have not always been sensitive enough to detect possible cases of different levels of intermediate dominance. In mero-diploids it has been possible to exploit the recessiveness of some *rif-r* mutations to isolate strains carrying lethal chromosomal mutations in the *rif* gene (*rif-O*) Some of them have been shown to be suppressed by amber suppressors (105, 107).

Effects on Phages and Plasmids

It has been shown that growth of some DNA phages is dependent on the host RNA polymerase or at least on its β subunit. In fact, rifampicin blocks the multiplication in *rif-s* strains of a number of *E. coli* phages such as T4 and λ (108–110), and of *B. subtilis* phages such as β 22 (111) and SPO1 (94), while it does not affect the development of the same phages in *rif-r* mutants. Some DNA phages such as T7 and T3 are rifampicin-sensitive only in the early stages of infection. It has been shown that the early genes of these phages are transcribed by the host polymerase and that the product of the early genes is a new phage-specific and phage-coded RNA polymerase which is rifampicin-resistant (112, 113) and which transcribes the late genes.

The conversion of single-stranded DNA of phage M13 into the double-stranded replicative form in *E. coli* is blocked by rifampicin in the rifampicin-sensitive host but not in a *rif-r* mutant (121). This observation has been interpreted as evidence that host RNA polymerase has a direct role in the initiation of M13 DNA replication, possibly through the synthesis of a primer RNA to which the new DNA molecule would be covalently attached (121). The other single-stranded DNA-phage ϕX174 seems, however, to behave differently (122). We have evidence that rifampicin does not interfere with the DNA replication of T4 phage (our unpublished data).

The development of RNA phages such as f2, Qβ, and MS2 in *E. coli* cells proceeds at a nearly normal rate if rifampicin is added four minutes after infection or later, despite the fact that host RNA and protein synthesis are completely shut off (82, 116–118). Addition of the drug before or in the first minutes after infection partially inhibits phage development. In order to explain these findings it has been suggested that the host RNA polymerase could use the double-strand replicative intermediate as a template for single-strand (plus) viral RNA synthesis (82). This hypothesis is supported by the fact that the growth of MS2 phage in *rif-r* mutants is not affected by the drug. It has also been observed that rifampicin prevents the release of MS2 phages from host bacteria, and that this effect can be separated from the inhibitory effect of the drug on RNA phage synthesis (119, 120). Phage release is unaffected when the host possesses a rifampicin-resistant RNA polymerase. These results indicate that a host-controlled protein plays an essential role in the release of RNA phage particles. Similar results have been reported in the case of Qβ phage assembly (143). The expression of some episomal

genes in *E. coli* is preferentially inhibited by rifampicin. The effect is at the level of transcription and concerns male-specific functions such as phage adsorption to F-pili, ability to promote conjugal transfer, and restriction of T7 phage (123). Curing of R factors by rifampicin at subinhibitory concentrations of the drug has been reported in *S. aureus* (125) and in *Salmonella* (126), and curing of F-episomes in *E. coli* (123, 124) has also been found. A possible link btween inhibition of F-gene expression and F-curing has been suggested (123). The curing effect is strictly concentration and temperature dependent (our unpublished data).

Studies on Bacterial Transcription

A certain number of authors have utilized rifampicin either to study or to confirm some aspects of transcription in bacteria. The exponential rate of decay of RNA synthesis following rifampicin treatment has been regarded as evidence for size heterogeneity of mRNA. The time required for full inhibition of transcription has been used to estimate the maximum size of such RNA molecules. According to these results, long operons such as the histidine operon with 13,000 nucleotides occur very infrequently (60). An analysis of the RNA synthesis after addition of rifampicin to exponentially growing cultures of *E. coli* (72) has led to the following conclusions: (*a*) the fraction of active polymerases engaged in mRNA synthesis is inversely related to the growth rate; (*b*) the number of active polymerases per unit dry weight increases with growth rate; (*c*) the rate of RNA chain elongation and the half-life of mRNA remain essentially constant at growth rates between 0.6 and 2.5 doublings/hour; (*d*) a pool of unengaged polymerases exists at low growth rates; (*e*) rRNA cistrons appear to be linked and at least part of tRNA is coded in polycistrons (72, 130). The independence of the rate of chain elongation of rRNA from growth rate in *E. coli* has been also observed by others (128).

The average chain length of the transcripts from which tRNAs are derived has been estimated to be between 130 and 210 nucleotides (129). Experiments involving rifampicin show that 5S rRNA is generated from a transcript that is between 13 and 30 times longer than the 5S molecule (62). *Rif-r* mutants of *E. coli* and of *S. aureus* bind smaller amounts of (^{3}H)-rifampicin than the corresponding *rif-s parents*. It has been suggested that the difference between the amount of drug bound by *rif-r* and *rif-s* strains represents rifampicin complexed with RNA polymerase (EC.2.7.7.6.). From these data an average number of 1500 RNA polymerase molecules per *E. coli* cell can be estimated (142).

Inhibition of initiation of transcription by rifampicin has been used to show that even if the elongation rate of mRNA of the tryptophan operon is not significantly affected by changes in the cell generation time it is, however, dependent on temperature (131). The results indicate that rifampicin blocks transcription initiation in vivo and that the block occurs at or close to the site

of tryptophan repression, thus confirming the close proximity of promoter and operator genes (132). On the other hand, it has been claimed that by using rifampicin it is possible to show that λ repressor interferes with the binding of RNA polymerase to promoter sites (133, 134). A similar result has been obtained with the *lac* repressor: when *lac* repressor is preincubated with *lac* DNA the formation of the rifampicin-resistant complex is inhibited (135). It has also been shown that rifampicin stops the process of induction of β-galactosidase at the same step as deinduction by inducer removal without any detectable effect on the expression of preformed messenger RNA (234). In a cell-free system both cyclic AMP (cAMP) and cyclic AMP receptor protein (CRP) are required during the preincubation period with *lac* DNA in order to form the rifampicin-resistant complex. Apparently both cAMP and CRP are required for the binding of RNA polymerase to *lac* promoter (135). Similar results have been reported for the in vitro transcription of the *Gal* operon (237).

A case has been reported in which rifampicin seems to fail in blocking the synthesis of an enzyme: the early rapid (phase 1) synthesis of the extracellular protease by *Bacillus amylolique faciens* is, in fact, insensitive to rifampicin. One possible explanation could be that the synthesis of this protein is not dependent on an RNA with a rapid turnover (136).

Experiments have been reported in which rifampicin not only fails to prevent the induction of acetylornithine transaminase by arginine in *E. coli,* but is able to induce it. In order to exert this activity, rifampicin has a target that is likely to be different from transcription (137). This effect could possibly be due to the interaction of the drug with the ribosome-arginine-regulatory-protein complex. In this connection it has been claimed that rifampicin affects the structure of ribosomes in *E. coli* growing cells, as revealed by the slower sedimentation velocity (138). A similar observation has been made when growing cells of *B. subtilis* were treated with rifamide (diethylamide of rifamycin B) (139, 140). In both cases, the ribosomes were not altered by treatment in vitro. It has also been observed that rifampicin inhibits the formation of phage-specific polyribosomes in R17-infected cells (149). Finally, it should be mentioned that preincubation of *E. coli* with rifampicin seems to increase markedly its sensitivity to gamma irradiation, thus suggesting a possible involvement of transcription in the repair mechanism (141).

Action on Eukaryotes

One of the most interesting aspects of rifamycins is that some of them, in addition to or alternative to their action on bacterial RNA polymerase, possess some activity against a certain number of nucleotide polymerizing enzymes present both in eukaryotes and in (or coded by) viruses. Furthermore, it has been claimed that rifamycins can interfere, by a different mechanism of action, with the maturation of some viruses. It is not easy to trace a clear picture of all these activities partly because only a few derivatives have been systematically screened in the various systems, and because in many cases the

test systems are not always sufficiently perfected to give a clear-cut answer. Furthermore, different systems have been used by different authors. It must also be kept in mind that in bacteria, enzymes other than DNA-dependent RNA polymerases could be affected by some rifamycins. In fact, while rifamycin B, rifampicin, and 3-morpholino derivative (XVI) of rifamycin SV are not active on *E. coli* DNA-dependent RNA polymerase (Kornberg enzyme) (55), rifamycin SV is reported to be active on it when tested with a synthetic poly d AT template (21). Many other rifamycins have yet to be tested on this enzyme, therefore caution must be exercised by those researchers who are currently using different rifamycin derivatives. Unfortunately, nothing is known at present about the nature of the binding of rifamycin SV to DNA polymerase and about the stage of the reaction that is blocked by the drug.

It has been claimed that 8-O-acetylrifamycin S (VI, Figure 2), which is inactive against bacterial RNA polymerase (1, 52), is active on trachoma agent infecting FL cells, but not when infecting embryonated eggs. This observation has been considered evidence for a second mechanism of action of some rifamycins against trachoma (144), as this product is inactive against RNA polymerase. However, there is no proof that inside the cells 8-O-acetylrifamycin S could not be converted, at least in part, into rifamycin S (VI, Figure 2). The problem of possible intracellular conversion of the administered molecule has to be kept in mind, in consideration of the possibility of a change of specificity of action. It has been written in a recent review dedicated to rifamycins that "in general, the nuclear RNA synthesis of eukaryotes is not affected by rifamycin" (15). This statement is correct for many rifamycins including those used in therapy, but other derivatives do show specific inhibitory properties on eukaryotic polymerases.

Rifampicin and rifamycin SV have been tested against crude extracts of DNA-dependent RNA polymerase from rat liver nuclei (145) and from ascites cells (64). Rifampicin has been shown to be inactive when tested on solubilized, DNA-dependent RNA polymerases from rat nuclei (146), from lymphoid tissue (147), and from human placenta (148).

Although rifampicin does not inhibit the DNA-dependent RNA polymerases purified from calf thymus (P. Chambon, personal communication), it was recently found that several oxime derivatives of rifamycin SV (among them AF/013, XXVIII, Table 1) do inhibit the above-mentioned enzymes by blocking the initiation of transcription. This has established that calf thymus (B) enzyme is able to recognize specific initiation sites on calf thymus DNA which are different from those recognized by enzyme A1 or by *E. coli* RNA polymerase (150). Very similar results have been obtained for the transcription of rat liver chromatin, using form A1 and form B RNA polymerases purified from rat liver (151). Endogenous RNA synthesis in chromatin of isolated nuclei is, however, insensitive to AF/013. Nuclear RNA polymerase partially purified from yeast is insensitive to rifampicin (152, 153). A specific reduction up to 60 percent of the ribosomal RNA synthesis in the presence of

rifampicin has, however, been observed on whole cells of *Saccharomyces cerevisiae,* permeabilized with amphotericin B (182). Di Mauro (personal communication) has isolated four nuclear and one mitochondrial RNA polymerases from cells of *S. cerevisiae,* some of them found to be sensitive to derivatives of rifamycin SV such as AF/013 and AF/ABDP (XX). AF/013 has also been found active on the three major nuclear RNA polymerases by Admen et al (171). The growth of blue-green alga *Anacystis montana is* inhibited by rifamycin B, rifamycin S, and rifampicin, but the same antibiotics do not affect the growth of the green alga *Chlorella pyrenoidosa* (154). In the first case, the main effect of the antibiotics is on RNA polymerase and on RNA synthesis. It has also been shown that rifampicin specifically inhibits the RNA synthesis in *Acetabularia mediterranea* (226). In *Chlamydomonas reinhardii,* rifampicin blocks, both in vivo and in vitro, the chloroplast incorporation of uridine but not the nuclear RNA synthesis (155). Rifampicin does not inhibit the RNA synthesis of macronuclei of the protozoon *Tetrahymena pyriformis,* suggesting that their enzyme is more closely related to the mammalian enzyme than to that found in bacteria (156). Rifampicin at concentrations greater than 50 μg/ml significantly inhibits the multiplication of the protozoon *Toxoplasma gondii* in L cell cultures, through a mechanism that is not clear since RNA synthesis in cell-free toxoplasma is not affected by extremely high concentrations of the drug (157). Rifampicin has been reported to be active in vivo on mice infected by *Plasmodium berghei* (202). The fungus *Blastocladiella emersonii* possesses three chromatographically distinct species of RNA polymerase; the activity of one of them (fraction III) is sensitive to rather high doses of rifampicin, thus suggesting the possibility that this enzyme might be similar to a procaryotic enzyme representing a transition in evolution (158). This hypothesis receives further support by a recent finding that the fraction III polymerase is mitochondrial (227). Rifamycin SV is a very weak inhibitor, if at all, of RNA synthesis in cells of higher plants (159). Coconut nuclei contain two different RNA polymerases (I, II) one of which is sensitive to rifampicin, but the addition of a protein factor from the nuclei makes it insensitive (160). It should also be mentioned in this context that rifampicin has been found to be an inhibitor (at 70 μg/ml) of antigen-stimulated RNA synthesis in rat peritoneal macrophages (161). The drug does not inhibit RNA synthesis in nonstimulated macrophages. These findings could be interpreted as an action of the drug on the macrophages RNA polymerase, but many alternative explanations could be put forward (161).

In most of the experiments on eukaryotes described above only one rifamycin derivative, namely rifampicin, has been used. However, many other derivatives are being tested in different systems and, in addition to the ones already mentioned that are active on mammalian RNA polymerases (150, 151), others are known to be active on the same system, for example, AF/05 (XXV), AF/015 (XXIX), Pr19 (IX) (P. Chambon and W. Keller, personal communication). Others again are potent inhibitors of other cellular

enzymes such as mammalian DNA polymerases (21, 164–166). It is therefore reasonable to foresee that in the near future other effects of rifamycin derivatives on eukaryotes will be discovered.

According to a recent report (163), certain rifamycin derivatives inhibit two RNA polymerase activities purified from isolated nuclei of HeLa cells. Some of these drugs were shown to inhibit RNA synthesis on intact cells by acting specifically on initiation. In this context an experiment should be mentioned in which it was found that streptovaricin D (a compound closely related to rifamycins) selectively inhibits the uptake of nucleosides into HeLa cells. Caution therefore should be exercised in interpreting data in which labeled nucleosides are used to monitor nucleic acid synthesis in the presence of the inhibitor (162).

A derivative of rifampicin, 2′,5′-dimethyl-N(4′)benzyl-N(4′)(desmethyl)rifampicin (AF/ABDP), has proved useful in studying ribosomal gene amplification during the early oogenesis of *Xenopus laevis* (164–166). This drug seems to inhibit preferentially the DNA synthesis involved in gene amplification. Since this compound is known to be an inhibitor of RNA-dependent DNA polymerase (20, 167), these results are consistent with the hypothesis that RNA-dependent DNA synthesis is involved in gene amplification. It is worth noting, however, that AF/ABDP seems to be active also on eukaryotic RNA polymerases. Coming to the question of the effect of rifamycins on cell organelles such as chloroplasts and mitochondria, it seems plausible that these drugs may be very useful tools for studying similarities between their RNA polymerases and the bacterial or the mammalian enzymes. Accordingly, many studies have been done on this subject both on purified enzymes and on whole particles. The data so far collected are neither conclusive nor clear-cut, mainly because of severe problems encountered in the purification of well-defined enzymes, and because of the limited permeability of the intact particles to the rifamycins tested so far.

Attempts to ascertain whether mitochondrial RNA polymerase responds to rifampicin have produced conflicting answers. In an early report (168), Shmerling obtained inhibition by rifampicin of RNA synthesis in intact rat liver mitochondria. Other authors, however, have found the same inhibition only in swollen (detergent-treated) rat liver mitochondria (169). More recently (170), a similar inhibitory effect in intact rat liver mitochondria has been described; furthermore, while the crude enzyme retains sensitivity to the drug a more purified preparation seems to loose sensitivity. These findings, however, have not been reproduced with mitochondria from yeast (171–173); furthermore, rifampicin does not inhibit the RNA polymerases purified from *S. cerevisiae* mitochondria as proved by experiments in which any bacterial or nuclear contamination seems to be ruled out (174). Puzzling results have also been obtained in mitochondria from *Neurospora crassa*. RNA synthesis is not affected by rifampicin in intact particles (173), but an electrophoretically pure, DNA-dependent enzyme preparation is sensitive to relatively low doses of rifampicin (6 μg/ml) (175). Finally, rather high doses of

rifampicin (>10 μg/ml) inhibit the synthesis of both stable and unstable RNA in heart mitochondria (176). In this report, however, the possibility is not ruled out that the method of mitochondrial isolation could alter the membrane permeability to rifampicin.

In vivo experiments with green algae have shown that growth in the presence of rifampicin causes bleaching (154). In *Euglena gracilis* the drug inhibits the incorporation of ^{32}P into chloroplast ribosomal RNA and also appears to promote the dissociation of chloroplast ribosomes into subunits (177). At a concentration of 250 μg/ml rifampicin blocks phototrophic growth in *Chlamydomonas reinhardii* while much higher concentrations are required to inhibit heterotrophic growth (500 μg/ml). If cells are allowed to grow in this latter condition for several generations their capacity to carry out photosynthesis is severely diminished, but cell division and chloroplast replication are not affected (178). One hundred μg/ml of rifampicin completely stops RNA synthesis in isolated chloroplasts through a mechanism that resembles that found in bacteria, namely, if the antibiotic is added after the RNA polymerization reaction has begun in vitro no inhibition is observed (155). As a result of the inhibition of rRNA synthesis, cells grown in the presence of rifampicin lack chloroplast ribosomes. These studies on chloroplasts have led to the following conclusions: (*a*) similarities between chloroplast and bacterial RNA polymerase stem from similarities between the nature and the status of the templates on which the two enzymes are working rather than from structural analogies between the two enzymes. The organization of the DNA in the chloroplast resembles that in bacteria rather than that found in the nucleus; (*b*) chloroplast ribosomal RNA genes are arranged in tandem in transcriptional units consisting of two or three pairs of 16S and 23S ribosomal RNA genes. The 16S ribosomal RNA gene is located first in every pair in relation to the promoter (229); (*c*) it appears that chloroplast DNA contains information for chloroplast rRNA but not for chloroplast DNA polymerase.

Effects on DNA Viruses

Poxviruses, which carry their own RNA polymerase inside the virion, have been studied for their sensitivity to rifampicin. Intracellular viral multiplication is inhibited by rather high concentrations of the drug (100 μg/ml). Conflicting interpretations of this effect have been given. By autoradiographic studies of infected and treated cells, Subak-Sharpe et al (183, 184) concluded that the drug affects the virus-directed RNA synthesis. The isolation of *rif-r* vaccinia mutants suggested that rifampicin affects a protein coded by the virus, probably the enzyme or a subunit of it involved in viral transcription. However, a number of different rifamycin derivatives active on the bacterial polymerase, such as rifazine, rifamide, desacetylrifamycin (IV, XII, V) and so on, were not inhibitory to vaccinia plaque formation. Rifamycin SV, on the other hand, is too toxic for the host cells to allow any conclusion to be drawn (184, 198).

When the virion-associated enzyme was tested in vitro it proved insensitive to rifampicin (184–186, 188, 189). Furthermore, incorporation studies in infected cells failed to show any effect on the molecular events occurring during the early stage of viral growth. Viral mRNA synthesis was not affected when tested 3 hr after infection. Viral DNA replication was partially affected and late viral proteins were completely blocked (187). Among the late proteins which are not synthesized (or are not active) there is the particulate RNA polymerase (190, 232). In rifampicin-treated cells no mature viral particles are formed, but after removal of the drug, at a time when in the controls mature virus is appearing, maturation takes place (191–195). There is no agreement among different authors about the requirement of additional protein synthesis for maturation after rifampicin removal (192, 194, 195). The authors who do not believe in the requirement for additional protein synthesis maintain that rifampicin inhibits the cleavage of a long polypeptide into smaller polypeptides which are the normal components of the mature virion (196, 197). It was observed by electron microscopy that the earliest event after the removal of rifampicin was the completion of spicule-coated membrane envelopes of the virus (192). It has been speculated that the lack of particulate RNA polymerase activity in treated cells (190) is a consequence of a lack of incorporation of the enzyme into the viral cores (192). Other authors have found that the particulate RNA polymerase which is made in infected cells (untreated) is sensitive to rifampicin in vitro. The same enzyme extracted from cells infected with a rifampicin-resistant mutant is not sensitive. They conclude therefore that the primary target of rifampicin is the late particulate RNA polymerase (195). It is not clear whether these different results can be attributed to the different systems employed.

It has been claimed that the hydrazone side chain of rifampicin is responsible for the anti-poxvirus activity (15, 198). Such a generalization cannot be made since there are also hydrazone derivatives which are inactive (198), and since only a small number of rifamycins have been tested. It has been reported that the side chains by themselves, 1-amino-4-methyl-piperazine and other N-aminopiperazines (199, 200), possess an antiviral activity, but also in this case other authors have obtained different results (201). Multiplication of the cytoplasmic DN Avirus of African swine fever is also sensitive to rifampicin (204). Large DNA viruses like herpes and pseudorabies were reported intensitive to rifampicin (183). Two picodna viruses have been reported to be inhibited by rifampicin (203). Treatment of arginine-deprived Burkitt lymphoblasts with AF/DMI [dimethylhydrazone of 3-formylrifamycin SV (XXI, Table 1)] prevented the synthesis of Epstein-Barr viral DNA; rifampicin is inactive on this system (224). 1-amino-2′-6′-dimethyl-4-benzylpiperazine, the side chain of AF/ABDP, inhibits the expression of viral antigens detectable by immunofluorescence in cell lines derived from Burkitt tumors (225).

The statement that the molecular requirements for viral and bacterial in-

hibition are certainly not identical (15) appears premature in view of the contradictory results so far reported. The argument that a higher concentration of drug is required for antiviral activity, and that the reversal of inhibition after removal of the drug is fast (15), is not conclusive, since the same is true for some *E. coli* rifampicin-resistant mutants (104) and for *Mycobacterium smegmatis* (96), at least insofar as rapidity of reversal is concerned.

Effects on RNA Tumor Viruses

Reviews are available on oncogenic viruses (205, 207) and on the enzyme which transcribes their RNA (RNA-dependent DNA polymerase) (22, 206, 218). There are also useful collections of papers on the same topics (14, 19). Rifampicin has been found to inhibit focus formation by different strains of Rous sarcoma viruses (RSV) (208–211). Different interpretations of these findings have been proposed. Diggelmann et al (208) suggested that rifampicin interferes with a step which is essential for transformation, but without significant effect on viral multiplication. Vaheri et al (209) confirmed the effect on focus formation but they claimed that both rifampicin and N-demethylrifampicin (XVIII) have no direct effect on cell transformation, but rather inhibit the growth of transformed cells more than that of normal cells. Finally, Robinson et al (211) observed an inhibition of proliferation of both normal and transformed cells and of virus multiplication. Also, focus formation by Moloney sarcoma virus (MSV) and viral multiplication are sensitive to rifampicin, to AF/ABDP (212), and to some streptovaricins (213). These authors hypothesize a mechanism of action mediated by RNA-dependent DNA polymerase. Vaheri et al warned against this conclusion since rifampicin itself is noninhibitory for this enzyme and the dosage of N-demethylrifampicin required for the inhibition of the enzyme is higher than that active on growth and viability of transformed cells (209). However, in a recent report (228), inhibition of RSV reverse transcriptase by rifampicin has been described. The discovery of RNA-dependent DNA polymerase in RNA tumor viruses (214, 215) raises the question of the role of this enzyme in virus replication and cell transformation, and of its possible function in neoplastic transformation (21, 216–222). The possibility that RNA viruses could be involved in human neoplasia is supported by the finding of RNA-dependent DNA polymerase in virus particles isolated from milk of relatives of patients with breast cancer (223). Specific inhibitors of this enzyme could be very useful in the analysis of its role in various cellular functions (in addition to a still hypothetical use in chemotherapy of viral diseases and cancer). Rifampicin does not inhibit the RNA-dependent DNA polymerase of many RNA tumor viruses, but lengthening of the 4-side chain on piperazine by substituting benzyl for the methyl group (AF/ABDP, AF/ABP XX, XIX) yields derivatives that are active against such enzymes. Removal of the 4-methyl group on piperazine in rifampicin gives demethylrifampicin (XVIII) that is a weak inhibitor of RNA polymerase. These results suggested that suitable chemical modifications of the basic molecule might

lead to stronger inhibitors of RNA-dependent DNA polymerase of tumor viruses. An extensive screening program of a great number of rifamycin derivatives for their activity against RNA-dependent DNA polymerase and other DNA polymerases found in both normal and malignant cells, has been undertaken in Gallo's (21) and in Green's laboratories (20). We will summarize here the results concerning the activities on the reverse transcriptase of tumor viruses. Detailed information on the activity against cellular DNA polymerases can be found elsewhere (21). A total of 182 derivatives were tested against the RNA-depndent DNA polymerase activity of MSV (M) disrupted by detergent. The results are discussed below with regard to the particular chemical modification of rifamycin derivatives active against the viral polymerase.

Rifamycin SV 3-iminomethyl derivatives.—Rifampicin, a 3-iminomethyl derivative of rifamycin SV in which the R′ substituent is 4-methylpiperazinoiminomethyl, is inactive against the MSV reverse transcriptase. But further lengthening of the 4-side chain on piperazine, i.e. by replacement of the methyl group by an octyl group, converts it into an effective inhibitor of the polymerase. Derivatives of rifampicin in which methyl is replaced by benzyl (XIX, XX) are also efficient inhibitors. It appears therefore that lengthening of the 4-side chain on piperazine of rifampicin by aromatic substituents may yield derivatives which inhibit MSV polymerase activity.

Rifamycin SV 3-hydrazonomethyl derivatives.—Hydrazone derivatives with large bulky side chains such as the cycloheptyl and cyclooctyl group are excellent inhibitors of MSV polymerase. Among these, diphenyl- (XXII) and dinitrophenyl- (XXIII) hydrazone derivatives and some of the large heterocyclic ring structure are especially active.

3-Formylrifamycin SV oxime derivatives.—The series of oxime derivatives illustrates especially well the correlation between the length of the alkyl-O substituent and the inhibitory activity. In fact, while methyl, ethyl, and propyl derivatives are completely inactive, further lengthening of the aliphatic side chain by a single C atom yields the butyl derivative (XXIV) that is a strong inhibitor of MSV polymerase. Further lengthening of the side chain in pentyl (XXVII) and octyl (XXVIII) substituents provides some of the most potent inhibitors of polymerase activity. As in the case of 3-iminomethyl and 3-hydrazonomethyl derivatives, benzyl (XXIV) and diphenylmethyl (XXV) substituents yield the strongest inhibitors. Rifamycin SV 3-substituted derivatives other than 3-iminomethyl, 3-formylhydrazone, and 3-formyloxime derivatives are either inactive or poorly active. Rifamycin SV 4-desoxy 3,4-substituted derivatives are inactive, indicating that the steric restriction imposed by the 3,4 substituent is incompatible with inhibitory activity. Rifamycin SV 4-desoxy derivatives and rifamycin B 4-substituted derivatives are inactive. Also streptovaricin A, C, and D do not inhibit enzyme (20). The ac-

tive rifamycin derivatives inhibit both the RNA-dependent DNA polymerase of disrupted viruses and the DNA-dependent DNA polymerase activity of the purified enzyme. This gives further support to the idea that both enzymatic activities are present in the same molecule.

Preliminary data indicate that rifamycin derivatives inhibit the MSV polymerase by combining with the enzyme and not with the template (20, 21). It should be noted that some of the most active derivatives inhibit the RNA-dependent DNA polymerase reactions at concentrations as low as 2–5 μg/ml. As for the activities of these products against other cellular enzymes, eight potent inhibitors from the oxime, imine, and hydrazone derivatives of 3-formylrifamycin SV bearing a ketone side group also show activity against DNA-dependent and DNA-RNA hybrid-dependent DNA polymerase, both viral and cellular. Some of them are relatively selective inhibitors of DNA polymerases from leukemic lymphoblasts (21).

Note added in proof: It has been reported that the accumulation of the regulatory nucleotide ppGpp (MS — 1) which is governed by the RC gene of *E. coli* does not occur in the presence of rifampicin (238). This fact was taken as evidence that the formation of ppGpp is somehow dependent on the presence of nascent RNA chains. In a more recent report, it was found that the effect of rifampicin is dependent on amino acid activation: when valyl-tRNA synthetase is specifically inactivated, then ppGpp accumulation proceeds in the presence of rifampicin despite complete block of RNA synthesis (239). The effect of rifampicin probably reflects the recharging of tRNA following the drop in the rate of protein synthesis. There is growing evidence for a pleiotrophic effect of some *rif-r* mutations. A class of *rif-r* mutants of *Salmonella typhimurium* (with an altered RNA polymerase) lysogenizes phage P22 at reduced frequency (240). The finding that a class of bacterial RNA polymerase mutants is lysogenized inefficiently supports the idea that the host RNA polymerase plays a role in the lysis-lysogeny decision.

Another author (241) has described an effect of some *rif-r* mutations on the suppression mechanisms in *E. coli.*

Finally, it has been observed in our laboratories that some *rif-r* spontaneous mutants of *E. coli* have an altered permeability to some antibiotics (manuscript in preparation). The requirement for transcription during development of the stalked bacterium *Caulobacter crescentus* has been studied by treating synchronous cultures of swarmer cells with rifampicin. When added at appropriate times, rifampicin blocks several steps of the development such as: loss of motility, initiation of DNA replication and cell division, indicating that RNA synthesis is required throughout the cell cycle for normal differentiation (242).

As for the effect on eukaryotes and viruses, it has been reported that in *S. cerevisiae,* rifampicin considerably enhances the petite mutagenic effect of ethidium bromide and depresses the mutagenic effect of acriflavine or nitrogen-source starvation, though having no mutagenic effect itself (243). Rifampicin is reported to have a significant prophylactic antiviral effect on

vaccinia virus in mice (244). Two rifamycin derivatives (AF/05 and AF/013; XXV, XXIII Table 1) which are powerful inhibitors of the RNA-dependent DNA polymerase of MSV inhibit virus production in chick fibroblasts transformed with Schmidt-Ruppin Rous sarcoma virus at concentrations which do not affect growth and cell transformation (245).

Concluding Remarks

In this review we have meant to give a complete picture of the effects of rifamycins on bacteria, viruses, and eukaryotic cells. From what has been reported here, it appears that the mechanism of action of these drugs is sufficiently understood only in bacteria. With regard to viruses and animal cells the results obtained are sometimes inconclusive and controversial; however, we believe that there are sufficient indications that some of these drugs will become useful tools for future work in these fields. We have intentionally omitted any reference to clinical applications of rifamycins. A complementary booklet containing over 1000 references of papers covering the various fields of application of rifamycins may be obtained from Gruppo Lepetit, Milan.

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