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Some recent developments in the chemotherapy of mycobacterial disease with special reference to some new oxadiazolones

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by

ARTHUR ERNEST WILDER SMITH

B.Sc., B.Sc., Fellow of the Royal Institute of Chemistry, London.
Doctor of Philosophy of the University of Reading, England.

British subject by birth

Accepted on the recommendation of:
Professor Dr. J. Büchi and Professor Dr. E. Hardegger

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This work is dedicated to my wife who, renouncing a promising career as cellist and organist, has given me constant companionship, encouragement and secretarial efficiency while bringing up four little children in four countries and thus made this venture possible.

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Chapter I

GENERAL INTRODUCTION

a) Tuberculosis

With the advent of streptomycin, p-amino-salicylic acid and isonicotinic acid hydrazide (INH) during the years between 1940 and 1952 there were many workers who prophesied that just as tuberculosis had been conquered by various means in animals (mostly non-chemotherapeutic!) in some countries, so now it was merely a question of time before human tuberculosis would be eradicated entirely. In fact, even before the advent of rational tuberculosis chemotherapy, the tuberculosis figures had been falling regularly for many years in developed countries, so that a deal of optimism has been shown. For example, Talcott Williams [1] stated in 1905 that: "One may unhesitatingly say that the child is now born who will live to see the year in which a well sprinkled, well swept, well watched and well sanatoriumed city will put as many difficulties in the way of the frequent local study of tuberculosis as now exists for the local study of smallpox or typhoid." As René Dubois fittingly remarks [2] tuberculosis is still in the process of disappearing since 1905!

The reason for this optimism lies in the falling mortality figures. Every new therapeutic advance accelerates this fall, but no single advance is entirely responsible for it. The mortality figures in the USA, thinks René Dubois, will perhaps sink to about 20 000 annually and the public will accept this as the lowest rate possible.

But, although the mortality rate has fallen, the morbidity rate has not fallen correspondingly. The reason is that tuberculosis has, by modern methods, been changed from a lethal disease to a chronic one. But a chronic disease is by no means a defeated disease. The terror of the lethalness of tuberculosis has been defeated, but not the disease itself.

The fact that tuberculosis has become chronic, rather than lethal, has made it a social problem too. Patients are sometimes hospitalised for long periods and cannot support themselves financially. The tendency to treat tbc patients at home ambulatorily has introduced, too, a further factor. Such patients constitute an infection risk to others. In earlier times, such patients would soon have died and infection spread would not have been a problem. But now these patients live and live to infect others. Thus the human reservoir of tuberculosis infection is ever increasing due to this transfor-

mation of a lethal disease into a chronic one and the percentage danger of infection rises from year to year as a result.

A further grave problem presents itself. Patients treated chronically with tuberculostatics do become drug resistant, especially if their management is faulty. Their chemotherapy resistant Mycobacteria tuberculosis are capable of infecting healthy subjects to produce a tuberculosis which is not sensitive to known chemotherapeutic agents. In fact, it is known that, if mice are infected with isoniazid resistant strains of Mycobacterium tuberculosis, treatment with INH renders the infection much more virulent. Since the infecting organism has become drug dependent, treatment with the drug activates the invading organism. Thus treatment of host organisms, infected with isoniazid resistant strains, may precipitate a more lethal form of the disease, at least in animals and in the case of isoniazid.

Thus it is clear that therapeutic surprises may await us as a result of the widespread chronic use and abuse of tuberculostatics and the conversion of the disease from a lethal to a chronic one.

For these reasons, although we possess active tuberculostatics in p-aminosalicylic acid, streptomycin, isoniazid and others, vigorous research on new tuberculostatics is urgently required. It is particularly necessary to understand better the processes underlying resistance emergence, so as to be better able to prevent or further inhibit its appearance. New tuberculostatics, towards which Mycobacterium tuberculosis shows a slower development of resistance emergence than that at present experienced with present day drugs and their combinations, would certainly be most welcome.

b) Leprosy

Leprosy is probably one of the oldest diseases described in history. Many references to it occur in the Old and New Testaments, although it is doubtful if the disease often translated with the word leprosy had, in fact, anything to do with our modern concept of the disease related to Hansens' bacillus (Mycobacterium leprae). Leviticus XIII and XIV describe a contagious condition, which is obviously little related to leprosy as we understand it today. Naaman (2 Kings V) was probably a case of scabies, but King Uzziah (2 Chron. XXVI, 19) was, judging by the description of the flushing of the facial capillaries, making the lesions stand out in relief, probably a case of lepromatous leprosy.

It is easily forgotten that leprosy was endemic in Europe and Scandinavia through-out the Middle Ages. It probably arrived in Europe about B.C. 500 and spread with the Roman Armies. The Spaniards and Portuguese took it with them to South America.

There are still three or four endemic leprosy cases in Bergen, Norway, but the disease in Scandinavia is rapidly dying out with improved living conditions and without chemotherapy. In certain parts of the Southern and Western United States the disease is endemic too, and the United States Public Health maintains a modern lep-rosarium at Carville. Spain and Portugal still have some endemic leprosy. It is thought that there are about 15 million lepers in the world at present, but exact fi-gures are difficult to arrive at, since patients fear to disclose their disease and of-ten hide it so long that effective therapy is no longer possible and mutilation unavoid-able.

The same sort of prophecies were made with regard to leprosy as to tuberculo-sis when the sulphones came into use as leprostatics replacing Chaulmoogra oil. Although no resistance of Mycobacterium leprae to dapsone or other sulphones has been noted generally, yet a diagnosis of lepromatous leprosy still entails five to se-ven years of intensive chemotherapy. Lifelong chemotherapy at a maintenance dose level is often indicated and relapses and failure to respond often occur. Senior lepro-logists have noted that the incidence of the disease seems to be actually increasing [3], particularly in India and other developing countries where poor nutrition and poor living conditions exist. Thus a parallel exists between the incidence of tuber-culosis and that of leprosy in certain parts of the world.

Over and above these difficulties, sulphones are toxic substances and in high doses, produce various undesirable effects, particularly that of anemia.

Thus, the active prosecution of research into the basic problems of leprosy and leprosy therapy is an urgent necessity.

The foundation of leprosy research was laid in 1879 when Dr. G. Armauer Hansen of Bergen, Norway, found Mycobacterium leprae to be the causative orga-nism of leprosy. It was fourteen years later that Koch isolated Mycobacterium tuberculosis. But, it must be remembered, in contrast to Mycobacterium tubercu-losis, Mycobacterium leprae has never been with certainty cultured in vitro. Until Dr. Shepherd's work on the mouse foot pad method was recently published [4] no certain culture of the organism outside the human body had ever been achieved. Dr. Shepherd's success in culturing human Myc. leprae in the mouse foot pad may considerably aid in the testing of leprostatic drugs and is therefore potentially a

great step forward. The achievement is probably due to the recognition of the fact that Myc. leprae grows in the human only in tissues showing reduced temperature, i. e. the testes, nasal septum, peripheral nerves, epithelium, etc. The mouse foot pad shows also a reduced temperature and proved suitable.

Innumerable attempts had been made to achieve this end by H a n k s and others without success. This fact alone has held up therapeutic and metabolic research on leprosy immensely. It is for this reason too that, although attempts have been made to grow Mycobacterium leprae in certain cold blooded animals, notably fish, most therapeutic efforts in leprosy research begin with testing the effect of prospective leprostatics on a culture of Mycobacterium tuberculosis grown on a synthetic medium. In fact, most leprostatics used today began life as potential tuberculostatics. The sulphones were first tried as tuberculostatics, but were dropped owing to toxicity due to excessive dosage and low activity compared with streptomycin etc. V a d r i - n e [5] (p-amino-salicylate of 2-pyridyl-1,3,4-oxadiazolone) similarly, was first investigated as a tuberculostatic and was found to be about as active in this disease as streptomycin. It was its high activity in miliary tuberculosis which led to its being tested in leprosy, with results which are well known today.

c) Realtionship between Tuberculosis and Leprosy

In general the only other human disease resembling leprosy at all is tuberculosis. But the resemblance does not go very far. The morphology of the causative bacilli is similar and certain skin lesions are comparable. It is the affinity of Mycobacterium leprae for peripheral nerve tissue which distinguishes it from Mycobacterium tuberculosis. Mycobacterium leprae leaves the lungs, intestine and central nervous system unaffected, in contrast to Mycobacterium tuberculosis. The chief clinical lesions associated with Mycobacterium leprae are found on the surface of the body and therefore easily investigated. No X-rays are necessary, in contrast to tuberculosis, for the diagnosis of leprosy. A feather or a piece of cotton wool are sufficient for at least a preliminary examination.

In spite of these differences in tuberculosis and leprosy a good deal of the chemotherapeutic progress achieved in leprosy in the past decade or so has been effected, for the reasons given above, via tuberculosis chemotherapeutic research. For this reason it is considered natural to treat together some of the chemotherapeutic advances in the two diseases.

d) Development of Theory and Practice in the Chemotherapy of Mycobacterial
Disease. Tuberculosis.

The first efforts at the chemotherapeutic control of infectious diseases were
carried out under the impression that the chemotherapeutic agent should specifical-
ly sterilise the host with respect to the offending invading organism. That is, the
idea motivating chemotherapeutic research was to be a practical utilisation of E h r -
l i c h 's "therapia magna sterilisans". Later experience showed that this belief was
inadequate to cover the facts. Salvarsan, for example, seems to have a stimulative
effect on the host as well as a possible direct inhibiting effect on the invading orga-
nism. It is probably, however, correct to say that today bacteriostasis is still over-
emphasised and the indirect effect on the host tissues underemphasised.

Before the "chemotherapeutic era", up to about 1935, the "Reiztherapie" held
sway in concepts behind tuberculosis therapy. Heavy metal salts, particularly gold
salts, were employed in an effort to stimulate the reticulo-endothelial system, lib-
erating Mycobacterium tuberculosis metabolites, which were then supposed to pro-
duce an immunising effect on the host.

Direct antimicrobial drug therapy was introduced in the later 1930s with the
advent of the sulphones. R i s t , B l o c h and H a m o n [6] found that 4, 4'diamino-
diphenyl-sulphone was active in avian tuberculosis. L a t e r , D o m a g d and his col-
laborators developed this line of thought and introduced the thio-semicarbazone drugs.
A result of metabolic studies on Mycobacterium tuberculosis was the introduction of
p-amino-salicylic acid as a tuberculostatic. As an extension of F l e m i n g 's work
on penicillin, streptomycin was introduced about the same time, both as a bacterio-
static and a tuberculostatic. But the real turning point in tuberculosis chemotherapy
was reached in 1952 when several laboratories discovered simultaneously the high
tuberculostatic activity of isonicotinic acid hydrazide (INH, isoniazid). This work is
particularly noteworthy in that it was a classical demonstration of a really interna-
tional collaboration. The immediate spate of scientific papers on INH from all parts
of the world testify to this fact. Many thousands of potential anti-tbc drugs were tried
and rejected until the activity of INH was discovered.

Today, chemotherapeutic work is still proceeding on turning up new drugs with
different anti-bacterial spectra to already known compounds, so as to be able to treat
patients infected with strains of Mycobacterium tuberculosis resistant to known tuber-
culostatics. The emergence of resistance to drugs is perhaps one of the most urgent

problems awaiting solution at present. A more complete understanding of drug me-
tabolism at a cellular level must be sought to solve this problem. As will be shown
later, tuberculostatic drugs with similar biological spectra can show varying rates
of resistance emergence with respect to Mycobacterium tuberculosis.

e) Development of Theory and Practice in the Chemotherapy of Mycobacterial
Disease. Leprosy.

      Almost every type of drug has been used in the past hundred years in the
treatment of leprosy. Such substances as potassium iodide, arsenic, antimony, cop-
per, sera, vaccines and aniline dyes have all been tried [7]. Later, sulphanil-
amides and penicillin were investigated and for a time diphtheria-formal toxoid was
advocated. Practically none of these drugs are now recommended. One drug, how-
ever, stood the test of time longer than most others, and in some parts of the world,
notably Russia, is still employed and has undoubtedly its indications. This drug is
chaulmoogra oil (hydnocarpus oil), which has been used for centuries as a folklore
medicine. The seeds of hydnocarpus fruit were eaten. Mouat introduced in 1854 the
use of the extracted oil as an anti-leprosy drug. "Antileprol", a mixture of the ethyl
esters of the crude chaulmoogra oil, was introduced in 1903 by Engel-Bay and was
claimed to be a cure for leprosy.

      When chaulmoogra oil had been extensively tried in leprosy about the time of
the First World War, the opinion was expressed that leprosy would be eradicated from
the British Empire within thirty years. As has already been mentioned similar pro-
gnoses are often made by the unwary and inexperienced with respect to modern tubercu-
losis and leprosy chemotherapy.

      One reason for the failure of the chaulmoogra campaigns of 1924-1935 lay in the
inadequacy of the dose given. Schujman S. [8] and Cochrane R.G. [7] both
showed that not less than 15 cc should be given weekly, half of the dose being given
intradermally. Even with the lower doses then used, about 50 % of the treated cases
were reported healed. But it was soon realised that the success was largely limited
to non-lepromatous and early lepromatous cases. The real test for activity lies in
success with lepromatous cases, where no natural tissue defence is present and which
do not resolve spontaneously. Particularly in the Caucasian race is the effect of hydno-
carpus oil poor. The relapse rate after initial success is also very high.

The first real turn in leprosy chemotherapy took place when F e l d m a n et alia [9] decided to try promin, which had been shown to be useful in animal tuberculosis, in leprosy. Promin is the sodium salt of p, p'diamino-diphenyl-sulphone-N:N' dextrose sulphonate, i.e. a soluble derivative of D.D.S. (p, p'diamino-diphenyl-sulphone). It is water soluble and reputedly less toxic than the parent sulphone. The success was immediate, although toxicity using sulphone therapy has been and still is a serious drawback.

Because of DDS toxicity, particularly hepatic toxicity, liver function must be in order before starting therapy and a good diet should be given. Anaemia is also easily precipitated under sulphone therapy, so that adequate iron intake must be assured. Internal parasites should also be eliminated, prior to commencing sulphone therapy, owing to their general debilitating effect. DDS shows a high toxicity in excessive dosage, particularly when given orally. The toxic signs appear usually during the first six months, so that care should be taken to start with low doses, gradually working up. After six months, chronic sulphone therapy is usually well tolerated.

Drug dermatitis due to sulphone therapy is one of the most distressing reactions and is due to drug sensitisation. Exfoliative dermatitis is not infrequently fatal. If dermatitis or other skin toxicity appears, sulphone therapy must be stopped at once. Cortisone, corticotrophin, prednisone or prednisolone are indicated in large doses.

Hepatitis may be accompanied by dermatitis and if present, sulphone therapy should be stopped immediately. Psychosis is sometimes seen, too, under sulphone therapy. It can be serious and can lead to suicide. Phenobarbital is used for treatment.

The above gives an idea of the value, but also of the drawbacks, of sulphone therapy in leprosy. Thus, further new drugs have been sought in the attempt to retain the advantages and to eliminate the dangers of sulphone therapy.

About 1958 a combination of isonicotinic acid hydrazide and streptomycin was introduced by P f i z e r and tested in leprosy by D r e i s b a c k and C o c h r a n e [10] with good results in cases showing intolerance to sulphone therapy. Thiosemicarbazone (TB 1, thiocetazone) was tried in leprosy in 1950 but, as in the case of tuberculosis, it was found to be active but toxic. Anaemia and agranulocytosis appeared occasionally.

A diphenylthiourea (CIBA 1906) has recently been introduced into leprosy therapy with good results but, if used alone, resistance is likely to appear in about twelve months.

In 1961 the author together with Dr. R.G. Cochrane and Dr. W.J. Jopling, Dr. R. Bréchet and Dr. D.S. Ridley introduced a pyridyl-oxadiazolone [11] (VADRINE) to leprosy therapy after more than seven years' clinical testing in leprosaria and tuberculosis clinics. Vadrine is non-toxic even using chronic high dose levels and rapidly active in lepromatous and other forms of leprosy. If used alone resistance is likely to emerge after about 12-18 months. In combination with the sulphones a marked synergism is observed in leprosy therapy.

Etisul (diethyl-dithio-isophthalate) was introduced recently by ICI Ltd. for leprosy therapy and is remarkable in that it is applied by inunction of the skin. A great deal of controversy is raging at the moment about the real value of this drug [12], some maintaining it to be inactive and some saying it is highly active. The truth would seem to lie midway. There is probably activity up to about six weeks to three months, after which resistance emerges.

At present it is impossible to say much about the precise mechanism of action of leprostatic drugs, whether they act directly on the Mycobacterium leprae or stimulate the host to defense in some way. By analogy with tuberculosis, where tuberculostatics can be shown to inhibit Mycobacterium tuberculosis growing in synthetic media, one may guess that the action in leprostasis is similar. But until Mycobacterium leprae can also be grown on a synthetic medium, formal proof of this point is lacking. Indeed, until Koch's postulates have been fulfilled in the case of leprosy, there is as yet not even formal proof that Mycobacterium leprae is the cause of human leprosy, though this assumption is practically universally accepted today.

Chapter II

MECHANISM OF ACTION

a) Resistance Emergence in Tuberculosis and Leprosy (General)

There are some leprologists who believe that no evidence for drug resistance exists in leprosy. Indeed, in the case of sulphones, until the present time but little albeit increasing evidence for resistance emergence has been found. It would be remarkable if Mycobacterium leprae should prove to be an exception compared with all other bacilli, all of which at least show some resistance emergence to antibiotics and chemotherapeutics. Indeed, there is a fair amount of evidence to show that resistance does emerge in leprosy, particularly to Etisul [12], where it would seem to occur after a few months of treatment. INH is believed by some leprologists to be inactive in leprosy simply because of extremely rapid resistance emergence. CIBA 1906 and VADRINE also apparently begin to lose their clinical efficiency, if used alone , after 12-18 months, thus probably indicating resistance emergence also.

In tuberculosis the same process can be demonstrated more easily, since Mycobacterium tuberculosis can be cultured on synthetic media. Drug resistant strains of tubercle bacilli arise in cultures exposed to isoniazid in vitro [13]. Isoniazid resistant cells have been found by many workers in Mycobacterium tuberculosis strains apparently not previously exposed to isoniazid and it is presumably from these initially resistant bacilli that resistant cultures arise in the presence of the drug. The sensitive cells are unable to multiply in the presence of the isoniazid, but the resistant cells are unaffected and replace the sensitive cells by multiplication. Cells initially resistant to 50 µg/ml isoniazid have been found occasionally, whereas normally a concentration of 0. 05 µg isoniazid/ml was sufficient to inhibit growth originally. In later years this figure has been raised by about 5 times owing to emergence of resistant forms generally. The longer the exposure of the bacillus to the drug the more resistant up to a certain point it becomes.

Hobby et alia [14] showed that in vitro and in vivo the emergence of resistance to isoniazid was inhibited to some extent by the presence of streptomycin in the culture medium and vice versa. But the ultimate emergence of resistance to either compound was not inhibited by this means. As is now well known, this observation was rapidly extended to include p-amino-salicylic acid and other tuberculostatics of

a different anti-bacterial spectrum to that of isoniazid. The application of two or three tuberculostatics of different biological spectrum is the universally applied method of overcoming or retarding clinical resistance to tuberculostatics in the clinic today.

In leprosy the use of combined therapy to prevent resistance emergence has not been developed as in the case of tuberculosis. In the first place, in view of the fact that one cannot culture Mycobacterium leprae on synthetic media, there is no laboratory method of ascertaining the inhibitory dose of leprostatic needed to check the growth of Mycobacterium leprae. Thus, there is no means of measuring the increase of the inhibitory dose with respect to time. Only clinical symptoms and the bacterial index in biopsies of human leprous tissue are left, both of which are tedious and may tend to vary from patient to patient. In the second place, as already remarked, no clinical resistance to sulphones in leprosy had been observed until fairly recently, when some leprologists became inclined to attribute late clinical ineffectiveness of sulphones to resistance emergence.

Some leprologists have observed [15, 16] an apparent synergism between Vadrine and sulphones which may possibly be due to resistance emergence inhibition towards Vadrine in the presence of sulphones. This one might expect in view of experience in combined therapy in tuberculosis. A similar effect may be perhaps observed using Etisul and other leprostatics.

A complication in the application of combined therapy should be pointed out at this stage. G r u n b e r g and S c h n i t z e r [17] noted an antagonism between isoniazid and streptomycin in mice when each substance was used in subeffective concentrations in experimental tuberculosis. These authors, therefore, stress the importance of the use of fully effective doses of each drug in clinical combined tbc therapy. Whether such considerations are of importance in leprosy remains to be seen.

A further factor must be considered, namely that of diffusibility of the drug in biological substrates. If poor diffusibility lowers the effective drug dose reaching the bacilli, then more rapid resistance emergence may be expected. Thus, isoniazid has been found highly effective in tubercle bacilli in phagocytic cells in culture. In contrast to results with streptomycin, isoniazid is as active on Mycobacterium tuberculosis in phagocytic cells as on bacilli suspended in nutrient fluid. Under the same conditions the concentration of streptomycin required was one hundred times higher. Lower effective concentrations of either drug in combined therapy may increase the resistance emergence rate to both.

b) Mechanisms of Resistance Emergence Inhibition

Few generalisations can be made at present on the mechanism by which one tuberculostatic drug inhibits resistance emergence towards another. Indeed, it is probable that each of a pair of drugs exercises its action in a different manner. So it is proposed here to cite a few examples, which have been more fully investigated.

p-Amino-salicylic acid and isoniazid

p-Amino-salicylic acid is absorbed from the gut easily and rapidly reaches comparatively high concentrations in the blood stream. It is, however, quickly excreted in the urine, mostly in the form of the acetylated derivative:

$$\text{COOH} \quad \text{OH} \quad \text{NH.COCH}_3$$
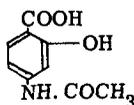
The majority is excreted within 24 hours. The tuberculostatic activity of the p-acetyl derivative is very much lower than that of the unconjugated parent substance. Little conjugation with glucuronic acid occurs [18]. Some conjugation with glycine to give p-amino-salicyluric acid takes place. This conjugate shows also a low bacteriostatic action. Between 14-33 % is excreted as free unchanged acid, 28-68 % as the p-acetyl-amino derivative and 0-26 % as the p-amino-salicyluric acid. The remainder is excreted as various amines.

The fact that p-amino-salicylic acid is mainly excreted as the acetyl conjugate means that it may enter into competition for acetylation with other substances, such as isoniazid, which are also eliminated by the same acetylation mechanism. The acetyl derivative of isoniazid is an important metabolite in the human subject and is of very low tuberculostatic activity. Thus, if large quantities of p-amino-salicylic acid are applied at the same time as isoniazid, the former appropriates to itself the majority of the acetylation process, with the result that a higher concentration of unconjugated, and therefore biologically active, isoniazid remains for a longer time in the serum. The higher concentration of isoniazid is more bacteriocidal than the lower concentrations, so that a larger proportion of the bacillus population is prevented from multiplying, if p-amino-salicylic acid is applied in the presence of isoniazid. This is referred to as the isoniazid sparing effect of p-amino-salicylic acid and is

due to metabolic competition. This may account for a good deal of the effectiveness
of p-amino-salicylic acid when combined with isoniazid, although p-amino-salicylic
acid itself is of low tuberculostatic activity, is rapidly excreted and in large doses
is poorly tolerated clinically.

An extended concept of competition in bacteriostasis and inhibition of resistan-
ce emergence applies, in fact, very widely today. When E h r l i c h instituted the
chemotherapy era he thought of "toxicity for bacilli" as a drastic chemical process
perhaps something like the coagulation or precipitation and denaturation of proteins.
Today, one imagines that competitive interference with the normal metabolism of
the bacterial cell takes place on introduction of the bacteriostatic. That is, that the
therapeutic agent so nearly resembles a substance essential for the normal metabo-
lism of the cell, that the drug is taken up by receptors of the growing bacterial cells
to the exclusion of the essential substance [19, 20]. The essential substance thus
blocked may be a vitamin, an essential amino acid or other essential metabolite.

Thus it is thought that substances showing identical biological spectra and there-
fore total cross resistance, as for example isoniazid and pyridyl-oxadiazolone (Va-
drine) block the same hypothetical essential metabolite. Where no cross resistance
is present, as for example in p-amino-salicylic and isoniazid, a different set of re-
ceptors will be occupied on the bacterial cell wall by a different drug and thus diffe-
rent essential metabolites will be blocked. If, then, two drugs are applied, each ha-
ving different points of attack on the cell receptor sites, then two different essential
metabolic cyclies will be blocked at the same time, giving more effective bacterio-
stasis and therefore less percentage chance of survival of the bacterial cells. All
this will result in reduced resistance emergence.

c) Resistance Emergence and Dependance in Single Substances

It has frequently been observed that Mycobacterium tuberculosis may show
rapid resistance emergence to some substances, but that the process may be slower
towards other drugs under similar conditions. In this process several factors may
be important. In the first place genetic factors must be considered, after which the
chemical structure of the drug plays a part.

The speed at which resistance emerges varies, but generally, with few excep-
tions, the lower the drug concentration and the larger the number of bacilli subjected

to its action, the faster the emergence of resistant variants [21]. Contrary to general belief, resistance develops more rapidly to streptomycin and isoniazid than to p-amino-salicylic acid. Resistance develops more quickly in the bacillus rich pulmonary lesions than in tuberculous meningitis.

In this process two major biological phenomena may be involved : (1) The original bacillary mixture contains naturally resistant variants. Under drug influence these are favoured by "natural selection" and a resistant variant rapidly replaces the sensitive strain (genetic variation). (2) "Phenotypic adaptation" of exposed sensitive variants under the influence of the drug, the new drug environment causing adaptive changes in the genetic make up. Bryson et alia [22], [23] believe that genetic variation is more important than phenotypic adaptation, that is that bacterial resistance emerges largely as a result of natural selection operating on spontaneously occurring mutants present in large populations of bacterial cells.

Two patterns of resistance emergence are distinguished : (1) the "penicillin type" in which resistance emerges through a series of multiple genetic steps; (2) emergence of high resistance in one step, such as is typical of resistance emergence to isoniazid and streptomycin in Mycobacterium tuberculosis. According to the Medical Research Council [24] such mutations tend to persist, but sometimes sensitive bacilli re-emerge in resistant cultures.

As an objection to the above explanation Abraham [25] points out that in the progeny of a single drug resistant bacterial cell drug sensitive and drug resistant strains appear.

Lenert and Hobby [26] have described a further remarkable development in the problem of drug resistance. Occasionally, under the influence of bacteriostatics, a drug dependent variant will develop, i.e. strains of tubercle bacilli which are dependent on the presence of a drug to grow properly. Spendlove et alia [27], Yegian et alia [28] and others have described certain streptomycin dependent strains of Mycobacterium tuberculosis. Some of these dependent forms are virulent and some non-virulent, although Brun [29] found all such dependent bacilli fully pathogenic.

In summary it may be said that most investigators have found streptomycin dependent strains pathogenic, but in vivo an enhancement of the disease as a result of streptomycin treatment does not seem to occur - in contrast to some experiments with some isoniazid dependent strains. A review of this subject has been compiled by Colestos [30].

d) Hypotheses on the Mechanism of Bacteriostatic Action at a Molecular Level

Streptomycin

There is, at present, no fully satisfying explanation of the bacteriostatic action of streptomycin, although an enormous amount of work has been done.

Henry and Hobby [31] divide up the bacteriostatic process as follows:

1. Drug fixation on the bacterial cell wall, with its consequences on wall permeability.
2. Absorption by the deoxyribonucleic acid of the cell.
3. A relation of streptomycin to the lag period of growth.
4. An antagonism of streptomycin to reducing substances, especially cysteine.
5. A competitive relationship with the lipositol of the bacillus.
6. Adverse effects of streptomycin on enzyme systems and bacillary metabolic reactions.

Since streptomycin (and other bacteriostatics) exercises its effect chiefly on cells in a state of rapid division and not on resting cells, it is thought that the process of division or bacterial synthesis necessary for cell division is blocked.

The following facts must be kept in mind:

a) Bacteriostatic action is proportional to concentration.
b) An alkaline pH favours bacteriostasis, an acid pH hinders the process.
c) Electrolytes, glucose and other reducing substances, greatly modify bacteriostasis.

Any hypotheses on mechanisms of action must take the above factors into account.

As in the case of Mycobacterium leprae under the influence of leprostatics, the morphology of Mycobacterium tuberculosis is modified under the influence of streptomycin and other tuberculostatics. Growth is inhibited without autolysis of the cell.

Detergents such as Tween 80 used in culture media abolish the tuberculostatic activity of many drugs but not that of streptomycin [37].

It has been postulated, but not proved, that streptomycin is an antimetabolite to lipositol. Indeed Stacey et alia [33] report an interference by streptomycin on the carbohydrate metabolism of other microorganisms. Oginsky et alia [34]

have shown that streptomycin interferes with the mechanism for oxalacetate-pyru-
vate condensation in the terminal respiratory metabolism of tubercle bacilli. Smith
et alia [35] found that resistant strains could dispense with this mechanism. The new-
ly recognised intermediate in metabolism 2-phospho-4-hydroxy-4-carboxy-adipic
acid is involved [36], the formation of which is specifically inhibited by streptomy-
cin.

The whole streptomycin molecule is necessary for bacteriostasis, the frag-
ments, streptidine and streptobiosamine applied separately or simultaneously have
no effect [37].

p-Amino-salicylic Acid

Here again, the precise mechanism of action of p-amino-salicylic acid is not
known. Studies with the C^{14} marked drug have shown that sensitive bacilli metabo-
lise some of it, but do not readily fix it. Resistant bacilli bind the drug firmly, but
do not metabolise it well [38]. Salicylates antagonise the bacteriostatic action of p-
amino-salicylic acid in vitro [39]; p-amino-benzoic acid antagonises p-amino-sali-
cylic acid in vitro and in mice [40, 41, 42, 43].

Methionine and biotin inhibit non-competitively the bacteriostatic action of p-
amino-salicylic acid and it would seem that the latter interferes with the synthesis
of the former [44].

p-Amino-salicylic acid is of low activity in experimental tuberculosis compared
with streptomycin and isoniazid [45, 46]. Bogen [47] found that in the guinea pig 2
gm daily or 3 times weekly were needed to protect. Feldman [48] used 125-500 mg
s. c. daily and obtained good protection in the guinea pig but the local effects of the
injection precluded continuance of this route of administration.

Isoniazid

Isoniazid shows a remarkably specific action on Mycobacterium tuberculosis
as compared with other bacterial groups. It acts on growing, not resting, cells and
there is a lag period before the growth inhibiting effect is noted. It is rapidly bacter-
iocidal to cells growing in a liquid medium containing glucose as the only source of
carbon, but inactive if no glucose was present, so that the cells did not divide [49].
A rapid loss of acid fastness occurs in the affected bacilli. Morphological changes
occur, too, under the influence of isoniazid [50]. During resistance emergence the
bacilli show reduced catalase function. Some degree of isoniazid resistance occurs

in all patients treated with isoniazid alone after 28 weeks and a substantial propor-
tion of the patients remain sputum positive. On combined therapy with p-amino-
salicylic acid or streptomycin some resistance to isoniazid did occur but most pa-
tients became sputum negative. The resistance emergence can be directly related
to catalase activity.

A number of suggested leads on the mechanism of action of isoniazid have
been obtained.

Sensitive tubercle bacilli fix isoniazid firmly, whereas resistant bacilli do not
[51]. The drug is fixed by physical adsorption forces rather than chemical bonds
[52]. The surface adsorption of the drug is probably by the hydroperoxidase of the
bacilli, followed by release of the isoniazid to deeper parts of the bacterial cell.
Resistant bacilli lack sufficient catalase to effect this initial adsorption and are
therefore not influenced by the drug.

The union between the cell and drug takes place slowly over 16-24 hours. Killed
bacilli do not adsorb the drug. Once bound to the cells, washing does not remove the
isoniazid. Bacilli made resistant by exposure to isoniazid did not adsorb it signific-
antly.

No unequivocal antimetabolite relationship for isoniazid has been established,
though many have been suggested. Pyridoxal is an effective antagonist of isoniazid
action [53] and it is thought that coenzyme systems involving pyridoxal phosphate
may be interfered with by isoniazid. Pyridoxal prevents the inhibitory action of iso-
niazid on sensitive bacilli but antagonises the emergence of resistant bacilli to iso-
niazid, which become sensitive again in the presence of pyridoxal.

Pyridoxal greatly increases the uptake of C^{14} labelled isoniazid by otherwise
sensitive bacilli [54] while other closely related compounds have no effect on the
isoniazid uptake. It would seem possible that there is a direct pyridoxal-isoniazid
reaction in vivo [55]. Certainly one would expect the aldehyde group in pyridoxal to
combine easily with the primary amino group of isoniazid under physiological condi-
tions. The resultant compound will probably show quite different physiological pro-
perties compared with isoniazid since its polarity is completely changed.

α-Ketoglutaric acid also antagonises the action of isoniazid, as do also a num-
ber of ketones and keto acids [56]. This may be due to simple combination with the
primary amino group of the drug.

Inhibitory effects of isoniazid on various transaminases, dehydrogenases and
diphospho pyridine nucleotidases [57, 58, 59, 60] have been described.

With respect to isoniazid action its relationship to catalase activity would seem to be most significant. There is no doubt that a correlation between bacillary resistance to isoniazid and catalase deficiency and nonvirulence exists. There is a general confirmation of the original findings of Middlebrook on this line [61, 62]. It is still debatable, however, whether catalase deficiency is fundamental to virulence. For example, much higher catalase activity has been noted in certain saprophytic mycobacteria than in mammalian tubercle bacilli. But an almost constant inverse relationship is found between catalase activity and drug resistance.

Ferric compounds render isoniazid unstable in culture media and catalase contains iron. Haemin, also an iron containing compound, is a growth promoting factor for Myc. tuberc. and has some catalase activity. Haemin is competitively antagonistic towards isoniazid. It has therefore been suggested that development of bacterial resistance to isoniazid is related to a loss of capacity to synthesise haemin. It may be that the hydrazid moiety of the isoniazid molecule chelates iron from enzyme systems in which pyridoxal is involved. Similar remarks apply to copper, with which isoniazid also combines to form a chelate. The metal chelate is thought to compete with hydrogen peroxide on the catalase molecule, thus allowing hydrogen peroxide to accumulate to toxic concentrations [63].

In addition to catalase, certain peroxidases may be concerned, too, in this problem. The peroxidase activity for some substrates is high in tubercle bacilli and if peroxidase is present, the bacillus is often susceptible to isoniazid [64]. Although no formal proof exists, since culturing experiments are not possible, it is thought that isoniazid has at least a temporary inhibitory effect on Mycobacterium leprae, but that resistance emerges very quickly.

It is not proposed here to go into the mechanism of action of other tuberculostatics such as pyrazinamide, the thiosemicarbazones, the sulphonamides and the sulphones. The subject is still in rapid development and the fields have been cited here in which perhaps the largest amount of research has been carried out.

Chapter III

RECENT WORK IN THE OXADIAZOLONE FIELD

a) Metabolic Pathway and Biological Spectrum

It was early found [65] that 2-pyridyl-1,3,4-oxadiazolone (S57) shows in vitro complete cross resistance with isoniazid, which was explained on the basis of the common metabolic pathway of the two substances:

2-pyridyl-1,3,4-oxadiazolone
(S57)

INH

isonicotinic
acid

isonicotinoylglycine

4-6 unknown products
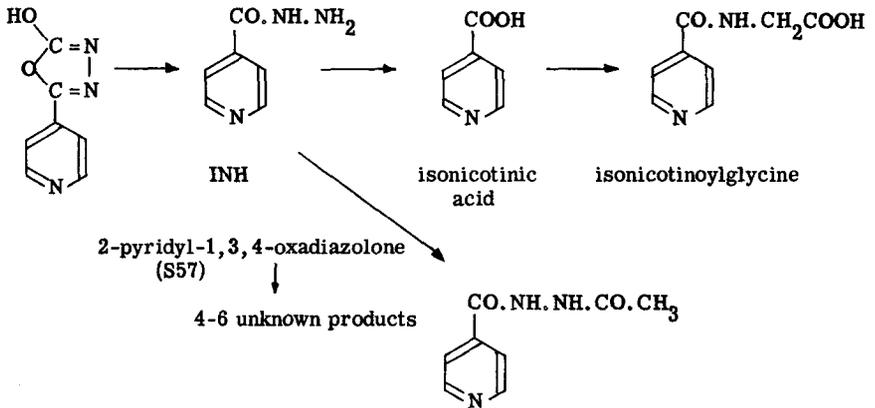
The following table (Table I) shows the cross resistance relationships between S57 and isoniazid.

Table I*

Clinical Sensitivity of Mycobacterium tuberculosis to INH and S 57

Patient	No.	Sensitive to μgm/ml	
		S 57	INH
B. V. J.	8435	0.4	0.1
B. V. J.	9454	0.4	0.1
B. V. J.	12/60	0.4	0.1
B. M.	9332	40	50
B. M.	72	40	50
B. M.	925/61	40	50
S. I.	10403/61	100	50
S. I.	582/62	-	10
K. S.	5139	40	50
K. S.	5441	40	50
K. S.	5873	40	50
K. S.	6368	40	50
G. L.	7268/61	0.4	0.1
L. J.	6065	40	50
O. K.	6064/61	40	50
O. K.	967/61	8	10
O. K.	33/61	8	10
O. K.	9036/61	8	10
O. K.	8534/61	40	10
O. K.	8078/61	8	50
O. K.	7459/61	8	10

* Courtesy Dr. G. Haukenes, Bergen

In the rat 2-pyridyl-1,3,4-oxadiazolone is metabolised to isonicotinic acid, isonicotinoylglycine and 1-isonicotinoyl-2-acetyl-hydrazine, together with 4-6 metabolites which could not easily be characterised [66]. In the same animal iso-niazid is metabolised to isonicotinic acid, isonicotinoylglycine and 1-isonicotinoyl-2-acetyl-hydrazine.

On the basis of the cross resistance between the two compounds and the similarity of metabolism, apart from the 4-6 unknown metabolites, which do not appear in the case of isoniazid, it was therefore considered that the clinical spectrum of the two substances would also be identical and that S 57 is merely a transport form of INH. But, on the other hand, codeine and heroine, for example, both have similar metabolic pathways to morphine, yet one would hardly risk maintaining that their clinical indications were identical 67 . However, in view of the low toxicity of 2-pyridyl-oxadiazolone it was tested in pulmonary and other clinical tuberculosis and found to be particularly active in cases of miliary tuberculosis 68, 69 .

Since 2-pyridyl-1,3,4-oxadiazolone possesses quite different physical and chemical properties to those shown by isoniazid - the former is a tertiary amine and an amide whereas the latter is a tertiary and a primary amine -, it may well be that the increased activity of the oxadiazolone is due to its different chemical and physical properties, which will modify its tissue affinities and diffusion characteristics. Thus, isoniazid is a moderately strong base and its primary amino group may probably be responsible for its euphoric properties. The primary amino group is lacking in pyridyl oxadiazolone and so are apparently some of the euphoric properties. Pyridyl-oxadiazolone is poorly soluble in water and has amphoteric properties, its isoelectric point lying at about pH 6. Isoniazid is relatively soluble in water and is also soluble in acids to give salts.

The reports on the high activity of 2-pyridyl-1,3,4-oxadiazolone in miliary tuberculosis led to a seven years' trial of the substance in leprosy, the results of which were reported by B r é c h e t and C o c h r a n e [70] and J o p l i n g and R i d - l e y [71]. The results, in summary, show that the oxadiazolone is rapidly active in lepromatous leprosy and is suitable for chronic chemotherapy, since very few side effects have been observed. After 12-18 months of chemotherapy, using the oxadiazolone and no other leprostatic, drug resistance, judged by a slowing down of the improvement in bacterial index and clinical symptoms, began to emerge. Except for the sulphones, drug resistance occurs with other leprostatics quite easily.

b) Differences in Resistance Emergence Rates in Tuberculostatics showing
Cross Resistance

Three explanations for the low leprostatic activity of isoniazid and the un-
expected leprostatic activity of pyridyl-oxadiazolone are possible:

(1) The biological spectrum of pyridyl-oxadiazolone is not one hundred per
cent superposable on the biological spectrum of INH. Pyridyl-oxadiazol-
one may be active against Mycobacterium leprae whereas INH is not.
It is not possible to test this hypothesis directly as one cannot yet culture
Mycobacterium leprae on synthetic media.

(2) INH possesses entirely different chemical and physical properties to those
of pyridyl-oxadiazolone. INH is a strong primary base, containing also a
tertiary nitrogen atom. Pyridyl-oxadiazolone contains a tertiary nitrogen
atom but no primary nitrogen atom and is an amphoteric substance. INH,
therefore, may not be able to diffuse to the site occupied by Mycobacterium
leprae and be transported across the cell wall surrounding it. Pyridyl-
oxadiazolone, possessing an aromatic and a pseudo-aromatic heterocyclic
ring and being amphoteric, may well be able to be transported in a manner
quite different to that of INH and to reach Mycobacterium leprae in situ.

(3) Many leptrologists think that INH does possess a very ephemeral action
in leprosy lasting about 3-6 weeks, after which rapid resistance super-
venes. The slow emergence of resistance to pyridyl-oxadiazolone compa-
red to the rapid resistance emergence towards INH may thus possibly
account for the leprostatic activity of the pyridyl-oxadiazolone.

Obviously, without special techniques and the use of radioactively marked
isoniazid and pyridyl-oxadiazolone in the human leprosy patient, it is not possible
to settle these points unequivocally. On the other hand, it is relatively easy to de-
termine the relative resistance emergence rates of isoniazid and pyridyl-oxadiazol-
one to Mycobacterium tuberculosis in in vitro cultures.

Two series of cultures were therefore set up and serial dilution was used to
determine the dilution of tuberculostatic just necessary to totally inhibit the growth
of Mycobacterium tuberculosis on liquid Dubos culture medium [72]. It was found
in experiment I that after 6 subcultures the minimal dose of isoniazid required to
just inhibit growth had increased 100 times compared with the initial inhibitory dose.
At the twelfth subculture the inhibitory dose required had risen 100 000 times. Ex-
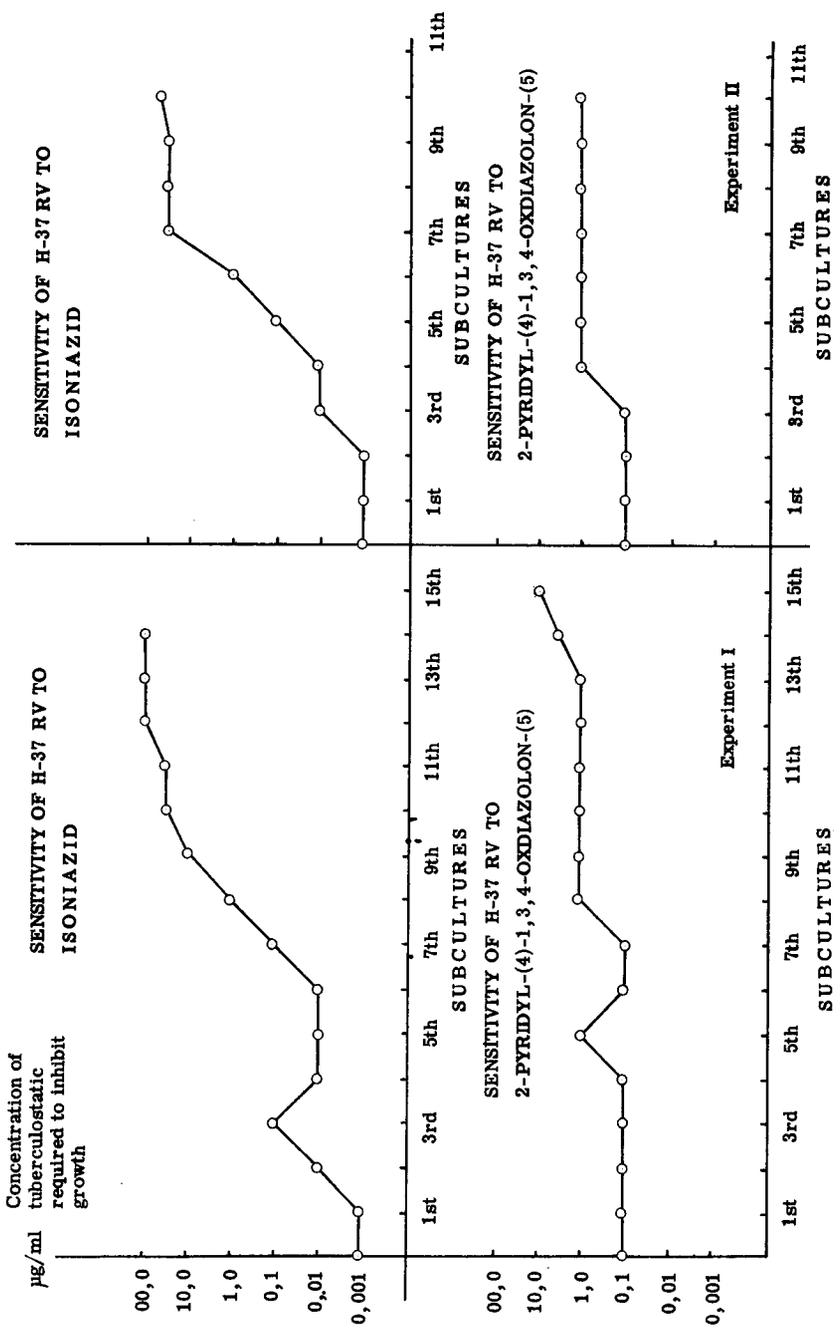
Figure I

periment II confirmed these results. The same experiment carried out using pyr-
idyl-oxadiazolone in the place of isoniazid showed a much slower emergence of
resistance. After 7 subcultures no detectable increase of inhibitory dose had oc-
curred in experiment I. At the 8th to the 13th subculture a 10-fold increase had
occurred and at the 14th to 15th subculture a 100-fold increase was noted. Similar
results are shown in experiment II. These results are given in graph form in Fig-
ure 1.

Thus, although cross resistance apparently does occur between isoniazid and
pyridyl-oxadiazolone yet differences in their respective resistance emergence rates
to Myc. tuberc. also occur, which might at least partly account for the leprostatic
action of pyridyl-oxadiazolone.

c) Resistance Emergence in Other Oxadiazolones

If, by the substitution of an oxadiazolone group for the hydrazide group on a
pyridine ring, a specific reduction in resistance emergence rate can be achieved,
it would be of interest to effect the same change in different tuberculostatic sub-
stances possessing chemical groups which allow this transformation to be carried
out.

It is known that p-amino-salicylic acid and its hydrazide (PAS-hydrazide)
show cross resistance to various strains of Mycobacterium tuberculosis. It is
possible to convert the hydrazide group in p-amino-salicylic acid hydrazide to the
oxadiazolone [73, 74].
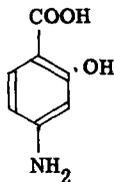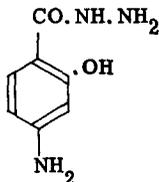
p-amino-salicylic acid   PAS-hydrazide   p-amino-o-hydroxy-phenyl-1.3.4-
                                                           oxadiazolone WS 127
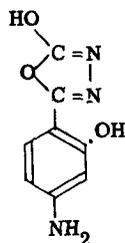
Figure II   Serial Dilution, liquid Dubos without addition of Tween. H 37 Rv inoculation carried out from the last tube which showed definite growth. Reading after 10-12 days' growth at 37°C

Serial Dilutions

INH + S 57 1 : 1

WS 127 =

WS 128 =

Serial Dilutions:
10 000
20 000
40 000
80 000
160 000
320 000
640 000
1 280 000
2 560 000
5 120 000
10 240 000
20 480 000
40 960 000

Key :   - = no growth
+ = growth of short cords, no serpentine filaments
++ = 10-50 % growth serpentine colonies   "
+++ = > 50 %   "   "   "

WS 127 proved to be tuberculostatically active at about the same or slightly higher concentration than p-amino-salicylic acid, depending on the strain of Mycobacterium tuberculosis used, but its resistance emergence rate was approximately the same as for that of p-amino-salicylic acid. That is, a slow resistance emergence rate is not specific for the oxadiazolone group in these two cases. Figures II shows the results obtained in resistance emergence experiments.

Thus, on the hypothesis that the leprostatic activity of pyridyl-oxadiazolone is due to its slow resistance emergence characteristics, WS 127 ought not to be active in leprosy. Clinical trials in leprosy have therefore been initiated to settle this point and results after six months show moderate leprostatic acitivity. It would thus seem that resistance emergence rate is not the deciding factor in leprostatic activity.

d) Toxicity of the p-Amino-o-hydroxy-phenyl-oxadiazolone and p-Amino-o-hydroxy-phenyl-oxadiazol-thione Groups

Before further animal work could proceed the acute and chronic toxicities of the p-amino-o-hydroxy-phenyl derivatives were investigated.

The first animal species used was the mouse and the acute toxicity determined orally and subcutaneously. The following results were obtained (Tables IX, X, XI, XII. Simultaneous toxicity tests using pyridyl-oxadiazolone and isoniazid are given for comparison. WS 174 is the N-acetyl derivative of WS 127):

Table IX

Dose i.p. in mg/kg	WS 127	Substance : WS 174	WS 176 N-acetyl PAS
500	0/4	0/4	0/4
600	0/4	0/4	4/0
700	1/3	0/4	4/0
800	0/4	0/4	./.

Key: The first figure means the number of animals (mice) dying and the second the number of animals surviving.

The N-acetyl derivative of p-amino-salicylic acid (WS 176) was used to make a direct comparison with the N-acetyl derivative of WS 127. Thus the p-amino-o-hydroxy-phenyl-oxadiazolone (WS 127) and its N-acetyl derivative (WS 174) are less toxic in the muose by the i.p. route than the corresponding p-amino-o-hydroxy-phenyl acid derivative (WS 176).

A similar test using the oral route in the mouse was then carried out to compare the toxicity of WS 127 with its sulphur analogue WS 128:

Table X

Acute toxicity of WS 127 and WS 128 per os in the mouse
Key as for Table IX

| Dose per os in mg/kg | Substance: | |
	WS 127	WS 128
500	0/3	0/5
1000	0/5	1/4
1500	1/4	0/5
2000	1/4	3/2

Thus, as far as the results for so few animals are significant, the sulphur derivative WS 128 is slightly more toxic than the oxygen analogue WS 127.

The toxicities of Pyridyl-oxadiazolone and isoniazid in various species are given for comparison.

Table XI

Acute toxicity S 57

guinea pig per os	LD_{50}	700 mg/kg
guinea pig s.c.	LD_{50}	750 mg/kg
rabbit per os	LD_{50}	1000 mg/kg
rabbit s.c.	LD_{50}	400 mg/kg
mouse per os	LD_{50}	2000 mg/kg
mouse s.c.	LD_{50}	2500 mg/kg

Table XII

Acute toxicity of INH

guinea pig per os	LD_{50}	450 mg/kg
guinea pig s. c.	LD_{50}	255 mg/kg
rabbit per os	LD_{50}	250 mg/kg
rabbit s. c.	LD_{50}	295 mg/kg
mouse per os	LD_{50}	200 mg/kg
mouse s. c.	LD_{50}	350 mg/kg

Comment

The acute toxicities of WS 127 and its N-acetyl derivatives and WS 128 compare favourably in the small number of animals used with that of isoniazid. On the basis of the small number of animals it is not possible to arrive at a reliable therapeutic index for comparison between WS 127 and p-amino-salicylic acid but the ratio LD_{50}/MIC would appear to be favourable for WS 127 and its derivatives.

MIC/N-acetyl-PAS   =   4 µgms/ml LD_{50} 550 mg/kg i. p. mouse
       = 176

MIC/N-acetyl-WS 127   =   7 µgms/ml LD_{50} 800 mg/kg i. p. mouse
       = 174

The activity relationships of the various derivatives against $H_{37}Rv$ are summarised in the following tables (Tables XIII and XIV).

Table XIII

Serial	dilutions	INH	PAS	N-acetyl PAS	WS 127	WS 128	S 57	WS 174
1 :	10 000	-	-	-	-	-	-	-
	20 000	-	-	-	-	-	-	-
	40 000	-	-	-	-	-	-	-
	80 000	-	-	-	-	-	-	-
	160 000	-	-	-	-	-	-	++
	320 000	-	-	+	-	-	-	+++
	640 000	-	-	++	-	-	-	+++
	1 280 000	-	+	++	+	+	++	+++
	2 560 000	-	+	+++	++	++	++	+++
	5 120 000	-	++	+++	++	+++	+++	+++
	10 240 000	+	++	+++	+++	+++	+++	+++
	20 480 000	++	++	+++	+++	+++	+++	+++
	40 960 000	+++	+++	+++	+++	+++	+++	+++
	81 920 000	+++	+++	+++	+++	+++	+++	+++

Key :   - no growth
         + slight growth
        ++ 50 % growth
       +++ 100 % growth
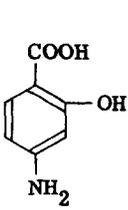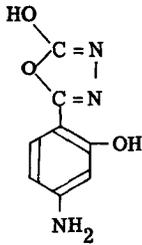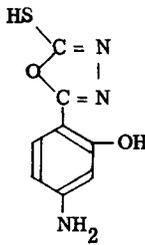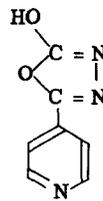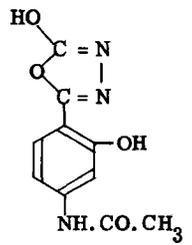
PAS   WS 127   WS 128   S 57   WS 174
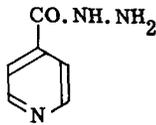
INH

The following table (Table XIV) shows the cross resistance relationship between PAS, Streptomycin and WS 127 in the tuberculosis clinic.

Table XIV

Clinical sensivity of Mycobacterium tuberculosis to WS 127, PAS and Streptomycin

Patient	No.	Sensitive to μgm/ml		
		WS 127	PAS	Streptomycin
B. V. J.	9454	1.0	0.4	1.0
B. M.	9332	100	40	100
B. M.	72/61	50	8	50
B. M.	925	50	8	50
S. I.	10403/61	50	8	50
S. I.	6871/62	50	40	10
S. I.	582/62	100	40	50
K. S.	5139/61	100	40	10
K. S.	5441/61	100	40	50
K. S.	5873/61	100	8	50
K. S.	6368/61	100	40	50
G. L.	7268/61	10	2.0	1.0
H. L.	11277/61	1.0	0.4	1.0
L. J.	6065/61	1.0	0.4	1.0
O. K.	6064/61	1.0	0.4	1.0
O. K.	967/61	1.0	0.4	1.0
O. K.	33/61	1.0	0.4	1.0
O. K.	9036/61	1.0	0.4	-
O. K.	8534/61	1.0	2.0	1.0
O. K.	8078/61	1.0	0.4	10
O. K.	7459/61	1.0	0.4	1.0
I	-	6	-	0.1
II	-	0.75	1.75	0.05
VIII	-	100	50	100
IX	-	10	50	10
X	-	10	50	10
XIV	-	1.0	0.4	1.0
XV	-	1.0	2.0	1.0
XVI	-	1.0	0.4	1.0

e) Resistance Emergence Rates in other Leprostatics

For the sake of theoretical interest the resistance emergence rate of Mycobacterium tuberculosis ($H_{37}Rv$) to Ciba 1906 (diphenylthiourea) was determined. Table XV gives the results.

Table XV

Serial dilutions and subcultures using Ciba 1906 (Diphenylthiourea) and $H_{37}Rv$. Liquid Dubos. * Subcultures approximately every 10 days.

		0	1	2	3	4	5	6
1::	10 000	-	-	-	-	-	-	-
	20 000	-	-	-	-	-	-	-
	40 000	-	-	-	-	-	-	-
	80 000	-	-	-	-	-	-	+
	160 000	-	-	-	-	-	-	++
	320 000	-	-	-	-	-	-	++
	640 000	-	-	-	-	-	+	++
	1 280 000	-	-	-	-	-	++	++
	2 560 000	-	-	-	-	-	+++	+++
	5 120 000	-	-	-	+	+	+++	+++
	10 240 000	-	-	-	++	++	+++	+++
	20 480 000	+	+	-	+++	+++	+++	+++
	40 960 000	++	++	+++	+++	+++	+++	+++
	81 920 000	+++	+++	+++	+++	+++	+++	+++
	Control	+++	+++	+++	+++	+++	+++	+++

subcultures

Key : - = no growth
       + = slight growth
      ++ = moderate growth
     +++ = heavy growth

* Courtesy Dr. H. B r o d h a g e , Lucerne

Thus Ciba 1906 shows similar resistance emergence properties to $H_{37}Rv$ to isoniazid and WS 127 so that it would appear unlikely that this property is the determining factor in whether a tuberculostatic will be leprostatic. The long ester side chain in the para-position will probably make the molecule more lipoid soluble than isoniazid, which factor might be useful in disease influencing nervous tissue and lipoproteins. This may be also a determining factor in the evaluation of the various side chain substituted oxadiazolones to be described.

For the sake of comparison the formula is given herewith:

$$CH_3 . CH_2 . CH_2CH_2 . O. \underset{}{\bigcirc} \text{-NH.} \underset{\underset{S}{\parallel}}{C} . NH \underset{}{\bigcirc} \text{-N}(CH_3)_2$$
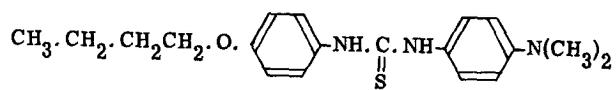
Diphenylthiourea (Ciba 1906)

Chapter IV

EXPERIMENTAL (SYNTHESES)

The melting points are uncorrected

Preparation of 2-Pyridyl-(4-)-1,3,4-oxadiazol-thione-(5) [73] = WS 202

Isoniazid (27.4 gms) was dissolved in distilled water (200 mls) and concentrated hydrochloric acid (10 mls) added to give a yellow solution in the cold. Thiophosgene (26.3 gms) dissolved in dioxan (100 mls) was added slowly with stirring at room temperature while passing a slow stream of nitrogen. A precipitate appeared early. The mixture was heated to 50^OC and filtered in the presence of a little decolourising charcoal. The precipitate was well washed with dilute hydrochloric acid at 90^OC and the filtrate combined with the original filtrate. The pH of the combined filtrate was then adjusted to 2.0 with concentrated ammonia solution when deep brown crystals appeared. Yield = 17.0 gms WS 202 (= 47.5 %), melting point = 251-254° (decomp.). No rise in melting point (decomp.) was achieved by dissolving in dilute hydrochloric acid and reprecipitating with ammonia.

Found N, 23.35, calc. $C_7H_5N_3OS$ N, 23.46 %.

Preparation of 2-(2'-hydroxy-4'-amino-phenyl)-1,3,4-oxadiazolone-(5) = WS 127

The starting material for the preparation of the above compound was p-nitro-salicylic acid which was prepared according to the method of McGhie [75].

Preparation of p-nitro-salicylic acid

(a) Preparation of 2:4-dinitro-phenyl-acetic acid

Phenyl-acetic acid (m.p. 66-67°) (250 gms) was added in small portions in finely powdered form to a mixture of concentrated sulphuric acid (920 mls) and fuming nitric acid (d 1.51, 400 mls) kept at exactly 60-65° in a water bath with stirring. The phenyl-acetic acid was added in small quantities so as to maintain the temperature at 60-65°. If too much is added at a time, the reaction runs away with the development of enormous quantities of nitrous fumes. It is as well to add urea (5-10 gms) to the nitrating acid to remove nitrous acid, which renders the reaction mixture unstable.

After the addition of the total phenyl-acetic acid, the reaction mixture was heated for 10 minutes to 70°, then cooled to 20° and poured slowly on to ice (6 kg) with vigorous stirring. The precipitate was washed well three times with ice water and filtered and dried. Crystallised from glacial acetic acid. Yield = 395 gms, m.p. 160-165°. Further crystallisation raised the melting point to 180°.

(b) Preparation of Methyl-2:4-dinitro-phenyl-acetate

2:4-Dinitro-phenyl-acetic acid (400 gms) was refluxed with absolute methanol (2.4 l) saturated with dry hydrogen chloride. On cooling to 0° the methyl ester crystallised. Yield = 400 gms, m.p. 80-81°.

(c) Oximation of Methyl-2:4-dinitro-phenyl acetate

Methyl-2:4-dinitro-phenyl acetate (176 gms) was dissolved in absolute methanol (1.5 l) at 60°, cooled rapidly to about 35° and butyl nitrite (100 mls freshly distilled, b.pt. $_{88 \text{ mms.}}$ 24-27°) added rapidly with stirring, then methanol (absolute, 500 mls) containing sodium (16.8 gms) was added immediately. The solution became black, then deep red. On placing in the refrigerator at 0°, fine yellow crystals were deposited. Product A. Yield = 152 gms, m.p. 128-131°. The melting point may be raised by recrystallisation from methanol to 140°.

Product A (Methyl ester)
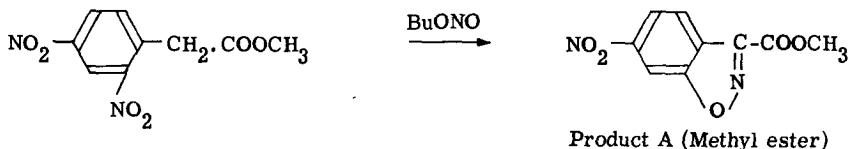
(d) Product A, m.p. 128-131° (85 gms), and 15 % aqueous potassium hydroxide (170 mls) were heated at 100° until solution occurred and then poured hot on to 6 N hydrochloric acid (200 mls), cold, containing 100 mls ethanol, and warmed under reflux until solution occurred. On cooling, a white precipitate was formed. After filtration and recrystallisation from methanol (200 mls) and water (150 mls) the m.p. was 155-157°. Yield = 60 gms. Product B.

Product A (Methyl ester)   Product B (nitrile)
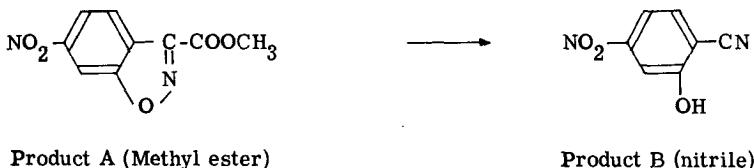
(e) Product B (m.p. 155-157°) (60 gms) was refluxed for 4 hours with concentrated sulphuric acid (334 mls), water (168 mls) and glacial acetic acid (480 mls). Then the solution was cooled to 0° and the product filtered on a fritted filter and recrystallised from hot water. Yield = 60 gms, m.p. 232-233°.

Product B   p-nitro-salicylic acid
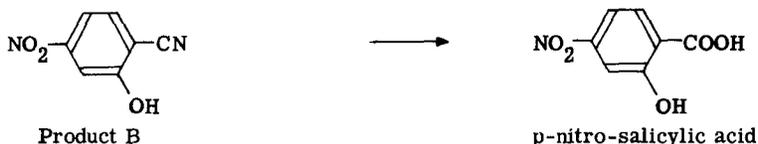
Preparation of 2-(2'-hydroxy-4'-amino-phenyl)-1,3,4-oxadiazolone-(5) (= WS 127) from p-nitro-salicylic acid

(a) The p-nitro-salicylic acid was first converted to its methyl ester in the following manner: 19 grams of p-nitro-salicylic acid (m.p. 228-230°) in 200 ml of absolute methanol, saturated with dry hydrogen chloride, was heated for 1 hour on a water bath under reflux and evaporated to dryness under reduced pressure. The methyl ester, crystallised from absolute methanol, was obtained.

Yield = 19 grams, m.p. 101-102°.

(b) The compound was then converted to the hydrazide thereof by the following method: 100 grams of the methyl ester prepared as above were heated 5 minutes on a steam bath stirring with 200 ml of 98-100 % hydrazine hydrate, the excess hydrazine being removed as quickly as possible under reduced pressure without excessive heating. The residue was dissolved in aqueous ammonia, filtered, and the filtrate acidified with concentrated hydrochloric acid to a pH of 6. Overheating with hydrazine must be avoided, otherwise the nitro group is reduced. The precipitate was filtered and recrystalised from dioxan and ethanol if necessary.

Yield = 97 gm, m.p. 224-226° (decomp. 227°).

Calculated for $C_7H_7O_4N_3$:   C 42,7   H 3,6   N 21,4 %

Found   C 42,8   H 3,8   N 21,4 %

(c) This hydrazide was then converted to the oxadiazolone by the following method: 10 grams of the above hydrazide were dissolved in 200 ml of pure dioxan and phosgene passed in a rapid stream through the hot filtered solution. The first formed precipitate almost completely dissolved after 1-2 hours. Meanwhile phosgene was passed

and the solution was heated to 70-80°C. The dioxan was removed under reduced pressure, the residue dissolved in slightly warm aqueous ammonia, filtered and the pH adjusted to 1 with concentrated hydrochloric acid. The precipitate was filtered and dried at 100°.

Yield = 9.5 gm, m.p. 245-247° (decomp.)

Calculated for $C_8H_5O_5N_3$:   C 43,0   H 2,2   N 18,8 %

Found :   C 43,2   H 2,2   N 18,9 %

(d) The above oxadiazolone was reduced to the p-amino-derivative in the following manner: 9.0 grams of the above p-nitro derivative, suspended in 80 ml of distilled water, 60 ml of concentrated hydrochloric acid and 40 g of tin powder, was stirred overnight. A further 50 ml of concentrated hydrochloric acid were added together with 20 g of tin powder, the mixture then being warmed to 100°C for 10 minutes, filtered hot and washed with concentrated hydrochloric acid. The filtrate was then let stand at 0° for 24 hours, when white plates precipitated, m.p. > 278° (decomp.).

Yield = 8.2 grams.

Calculated for $C_8H_8O_3N_3Cl$:   C 41,9   H 3,5   N 18,3 %

Found :   C 42,1   H 3,4   N 18,2 %

The above reduction may also be effected by using Adam's catalyst.

Preparation of 2-(2'-hydroxy-4'-nitrophenyl)-1,3,4-oxadiazol-5-thione

(a) 2.0 grams of the above hydrazide in 150 ml of pure dioxan were filtered hot and 2 grams of thiophosgene in 5 ml of dioxan were added thereto with stirring. A slight cloudiness occurred which cleared up on warming. The dioxan was removed under reduced pressure and the residue taken up in dilute aqueous ammonia, filtered and the pH adjusted to 3 with concentrated hydrochloric acid. The precipitate obtained was filtered and dried at 100°.

Yield = 1.9 gm, m.p. 190-193° (no decomp.)

Calculated for $C_8H_5O_4SN_3$:   N 17.6 %

Found :   N 17,7 %.

(b) The above compound was reduced to the p-amino derivative W 128 as follows: 3 grams of the p-nitro derivative was suspended in 50 ml of distilled water and 50 ml of concentrated hydrochloric acid together with 20 g of tin powder added with stirring. The mixture was left overnight with constant stirring and then heated to 100° for 10 minutes and filtered hot. Crystals of the hydrochloride separated in a

yield of 100 milligrams. The filtrate was brought to pH 5 with ammonia and the free base precipitated.

Yield = 1 g. The free base has a melting point of 234-235° (decomp.) while the hydrochloride has no sharp melting point but becomes rather soft at 250°.   WS 128.

Calculated for $C_8H_8O_2SN_3Cl$:   C 39,1   H 3,3   N 17,1 %

Found :   C 39,3   H 3,5   N 17.3 %

Preparation of 2-(2'-hydroxy-4'-amino-phenyl)-1,3,4-oxadiazolone-(5) (= WS 127) from PAS-hydrazide directly

Phosgene gas was passed into 200 ml absolute dioxan until about 10 g had been absorbed, then a solution of 1 g PAS hydrazide in 100 ml of dioxan was added slowly with vigorous stirring at room temperature. A slight precipitate was formed. After allowing to stand for 15 minutes the dioxan was distilled off under reduced pressure and the residue warmed with 60 ml of 1 N HCl for 10 minutes on the water bath. The mixture was then boiled one minute and filtered, and to the filtrate 100 ml of concentrated hydrochloric acid added. After a few minutes fine crystals of the hydrochloride of 2-(2'-hydroxy-4'-amino-phenyl)-1,3,4-oxadiazolone-(5) (WS 127) appeared.

Yield = 1 gm, m.p. > 270°. A mixed melting point taken with authentic material showed no depression.

Preparation of 2-(2'-hydroxy-4'-amino-phenyl)-1,3,4-oxadiazolone-(5) from PAS-hydrazide indirectly

(a) Preparation of methyl-p-amino-salicylate

100 grams of PAS monohydrate, 1 litre methanol (absolute) and 110 ml conc. H_2SO_4 were refluxed for 12 hours. Excess methanol was distilled off on the oil bath at 110-120° and the residue poured on to excess aqueous sodium bicarbonate ($NaHCO_3$) suspended in 1 litre of water. The pH was 7 at the end of the reaction. The methyl ester was filtered off and crystallized from methanol.

Yield = 84 gm, m.p. 116-119°.

(b) Preparation of methyl-p-acetamido-salicylate

Methyl-p-amino-salicylate (83.5 g) was dissolved in 150 ml of pyridine with stirring and 50 grams of acetyl chloride was slowly dropped in over 1 hour. Pyridine was distilled off under reduced pressure. The residue was taken up in hot ethanol and poured into 1 litre water, filtered after 1 hour and crystallized from methanol.

Yield = 88 gm, m.p. 144-146°.

(c) Preparation of p-acetamido-salicylic acid hydrazide hydrochloride

30 grams methyl-p-acetamido-salicylate m.p. 141-144° and 70 ml hydrazine hydrate 98-100 % were allowed to react together at room temperature until the salicylate completely dissolved (40-60 minutes). The hydrazine hydrate was then lyophilised off at approximately 0° under high vacuum, using a dry ice trap for hydrazine hydrate recovery. One litre water was then added to the residue at 80-90° and concentrated hydrochloric acid added dropwise until solution just occurred (35 ml conc. HCl). The solution was filtered, 200 ml conc. hydrochloric acid were added to the cool filtrate and the mixture was cooled to 15°. After 1 hour the crystals were filtered off and dried at 100°C.

Yield = 34 gms, m.p. 249-251° (decomp.)

Calculated for $C_9H_{12}N_3O_3Cl$:   N 17,0 %

Found :   N 17,1 %

(d) Preparation of 2-(2'-hydroxy-4'-acetamido-phenyl)-1,3,4-oxadiazolone-(5) (WS 174)

1 gram p-acetamido-salicylic acid hydrazide hydrochloride m.p. 249-251° (decomp.) was dissolved in 20 ml glacial acetic acid and 80 ml warm water added to give a clear solution. Phosgene was passed for 10 minutes and the precipitate filtered after standing for 30 minutes.

Yield = 50 gms, m.p. 250° (soft) 255° (decomp.).

The above product was practically insoluble in dilute hot acetic acid and the filtrate gave no precipitate with a hot aqueous solution of benzaldehyde. Under the same conditions the hydrazide hydrochloride gave a heavy precipitate with hot aqueous benzaldehyde.

Calculated for $C_{10}H_9N_3O_4$:   N 17,9 %

Found :   N 18,0 %

Hydrolysis to the free amino compound WS 127 is effected by refluxing the acetamido compound with 1 N HCl during one hour and precipitating the hydrochloride of the desired WS 127 by the addition of excess concentrated HCl in the cold. The yield is almost theoretical.

Substituted Pyridyl-Oxadiazolone Derivatives

I. Formaldehyde Derivatives

(a) Methylidene Derivative of Isoniazid (WS 234)

Initially this condensation presented some difficulties in view of the solubility relationships. An oily product was obtained, soluble in water. The method given by Roche [77] did not give reliable results in our hands.

After a good deal of experimentation the following simple method was developed and always gave reliable results and good yields.

Isoniazid (30 gms) was dissolved in 40 % formalin solution (50 ml) warm and allowed to stand at room temperature with occasional shaking and scratching for 4 days. The white crystals were filtered, washed with a little water and dried at 100°.

Yield = 26 gms (80 %), m.p. 190-192° (decomp.). WS 234. The product was used without further purification.

(b) Reduction of Methylidene Derivative of Isoniazid (WS 234)

WS 234 (10 gms) dissolved in water (50 mls) and concentrated hydrochloric acid (5 ml). Platinum oxide (Adam's catalyst) (200 mgs) was added to the mixture and reduced during 24 hours at room temperature and 120 atmospheres hydrogen. The solution was decanted from the catalyst and evaporated to dryness in vacuo. The solid remaining is hygroscopic and difficult to obtain crystalline. It was dissolved in absolute ethanol (20 mls) and a slight excess of ether added, scratched and cooled. White hygroscopic crystals.

Yield = 10 gms (83 %).
M.p. WS 242.2 HCl (Methyl-isoniazid) = 225-228° (decomp.).
M.p. WS 234.HCl = 230° (brown), 245-246° (decomp.).
Mixed m.p. WS 234.HCl/WS 242.2 HCl = 188° (soft), 200-205° (decomp.).

The WS 242.2 HCl was used without further purification.

(c) Preparation of 2-Pyridyl-(4)-4-methyl-1,3,4-oxadiazolone-(5) (WS 245)

Methyl isoniazid (WS 242.2 HCl, m.p. 224-228° decomp.) (3.0 gms) was dissolved in water (50 mls) and dioxan (100 mls). Phosgene gas was passed through the solution slowly at 50-60° during one hour, during which time the colour changed

to pale green. After standing for half an hour to allow the excess phosgene to de-compose, the solution was evaporated to dryness in vacuo, the solid residue taken up in 10-20 mls hot water, filtered with a trace of decolourising charcoal and a slight excess of ammonia solution (25 %) added. On cooling to 0° and scratching the oxadiazolone crystallized.

Yield = 1.7 gms (74 %). Two crystallisations from aqueous methanol gave a constant m.p. of 137-139°.   WS 245.

Calculated for $C_8H_7N_3O_2$:   C 54,2   H 4,00   N 23,7 %

Found :   C 54,31   H 4,10   N 23,6 %

(d) Preparation of 2-Pyridyl-(4)-4-methyl-1,3,4-oxadiazol-thione-(5) (WS 287)

Methyl isoniazid (WS 242.2 HCl, m.p. 225-228° decomp.) (3.0 gms) was dissol-ved in water (50 mls) and dioxan (100 mls) and a stream of nitrogen passed through the solution for 10 minutes. Thiophosgene (1 ml) was added and the solution heated to 50-60° under reflux for 1 hour. Then a further addition of thiophosgene (2 mls) was made and the solution heated a further 2 hours at 50-60°. After evaporating to dryness in vacuo the residue was taken up in hot 2 N HCl (100 mls), treated with a trace of decolourising charcoal and filtered. A slight excess of aqueous ammonia (25 %) was added and the yellow crystals filtered.

Yield = 2.0 gms (78 %). WS 287 m.p. 127-128°. After two further crystalli-sations from aqueous methanol the melting point was constant at 128-129°, straw yellow crystals.

Calculated for $C_8H_7N_3OS$:   C 49,7   H 3,63   N 21,8 %

Found :   C 49,9   H 3,73   N 21,7 %

II. Acetaldehyde Derivatives

(a) Ethylidene Derivative of Isoniazid (WS 220)

This reaction was carried out as described by Roche [76, 77] but with slight modifications.

Isoniazid (30 gms) was refluxed with isopropanol (400 mls) and acetaldehyde (40 mls) on the steam bath until complete solution was effected (one hour). On cool-ing in ice fine white needles were deposited and were filtered and dried at 100°.

Yield = 35.2 gms (99 %). WS 220 m.p. 178-179°.   WS 220.

The material was used without further purification.

(b) Ethyl Isoniazid (WS 229). Reduction of Ethylidene Derivative (WS 220).

WS 220 (15 gms) was dissolved in 200 mls glacial acetic acid and platinum oxide (Adam's catalyst) (300 mgs) was added and the solution reduced at 90 atmospheres with hydrogen for 24 hours at room temperature. After decanting off from the catalyst, the solution was evaporated to dryness under reduced pressure, the residue taken up in isopropanol (100 mls) and ethanol satured with dry hydrogen chloride (100 mls) was added. The precipitate was filtered and dried at 100°.

Yield = 20 gms (91 %). WS 229. 2 HCl m. p. 212° (soft), $220\text{-}222^{O}$ (decomp.).

(c) Conversion of Ethyl-Isoniazid-hydrochloride (WS 229. 2 HCl) to the free base (WS 229)

WS 229. 2 HCl (15 gms) was dissolved in water (30 mls) and the pH of the solution adjusted to 6 with aqueous ammonia. After saturating with solid sodium chloride the solution was extracted four times with chloroform, the extracts combined and the chloroform distilled off. The resulting base was crystallized from benzene and a trace of petroleum ether (b. p. $60\text{-}80^{O}$).

Yield = 8.0 gms (77 %). WS 229 m.p. $110\text{-}111^{O}$.

(d) Preparation of 2-Pyridyl-(4)-4-ethyl-1, 3, 4-oxadiazolone (5) (WS 236)

Ethyl isoniazid (WS 229, m.p. $110\text{-}111^{O}$) (3.0 gms) was dissolved in dioxan (50 mls) and phosgene passed slowly through the solution at $50\text{-}60^{O}$. A yellow oily precipitate was formed. Water (50 mls) was added to form one phase and phosgene passed during a further 2 hours at $50\text{-}60^{O}$. After evaporating to dryness in vacuo, the residue was taken up in water (50 mls), treated with a trace of decolourising charcoal, filtered hot and excess aqueous ammonia added. After cooling to 0° the shining white crystals were filtered and dried at 50°.

Yield = 2 gms (58 %). WS 236 m.p. $104\text{-}105^{O}$. WS 236. After two crystallisations from aqueous methanol the melting point was constant at $106\text{-}107^{O}$.

Calculated for $C_9H_9N_3O_2$:   C 56,6   H 4,70   N 22,0 %
Found :   C 56,6   H 4,98   N 21,9 %.

(e) Preparation of 2-Pyridyl-(4)-4-ethyl-1, 3, 4-oxadiazol-thione (5) (WS 288)

Ethyl isoniazid (WS 229, m.p. $110\text{-}111^{O}$) (3 gms) was dissolved in dioxan (150 mls) and nitrogen passed for 10 minutes, then thiophosgene (2 mls) was added and

the mixture heated under reflux for 1 hour at 60-70°. A further addition of thio-
phosgene (1 ml) was made and the solution heated during 2 hours at 60-70°. After
evaporation to dryness in vacuo the residue was taken up in hot 1 N hydrochloric
acid (50 mls), heated with a trace of decolourising charcoal and filtered. The pH
of the filtrate was adjusted to 10 with aqueous ammonia. The resultant oil crystal-
lised on standing at 0°.

Yield = 3.0 gms (79.5 %).   WS 288.

M.p. 88-89°. Sublimes slowly at 80°. After three further crystallisations
from aqueous methanol straw yellow crystals were obtained m.p. 93-94°.

Calculated for $C_9H_9N_3OS$:		C	52,2	H	4,3	N	20,1	%
Found :		C	52,3	H	4,25	N	19,97	%

III. Propionaldehyde Derivatives

(a) Preparation of the Propylidene Derivative of Isoniazid (WS 221)

This reaction was carried out with some modifications, as described by
R o c h e [76, 77].

Isoniazid (30 gms), isopropanol (400 mls) and propionaldehyde (25 mls) were
refluxed on a steam bath. A clear solution was rapidly obtained and refluxed for
a further hour. On cooling, no crystallisation occurred. The solution was evapo-
rated under reduced pressure to one half the volume and ether added until incipient
crystallisation.

Yield = 37 gms (96 %). WS 221 m.p. 137-140°. WS 221.

The product was used without further purification.

(b) Preparation of n-Propylisoniazid (W 230). Reduction of the propylidene
derivative of isoniazid (WS 221).

WS 221 (15 gms) and methanol (absolute) (300 mls) and platinum oxide (100
gms) (Adam's catalyst) were hydrogenated under 95 atmospheres hydrogen for 24
hours in a rotating stainless steel autoclave at room temperature. The solution was
decanted from the catalyst and evaporated to dryness under reduced pressure. The
residue was dissolved in isopropanol (300 mls) and ethanol (100 mls) saturated with
dry hydrogen chloride added. After filtering and drying yield = 13.5 gms (63 %),
m.p. 210-218°. WS 230.HCl.

The material was used without further purification.

(c) Preparation of 2-Pyridyl-(4)-4-n-propyl-1,3,4-oxadiazolone-(5) (WS 239).
Reaction of n-propylisoniazid (WS 230) with phosgene

WS 230.HCl (15 gms) and 25% aqueous acetic acid (100 mls). A clear solution
was obtained and a slow stream of gaseous phosgene was passed through for one
hour, during which time the colour almost disappeared. The solution was evapora-
ted to dryness under reduced pressure, dissolved in water (50 mls) and the pH ad-
justed to 6-7 with dilute ammonia. The precipitate was filtered and dried.

Yield = 6.3 gms, m.p. 78-81°. WS 239.

May be crystallised from aqueous methanol.

Calculated for $C_{10}H_{11}N_3O_2$:   N   20,5   %

Found :   N   20,22 %.

(d) Preparation of 2-Pyridyl-(4)-4-n-propyl-1,3,4-oxadiazol-thione-(5)
(WS 282). Reaction of n-propylisoniazid with thiophosgene

WS 230 HCl (2.8 gms), water (100 mls) and thiophosgene (2 mls) were allowed
to react at room temperature in the presence of a stream of nitrogen for 2 hours.
The mixture was then allowed to stand at room temperature with occasional shaking
for 2 days, then warmed and filtered after adding a little decolourising charcoal.
The solution was then made alkaline with concentrated ammonia solution and the
crystals separated after 1 hour at 0°.

Yield = 1.2 gms (50 %). Yellow needles m.p. 92-93°. WS 282. Crystallised
three times from aqueous methanol to constant m.p. 94-95°.

Calculated for $C_{10}H_{11}N_3OS$:   C   54,3   H   5,0   N   19,0   %

Found :   C   54,46   H   5,06   N   18,92 %

IV. Acetone Derivatives

(a) Isopropylidene Derivative of Isoniazid

Isoniazid (20 gms), isopropanol (100 mls) and actone (40 mls) were refluxed
together for 1 hour and allowed to stand at room temperature overnight. The white
crystals were filtered and then dried at 100°.

Yield = 23 gms (89 %), m.p. 163-166°. WS 231. Mixed melting point with
isoniazid = 135-140°.

The product was used without further purification.

(b) Isopropyl-isoniazid (Marsilid). Reduction of Isopropylidene Derivative

WS 231 (15 gms), methanol (300 mls) and platinum oxide (Adam's catalyst) (100 mgs) were reduced for 24 hours at 110 atmospheres hydrogen in stainless steel rotating autoclave at room temperature. After decanting from the catalyst the solution was evaporated to dryness under reduced pressure. The substance was difficult to crystallise but eventually came out from benzene.

Yield = 10 gms (66 %), m.p. 111-112°. WS 233.

The substance was used without further purification.

(c) Preparation of 2-Pyridyl-(4)-4-isopropyl-1,3,4-oxadiazolone-(5) (WS 235). Reaction of Isopropyl-isoniazid (Marsilid) with phosgene

The phosphate known as Marsilid was used for this reaction. Marsilid (10 gms) was dissolved in 25 % aqueous acetic acid (100 mls) and phosgene passed through at room temperature until the green colour was almost discharged. The reaction mixture was then evaporated to dryness under reduced pressure to yield an oil which was taken up in water (100 mls) and the pH adjusted with ammonia solution to 7. The glistening white needles were filtered and dried at 100°.

Yield = 6.5 gms (88%), m.p.: sinters at 130°, melts sharply at 168-170°.

Calculated for $C_{10}H_{11}N_3O_2$:   C 58,5   H 5,3   N 20,5 %

Found: C 58,43 H 5,3   N 20,38 %

(d) Preparation of 2-Pyridyl-(4)-4-isopropyl-1,3,4-oxadiazol-thione-(5) (WS 285). Reaction of Isopropyl-isoniazid (Marsilid) with thiophosgene

For this reaction the free base, isopropyl-isoniazid was used, m.p. 111-112°. Marsilid base (3 gms) and dioxan (250 mls) were heated together under a stream of nitrogen and thiophosgene (2 mls) was added. The solution was kept under reflux for 2 hours in an atmosphere of nitrogen. The resulting mixture was very dark and was cooled to room temperature and water (150 mls) was added and allowed to stand at room temperature until the excess thiophosgene had decomposed. The solution was warmed with a trace of decolourising charcoal, then filtered and to the filtrate a slight excess of concentrated ammonia solution was added. The precipitate was filtered and dried at 100°. Brown crystals.

Yield = 3 gms (81 %).

The substance was purified by crystallisation from methanol and water. Constant m.p. at 125-126°. WS 285.

Calculated for $C_{10}H_{11}N_3OS$:   C 54,3   H 5,0   N 19,0 %

Found :   C 54,22   H 5,08   N 18,86 %

V. n-Butyraldehyde Derivatives

(a) Preparation of n-Butylidene Derivative of Isoniazid

Isoniazid (30 gms), isopropanol (300 mls) and n-butyraldehyde (40 mls) were refluxed together for 1 hour when a clear solution resulted. The solution was evaporated under reduced pressure to one quarter of the volume and an equal volume of ethyl ether added. The white crystals were filtered and dried at 80°.

Yield = 40 gms (96 %). WS 225 m.p. 115-118°.

The substance was used without further purification.

(b) Preparation of n-Butyl-isoniazid. Reduction of n-Butylidene Derivative (WS 225)

WS 225 (25 gms), methanol (250 mls) and platinum oxide (Adam's catalyst) (200 mgs) were reduced together at 80 atmospheres hydrogen at room temperature during 24 hours. After decanting from the catalyst the solution was evaporated under pressure, the residue taken up in isopropanol (300 mls) and ethanol (100 mls) saturated with dry hydrogen chloride added. The precipitate was dried at 100°.

Yield = 34 gms (97 %), m.p. 245° (decomp.) WS S. 241.

(c) Preparation of 2-Pyridyl-(4)-4-n-butyl-1,3,4-oxadiazolone-(5). Reaction of n-butyl-isoniazid (WS 241) with phosgene

WS 241 (15 gms) was dissolved in 25 % aqueous acetic acid (100 mls) to give a clear yellow solution through which gaseous phosgene was passed during 1 hour at room temperature. After adding a trace of decolourising charcoal and warming, the solution was filtered and evaporated to dryness. Distilled water (200 mls) was then added and the pH adjusted to 10 with ammonia. After standing at 0° for 24 hours the oil crystallised.

Yield = 10 gms (81 %) WS 244. The substance was recrystallised from aqueous methanol four times to constant m.p. 61-62°.

Calculated for $C_{11}H_{13}N_3O_2$:   C 60,3   H 5,94   N 19,20 %

Found :   C 60,38   H 6,20   H 19,23 %

(d) Preparation of 2-Pyridyl-(4)-4-n-butyl-1,3,4-oxadiazol-thione-(5)
(WS 286). Reaction of n-butyl-isoniazid (W 241) with thiophosgene

WS 241. HCl (7.0 gms) and water (30 mls) and dioxan (70 mls) were placed in a round bottomed flask of 250 mls capacity and nitrogen passed through for 10 minutes. At room temperature thiophosgene (2 mls) was added and the solution kept at 60° during 1 1/2 hrs. A trace of decolourising charcoal was then added, the solution heated to 80° and filtered. The filtrate was evaporated to dryness in vacuo. The residue was taken up in water (100 mls) and 2 mls conc. HCl, and filtered. Concentrated ammonia was then added together with 10 mls of distilled water until the pH = 9. The precipitate was filtered and dried at 50°.

Yield = 4.4 gms (71 %) WS 286. Crystallised to constant m.p. from Methanol/H_2O m.p. = 71-72°.

Calculated for $C_{11}H_{13}N_3OS$:   C 56,2   H 5,6   N 17,9 %
Found :   C 56,48   H 5,61   N 17,8 %

VI. Iso-butyraldehyde Derivatives

(a) iso-Butylidene Derivative of Isoniazid ("S 223)

Isoniazid (20 gms) and isopropanol (100 mls) and iso-butyraldehyde (40 mls) were refluxed together during 1 hour and rapidly gave a clear solution. The solution was then evaporated to dryness under reduced pressure and the resulting oil poured on to 200 mls of distilled water. After several days at room temperature the crystals were filtered. The oil may also be directly crystallised from ether, if necessary, in which it is somewhat soluble.

Yield = 18 gms (65 %) WS 223. m.p. 125-130°.

The product was used without further purification.

(b) iso-Butyl-isoniazid. Reduction of iso-Butylidene Derivative (WS 223)

WS 223 (15 gms), methanol (200 mls) and Adam's catalyst (platinum oxide) (200 mgs) reduced at 100 atmospheres hydrogen at room temperature during 24 hours. After decanting from the catalyst and evaporating to dryness under reduced pressure the residue was dissolved in isopropanol (100 mls) and ethanol (100 mls) saturated with dry hydrogen chloride was added. The white crystals were filtered and dried at 100° during 4 hours.

Yield = 19.4 gms (93.4 %) WS 237. HCl. M.p. darkened 225°, decomp. 235°.
This product was used without further purification.

(c) Preparation of 2-pyridyl-(4)-4-isobutyl-1,3,4-oxadiazolone-(5) (WS 238).
    Reaction of iso-Butyl-isoniazid (WS 237) with phosgene

WS 237 (15 gms) was dissolved in 25 % aqueous acetic acid (100 mls) and gave
a clear solution through which gaseous phosgene was slowly passed during 1 1/2 hours
at room temperature, after which time the greenish colour was almost discharged.
The solution was then evaporated to dryness under reduced pressure and the residue
taken up in distilled water (50 mls). The pH was adjusted to 6.5 with ammonia. The
white crystals were filtered, washed with a little water and air dried.

Yield = 11 gms (89.5 %) WS 238. m.p. 56-59°. The product may be recrystalli-
sed from aqueous methanol.

Calculated for $C_{11}H_{13}N_3O_2$:   N   19,2   %
Found :   N   19,16 %

(d) Preparation of 2-Pyridyl-(4)-4-isobutyl-1,3,4-oxadiazol-thione-(5). (WS 284).
    Reaction of isobutyl-isoniazid (WS 237) with thiophosgene

WS 237 (2.8 gms) was dissolved in distilled water (100 mls) and dioxan (100 mls)
and nitrogen passed for 5 minutes. Thiophosgene (1 ml) was added and the mixture
allowed to stand 5 days at room temperature with occasional hand shaking until the
excess thiophosgene was hydrolysed. This reaction may also be carried out in aqueous
dioxan at 60-80° in which case the thiophosgene is more rapidly hydrolysed.

Distilled water (200 mls) and ethanol (200 mls) were added and the solution heated
to 80° with a trace of decolourising charcoal and filtered. The solution was evaporated
to dryness under reduced pressure and the residue taken up in 50 mls dilute hydrochlo-
ric acid and the pH adjusted to 9 with ammonia and cooled to 0° when fine white needles
appeared. After filtering and drying the yield was 1.3 gms (48.5 %) WS 284, m.p. 87-
88°. Crystallised three times from aqueous methanol, m.p. 87-88°.

Calculated for $C_{11}H_{13}N_3OS$:   C   56,2   H   5,5   N   17,9   %
Found :   C   56,35 H   5,55 N   17,78 %

VII. Benzaldehyde Derivatives

(a) Benzylidene Derivative of Isoniazid (WS 222)

Isoniazid (30 gms), isopropanol (500 mls) and benzaldehyde (30 mls) were refluxed together for 2 hours when a clear solution resulted. The solution was allowed to stand at room temperature for a further 2 days and the white needles were filtered and dried at 100°.

Yield = 45 gms (91.2 %) WS 222, m.p. 202-204°.

The product was used without further crystallisation.

(b) Preparation of Benzyl-isoniazid. Reduction of Benzylidene Derivative (WS 222)

WS 222 (10 gms) dissolved in water (100 mls), dioxan (75 mls) and concentrated hydrochloric acid (3 mls) were added to give an intensely yellow solution. Platinum oxide (Adam's catalyst) (200 mgs) was added and the whole was reduced during 24 hours at 90 atmospheres hydrogen at room temperature. After decanting from the catalyst the solution was evaporated to dryness and the residue treated with hot methanol (150 mls) to effect solution, then ethanol (100 mls) saturated with hydrogen chloride was added to give a clear solution. An equal volume of isopropanol was then added and the crystals filtered after allowing to stand at 0° for 4 hours.

Yield = 10 gms m.p. 231-235° (decomp.)

The mother liquor was then evaporated and yielded 0.5 gms product.

Yield = 10.5 gms (80 %) WS 240. HCl m.p. 236-238° (decomp.)

(c) Preparation of 2-Pyridyl-(4)-4-benzyl-1,3,4-oxadiazolone-(5) (WS 243).
Reaction of Benzyl-isoniazid (WS 240. HCl) with phosgene

WS 240. HCl (6 gms) was dissolved in 25 % aqueous acetic acid (50 mls) and gaseous phosgene passed through slowly for 30 mins. until the colour was almost discharged. The solution was then evaporated to dryness under reduced pressure and the residue taken up in distilled water (100 mls). pH was then adjusted with ammonia solution to 10 and the precipitate filtered and dried at 50°.

Yield = 5 gms (99 %) WS 243. The yield was crystallised from absolute methanol four times until the m.p. was constant at 115-117°.

Calculated for $C_{14}H_{11}N_3O_2$:   C 66,4   H 4,35   N 16,6 %

Found :   C 66,21   H 4,61   N 16,46 %

(d) Preparation of 2-Pyridyl-(4)-4-benzyl-1,3,4-oxadiazol-thione-(5) (WS 283).
Reaction of Benzyl-isoniazid (WS 240) with Thiophosgene

WS 240. HCl (2.0 gms) was dissolved in distilled water (100 mls) and dioxan
(100 mls) and thiophosgene (1 ml) was added at room temperature after having satur-
ated the system with nitrogen. The mixture was heated to 80° and allowed to stand at
room temperature for 3 days with occasional hand shaking until all the excess thiophos-
gene had been hydrolysed. There was a considerable precipitate, insoluble on heating.
The solution was heated to 80°, a trace of decolourising charcoal added and then filtered
and ammonia added to the filtrate in a slight excess. The precipitate was filtered and
dried at 100°.

Yield = 1.0 gm (56 %) WS 283, m.p. 135-138°.

The crystals may be purified by crystallisation from ethanol and the constant
m.p. was reached at 145.5-146°.

Calculated for $C_{14}H_{11}N_3OS$:   C 62,5   H 4,1   N 15,6 %
Found :   C 62,61 H 4,23 N 15,41 %

Chapter V

NEW OXADIAZOLONE DERIVATIVES

One may envisage the mechanism of attachment of a molecule of active substance to the receptor sites in the Mycobacterium cell as follows:

There are possibly three active centres (marked I, II and III) which may be the deciding factors in isoniazid and possibly four (marked I, II, III and IV) in the case of p-amino-salicylic acid, to mention the simpler and better known tuberculostatics.

isoniazid

p-amino-salicylic acid
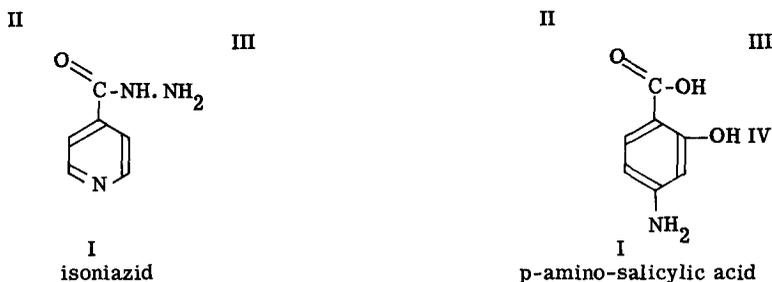
Conversion of the tertiary nitrogen atom in isoniazid to a quarternary salt destroys the tuberculostatic activity, so that we may assume that the p-tertiary nitrogen atom is essential for activity.

Removal of the C=O group at II similarly destroys activity; hydrazines, as opposed to hydrzides, are of low activity. Substitution in the primary amino-group, however, does not destroy activity, although it may be reduced. Many hydrazones, even though the primary nature of the nitrogen atom is changed, and alkyl substituted hydrazides, such as Marsilid (N-isopropyl-isoniazid) are active in vitro (possibly by hydrolysis in the case of the hydrazones) and some have been tested clinically against tuberculosis. In doses much smaller than those applied in the therapy of tuberculosis, Marsilid has proved to be an active mono-amine-oxidase inhibitor, useful in psychiatry. For clinical activity in tuberculosis much higher doses of Marsilid are required than those used in ' psychiatry. In sum, therefore, substitution at III does not necessarily result in loss of tuberculostatic activity, although loss of the primary atom itself does. A loss of the amido-nitrogen atom adjoining III does, however, entail loss of activity.

In p-amino-salicylic acid substitution on the primary amino group sharply reduces tuberculostatic acitivity as in benzoyl or acetamido-PAS. Conversion of the carboxy

group to a hydrazide as in PAS hydrazide does not disturb the tuberculostatic activity. Reaction of the latter with phosgene or thiophosgene gives an active oxadiazolone or oxadiazolthione (WS 127, WS 128, etc.).

On the basis of these considerations cyclisation of the hydrazide side chain in isoniazid by means of phosgene or thiophosgene, or other longer chain diacylhalides, to give the oxadiazolone, oxadiazolthione or similar larger ringed derivatives, might be expected to reduce the CNS stimulating properties often resident in amines of the hydrazide or hydrazine type, while retaining the tuberculostatic properties. Pyridyl-1,3,4-oxadiazolone would then be expected to show reduced toxicity and less euphoric properties, while retaining the tuberculostatic properties of the parent compound. In fact, unforeseeably, the ring closure confers additional leprostatic properties on the molecule not present in the open chain compound, as we have already mentioned. Experiment has borne out the first conclusions [67]:

Isoniazid   2-Pyridyl-(4)-1,3,4-oxadiazolone-(5)

PAS-hydrazide   WS 127   WS 128
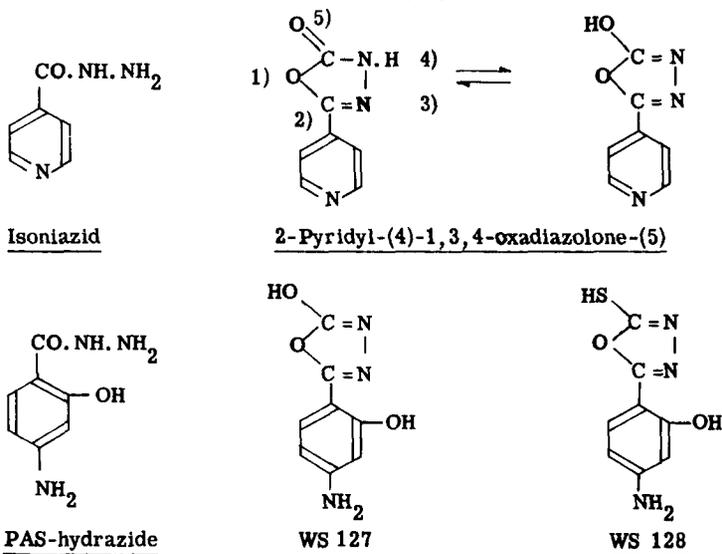
Continuing these considerations, it would not be expected that substitution of the hydrogen atom at position (4) on the oxadiazolone ring would destroy tuberculostatic activity, since a number of active dialkyl substituted isoniazid type on compounds are known and since Marsilid is also a highly active tuberculostatic. Similarly, changing the nature of the primary nitrogen atom in isoniazid to an amide in pyridyl-oxadiazolone should not result in activity loss since a number of hydrazones, in which the nitrogen atom is no longer primary and basic, are active.

<u>A</u>. - A series of new oxadiazolones was therefore conceived, in which the hydrogen at position 4 in the oxadiazolone ring was substituted by various alkyl groups (R). There is, in view of the possibility of tautomerism shown above, the possibility that R may be situated in the position 4 on the nitrogen atom, or in position 5 on the oxygen atom. It is generally conceded on spectrographic data in similar ring systems that in the parent oxadiazolone the favoured tautomer is the one involving hydrogen situated on the nitrogen atom under neutral conditions, so that we have followed this convention, though, at the same time, not wishing to exclude the other tautomeric possibility.

The following table (Table XVI) shows the compounds prepared in this series and gives their minimum inhibitory concentrations in µgms per millilitre (MIC/µgms/ml) tested against Mycobacterium tuberculosis (strain H37Rv) by Dr. Hans B r o d h a - g e , Cantonal General Hospital, Lucerne.

Table XVI

Tuberculostatic activity (MIC/ugms/ml) of some new Oxadiazolones tested against Mycobacterium tuberculosis (H37Rv) (liquid Dubos)
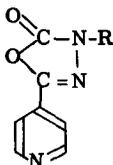
R	MIC in µgms/ml	WS Number
H	0.4	WS 57
CH_3	0.2	WS 245
C_2H_5	12.5	WS 236
n-C_3H_7	25.0	WS 239
n-C_4H_9	0.2	WS 244
i-C_3H_7	6.25	WS 235
i-C_4H_9	3.13	WS 238
$C_6H_5 \cdot CH_2$	0.8	WS 243
Isoniazid	0.02 - 0.1	-

MIC = minimum inhibitory concentration.

Comment

The in vitro activity against Mycobacterium tuberculosis of some of the new compounds approaches and even exceeds the activity of the parent oxadiazolone but does not surpass that of isoniazid. The methyl, n-butyl and even benzyl group all confer high activity.

Further experimental work in animals will now have to be carried out, at least on the methyl and n-butyl derivatives, to ascertain whether their high activity, together er with their enhanced fat solubility, would be useful in comparison to known compounds. In the chemotherapy of leprosy involving lipoprotein solubility, the long side chain present in CIBA 1906 has shown itself useful. Thus, we would guess that the n-butyl compound is potentially the most promising in this group.

B. - In view of the fact that leprostatic properties arise on converting isoniazid to pyridyl-oxadiazolone and also of the fact that most leprostatics contain a sulphur atom in the molecule (compare Table XVII: Dapsone, Diazone, Promine, Etizul, CIBA 1906, Bis(p-hydrazino-phenyl)-sulphone, Promizoel, 4,4'-diaminodiphenylsulphoxide, Promacetin, Sulphetrone, Hydnosulphone, etc.) it was thought to be of interest to introduce a sulphur atom into the pyridyl-oxadiazolone molecule.
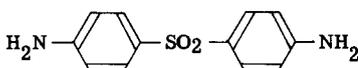
Table XVII

NH_2—⟨⟩—$SO_2 . NH_2$

Sulphanilamide

H_2N—⟨⟩—SO_2—⟨⟩—NH_2

4:4'-diaminodiphenyl sulphone

$H_2N . NH$—⟨⟩—SO_2—⟨⟩—$NH . NH_2$

Bis(p-hydrazinophenyl)-sulphone

H_2N—⟨⟩—SO_2—⟨⟨N / S⟩⟩—NH_2

Promizole

H_2N—⟨⟩—SO_2—⟨⟩—NH_2

4:4'-diaminodiphenylsulphoxide

H_2N—⟨⟩—SO_2—⟨⟩—NH_2
$SO_2 . N . CO . CH_3$
Na

Promacetin

$OHCH_2 . (CHOH)_4 . CH . HN$—⟨⟩—$SO_2$—⟨⟩—$NH . CH . (CHOH)_4 - CH_2OH$
SO_3Na SO_3Na

Promin

⟨⟩$CO . SC_2H_5$ / $CO . SC_2H_5$

Etisul (Ditophal)

$CH_2 . NH$—⟨⟩—SO_2—⟨⟩—$NH . CH_2$
SO_3Na SO_3Na

Diasone

⟨⟩—$CH . CH_2 . CH . HN$—⟨⟩—SO_2—⟨⟩—$NH . CH . CH_2 . CH$—⟨⟩
SO_3Na SO_3Na SO_3Na SO_3Na

Sulphetrone

$CH_3 . CH . HN$—⟨⟩—SO_2—⟨⟩—$NH . CH . CH_3$
SO_3Na SO_3Na

2196

⟨⟩—$(CH_2)_{10} . CO . HN$—⟨⟩—SO_2—⟨⟩—$NH . CO . (CH_2)_{10}$—⟨⟩

Hydnosulphone

$CH_3 . CH_2 . CH_2 . CH_2 . O$—⟨⟩—$NH . C . NH$—⟨⟩—$N(CH_3)_2$
$\overset{\parallel}{S}$

Diphenylthiourea (Ciba 1906)

This latter compound is among the first generally available synthetic lepro-statics lacking a sulphur atom. The introduction of a sulphur atom might hold out a chance of increasing its leprostatic activity.

To this end isoniazid was reacted with thiophosgene to give the desired thione:
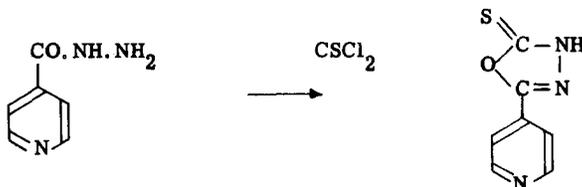
2-Pyridyl-1,3,4-oxadiazol-thione (5)
(WS 202)

This compound has been prepared before [67], with a view to testing its tuber-culostatic activity. As it did not show any particular advantages in tuberculosis over other known tuberculostatics, it was abandoned.

In serial dilution tests WS 202 shows activity against Mycobacterium tuberculosis ($H_{37}Rv$) comparable to that of pyridyl-oxadiazolone (Table XVIII).

Table XVIII

Comparative Acitivity of pyridyl-oxadiazolone and pyridyl-oxadiazolthione against Mycobacterium tuberculosis (H37Rv) on liquid Dubos medium Minimum inhibitory concentration in µgms/ml (MIC/ml)

Substance	MIC in µgms/ml
Pyridyl-oxadiazolone	0.4
Pyridyl-oxadiazolthione	1.0
Isoniazid	0.02-0.1

In view of the slow development of resistance towards $H_{37}Rv$ in serial dilution and subculturing tests shown by pyridyl-oxydiazolone compared with isoniazid under identical conditions, the rate of resistance emergence of $H_{37}Rv$ to pyridyl-oxadiazol-thione was determined (Graph I).

Serial Dilutions
(Mother Soln. = 1000 μgm/ml)   Subcultures every 2 weeks
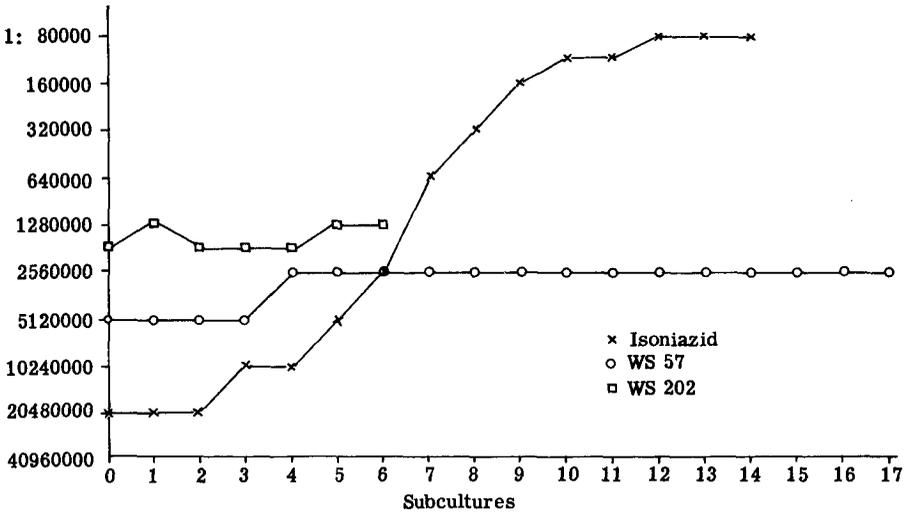
Graph I

Rate of resistance emergence to H37Rv shown by isoniazid, pyridyl-oxadiazolone and
pyridyl-oxadiazolthione. Liquid Dubos.

In view of the similarity of tuberculostatic properties shown by the oxygen and
sulphur derivatives, it was resolved to investigate WS 202 in clinical lepromatous
leprosy. Animal acute and chronic toxicity experiments in several species showed
low toxicity (Tables XIX and XX).

Table XIX

Acute subcutaneous toxicity of 2-pyridyl-1, 3, 4-oxadiazolthione-(5) (WS 202) in the mouse. Solution in NaOH pH 7

Mouse weight in gms	Dose mg/kg subcutaneous	Remarks	No. of deaths in 24 hrs.	Total deaths in 24 hrs.
31	250	no effect	0	
28	250	no effect	0	0
20	250	no effect	0	
29	500	no effect	0	
29	500	no effect	0	0
30	500	no effect	0	
28	750	sleep after 30 mins.	1	
28	750	"	0	
33	750	"	0	
27	750	"	0	
27	750	moribund after 4 hrs.	1	3
27	750	sleep after 30 mins.	0	
31	750	"	0	
33	750	"	1	

Comment

The LD_{50} s.c./mouse = > 750 mgs/kg.

More animals would be needed for an acurate LD_{50}.

Table XX

Acute subcutaneous toxicity of 2-pyridyl-1,3,4-oxadiazolthione(5) (WS 202) in the guinea pig. Solution in NaOH pH 7

Guinea pig weight in gms	Dose mg/kg subcutaneous	Remarks	No. of deaths in 24 hrs.	Total deaths in 24 hrs.
815	250	no effect	0	
685	250	no effect	0	0
638	250	no effect	0	
505	500	no effect	0	
472	500	no effect	0	0
422	500	no effect	0	
340	750	tranquillised after 30 mins.	1	
343	750	"	0	1
411	750	"	0	

Comment

The acute LD_{50} (mouse, guinea pig) lies somewhere about 750 mgs/kg subcutaneously. More animals would be needed for an acurate figure. The acute s.c. toxicity is comparable in the mouse and guinea pig.

Chronic toxicity tests were carried out by Bayer & Co., Leverkusen (courtesy Dr. Querfeld) using pyridyl-1,3,4-oxadiazolthione in rats and dogs. The oral route was used in each case, the substance being mixed with the food in the case of the rat.

In the rat a dose rate of 0.05, 0.1 and 0.5 % was used giving a maximal dose of about 250 mgs/kg/die. After 20 weeks no signs of intolerance were noted and the weight graphs were indistinguishable from those of control animals. There were no changes in blood picture.

In the dog two animals were used and given 5 times weekly per os in gelatine capsules. No toxic signs were noted in the urine or blood. The test is continuing at present.

<u>C</u>. - Clinical trial of pyridyl-oxadiazol-thione in leprosy

Trials were begun in a leprosarium in Angola. After six months trial in lepromatous leprosy cases at between 500 mgs and 2000 mgs daily per os, biopsies showed a substantial reduction in bacterial index and clinical improvement occurred at the same time. Above 2000 mgs per os daily slight gastric disturbance had a tendency to appear. Otherwise no other toxic symptoms were observed. It will require a further year's clinical work to evaluate the leprostatic usefulness of pyridyl-oxadiazol-thione (WS 202) which work is now proceeding.

<u>D</u>. - Some new substituted pyridyl-oxadiazol-thiones

Since pyridyl-oxadiazol-thione is also leprostatic, it was resolved to prepare a further series of this type of thio-compound. Substitution at position 4 on the oxadiazol-thione ring was carried out as for the corresponding oxygenated series of analogues.

The corresponding substituted isoniazid derivatives were prepared as before and reacted in aqueous or organic media with thiophosgene, according to the scheme:
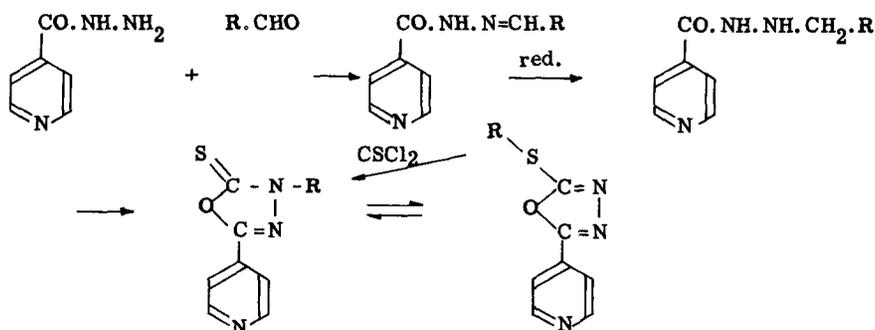

where $R = H$, CH_3, C_2H_5, $n\text{-}C_3H_7$, $n\text{-}C_4H_9$, $C_6H_5.CH_2$, $i\text{-}C_3H_7$, $i\text{-}C_4H_9$.

These sulphur derivatives were all tested in vitro against Mycobacterium tuberculosis ($H_{37}Rv$) with the following results (Table XXI):

Table XXI

Tuberculostatic acitivity (MIC/ml) of some new pyridyl-oxadiazol-thiones tested against Mycobacterium tuberculosis (H_{37}Rv). Liquid Dubos.

$$S \diagdown \atop O \diagup \overset{\displaystyle C - N - R}{\underset{\displaystyle C = N}{\big|}}$$
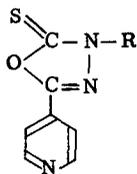
R	MIC in µgms/ml	WS Number
H	1.0	WS 202
CH_3	0.2	WS 287
C_2H_5	0.8	WS 288
$n-C_3H_7$	0.2	WS 282
$n-C_4H_9$	3.0	WS 286
$i-C_3H_7$	0.4	WS 285
$i-C_4H_9$	0.2	WS 284
$C_6H_5 . CH_2$	0.2	WS 283
Isoniazid	0.02-0.1	-

Key: MIC = minimum inhibitory concentration

Comment

In this series of thiones there are one or two surprises compared to the corresponding oxadiazolone series. WS 202 is slightly less active than WS 57, but the methyl derivatives of both series are equally highly active at $0.2\,\mu gm/ml$. The ethyl-oxadiazolone is a good deal less active than the corresponding ethyl-oxadiazol-thione. It is surprising that the ethyl derivative in the oxadiazolone series is relatively speaking inactive while it is quite active in the oxadiazol-thione series. There is little doubt about this finding as it was twice repeated. The same remarks apply to the n-propyl derivatives in the two series. In both series the n-butyl group confers high tuberculostatic activity. The iso-propyl-oxadiazol-thione is a good deal more active than its oxygen analogue, as also applies to the iso-butyl and benzyl derivatives of both series.

Thus in the oxadiazol-thione series the following derivatives are eligible for further investigation in animal toxicity and therapeutic tests: CH_3 (WS 287), $n-C_3H_7$ (WS 282), $iso-C_4H_9$ (WS 284) and $C_6H_5.CH_2$ (WS 283). The presence of the side chain, increasing fat solubility, and the presence of the sulphur group may mean that new and useful leprostatics are to be evaluated in the substituted 2-pyridyl-(4)-4-R-1,3,4-oxadiazol-thione type of compound. It is proposed to carry out first the acute and chronic toxicity determination in several species of animals before considering whether clinical trials be undertaken.

Summary

A review is given on the present state of the chemotherapy of Mycobacterial disease. Some theories of the mechanism of action of chemotherapeutic agents active in this area are discussed.

The tuberculostatic activity of isoniazid is compared with the tuberculostatic and leprostatic activity of the corresponding oxadiazolones and oxadiazol-thiones and the role of the sulphur atom in leprostatics mentioned.

Two new series of substituted pyridyl-oxadiazolones and oxadiazol-thiones are described from the synthetical and bacteriological aspect. The activities against Myco-bacterium tuberculosis ($H_{37}Rv$) are reported.

In the 4- substituted 2-pyridyl-(4)-1,3,4-oxadiazolones the most active tuber-culostatic compounds are those in which R = methyl and n-butyl. The minimum inhib-itory concentration against $H_{37}Rv$ in liquid Dubos medium is in these two cases 0.2 µgm/ml.

In the 4- substituted 2-pyridyl-(4)-1,3,4-oxadiazol-thiones the most active tuberculostatics are found where R = methyl, n-propyl, iso-butyl and benzyl. All these derivatives are active at 0.2 µgm/ml against $H_{37}Rv$ in liquid Dubos.

Thus, the above mentioned 2-pyridyl-oxadiazolone and 2-pyridyl-oxadiazol-thione derivatives are, under the test conditions used, somewhat more active than the parent oxadiazolone and will be further investigated in animal pharmacology and bacteriology.

Zusammenfassung

Das erste Kapitel bringt eine Beschreibung der Geschichte der Behandlung mycobacterieller Infektion nebst einer Besprechung des heutigen Standes der Theorie und der Praxis auf diesem Gebiet.

Kapitel II behandelt das Problem der Wirkungsweise von Chemotherapeutika in Tuberkulose und Lepra. Die Entwicklung von Resistenz und die Verhinderung derselben wird eingehend behandelt.

Kapitel III beschreibt die neueren Arbeiten auf dem Gebiet der Oxdiazolone und behandelt das Verhältnis des Stoffwechsels im Organismus zum antibacteriellen Spektrum in vitro und in vivo. Die Geschwindigkeit der Entwicklung von Resistenz bei Substanzen, die Kreuzresistenz aufweisen, wird besonders behandelt. Wie ist es möglich, dass zwei Substanzen wie Isoniazid und 2-pyridyl-1,3,4-oxdiazolon, die totale Kreuzresistenz bei tbc in vitro aufweisen, verschiedenartige Aktivität in Lepra zeigen? Isoniazid ist praktisch inaktiv in Lepra, wogegen das Oxdiazolon aktiv ist.

Die Extrapolierung der ausgeführten Ueberlegungen auf p-Amino-o-hydroxyphenyl-1,3,4-oxdiazolon wird durchgeführt und letztere Verbindung erwies sich aktiv als Leprostatikum, obwohl p-Amino-salizylsäure in dieser Indikation inaktiv ist und doch vollkommene Kreuzresistenz mit dem p-Amino-o-hydroxy-phenyl-1,3,4-oxdiazolon an den Tag legt.

Die Toxizitäten verschiedener neuer Oxdiazolone wurden an einigen Tierspecies bestimmt.

Kapitel IV beschreibt die organisch-chemischen Synthesen der neuen Oxdiazolone.

Kapitel V geht auf die Wirkungsweise und auf die in vitro Aktivität der neuen 2-Pyridyl-1,3,4-oxdiazolone-(5) und 2-Pyridyl-1,3,4-oxdiazol-thione-(5), die in Position 4 mit verschiedenen Resten substituiert sind, ein. Einige dieser Verbindungen weisen eine Aktivität von M.I.C. 0,2 µgms/ml bei H37Rv auf, so dass Tierversuche mit diesen Verbindungen indiziert sind.

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Curriculum vitae

Born on December 22nd, 1915 as the fourth child and eldest son of five I was brought up on my parents' estate in Cholsey, near Oxford, England. After attending the Grammar School at Wallingford from 1921-1930, my school education was completed at Taunton, Somerset, England, where I obtained the Cambridge School Leaving Certificate with distinction in seven subjects, in 1932. After one year at St. Edmund's Hall, Oxford, I took two B.Sc. degrees at Reading in 1937 and 1938 and proceeded to the Ph.D. degree of that University in 1941.

During the war 1939-1945 I was engaged on fundamental organic research at the Imperial Chemical Industries at Billingham, England. In 1945 I returned to University life at London University to work with Professor Sir Charles Dodds F.R.S. on Cancer Research with the British Empire Cancer Campaign at Middlesex Hospital, London University.

After marrying in 1951 I returned to industry for four years and worked on local anaesthetics, leprostatics, tuberculostatics, etc. I was then nominated P.D. (med.) at Geneva in 1956, since which time I have been active at that University. In 1957-58 I was seconded to be Visiting Assistant Professor of Pharmacology at Chicago, USA, and from 1960-62 was seconded to take over the functions of the Professor of Pharmacology at the Faculty of Medicine, Bergen, Norway.

During the years 1962 and 1963 I took advantage of the hospitality of the Pharmaceutical Institute directed by Professor J. Büchi at the Swiss Federal Institute of Technology, Zürich, to carry out the above described work. My sincere thanks go to Professor J. Büchi and his staff for their kind hospitality.