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Actions of the Rifamycins

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INTRODUCTION

The rifamycin antibiotics are fermentation products of *Streptomyces mediterranei* sp. n. (140) which were discovered in 1957. [In early literature, the name "rifomycin" was used. In this review, the term "rifamycin(s)" stands for the whole class of the rifamycins. For structural formulae, see Fig. 5.] Since rifamycin B, the compound originally isolated, has no antibacterial activity in its own right, they might easily have escaped attention altogether but for the fact that rifamycin B is readily degraded to the very active derivative rifamycin S, which inhibits the growth of gram-positive bacteria at concentrations as low as 0.0025 μg of antibiotic per ml (145). Clinically, the rifamycins proved to be a very valuable class of antibiotics, especially for the treatment of tuberculosis, but the naturally occurring compounds had the disadvantage of not being orally active.

In 1963, Prelog and co-workers determined the chemical structure of various rifamycins (122) and opened up the way for the synthesis of a vast number of semisynthetic derivatives. Lepetit and Ciba succeeded in finding orally active compounds, one of which, rifampin, is now in widespread clinical use. (Unfortunately, two generic names exist: rifampin in the U.S., rifampicin elsewhere; trade name of Ciba-Geigy; Rimactane; trade name of Lepetit: Rifadin.) Only in the last few years, however, since Hartmann et al. (53) and Umezawa et al. (171) discovered that the rifamycins inhibited bacterial deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase, has the mechanism of action of the rifamycins in bacteria been elucidated. Since this inhibition is highly specific, the rifamycins have become an important tool in the study of RNA biosynthesis and metabolism. Furthermore, the possibility of specifically blocking bacterial RNA synthesis has prompted many investigators to study the general function of the bacterial cell and the viruses depending on it.

Inhibition of bacterial RNA synthesis is, however, not the only effect displayed by this class of antibiotics. Some derivatives of rifamycin have also been reported to inhibit the growth of certain

mammalian DNA viruses. Recently, effects on the growth of RNA tumor viruses were observed, and it has been found that some derivatives inhibit the RNA-dependent DNA polymerase of such viruses.

In this review, an attempt is made to describe the various effects of the rifamycins and to evaluate their significance in relation to various biochemical problems.

ACTION ON BACTERIA

General Effects of Rifamycins In Vivo and In Vitro

Early experiments (21, 40, 41) indicated that some rifamycins inhibited the cell-free protein synthesis of *Bacillus subtilis*. No such effect was evident, however, in a similar system from *Escherichia coli* (53) or *B. stearothermophilus* (179). In intact cells of *B. subtilis*, Calvori et al. (21) observed that the uptake of uracil was greatly inhibited by some rifamycin derivatives.

Hartmann and co-workers (53), who found that the inhibition of uracil uptake by rifampin was even more pronounced in cells of *Staphylococcus aureus*, were the first to suggest that the rifamycins might act on RNA synthesis. Experiments in vitro with DNA-dependent RNA polymerase from *E. coli* clearly demonstrated that this enzyme was inhibited by rifampin concentrations as low as 2×10^{-8} M (0.02 μg/ml) (53, 171, 174). Similar results have been obtained with RNA polymerase isolated from *S. aureus* (175), *Micrococcus luteus* (156), *B. subtilis* (48), *Azotobacter vinelandii* (89), and *B. stearothermophilus* (127). In contrast, DNA-directed DNA polymerase from *E. coli* was not affected even by concentrations of rifampin 5,000 times higher (53). This highly specific action of the rifamycins differs from the specificity of other potent inhibitors of nucleic acid synthesis such as actinomycin, chromomycin, etc. These compounds inhibit both DNA-dependent DNA and RNA synthesis, although to a different extent, by complexing with the DNA template (80). The rifamycins, however, do not interact with the template but they affect the RNA polymerase directly (174).

Further in vivo studies with *E. coli* and *B. subtilis* clearly indicated that the inhibition of RNA synthesis is the primary action of the rifamycins and that their effect on protein and DNA synthesis is only a consequence of the inhibition of RNA synthesis (86, 87, 126). In the following subsections, a detailed account of the interactions between bacterial RNA polymerase and rifamycins will be given.

Interaction of Rifamycins with DNA-Dependent RNA Polymerase (EC 2.7.7.6)

Characterization of RNA polymerase. RNA polymerase is the enzyme responsible for the transcription step. With DNA as template, it catalyzes the polymerization of four ribonucleoside triphosphates into RNA molecules and thus transfers the genetic information stored in the very large DNA molecule into relatively small RNA molecules which are used by the cells in various ways. The general equation of the reaction is given in Fig. 1. RNA polymerase was independently discovered by Hurwitz et al. (63*a*), by Stevens (155*a*), and by Weiss and Gladstone (180) in 1959. Since then numerous studies have been done with the enzyme isolated from various organisms [see reviews, references 23*a*, 49, 64, 129; volume 35 of the Cold Spring Harbor Symp. Quant. Biol. (1970) contains a large number of excellent papers on the transcription of genetic material].

In the last 2 years, quite a number of exciting new facts concerning RNA polymerase have come to light. One of the most interesting is that RNA polymerase can exert a positive control function on the transcription of DNA (19, 159, 168, 169, 195). In this review, we will limit ourselves to those aspects of RNA polymerase that are important for the understanding of the mode of action of the rifamycins. Unless otherwise stated, the studies described below are related to the enzyme of *E. coli*.

Various steps of the RNA polymerase reaction. In bacterial DNA-directed RNA synthesis, the following enzymatic steps can be distinguished (23*a*). (i) *DNA binding*. The free enzyme binds to the DNA functioning as template. A variety of different binding sites and states exist. (ii) *Chain initiation*. The enzyme forms a ternary complex with DNA and the nucleoside triphosphate which forms the 5′-terminus of the RNA chain. The binding of a second nucleoside triphosphate to the enzyme followed by elimination of pyrophosphate leads to a dinucleoside tetraphosphate. (iii) *Chain elongation*. Nucleoside monophosphates are added sequentially to the 3′-end of the growing RNA chain. A nucleotide sequence complementary to the DNA template is obtained. (iv) *Chain*

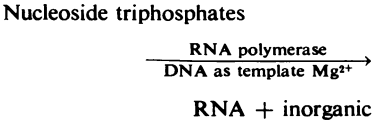


FIG. 1. *Transcription step catalyzed by RNA polymerase.*

TABLE 1. *Composition of ribonucleic acid polymerase (19, 24)*

Subunit	Molecular weight	No. of subunits	
		In core enzyme	In holo enzyme
α	40,000	2	2
β	145,000	1	1
β'	160,000	1	1
σ	85,000		1

termination. The RNA chain growth stops, and the enzyme-DNA-RNA complex dissociates. Various modes of chain termination have been found.

Structure of RNA polymerase. RNA polymerase has a rather complicated structure. Burgess (19) found that the enzyme consists of several subunits (Table 1). Four of them, two α, one β, and one β' subunit, constitute the core enzyme and catalyze RNA synthesis with certain templates in an unspecific way. A further protein, σ, can combine with the core enzyme yielding the holo enzyme; σ regulates the transcription in such a way that specific genes of certain DNA species are transcribed. It reacts catalytically and only during chain initiation and is detached during chain elongation (168). More recently, additional factors regulating the transcription step have been identified: the Ψ-factor which is apparently responsible for ribosomal-RNA synthesis (169); the catabolite gene activator protein (CAP factor) (195), mediating together with cyclic adenosine monophosphate the transcription of catabolite-sensitive genes; and the ρ factor (131), which seems to be related to RNA chain termination.

Which enzymatic step is inhibited by rifamycins? Rifamycins can, in very low concentrations, inhibit the polymerization of nucleoside triphosphates completely. Inhibition does not take place immediately, however, unless the drug is present at the beginning of the reaction. If rifamycins are added after RNA synthesis has begun, it continues for some time (149, 171). Furthermore, after the formation of an initiation complex by preincubation of enzyme, DNA, and purine triphosphates, chain elongation with all four triphosphates is practically unaffected by rifampin (149). The drug, therefore, seems to affect the enzyme before chain elongation.

Studies of the initial complex formation between enzyme and DNA in the presence and absence of rifamycins have shown that the antibiotic does not influence this interaction (113, 171) and that the formation of the initial DNA-enzyme complex does not yield protection against rifamycin inhibition. However, incubation of the DNA-enzyme-complex at temperatures above 17 C yields a transformed DNA-enzyme complex which now is protected against the action of rifampin (6, 93, 150). This transformation occurs in the absence of nucleoside triphosphates and seems to be related to the DNA melting reaction described for double-stranded DNA (173, 192). However, not only the DNA but also the enzyme conformation seems to change, since the activation temperature varies with different enzymes (139).

When using natural templates, such as phage DNA, the transformed DNA-enzyme complex resistant against rifampin has only been obtained in the presence of initiation factor σ. Since σ is thought to direct RNA polymerase to the genuine initiation (promoter) sites on the DNA molecule, this result has been taken as evidence that only enzyme molecules attached at DNA-promoter sites undergo this transformation and are thus rendered resistant to rifampin (6). The finding that there are about two orders of magnitude difference between the rate of dissociation of the transformed DNA-enzyme complex and the rate of its inactivation by rifamycin (193) has lead Travers (169*a*) to postulate that two forms of activated DNA-enzyme complexes exist. Further studies, especially on the binding of rifamycin to the transformed DNA-enzyme complex(es), are required to prove this hypothesis. With synthetic templates, such as poly d(A-T), conflicting results as to the protection of the enzyme against rifampin have been obtained (150, 152), which seem to be related to the rates of formation and the stability of the various complexes.

From all of the data available, it seems clear, however, that the rifamycins block RNA chain initiation. After the intial binding of RNA polymerase to DNA, this chain initiation occurs in three steps: (i) transformation to an activated DNA-enzyme complex, whereby enzyme and DNA change their conformation and the enzyme can bind to specific promoter sites on the DNA; (ii) binding of the first triphosphate, yielding the ternary enzyme-DNA-nucleoside triphosphate complex; and (iii) binding of the second nucleoside triphosphate and formation of the first phosphodiester bond.

When a natural template is transcribed, whereby RNA polymerase attaches to genuine promoter sites, rifamycins apparently affect the first step. With synthetic polynucleotides as templates, which of the three steps is inhibited by the drug seems to depend on the rates of formation and the stability of the various complexes under the given experimental conditions.

Complex formation of rifamycins with RNA polymerase. In studies of the interaction between RNA polymerase and rifamycins, it has been found that, by simply mixing the free enzyme with the antibiotic, a complex is formed that can be isolated by passage through a Sephadex column (176). In contrast to the DNA-enzyme complex, the antibiotic-enzyme complex is formed to the same extent at salt concentrations as high as 1 M. Quantitative measurements have shown that 1 mole of holo enzyme ($\alpha_2\beta\beta'\sigma$) binds 1 mole of rifampin (178). The same amount of antibiotic is bound by the core enzyme ($\alpha_2\beta\beta'$), which is also inhibited to the same degree as the holo enzyme (33, 178). Thus, it is not σ that binds rifampin. Experiments with isolated subunits have proved that rifampin binds to the β subunit (193). This result has been confirmed by experiments with RNA polymerase from rifamycin-resistant cells (*see below*). The formation of the complex is very rapid: with 1.5 nmoles of enzyme per ml, a threefold excess of rifampin yields 95% of the complex at 0 C in less than 2 min (Fig. 2). The stability of the complex varies according to the temperature and can be determined by measuring the exchange rate between unlabeled, complexed rifampin and ^{14}C-labeled, free rifampin (176, 178). Furthermore, during enzyme purification, the stability of the complex diminishes. Whereas a crude enzyme preparation forms a complex with rifampin that is practically stable for 1 hr at 37 C, a complex with a pure enzyme is decomposed to the extent of 90% under the same conditions (Fig. 3). The decrease of the complex stability due to enzyme purification

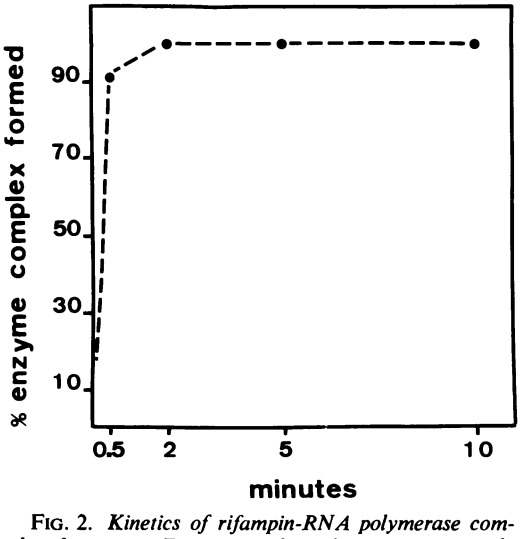


FIG. 2. *Kinetics of rifampin-RNA polymerase complex formation. Experimental conditions as previously described (176).*

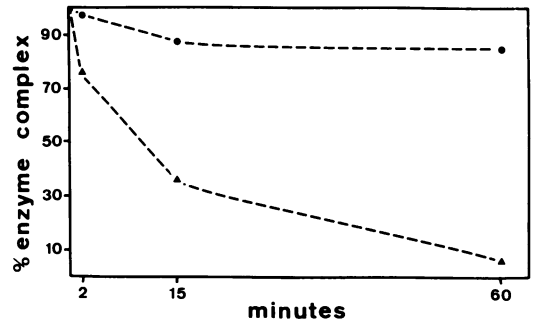


FIG. 3. *Stability of the complex between rifampin and crude (●) or purified (▲) RNA polymerase. In a first step, the enzyme-^{14}C-rifampin complex was formed and isolated as previously described (176). The stability of this complex was measured either by determining the exchange of the labeled rifampin with an excess of added cold rifampin (178) or by adsorption of dissociated ^{14}C-rifampin to charcoal and measuring the remaining rifamycin-enzyme complex (Wehrli et al., in preparation).*

cannot be explained yet, but it could be assumed either that the β subunit that is responsible for the binding of rifamycin is somehow modified or that other factors that are lost during purification contribute to the stability of the complex. Modifications of the enzyme by adenylation (24) or phosphorylation (100) have recently been described.

Resistance Against the Action of Rifamycins

In an overnight culture of bacteria, cells resistant to rifamycins can be observed. The mutation rate for this resistance is 1.3×10^{-8} in the case of *E. coli* at a selection concentration of 100 μg of antibiotic per ml, and the degree of resistance varies especially at low doses of drug. With high rifamycin concentrations, mutants showing complete resistance to the drug can be selected (76). To find the reason for this resistance, RNA polymerase from totally resistance strains of *E. coli* and *S. aureus* have been isolated and tested in respect to their interaction with rifampin. The resistance proved to be due to a modified RNA polymerase that was insensitive to the inhibitory action of rifampin (175). Concentrations up to 1,000 times higher than those inhibiting a sensitive RNA polymerase showed no effect on the resistant enzymes, whereas actinomycin inhibited both sensitive and resistant enzymes to the same degree (175). Inactivation of rifamycins by destruction or binding to factors in the mutant extract could be excluded, since in a mixture of sensitive and resistant enzyme a normal inhibition of the sensitive enzyme was found (37). These experiments validate the conclusion that the rifamycins interact specifically with RNA poly-

merase and not with some factors unrelated to the enzyme.

With *B. subtilis* it has been discovered (16) that mutants resistant to rifampin in vivo, but with a sensitive RNA polymerase, can be obtained. In this case, resistance is perhaps related to changes in cell permeability. However, no experiments capable of proving this hypothesis have been done.

Whereas a sensitive enzyme forms a very stable complex with the drug, a completely resistant enzyme forms no complex at all (176). Experiments with partly resistant enzymes obtained from strains selected at low concentrations of rifampin revealed that complex stability and the extent of enzyme inhibition are closely related: the less stable the complex, the less sensitive is the enzyme to the drug (Fig. 4*a* and *b*). Since conversion to rifamycin resistance is apparently due to a one-step mutation (2, 76), it seems that rifamycin is bound to a very specific site of the enzyme and that substitution of single amino acids alters this site in such a way that binding

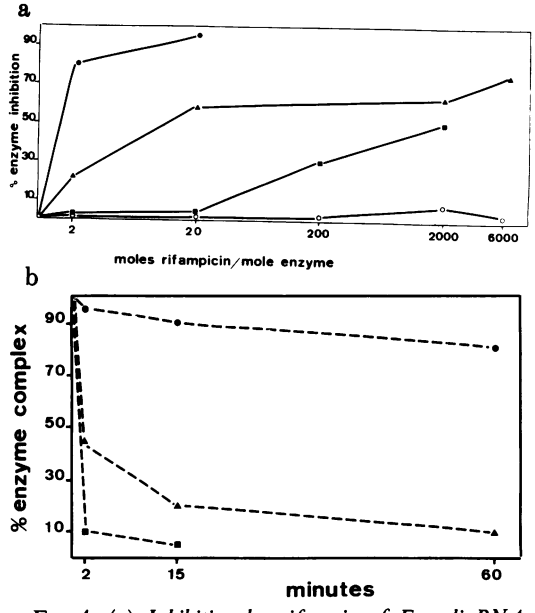


FIG. 4. (*a*) *Inhibition by rifampin of E. coli RNA polymerase with varying sensitivity towards the drug. Assay conditions as described previously (174). Symbols:* ●, *normal sensitive enzyme;* ▲, *enzyme from strain selected at 50 μg of rifampin per ml;* ■, *enzyme from strain selected at 100 μg of rifampin per ml;* ○, *enzyme from strain selected at 1,000 μg of rifampin per ml. (b) Stability of the complex between rifampin and E. coli RNA polymerase with varying sensitivity towards the drug. For experimental conditions, see Fig. 3. Symbols as in Fig. 4a. An enzyme from strain selected at 1,000 μg of rifampin per ml does not form a complex.*

with the antibiotic becomes more difficult, if not impossible. Heil and Zillig (57) determined which of the subunits of RNA polymerase is responsible for the resistance against the drug. They isolated an enzyme from a resistant *E. coli* mutant that was not affected by the antibiotic and found that the β subunit behaved differently upon electrophoresis on cellulose acetate sheets (125). Experiments involving mixed reconstitution of the subunits of this enzyme with those of a sensitive enzyme clearly showed that the rifamycin resistance is related to the β subunit: an enzyme containing the α and β′ subunits from the sensitive enzyme and the β subunit from the resistant one was resistant to rifampin, whereas in the reverse case, i.e., with all subunits from the resistant enzyme except β, the reconstituted enzyme was rifampin-sensitive (57). These results indicate that the β subunit is directly involved in the action of the rifamycins; however, it cannot be excluded that α and β′ or other unknown subunits or enzyme modifications such as phosphorylation might have some influence. In fact, the change in the stability of the enzyme-drug complex during enzyme purification (Fig. 3) would seem to indicate that this is so.

Rifamycin-resistant *E. coli* mutants have been used to map the gene(s) of the RNA polymerase subunit(s) responsible for the resistance. It has been found that rifamycin resistance in *E. coli* maps between met B and arg H (2, 3, 37, 73, 78, 167). Since only rifamycin resistance due to mutations in the β subunit has thus far been encountered, no conclusions as to the location of genes for the other subunits can yet be drawn. Dominance studies with merodiploid strains of *E. coli* containing a rifamycin-sensitive and a rifamycin-resistant gene have shown that both dominance of the sensitive or the resistant gene can be found (2, 78, 189). No explanations of these differences in gene expression can be given at present. Austin, who found dominance of the sensitive gene, has made use of this property to select for mutants with a gene yielding a nonfunctional RNA polymerase (2).

An interesting property related to rifamycin resistance has been noted in *B. subtilis*. Mutants have been isolated, which, in a single mutational event, acquire drug resistance and lose the ability to sporulate, although their vegetative growth is not changed. In normal spores, one β subunit has a molecular weight of only 110,000 as against 155,000 in a vegatative cell. Since in *E. coli* the β subunit is involved in the mutation leading to rifamycin resistance, it is argued that, in drug-resistant strains of *B. subtilis*, the β subunit must be changed in such a way that it cannot be

cleaved to the low-molecular-weight form so that sporulation does not take place (95, 154). Another rifamycin-resistant mutant of *B. subtilis* can still form spores, but they have an altered morphology (34). It therefore seems that mutations in RNA polymerase that manifest themselves in rifamycin resistance can affect sporulation specifically and that RNA polymerase plays a part in differentiating between growth and sporulation.

Relationship Between the Chemical Structure and Biological Activity of the Various Rifamycin Derivatives

The rifamycins are ansa compounds consisting of a chromophoric naphthoquinone or naphthohydroquinone ring which is spanned by a long aliphatic bridge. One of the substances originally produced by *S. mediterranei* sp. n. is rifamycin B (140). Its structure has been determined by

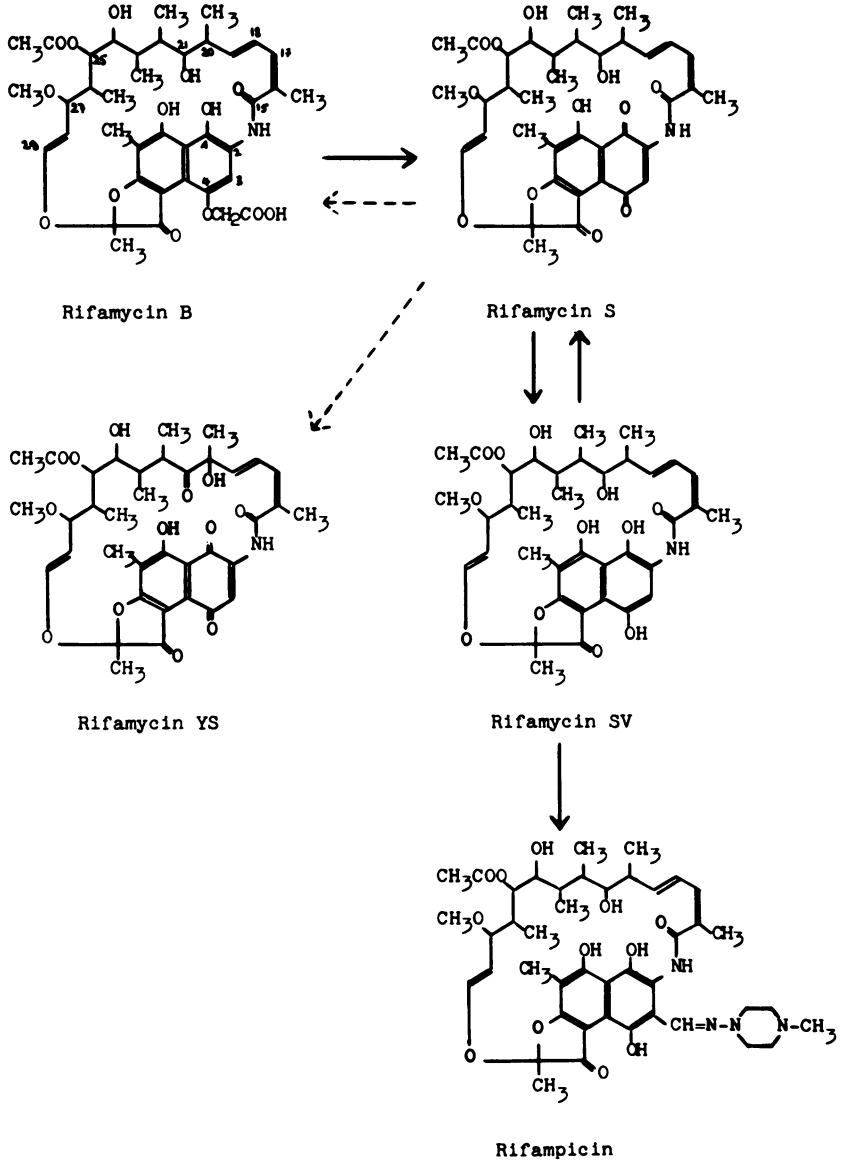


FIG. 5. *Structural formulas of various rifamycin derivatives. Symbols: →, chemical reactions; --→, biosynthetic pathways (17, 84, 85, 81, 92).*

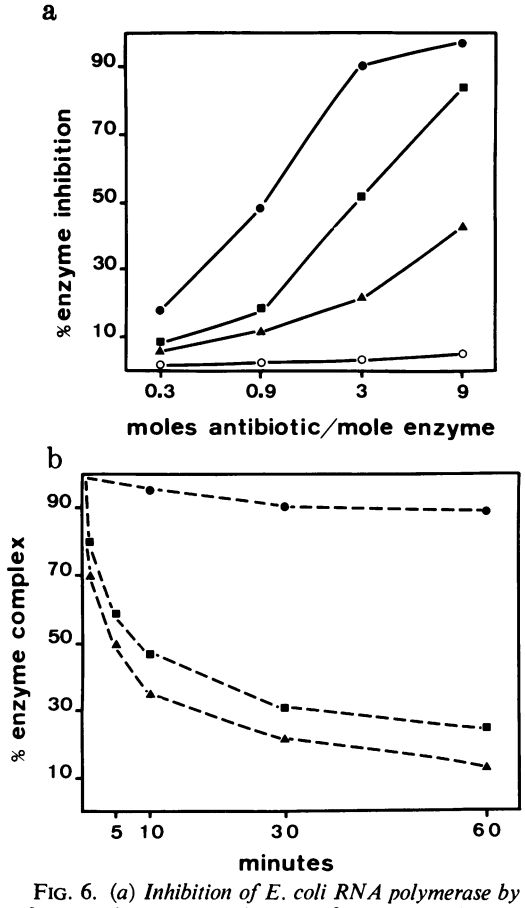


FIG. 6. *(a) Inhibition of E. coli RNA polymerase by rifamycin derivatives with a modified aliphatic bridge. For experimental conditions, see Fig. 4a. Symbols: ●, rifampin; ■, 16, 17, 18, 19-tetrahydrorifamycin SV; ▲, 16, 17, 18, 19, 28, 29-hexahydrorifamycin SV; ○, rifamycin YS. (b) Stability of the complex between RNA polymerase and rifamycin derivatives with modified aliphatic bridge. For experimental conditions, see Fig. 3. Symbols as in Fig. 6a. Rifamycin YS does not form a complex.*

chemical (115) and X-ray analysis (17, 90). Rifamycin B can be oxidized and hydrolyzed to rifamycin S, a naphthoquinone derivative; reduction yields the naphthohydroquinone derivative rifamycin SV (45, 122, 141–143; for structural formulae, *see* Fig. 5).

To obtain substances possessing greater antibacterial activity, a vast number of semisynthetic derivatives have been prepared. In many cases, rifamycin S or SV was modified by the introduction of substituents in position 3, which contains the only aromatic hydrogen (14, 96, 145). Rifampin, the compound most widely used for both clinical and biochemical purposes, is a 3-(4-methyl-piperazinyl)-iminomethyl derivative of rifamycin SV (96, 97, 117). Other positions of the rifamycin molecule that have been chemically modified include the carboxyl group of rifamycin B (144, 145) and the aliphatic bridge (14, 145).

The influence of the various derivatives on bacterial growth and on *E. coli* RNA polymerase has been compared to determine which part of the rifamycin molecule is responsible for its specific action (177). It has been found that changes in the aromatic part of the molecule, such as occur in rifamycin S, SV, and B and rifampin (Fig. 5), have little effect on the inhibition of the isolated enzyme, although the influence on bacterial growth in vitro and on the chemotherapeutic activity of these substances varies considerably (14, 81): rifamycin B for instance, although very active on the enzyme, does not inhibit the growth of bacteria. This seems to be due to differences in cell permeation (98). On the other hand, most modifications of the aliphatic bridge diminish the capacity of the substance to inhibit the enzyme activity, to form a stable complex with the enzyme, and to affect bacterial growth (177). In this context, it is interesting to note that gradual changes, such as successive hydrogenation of the three double bonds, result in a gradual decrease of both enzyme inhibition and complex stability (Fig. 6*a* and *b*). It can be thus concluded that these changes alter the stereochemistry of the ansa ring step by step in such a way as to impair the fit of the molecule to the acceptor site on the enzyme molecule. However, not all modifications lead to a gradual loss of activity. Rifamycin YS, whose structure is identical with that of rifamycin S except for a keto instead of a hydroxyl group in position 21 and an additional hydroxyl group in position 20 (Fig. 5; references 18, 91), is completely inactive as regards both enzyme binding and inhibition (Fig. 6*a* and *b*). Thus, besides the correct shape of the ansa ring, the structure in position 20 and 21 seems to be crucial for the effects of the rifamycins.

Although the action of the many rifamycin derivatives on bacterial RNA polymerase varies quantitatively, there is no evidence to suggest a qualitative difference. Therefore, it seems reasonable to assume that the mechanism of action on RNA polymerase is the same for all derivatives, although many studies have been done with rifampin only. This assumption certainly does not apply to the various actions of rifamycin derivatives on viruses, as will be discussed in the section on antiviral activities.

However, even in bacteria some rifamycin derivatives seem to have additional effects. Knüsel and co-workers (77) have shown that certain derivatives with amine substituents in position 3

of the naphthohydroquinone ring inhibit the in vitro growth of gram-positive bacteria resistant to rifampin. Although these derivatives inhibit an RNA polymerase isolated from a sensitive strain, they do not affect a resistant enzyme. So it is conceivable that they exert an additional, as yet unknown, effect on the metabolism of gram-positive bacteria. This hypothesis is supported by the observation that the mutation rate for resistance against 3-amino derivatives is much smaller than that for rifampin.

Rifamycins as a Tool in Biochemical Studies

The specific inhibition of bacterial DNA-dependent RNA polymerase by the rifamycins has been utilized in many studies of RNA metabolism and biosynthesis. A few examples are given below to show how the use of rifamycins has contributed to the understanding of certain biochemical events. The action of rifamycins on bacterial and mammalian viruses will be discussed in a subsequent section.

Quite a number of studies have been done of the stability of messenger RNA (mRNA) by stopping RNA synthesis with rifampin and following the fate of the already synthesized RNA (118, 137, 160). The great advantage of the rifamycins over actinomycin D is their specific effect on RNA synthesis.

The fact that the rifamycins completely stop RNA synthesis in *E. coli* has been taken as evidence that at least the β subunit of RNA polymerase is involved in all *E. coli* RNA syntheses. Studies in vivo have confirmed the in vitro findings that the rifamycins inhibit RNA chain initiation but not chain elongation (35, 56, 108).

Yanofsky and co-workers have taken these results as the starting point in their experiments on the mRNA transcribed from the tryptophan operon. After the addition of rifampin, the initiation of new mRNA is inhibited immediately. However, elongation of already initiated mRNA was unaffected. Elongation rates could thus be measured and were found to be dependent on growth temperature: at 37 C, the elongation rate was 37 to 45 nucleotides per sec but at 25 C only 16 to 17 nucleotides per sec (133). Growth rate had practically no effect on the elongation rate (118, 133). However, the number of active RNA polymerase molecules per cell increased with growth rate, although the fraction of active enzyme engaged in mRNA synthesis was inversely related to growth rate (118).

The repressor of the tryptophan operon seems to block RNA polymerase from binding to the promotor region, since in the presence of rifampin and after derepression no RNA synthesis occurred (108). A similar result has been obtained in respect to the repression of phage lambda (55). The kinetics of the inhibition of RNA synthesis in a derepressed culture of *E. coli* by tryptophan and rifampin are very similar, indicating that the site of tryptophan repression is the same as or close to the site at which rifampin blocks initiation.

In studies of the position of stable RNA cistrons, Pato and Meyenburg (118) suggest that the three cistrons for 16, 23, and 5*S* RNA are linked, since their results are consistent with the assumption that, after initiation of the 16*S* RNA cistron, the 23*S* and 5*S* RNA cistrons are transcribed in presence of rifampin. In keeping with this interpretation, Doolittle and Pace (35) found, upon analogous use of rifampin, that the 5*S* RNA is generated from a transcription unit carrying between 13 and 30 times the amount of genetic information necessary for the production of one molecule of 5*S* RNA.

Mode of Interaction Between Rifamycins and Bacterial RNA Polymerase

Nature of the enzyme-rifamycin complex. The rifamycin-RNA polymerase complex is so stable that the question arises as to the nature of this complex. At first sight, the most plausible explanation of its stability would appear to be that enzyme and antibiotic are linked by a covalent bond. However, the experiments performed with various rifamycin derivatives show that appropriate substitution results in a gradual loss of complex stability (Fig. 6*b*; reference 177). The same gradual decrease of complex stability is observed when enzymes with increasing resistance to rifamycin are tested (Fig. 4*b*). In both cases, complex formation proceeds at an equal or slower rate. Yet, if there would be a covalent bond, the reverse would be expected: if owing to steric hindrance the complex were formed more slowly, it would be more stable. Furthermore, although less active rifamycin derivatives do bind to the enzyme, they are readily displaced by more active ones, the rate of displacement being dependent on the temperature (178). These data thus indicate that the stability of the rifamycin-RNA polymerase complex is not due to a covalent bond but that other forces, such as hydrogen bonds and π-π bond interactions between the naphtoquinone ring and aromatic amino acids of the enzyme, must be involved.

Relation between rifamycin-enzyme complex and enzyme inhibition. In general, the stability of the rifamycin-enzyme complex determines the degree of enzyme inhibition. This applies to the various resistant enzymes (Fig. 4*a* and *b*) and to the rifamycin derivatives with changes in the ansa ring (Fig. 6*a* and *b*). In both cases, the decrease

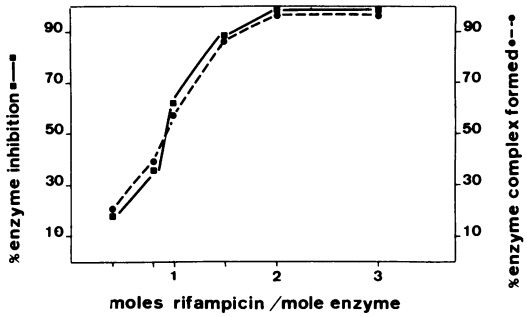


FIG. 7. *Relationship between extent of complex formation and inhibition of RNA polymerase (176).*

in complex stability coincides with a lesser degree of enzyme inhibition. Furthermore, it can be shown that the extent of complex formation also corresponds to enzyme inhibition (Fig. 7): when limiting amounts of rifampin are used, the amount of complex formed runs parallel to the extent of enzyme inhibition. In all of these experiments on complex formation and inhibition, rifampin was added at the beginning of the reaction to the free enzyme. As described above, RNA chain elongation is not inhibited by rifampin, and preincubation with DNA in the presence or absence of purine triphosphates protects the enzyme against drug inhibition. Can a protected enzyme still form a complex with rifampin? Studies of the complex formation during chain initiation and elongation show (Davies and Wehrli, *in preparation*) that an enzyme-DNA-purine triphosphate initiation complex that is protected to the extent of about 80% against rifampin inhibition only forms about 30% complex with the drug, compared with a free enzyme. On the other hand, during chain elongation an enzyme which is protected against inhibition can form a complex to a greater extent, although the rate of complex formation is much slower than with a free enzyme. These results indicate that an initiating enzyme does not bind rifampin, whereas during chain elongation a slow binding is possible. Thus, it seems that the rifamycin binding site is not dramatically changed during RNA synthesis but that in some stages its accessibility is more or less reduced. The initiating purine triphosphates might in fact keep rifampin from binding to the enzyme. This would be consistent with the finding of Wu and Goldthwait (185, 186) that rifampin inhibits the binding of purine triphosphates to the enzyme. However, their experiments were performed in the absence of DNA which is likely to have a considerable influence on the triphosphate binding. The results obtained by Sippel and Hartmann (150) and Bautz and Bautz (6) furthermore indicate that the action of rifampin can be prevented in the absence of triphosphates. Thus, some additional factors must play a role. A study of complex formation between rifampin and the DNA-enzyme complex under various conditions and in the absence of purine triphosphates might shed some light on these events.

To sum up, the rifamycins specifically inhibit the RNA chain initiation step of RNA polymerase by binding tightly to the β subunit in a molar ratio of 1:1. Therefore, only very small amounts of antibiotic are necessary to affect the enzyme, a twofold excess of the drug producing 80 to 90% inhibition. Changes of single amino acids of the enzyme through mutation can diminish the affinity of the binding site for rifamycins and thus lessen the enzyme inhibition to a varying degree. Mutation can even yield a completely resistant enzyme that no longer forms a complex. A less stable complex and a correspondingly lesser degree of enzyme inhibition can also be obtained by chemical modification of the rifamycin molecule. The ansa ring proved to be the crucial part responsible for enzyme binding and inhibition, whereas the aromatic ring system can be modified to some extent without a change in activity. The way in which the rifamycins interact with RNA polymerase during RNA synthesis is not yet quite clear. However, the fact that an elongating enzyme that is not inhibited by the drug can still bind the antibiotic indicates that enzyme inhibition is not a direct consequence of complex formation. But depending on the state of the enzyme, complex formation could either induce or prevent a change in its properties, perhaps a change of the allosteric conformation.

ACTION ON EUKARYOTES

The rifamycins have been shown to have a very low toxicity for mammalian organisms. The question thus arose whether this effect is due to the fact that the rifamycins cannot reach their site of action or whether they are ineffective against mammalian RNA polymerases. Studies with RNA polymerase from rat liver nuclei (174) and ascites cells (171) showed that they contained an enzyme insensitive to rifamycins. These results have been confirmed with solubilized RNA polymerase isolated from rat liver, human placenta nuclei, and lymphoid tissue; the activity of these enzymes depended completely on the addition of DNA (43, 65, 172). Furthermore, both RNA polymerase A and B isolated from calf thymus were not affected by the antibiotic (72). The same resistance against various rifamycin derivatives has been found with nuclear RNA polymerases isolated from a variety of eukaryotic cells, such as yeast (31, 183), green algae (132, 161), pro-

tozoans (20), and plant roots (111, 112). Coconut nuclei contain two RNA polymerases, one of which is reported to be inhibited by rifampin (99). This inhibition can be prevented by the addition of a protein factor. Furthermore, in the water mold *Blastocladiella emersonii*, three RNA polymerase activities were found, one of which is inhibited by high concentrations of rifampin (62). The data available give no indication of the significance of these inhibitory effects of rifampin. However, it seems clear that, in general, the nuclear RNA synthesis of eukaryotes is not affected by rifamycin.

But RNA synthesis also occurs in certain other cell particles, such as mitochondria and chloroplasts. In many respects, protein synthesis in these particles resembles that of bacteria, and inhibitors such as chloramphenicol, which specifically affect protein synthesis in bacteria but not in the cytoplasma of eukaryotes, do inhibit mitochondrial and chloroplast protein synthesis (7). The rifamycins would be an ideal tool for testing for similarities between bacterial and mitochondrial or chloroplast RNA polymerases. Quite a number of studies have been undertaken along these lines, but the results obtained thus far do not allow a clear-cut conclusion. The main difficulty seems to be the isolation of a well-defined RNA polymerase whose activity is dependent on DNA.

Tests on intact mitochondria or chloroplasts are not conclusive because of permeation problems. Various authors claim to have obtained enzyme inhibition, but the amounts of rifamycin used are 100 to 1,000 times higher than those needed to inhibit bacterial RNA polymerase. In vivo experiments with green algae have shown (132, 161) that rifampin causes bleaching, i.e., impairs the function of chloroplasts. Furthermore, incorporation of ^{32}P into chloroplast RNA is inhibited, and the drug seems to promote the dissociation of chloroplast ribosomes into subunits (15). Cells that were grown in the presence of rifampin for several generations still contained chloroplasts, although they showed a great deal of internal disorganization and could no longer fix CO_2. In vitro RNA synthesis in isolated chloroplasts was inhibited by concentrations of 100 μg/ml, whereas 50 μg/ml had very little effect (161). It thus seems clear that rifampin has some effect on chloroplasts; however, the data do not warrant the conclusion that chloroplast RNA polymerase is inhibited by the antibiotic in a way comparable to bacterial RNA polymerase.

In vitro RNA synthesis in swollen mitochondria of yeast and rat liver was not inhibited by concentrations of rifampin as high as 50 μg/ml, whereas the same concentration of actinomycin caused 90% inhibition (183, 188). Thus, a permeation effect does not seem likely in this case. An analogous result has been obtained with *Neurospora* mitochondria (59). Furthermore, a mitochondrial RNA polymerase from a cytoplasmic "petite" mutant, whose activity was dependent on added DNA, was not affected by rifampin concentrations as high as 16 μg/ml (184). Just recently, two forms of mitochondrial RNA polymerase were isolated from *Saccharomyces cerevisiae*: both were dependent on DNA, but rifampin had no inhibitory effect in concentrations as high as 40 μg/ml (170), which is more than a 1,000-fold higher concentration than required for the inhibition of bacterial RNA polymerase. On the other hand, there have been some reports which state that rifampin inhibits mitochondrial RNA synthesis in rat liver or bovine heart (44, 47, 146). Since very high concentrations of the drug were needed in these cases to obtain an effect and since the enzymes used were not well characterized, no definitive conclusion as to the action of rifampin on mammalian mitochondrial RNA synthesis can be drawn. However, the experiments with yeast mitochondrial RNA polymerase do make it clear that the theory that, in general, mitochondrial RNA polymerase is a bacteria-like enzyme cannot be upheld.

ANTIVIRAL ACTIVITIES

Since prokaryotic and eukaryotic cells differ so much in their sensitivity to rifamycin, it is clear that the effects to be expected on the corresponding viruses will likewise be quite different. Any involvement of host bacterial RNA polymerase in phage growth would lead to the inhibition of virus development. Furthermore, the development of bacterial viruses insensitive to rifamycins could conveniently be studied by blocking host RNA synthesis with rifamycin. On the other hand, since mammalian RNA polymerase is insensitive to the antibiotic, the possibility has been raised that the rifamycins might prove to be selective agents against mammalian viruses. In the following sections, the various actions of the rifamycins on bacterial and mammalian viruses will be discussed.

Effects on Bacterial Viruses

Since the rifamycins are such potent inhibitors of bacterial RNA polymerase, they have been used in attempts to find out what role the drug-sensitive part of the host RNA polymerase plays in the development of the various bacterial viruses.

The growth of several DNA phages, such as SP01 (48), β 22 (63), T4 (54, 105), and λ (162), has been shown to remain sensitive to the rifamycins throughout phage infection. In drug-resistant cells, the phages develop in a normal way even in the presence of the antibiotic. This has been taken as an indication that the rifamycin-sensitive part of the host RNA polymerase is required for the transcription of the entire genome. However, studies with phage T7 have revealed that the growth of this virus becomes resistant to rifamycin inhibition after 5 min, coinciding with the appearance of a specific T7 RNA polymerase (23) that is responsible for the transcription of late bacteriophage functions. This enzyme is not affected by rifampin (23, 50). At least one new phage protein is also directly required for late transcription in both T4 and λ (123, 151). If these proteins were enzymes similar to that found in T7, then the sensitivity to rifamycins of late transcription in the phages could be explained by assuming that host RNA polymerase is required for some other phage functions (23).

Studies with RNA viruses have demonstrated that the growth of phages such as f2 (39) and Qβ (66) is not substantially affected by rifampin added 4 to 5 min after infection, although under these conditions host-specific RNA synthesis is inhibited by more than 95%. However, when the drug is added before or shortly after phage infection, the virus development is severely inhibited. No clear explanation for this inhibition has yet been found, although it has been proved that phage synthesis is not affected at the enzymatic level (5).

Effects on Mammalian Viruses

In the last few years, many effects of rifamycins on mammalian virus growth have been reported. In contrast to the bacteria and their viruses, in which the action of the rifamycins is reasonably well understood, the effects on mammalian viruses are very heterogeneous and no general picture of the mechanism of action has yet emerged. It seems clear, however, that the action of the antibiotic on these viruses is quite different from that on bacteria. To mention just two differences, only certain specific derivatives are markedly active, and very high concentrations of antibiotic are required to obtain any effects. It has not yet been possible, therefore, to employ the available derivatives for therapeutic purposes.

On the other hand, various rifamycin derivatives have proved to be very valuable tools for the elucidation of some biochemical events during virus development. Since quite divergent results are obtained, depending on the type of nucleic acid present in the virus, the various effects are discussed in two sections on DNA and RNA viruses.

Effects on DNA viruses

The finding that poxvirus carries its own RNA polymerase (68, 109) prompted two groups of investigators (58, 157) to study the effect of rifamycins on the growth of this class of viruses in vitro. They found that high concentrations (100 μg/ml) of rifampin inhibited the growth of poxvirus but not of herpesvirus and pseudorabies virus. Furthermore, they showed that this activity was restricted to rifampin and that several other rifamycin derivatives with antibacterial activity were inactive. They were also able to select mutants of vaccinia virus that were resistant to rifampin and grew equally well in the presence of 100 μg of the antibiotic per ml.

Despite the numerous studies undertaken to find out how rifampin affects the multiplication of vaccinia virus, no definite answer to this question has emerged. Two periods of mRNA synthesis can be distinguished during virus replication: early and late mRNA. Neither is affected by rifampin, nor is the formation of viral DNA inhibited (12, 101, 106, 107). Early and late proteins are also produced, although synthesis of the latter declines sooner than in the absence of the antibiotic (12, 106, 107, 158), and some variations occur, depending on the virus strain (119, 163). Rifampin does not inhibit the RNA polymerase activity of purified vaccinia virus particles (26, 101, 107, 158). The only effect that could be detected was the lack of a particulate RNA polymerase activity, which appears late during virus infection in untreated cells (101). However, mature virus particles are not formed in the presence of rifampin (51, 106), but on removal of the antibiotic maturation to infective viruses can occur (106, 158). This has been confirmed by electron microscope studies (51, 71, 110, 120). Efforts have therefore been concentrated on the study of the events in virus development after the removal of rifampin. Moss and co-workers (106) found that even in the presence of cycloheximide, which inhibits protein synthesis, this maturation can occur. But Nagayama et al. (110), using other inhibitors, have come to the conclusion that some RNA and protein synthesis is required for the final steps of virus assembly. These authors have shown that the synthesis of four defined enzymes that are found in the virus core was blocked by rifampin. In rifampin-resistant mutants, these enzymes were synthesized in a normal way. Pennington et al. (120) have also found that protein synthesis is necessary for the final maturation steps. Katz et al. (71) demonstrated by electron microscopy that

the first event after the removal of rifampin was the conversion of the irregular membrane precursors to coated envelopes. This occurred during the first 10 min after rifampin removal, and only afterwards could the appearance of the late particulate RNA polymerase activity be demonstrated. The appearance of polymerase was nearly coincident with the formation of DNA-containing virus particles, which seems to indicate that integration into particulate form is required before this enzyme activity can be detected.

The development of cores within the envelopes could also be detected only after 30 to 60 min (69). Furthermore, Katz and Moss (69, 70) have found that rifampin prevents the formation of a core polypeptide by inhibiting the cleavage of a longer precursor molecule. The function of this polypeptide is not yet known. The sequence of events after the removal of the antibiotic suggested that cleavage of the precursor occurs during the formation of the virus core. Rifampin seems to act by interrupting earlier maturational events that precede the formation of the core polypeptide.

Antiviral activity is restricted to rifampin and a few other hydrazone derivatives. Other rifamycins that are equally active on bacterial RNA polymerase (191) have no effect. Recently, it was found (124) that an unknown component of the streptovaricin complex (not streptovaricin A or C; *see below*) inhibited the growth of cowpox virus but was much less active against vaccinia or rabbit pox virus. The substituents of rifampin itself, 1-methyl-4-amino-piperazine (166) and other *N*-amino-piperazines (88), were also reported to show antiviral activity. However, inhibition by 1-methyl-4-amino-piperazine could not be confirmed in recent studies by Follett and Pennington (38). In bacteria, this substance has no effect (166); in fact, it has been shown that the ansa ring is the crucial part of the molecule for activity on bacterial RNA polymerase and that substituents in position 3 have practically no influence (177). Which parts of the rifamycin molecule are responsible for the anti-poxvirus activity is not clear at present, but the molecular requirements for viral and bacterial inhibition are certainly not identical.

These differences, together with the fact that concentrations 1,000 to 10,000 times higher than are needed for bacterial inhibition are necessary to affect virus growth and that this inhibition is readily reversible, clearly show the different mode of action in bacteria and viruses. The large amounts of rifampin required naturally preclude any clinical application of the drug as an antiviral agent. It may be relevant in this respect that Engle et al. (36) found rifampin inactive in the mouse against vaccinia virus, even when administered to mice in maximally tolerated doses, whereas a partial inhibitory action of the drug was demonstrated in H-1 virus infections of hamsters.

Effects on RNA viruses

Whereas some DNA viruses can be inhibited by rifampin, RNA viruses such as vesicular stomatitis virus and reovirus were not affected by the antibiotic (58, 157). However, some in vitro effects on the class of oncogenic RNA viruses, such as Rous sarcoma virus have recently been found. Diggelmann and Weissmann (32) have shown that the formation of foci in chick fibroblast cell cultures infected with Rous sarcoma virus is inhibited by rifampin but not by other derivatives. As in the case of the DNA viruses, large concentrations of the drug are necessary to produce the effect. In the oncogenic RNA viruses, a new enzyme, an RNA-dependent DNA polymerase, has been identified (4, 155, 165). A similar enzyme was also detected in the lymphoblasts of patients with acute lymphoblastic leukemia (46). Whereas these enzymes are not or are only slightly inhibited by rifampin, *N*-desmethyl-rifampin was shown to be partially active at a concentration of 50 μg/ml. Much higher concentrations are required for complete inhibition (46). Similar results have been reported by Green and co-workers for a variety of rifamycin derivatives with modified aminopiperazine side chains (52).

If the presence of such an enzyme is indeed required for the proliferation of leukemic or other neoplastic cells, its specific inhibition would also be very interesting for possible clinical application. However, derivatives that are active in much smaller concentrations would be required. Moreover, recently a DNA polymerase was isolated from normal mammalian cells that was also inhibited by rifamycin derivatives (138). A first hypothesis that this enzyme was closely related to the viral reverse transcriptases proved to be wrong, since it could be clearly distinguished from the viral enzyme by chromatographic and immunological methods (133*a*). The fact that both enzymes, although physically different, are inhibited by rifamycins raises some doubt about the specificity of the action of rifamycins in this case.

Effects on Trachoma Agent

The infectious elementary bodies of trachoma agent belong to the *Chlamydozoaceae* which are considered unusually small bacterial cells. They contain DNA, RNA, and ribosomal subunits and grow as parasites in mammalian cells (134).

Becker and co-workers (8) studied the effect of

rifampin in the developmental cycle of trachoma and psittacosis agent in mammalian cells and embryonated eggs. They found that the multiplication of the trachoma agent, and to a lesser degree of the psittacosis agent, was inhibited. As in the case of the DNA-viruses, derivatives with a hydrazone side chain proved to be the most active ones; however, some differences in their specificity as compared with the viruses were noted (10). Even a therapeutic effect upon local treatment of a monkey's eye has been reported (9), but very high concentrations were required.

CLINICAL APPLICATIONS

Rifamycins have a wide spectrum of antibacterial activity, but the susceptibility of the various organisms differs considerably. The minimal inhibitory concentration actually ranges from 0.0005 to over 20 μg/ml according to the bacterial species (76). Particularly sensitive are gram-positive organisms and mycobacteria. Among the gram-negative organisms, *Neisseria* and *Haemophilus* are also quite sensitive, with minimal inhibitory concentrations of less than 1 μg/ml (1, 13).

Accordingly, the clinical efficacy of the orally active semisynthetic derivative rifampin (42) was proven to be greatest in infections caused by the most sensitive organisms. The antimycobacterial activity in vitro and in vivo has been well documented (22, 25, 94, 116), and numerous publications on its clinical use as first line drug in tuberculosis have appeared (22, 29).

Since about one organism in 10^8 spontaneously develops resistance to rifamycins (76, 82, 83), the emergence of resistant bacteria is related to the inoculum size (60, 82). As there is no cross-resistance with other antibiotics (83), combination therapy in tuberculosis can be carried out with any other class of antibiotics (61). Over 100 reports on the effect of rifampin against nontuberculous infections have been published (29), the agent being most clearly defined in the treatment of gonorrhoea (182), meningococcal carriers (27, 30), and urinary (79, 121), dermatological (102), and respiratory infections (11).

Besides its antibacterial activity, rifampin has a variety of effects on viruses, and it has even been referred to as "wonder drug." However, the term "drug" has to be used with caution when it is applied to the effects found with viruses and some forms of cancer. The quantities of the rifamycin derivatives thus far known that are needed for an inhibitory effect absolutely preclude any clinical use as systemic drug against viruses and cancer. The chief value of the rifamycins in these fields has thus far been as a tool in elucidating the biochemical events on which they have an influence.

COMPOUNDS RELATED TO THE RIFAMYCINS

Three groups of antibiotics that are chemically very similar to the rifamycins have been described: the streptovaricins (128, 130, 148, 181, 187), the tolypomycins (67, 74, 75), and geldanamycin (28, 135). Like the rifamycins, they all contain an aromatic ring system spanned by an aliphatic bridge (Fig. 8). Owing to this characteristic structure, the whole group of antibiotics has been termed ansamycins according to a proposition of V. Prelog.

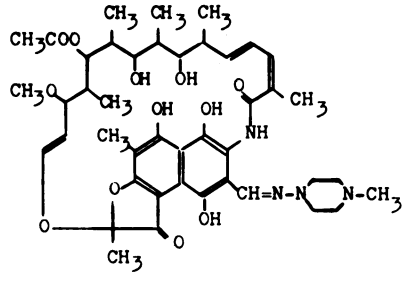
The streptovaricins and tolypomycins have biochemical properties closely resembling those of the rifamycins, as would be expected from their chemical similarity. They inhibit the initiation step of bacterial RNA polymerase (103, 104), although to a considerably lesser degree than the most active rifamycins. This is due to the fact that they form a less stable complex with RNA polymerase (Wehrli et al., *in preparation*). Both drugs competitively inhibit the binding of rifamycins to the enzyme, indicating that the binding site is the same for all three groups of antibiotics. Resistance to streptovaricins is also due to an altered RNA polymerase (114, 189, 190).

However, mapping of the locus for streptovaricin resistance yields a result slightly different from that found with the rifamycins (189). The available data are not sufficient to show whether this difference is real or an artifact due to experimental conditions, e.g., the use of different bacterial strains. But the fact that the streptovaricins compete with the rifamycins for a binding site on the enzyme, together with the finding that thus far no rifamycin-resistant RNA polymerase has been isolated that is inhibited by streptovaricin (16), suggests that the two antibiotics attach to the enzyme at the same site.

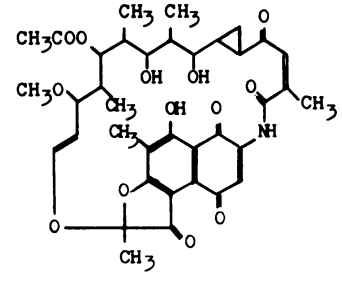
Mammalian RNA polymerase has been shown to be unaffected by the streptovaricins (103). However, streptovaricin D, but neither A nor B, inhibits the incorporation of nucleosides into HeLa cells (164). One derivative of the streptovaricin complex (not A nor C) was found to inhibit the replication of poxvirus (124; *see above*). This selective activity corresponds to that of the rifamycins, in that only rifampin and a few other compounds affect viral growth.

Geldanamycin inhibits bacteria only in rather high concentrations (27). No biochemical data have thus far been reported, but it is interesting to note that extensive simplification of the chromophoric ring system still yields a substance with antibiotic activities.

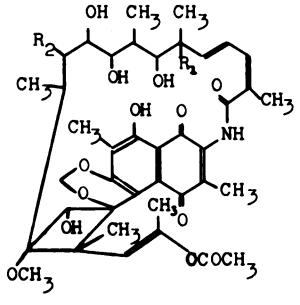
Another noteworthy compound is streptolydigin. It does not belong to the ansamycin group, and its mechanism of action is different. It also inhibits bacterial RNA polymerase, although it is not RNA chain initiation but the RNA chain elongation step that is affected (22*a*, 147). Rather high concentrations of streptolydigin are needed to obtain inhibition. Resistance to this substance is due either to changes in cell permeability or to a modified RNA polymerase (136, 153). In contrast to rifamycin, however, complete resistance to streptolydigin has not been observed. Heil and Zillig (57) have demonstrated that, like rifampin, it attaches to the β subunit of RNA polymerase. Thus, marked similarities do exist between streptolydigin and the rifamycins, although their sites on the RNA polymerase are obviously not identical. However, comparison of the chemical structure of the two compounds reveals striking analogies (Fig. 8): streptolydigin contains a great part of the ansa ring of the rifamycins, including the two oxygen functions at positions 21 and 23 and the tetragonal carbon atom 21, which has been shown to be crucial for the rifamycin activity. Thus, it could be maintained that the sites for streptolydigin and rifamycin on the RNA polymerase partly overlap and that a substituted carbon-hydrogen chain from C atom 15 to 27, such as is present in all the ansamycins and in streptolydigin, is a partial requirement for binding to RNA polymerase and thus for enzyme inhibition. However, it is clear that not every



Rifampicin

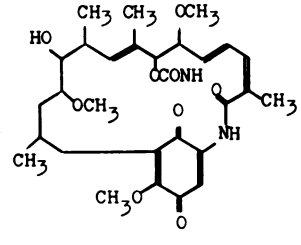


Tolypomycinon

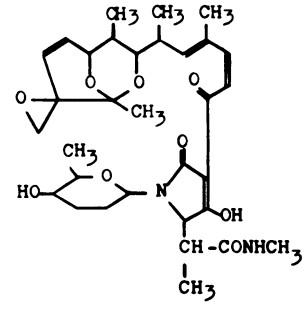


Streptovaricin

	R_1	R_2
A:	OH	OAc
B:	H	OAc
C:	H	OH
D:	H	OH *



Geldanamycin



Streptolydigin

FIG. 8. *Structural formulas of various ansamycins and streptolydigin. * Streptovaricin D does not contain a cyclobutane ring because the C atoms carrying the —OCH_3 and —CH_3 groups are not linked (164).*

substituted ansa ring can affect the enzyme, since rifarubin, e.g., a rifamycin derivative with an ansa ring cleaved at position 29, is completely inactive.

SUMMARY AND CONCLUSIONS

The primary effect of the rifamycins on bacteria seems to be clear, at least in general: they specifically inhibit DNA-dependent RNA polymerase by blocking the RNA chain initiation step. The specificity of this action is due to the fact that the drug binds very tightly to the enzyme and therefore is effective at very low concentrations. Mutation of single amino acids in the enzyme can diminish or completely abolish this binding capacity, yielding a drug-resistant enzyme and consequently a resistant organism. The crucial part of the rifamycin molecule responsible for its inhibitory action on bacterial RNA polymerase is the ansa ring. The aromatic ring system, however, can be modified to some extent without detracting from the activity of the substance. Under conditions in which bacterial RNA polymerase is completely inhibited, the corresponding mammalian enzymes are not affected. Some rifamycin derivatives, especially the orally active derivative rifampin, have therefore proved to be clinically very valuable antibiotics.

Besides the specific inhibition of bacterial DNA-dependent RNA polymerase, the rifamycins display a variety of other effects. These other actions, however, require a 100- to 10,000-fold higher concentration of the antibiotic. All results must, therefore, be very carefully evaluated to exclude possible unspecific reactions. This applies especially to the various observations made with eukaryotic cells, for example, those with mitochondria and chloroplasts. These have usually been explained, by analogy with bacteria, as being due to inhibition of RNA polymerase. However, in most instances the enzymes were not well defined. In the case of yeast, it was possible to isolate and characterize two mitochondrial RNA polymerases that were DNA-dependent, but neither enzyme was inhibited by rifampin concentrations 1,000 times higher than those required to inhibit the bacterial enzyme (170). Thus, it is clear that mitochondrial RNA polymerase is not as bacteria-like as it has often been asserted to be.

The effects of the rifamycins on viruses are rather heterogeneous. In the case of bacterial viruses, the drug has mainly been used to determine the role played by the host RNA polymerase during virus development. Mammalian viruses are, in general, unlikely to be influenced by rifamycins. However, the multiplication of poxviruses has been shown to be sensitive to certain rifamycin derivatives, especially rifampin. Although very high concentrations of antibiotic are needed, the effect is specific, since rifampin-resistant strains could be selected. The mechanism of action is not clear as yet; however, it seems to be different from that observed in bacteria.

Most recently, the observation that selected rifamycin derivatives affect the RNA-dependent DNA polymerase found in tumor-inducing RNA viruses and in leukemic cells has led to speculations on the possibility that some forms of cancer might be affected by the rifamycins. However, the very large amounts of antibiotic required to produce enzyme inhibition rule out the therapeutic use of any of the known derivatives. Furthermore, the recent discovery in normal mammalian cells of an enzyme different from the one found in RNA viruses but also inhibited by certain rifamycins (133*a*, 138) raises some doubt about the specificity of the effects on the viral enzyme.

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