The Chemical Approach to the Control of Tuberculosis[1]

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ALTHOUGH THE VIVIDLY DESCRIPTIVE NAME "white plague" has long since gone out of fashion in designating tuberculosis, it is worth remembering that the disease itself is still very much with us. In disclosing his discovery of the tubercle bacillus in 1882, Koch (*1*) pointed out that tuberculosis was the greatest killer of human beings among all the diseases. What Koch said then is still true today, despite the tremendous strides made in the chemotherapy of bacterial infections. It is estimated that 5–6 million people are killed yearly throughout the world by the tubercle bacillus.

The tuberculosis problem. Under the impetus of such a tremendous spur, it might seem strange that so little has been accomplished in controlling the disease, but the uncomfortable fact is that the fungus-like organism has proved to be practically immune to the many chemotherapeutic agents that are so spectacularly effective against other bacteria. The common antibiotics such as penicillin, aureomycin, terramycin, and chloramphenicol, as well as the host of active "sulfa" drugs, are without significant effect in tuberculosis. In this intractableness, tuberculosis is almost unique among bacterial diseases. It is difficult to overemphasize this point, because the whole problem of its chemotherapy has hinged upon this outstanding and deplorable characteristic. It has also been responsible, in part, for a major misconception concerning the structure of the tubercle bacillus which has plagued workers engaged in the search for antitubercular agents. According to the generally accepted concept, the intractable character of the tuberculous infection and the resistance of the tubercle bacillus to chemotherapeutic agents were due to a waxy capsule surrounding the organism (*2, 3*). On the assumption that penetration of the organism by the drug was an essential prerequisite for antitubercular activity, it seemed reasonably certain that the desired therapeutic effect could be achieved only with fat-soluble materials, which could penetrate through the waxy envelope. We know now that fat-solubility is not required. Indeed, all the effective tuberculostats known to date are either water-soluble or are highly polar molecules of the type associated with water-solubility rather than fat-solubility. Nonetheless, some experimentalists still stress the positive influence of a high lipid/water distribution ratio on the inhibitory action of their compounds.

Another difficulty inherent in the tuberculosis problem is the fact that the host-parasite relationship is different from that found in the common bacterial infections. In the latter type, the struggle between the host and the parasite is an acute, all-or-none affair, with one or the other quickly succumbing. Since the advent of modern chemotherapy, the struggle has been shortened still further, and the issue is generally decided in favor of the host. On the other hand, most tuberculosis is chronic in character, and the tubercle bacillus can remain viable in the host for long periods without provoking a fulminating, all-or-none struggle. Moreover, the host's defensive mechanisms seem slower and less certain, so that even though the disease process has been slowed or suppressed by means of a tuberculostat the host is not capable of rapidly destroying the invading organism.

The importance of the chronicity of an infection on its susceptibility to treatment was recently stressed by Florey (*4*), who pointed out that staphylococcic osteomyelitis, upon early diagnosis and treatment, before tissue destruction has occurred, can be controlled by penicillin alone. In the later stages, when an abscess is present, the pus must be drained off before a satisfactory penicillin effect can be obtained. When, still later, the bone becomes chronically infected and dead bone is present, sterilization with penicillin is impossible and surgical removal of the dead tissue is essential. Florey further stated:

> The reason for the failure of penicillin to sterilize slough or dead tissue is not clear, but the fact is worth keeping in mind as we go on to consider the possibilities of chemotherapy of tuberculosis, for penicillin in its own field is a very powerful bactericidal agent compared with agents used against mycobacteria, and the lesions of tuberculosis nearly always contain necrotic tissue.

It would therefore seem that, even though the old concept of a waxy, protective capsule around the tubercle bacillus is untenable, the problem of getting the drug to the organism is still salient because of the mechanical barriers imposed by necrotic and fibrotic tissue, caseation, and incorporation of the bacilli in phagocytes.

As an end result of these difficulties, it is very unlikely that any drug will be found which will cure chronic tuberculosis with the same speed and dispatch

[1] Contribution No. 299 from the Research Laboratories of Hoffmann-La Roche Inc.

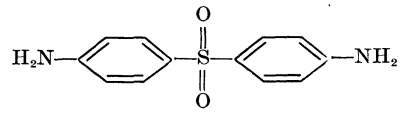
as penicillin, for example, disposes of many acute bacterial infections. Even if the drug were powerfully tuberculocidal, clinical cure would still be relatively slow because of the extensive tissue destruction that accompanies the disease, and because healing and tissue regeneration are in themselves slow processes. Since it is patently obvious that no drug can be expected to restore a destroyed lung, it becomes axiomatic that early diagnosis and treatment are essential corollaries to successful chemotherapy.

The antibiotics. The clinically effective chemotherapeutic agents for tuberculosis may be divided into two principal classes: the antibiotics and the synthetics. Until very recently the most important and most active tuberculostats belonged to the antibiotic class. These were streptomycin, first isolated in 1944 by Schatz, Bugie, and Waksman (*5*), and its hydrogenated derivative dihydrostreptomycin. The latter compound was developed (*6*) in what proved to be an essentially unsuccessful effort to find a less toxic substitute for streptomycin. Both compounds are about equally effective in the treatment of miliary tuberculosis, pulmonary tuberculosis, and tuberculous meningitis and, despite initial expectations, are about equally toxic. The list of toxic reactions attending the use of these drugs has assumed formidable proportions over the years, but perhaps their most serious disadvantage lies in the rapidity with which resistant strains emerge. This phenomenon has militated against their use in minimal cases of tuberculosis on the theory that most such cases would recover with the more conventional regimen, and that it would therefore be unwise to run the risk of developing in them a streptomycin-resistant strain. The necessity of withholding streptomycin or dihydrostreptomycin in the early stages when the disease is most susceptible to chemotherapy is, of course, particularly painful to the clinician, because it is entirely contrary to his concept of what is generally regarded as the ideal practice. More recently, it has been found that the emergence of resistant strains can be delayed by the concomitant administration of *p*-aminosalicylic acid (*7, 8*). An additional disadvantage of streptomycin lies in the fact that some cases, which are apparently suitable for chemotherapy, fail, for some obscure reason, to respond to the drug. These are the so-called intractable cases.

The other antibiotics that have been clinically explored are neomycin (*9*) and viomycin (*10, 11*). Little need be said of these other than that they are less active than streptomycin and hold little promise for the future (*12*).

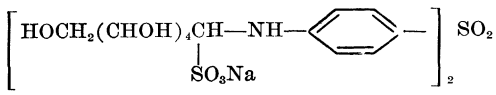
The synthetic tuberculostats. The synthetic tuberculostats may be divided into four principal categories: the sulfones, the aminohydroxybenzoic acids, the thiosemicarbazones, and the pyridine carboxylic acid derivatives.

The sulfones are chronologically the first of the modern synthetic tuberculostats. The tuberculostatic activity of 4,4′-diaminodiphenylsulfone, the parent substance of this group, was first discovered in 1939 (*13*), and since then many attempts have been made to develop sulfones with decreased toxicity and increased solubility and activity. No notable success in

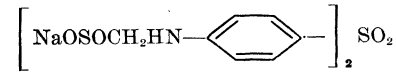


4,4′-Diaminodiphenylsulfone

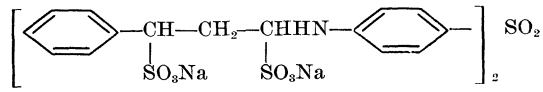
this direction has yet been achieved. Derivatives, such as promin (*14*), diasone (*15, 16*), and sulphetrone (*17*), although more soluble and less toxic, are also less active than the parent substance.



Promin

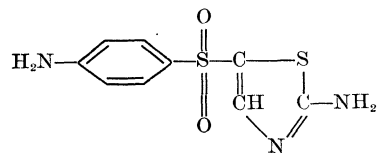


Diasone



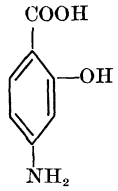
Sulphetrone

It has been suggested, but not proved, that these compounds are active, because they are degraded in the body to 4,4′-diaminodiphenylsulfone. Promizole (*18*), a slightly later development in the sulfone field, appears to be somewhat better than the others (*19*), but clinically all the sulfones leave much to be desired. They are all quite toxic, and none of them is sufficiently tuberculostatic to serve effectively as the sole chemotherapeutic agent in the treatment of clinical tuberculosis. It is interesting to note, however, that promin has been used with some success in Hansen's disease (*20*).



Promizole

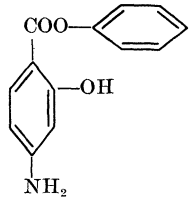
The tuberculostatic activity of *p*-aminosalicylic acid (PAS) was discovered by Lehmann (*21, 22*) in 1944 on the basis of the earlier observations of Bernheim (*23, 24*) that the oxygen uptake of the tubercle bacillus increased under the influence of benzoates and salicylates. The activity of PAS is much lower than that of streptomycin and is limited to pulmonary tuberculosis and tuberculosis of the mucous membranes, but because it is relatively atoxic and can be given safely in large doses, it has found clinical acceptance. Unfortunately, it is rapidly absorbed and



PAS

excreted so that, to maintain adequate blood levels, it must be given in large doses at frequent intervals. Lately, despite its low order of activity and limited usefulness alone, it is being ever more widely used, because, as was pointed out before, there is evidence to show that the appearance of resistant strains is retarded if PAS is given along with streptomycin (*7, 8*).

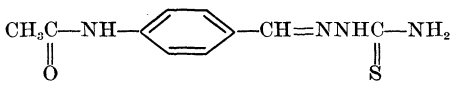
In the normal course of events, many variants of PAS have been prepared and studied, but with one possible exception, none of them has proved to be superior to the parent substance. In 1951, Freire, Rist, and Grumbach (*25*) announced the discovery of FR7 (phenyl *p*-aminosalicylate), a compound that



FR7

was designed with a view to slowing up the overly rapid absorption and elimination characteristic of PAS. According to them, FR7 given orally is no more active than PAS, but on subcutaneous administration in mice in either oil solution or aqueous suspension, it is ten times more active than PAS and is about equal to streptomycin.

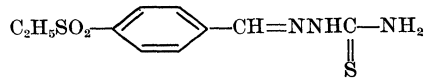
Until very recently the most active of all the synthetic tuberculostats were the thiosemicarbazones. Their discovery by Domagk and his co-workers (*26–29*) constituted a great forward step in the conquest of tuberculosis. Tibione (*p*-acetaminobenzaldehyde thiosemicarbazone), the most prominent mem-



Tibione

ber of this class, is now widely used in Europe, where it has been applied with some success in most forms of tuberculosis. In this country, it has not received much attention, because its use is accompanied by a high incidence of severe side reactions, which include gastric disturbances, anemia, and liver and kidney damage. Another probable reason for its lack of acceptance here is the ready availability of streptomycin, which is generally regarded as a much superior tuberculostat.

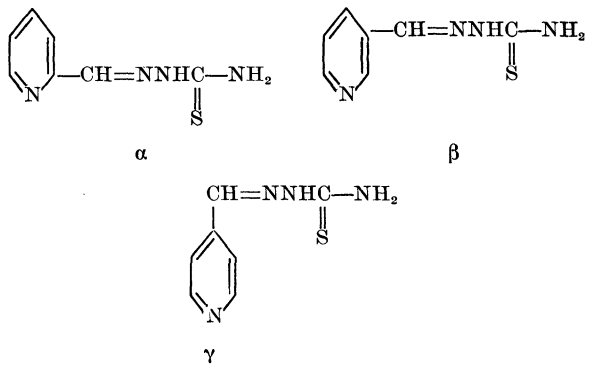
Many variations of the Tibione structure have been made, most of which have involved the character and position of the subordinate grouping on the benzene ring. None of these is superior to Tibione, with the possible exception of the *p*-ethylsulfonyl derivative (Tb III). This compound is not regarded with par-



Tb III

ticular favor by its originators, but some English workers consider it to be more promising than Tibione (*30, 31*).

Replacement of the benzene ring with pyridine results in the pyridine analog of Tibione. The three isomeric forms possible to this structure—picolinaldehyde thiosemicarbazone, nicotinaldehyde thiosemicarbazone, and isonicotinaldehyde thiosemicarbazone —have been prepared and studied, at least preliminarily. The α-isomer (picolinaldehyde thiosemicarba-

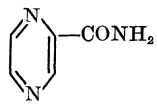


zone) was prepared by the author (*32*) and is too toxic for use. The β-isomer (nicotinaldehyde thiosemicarbazone) was prepared independently in Switzerland (*33*), France (*34*), and the United States (*35*) and, according to the French workers, is much superior to Tibione in animal studies (*34, 36, 37*). These results have been partially confirmed in this country (*38*). The γ-isomer (isonicotinaldehyde thiosemicarbazone) was prepared by the author (*32*) and was found to be comparable in activity to the β-isomer (*38*). In effect, therefore, the β- and γ-pyridylaldehyde thiosemicarbazones were the most potent synthetic tuberculostats known up to that time—particularly if judged by their marked antitubercular efficacy in the intranasal type of infection in mice.

The pyridine carboxylic acid derivatives constitute a relatively new category in the realm of synthetic tuberculostats, and their erstwhile most important member, nicotinamide, has been largely ignored by chemotherapists and tuberculotherapists in this country. The antitubercular activity of nicotinamide equals that of PAS, but it has not gained favor here, perhaps because it is generally anticipated that in the large doses required it would prove too toxic. It is,

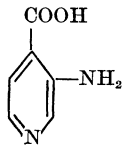
however, being tested more extensively in Europe.

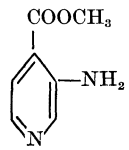
The discovery of the tuberculostatic activity of the vitamin nicotinamide was made by Chorine (*39*) and by Huant (*40*) in 1945. Chorine showed at the time that nicotinic acid, despite its vitamin activity, is not tuberculostatic and thus conclusively proved that there is no relationship between the two types of activities. Oddly enough, this very significant discovery seems to have completely escaped notice in this country. In 1948, the activity of nicotinamide in tuberculosis was rediscovered here by McKenzie and Kushner and their co-workers (*41, 42*) who, in addition, postulated that the activity against the tubercle bacillus is a function of its vitamin activity. This postulate was based on their observation that all the derivatives of nicotinamide that are tuberculostatic also have vitamin activity, whereas those derivatives devoid of one activity are devoid of the other. Perhaps because none of the active derivatives that they prepared were as good as nicotinamide, they appear to have dropped their investigation in this direction. Recently, Kushner *et al.* (*43*) announced the discovery of pyrazinamide (Aldinamide) as a tuberculostat with an activity about three times greater than that of PAS or nicotinamide. Preliminary reports indicate, however, that it quickly produces resistant strains—a fact that seems to rule it out as an effective clinical agent, at least when used alone (*44–48*).



Aldinamide

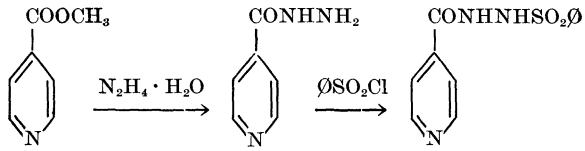
Intrigued by the postulate of McKenzie, Kushner, *et al.* and unaware of Chorine's work negating it, the author decided to investigate pyridine carboxylic acid derivatives closely related to nicotinamide in the hope of uncovering compounds of greater activity. The first results obtained seemed to confirm the postulate, but as the work progressed two compounds were discovered that proved to have tuberculostatic activity without vitamin activity. These compounds, 3-aminoisonicotinic acid and its methyl ester (*49*), were, to the author's knowledge, the first in the pyridine field,



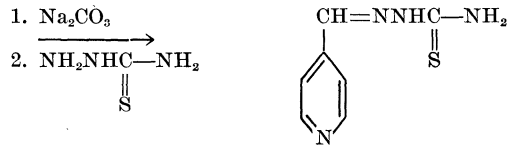


other than nicotinamide and its immediate derivatives, which exhibited *in vivo* antitubercular activity. Although both compounds are only about one half as active as nicotinamide and are of no interest clinically, they served to show that antitubercular activity in the pyridine field is not necessarily limited to derivatives of nicotinamide or to compounds with vitamin activity. This meant, in effect, that the field was wide open and that, theoretically, at least, tuberculostatic activity might exist in any pyridine structure.

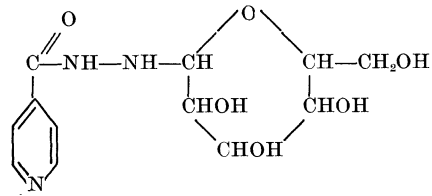
This concept was confirmed subsequently by the marked activity of the β- and γ-pyridylaldehyde thiosemicarbazones. It was in the preparation of the latter compound by the author that the discovery was made of a new class of antitubercular agents of remarkable *in vivo* activity. In preparing isonicotinaldehyde thiosemicarbazone by a modification of the McFadyen-Stevens reaction (*32*), methyl isonicotinate was converted to isonicotinylhydrazine. The latter, in turn, was treated with benzenesulfonyl chloride to give 1-isonicotinyl-2-benzenesulfonylhydrazine, which yielded the desired thiosemicarbazone on alkaline decomposi-



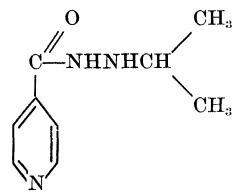
Rimifon



tion in the presence of thiosemicarbazide. Since both the isonicotinic acid hydrazide and its benzenesulfonyl derivative were pyridine carboxylic acid derivatives and therefore closely related to the structures under investigation, they were submitted for testing to the Chemotherapy Laboratories of Hoffmann-La Roche Inc. The benzenesulfonyl derivative was shown to be inactive. On the other hand, the isonicotinic acid hydrazide (Rimifon[2]) proved to have an *in vivo* anti-



1-Isonicotinyl-2-d-glucosylhydrazine



Marsilid

tubercular activity that far exceeded that of any other known substance—whether synthetic or antibiotic. Further investigation of this new type of tuberculostat resulted in the synthesis of 1-isonicotinyl-2-d-glucosylhydrazine and 1-isonicotinyl-2-isopropylhy-

[2] Trade mark of Hoffmann-La Roche Inc.

drazine (Marsilid[2]). Both these compounds were remarkably potent, but the glucosyl derivative proved to be relatively unstable and was withdrawn after brief clinical testing (*50*).

Chemotherapeutic studies by Grunberg and Schnitzer (*51*) indicate that mice infected intravenously with 0.5 ml of a 10^{-1} dilution of a 7–10-day-old culture of *Mycobacterium tuberculosis* in Dubos medium are protected in 50 per cent of the cases (PD_{50}) by a daily dose of 4.6 mg/kg Rimifon in the diet, whereas the PD_{50} is 6.2 mg/kg in mice infected intranasally with 4 drops of a 10^{-1} dilution of a 7–10-day-old culture of *M. tuberculosis* in Dubos medium. When the drug is administered subcutaneously, the PD_{50} for the intravenous infection is 1.86 mg/kg as against 1.77 mg/kg for the intranasal infection.

Similarly, the PD_{50} of Marsilid given in a medicated diet to mice with the intravenous and intranasal type of infection is 7.3 mg/kg and 10.7 mg/kg, respectively, whereas by subcutaneous administration the respective figures for the PD_{50} are 5 mg/kg and 3.3 mg/kg.

Protection of all mice, shown by the absence of characteristic lesions, was consistently achieved in an unusually low dosage range (10 mg/kg/day or less); at about twice this dosage, cultures from the lungs were negative. No comparable results could be obtained with other known antitubercular agents, such as streptomycin, PAS, and the thiosemicarbazones, even if high doses were used. Steenken and Wolinsky (*52*) observed that infected guinea pigs, which gave a positive tuberculin reaction initially, gave an almost negative test after treatment with Marsilid but remained positive after streptomycin treatment.

The acute toxicity studies in mice (*51*) show that Rimifon has an LD_{50} of 203 mg/kg orally or subcutaneously and an LD_{50} of 171 mg/kg intravenously. Marsilid, which in mice is considerably the less toxic of the two, has an oral LD_{50} of 920 mg/kg and an LD_{50} of 689 mg/kg intravenously. The pharmacology of the compounds has been described by Benson *et al.* (*53*). Zieper and Lewis (*54*) administered Rimifon to a *Macacus rhesus* monkey with clinical tuberculosis and appeared to have obtained a clinical arrest, which was confirmed by post-mortem examination.

The superiority of Rimifon and Marsilid over streptomycin in mouse infections is illustrated in Table 1[3], where the efficacy of the three drugs by the subcutaneous route is compared. On this basis it is apparent that Rimifon and Marsilid are active at one thirteenth and one fifth the dose of streptomycin, respectively, in the intravenous type of infection; in the intranasal type of infection the corresponding dosage ratios are 1 : 56 and 1 : 30.

Unlike streptomycin, both Rimifon and Marsilid are practically as effective orally as they are parenterally. A comparison of the efficacy of the two, as against Tibione, given per os in mouse infections, is shown in Table 2.[3]

TABLE 1

Compound	Route	LD_{50} (mg/kg) *a*	Intravenous type	Intranasal type	Therapeutic ratio	
			PD_{50} (mg/kg) *b*	PD_{50} (mg/kg) *c*	*a/b*	*a/c*
Streptomycin	Subcutaneous	970	25	100	38.8	9.7
Rimifon	"	203	1.86	1.77	109	115
Marsilid	"	732	5	3.3	150	222

TABLE 2

Compound	Route	LD_{50} (mg/kg) *a*	Intravenous type	Intranasal type	Therapeutic ratio	
			PD_{50} (mg/kg) *b*	PD_{50} (mg/kg) *c*	*a/b*	*a/c*
Tibione	Per os	825	50	829	16.5	1.0
Rimifon	" "	203	4.6	6.2	44.1	32.7
Marsilid	" "	920	7.3	10.7	126	86

Preliminary clinical investigations of both drugs show them to have unusual antitubercular activity without severe toxic reactions (*50, 55, 56*). In a concluding statement, Robitzek and his co-workers (*50*) stated:

> The systemic ravages of the tuberculous process are rapidly halted; there is loss of toxicity, return of temperature to normal, recovery of appetite and remarkable weight gain. This occurs with a rapidity, a certainty and to a degree which we have never observed in other chemotherapeutic or antibiotic agents. In limited studies, we have also observed a marked effect on the local, anatomical pulmonary lesion, evidenced by some radiological changes, marked reduction in cough and expectoration in about one-third of the cases, and an apparent, at least temporary, conversion of the sputum to negative on bacteriological examination.

Conclusion. In a discussion of the chemotherapy of

[3] These tables are based on the published and unpublished work of Schnitzer and Grunberg.

tuberculosis, Florey (*4*) listed some of the features to be sought in an antitubercular agent. Among these were small molecular size with concomitant easy diffusibility to the site of infection, bactericidal rather than bacteriostatic activity in low concentrations, a slow rate of production of resistant strains, and a relative atoxicity to the host in general and to the cells of the kidney, liver, and other organs where the drug might be concentrated. At the current state of our knowledge, the isonicotinylhydrazines, as exemplified by Rimifon and Marsilid, appear to fulfill these requirements with fidelity. They are small molecules, very soluble in water, and they probably diffuse with great ease through the body tissues. They are highly active, and at certain dose levels, there is some evidence to indicate that they are bactericidal rather than bacteriostatic. From the preliminary observations of Robitzek and his co-workers (*50*), it would appear that drug resistance does not readily develop. They are relatively atoxic with a very favorable therapeutic ratio and, finally, they are effective orally and are within the economic reach of most of the civilized world.

Whether these drugs, in combination with early diagnosis and treatment, will provide the answer to the problem of tuberculosis remains to be seen. Much work must be done, and some time must elapse before we can correctly evaluate them and see them in the proper perspective. This much, however, is certain—whether with these drugs or with others—the problem will be solved; the answer will be found.

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