ANTITUBERCULOSIS DRUGS

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I. INTRODUCTION

Following the discovery of streptomycin by Waksman in 1944, advances have been made in the field of chemotherapy which have revolutionised the treatment of tuberculosis. This seems a suitable time to review what has been achieved and also to point out what remains to be done. In important respects, the drug treatment of tuberculosis is at present at a stage similar to that achieved for the treatment of syphilis previous to the discovery of penicillin, which in itself is a heartening thought. In both cases, *i.e.*, tuberculosis at present and syphilis before the 1950's, cure is and was probable, particularly with an early diagnosis, but at the expense of prolonged treatment with a combination of drugs. When the disease has run a more prolonged course and done more damage, complete

cure becomes more difficult, or even impossible, and additional forms of treatment not infrequently become necessary. A notable example of this is the use of surgery to remove areas which are badly damaged by the tuberculous process and which may moreover provide a nidus from which the disease may flare up again.

The use of penicillin in early syphilitic infections represents almost a *therapia magna sterilans*, involving the elimination of all infecting organisms within a comparatively short time and without the development of resistance. No such advance has as yet been made in tuberculosis. It is well known that the application of penicillin to the treatment of syphilis was the result of a chance discovery and not the result of research into the properties of the spirochaete and of the special lesions which are the consequences of spirochaetal infections. We shall, nevertheless, examine these factors in tuberculosis, *i.e.*, the properties of the infecting organisms and of the resultant lesion, not only because of the light this may throw on the action of existing chemotherapeutic agents, but also in the hope that some hint may be obtained about the properties of new and more rapidly effective substances.

II. SHORT HISTORICAL SURVEY

The older drugs were reviewed by Long (197); it is of interest to note that Koch actually investigated a number of substances, including gold. It is most interesting that Koch claimed that he had tested a substance, the nature of which was not given, capable of preventing completely the further development of an already established infection in guinea pigs (177). It is sad to think that Heidelberger had a somewhat similar experience when he investigated a number of sulphonamide derivatives in 1917 (143).

The more modern work really started with the observation of Rich and Follis (259) that large doses of sulphanilamide produced a beneficial effect on the development of experimental tuberculosis in the rabbit, a result which was confirmed by Buttle and Parish in the same year (64). Other sulphonamides were also tried but the results were not appreciably better. 4,4′-Diaminodiphenylsulphone did, however, represent an advance over the conventional sulphonamides, as was first shown by Rist *et al.* (265); this compound and several derivatives were also investigated clinically, but believed to be too toxic. It is interesting that the toxicity was probably due to the use of too large doses. The sulphones are at present the most effective drugs known for the treatment of leprosy and there is renewed interest in the use of diaminodiphenylsulphone as a possible alternative drug in tuberculosis.

However, at that time (*i.e.*, in the 1940's) further work on the sulphones was checked by the discovery of streptomycin by Waksman and his colleagues (166). This did indeed represent a major advance in the chemotherapy of tuberculosis, since the drug is effective in a variety of tuberculous conditions, including the miliary and meningeal types. It soon became apparent that streptomycin could produce serious toxic effects, and dihydrostreptomycin, which at first appeared to be more satisfactory, ultimately proved to be even more toxic.

The next useful discovery was *p*-aminosalicylic acid (PAS), which arose out of work on the effect of various substances, particularly *p*-aminobenzoic acid, on the metabolism of the tubercle bacillus. This led to the testing of various substances *in vitro* and *in vivo* and to the finding that PAS was the most effective (186). Shortly afterwards, Domagk, who had investigated a number of sulphonamides and related compounds, found that thiosemicarbazones were effective in experimental tuberculosis (96). One of these compounds (Thiacetazone, TB1) was investigated extensively in Germany and later in the United States, but was found to be relatively toxic.

The most spectacular advance in the chemotherapy of tuberculosis came with the discovery of isoniazid in 1952. Here was a drug which was not only much more potent than any previously used, but also relatively free from side-effects, particularly serious ones. At first isoniazid, because of its great potency, was used by itself in the treatment of human tuberculosis, but it soon became obvious that this was undesirable because of the development of resistant organisms, and it became accepted that to prevent this at least two drugs had to be combined. Since then, isoniazid, PAS, and streptomycin, in various combinations, have been the mainstay in the treatment of the disease.

No further major advance has been made since then. Several drugs are available in cases where the classical treatment has, for some reason, failed, *e.g.*, neomycin, cycloserine, and pyrazinamide, but they are not particularly active and are likely to produce toxic effects which can be serious. It is possible that ethambutol may prove an additional efficacious drug, but this awaits extensive clinical trials. The phenazines, highly active in experimental tuberculosis, have proved disappointing in clinical trials and this mysterious discrepancy still remains to be elucidated.

III. AIMS OF ANIMAL STUDIES OF ANTITUBERCULOSIS DRUGS

The aim of such studies is, of course, to obtain drugs which will be of value in the treatment of human tuberculosis, and to gain information which will lead to their most effective use in human disease. Any such studies always present the difficulty that data obtained in various animal species require careful interpretation and are not necessarily applicable to man. Thus, the absorption, metabolism, and excretion of drugs may show important variations in different species, and unexpected results may arise when the drug is used clinically even after extensive animal experimentation. The same applies to toxicity, for it is not unusual for a drug to show toxic effects in man which could not be prophesied from the results of animal experiments.

Because of the changing pattern of tuberculosis as a world health problem, there are certain features about future antituberculosis drugs which would have been regarded as of only secondary importance ten years ago. The major problems of treatment today are in the underdeveloped countries where expense and frequency and route of administration are primary considerations. Those concerned in screening drugs as potential antituberculosis agents should therefore bear in mind that among the desirable properties to be considered is the

cost of the new substance. It should certainly not exceed that of the cheaper drugs available against tuberculosis today. Secondly, the drug should be active when taken by mouth so that patients may be given treatment under conditions where poor medical services exist. A property which would be acceptable, or even desirable, in an antituberculosis drug is that of slow excretion, with persisting high blood levels for long periods, provided of course that this did not lead to any excessive toxicity. Thus compounds like the phenazines which, in animals at any rate, may persist in the body for weeks following one single dose, may be acceptable under the special conditions appertaining in the treatment of tuberculosis, whereas they might be considered undesirable in the treatment of acute infections.

Among the other desirable features which are rather special to antituberculosis drugs are penetration into cells (especially macrophages), into the cerebrospinal fluid (for the treatment of meningitis), and into non-vascularised caseous material, where the majority of the organisms may be localised. The acceptability of the product is also of special importance with regard to taste, amount to be taken, and the incidence of gastrointestinal upsets, especially nausea. These are important since patients are normally treated with a combination of two drugs to prevent the emergence of resistant bacteria. If the patient fails to take one of them the physician may not be aware of the fact until the course of the disease changes with the emergence of resistant organisms. It has been shown with PAS, for example, that a high percentage of out-patients, as many as 49% in one series reported by Wynn-Williams and Arris (353), do not in fact take the drug or take it only sporadically.

IV. TESTS USED FOR SCREENING ANTITUBERCULOSIS DRUGS

We may next consider tests that have been used in an attempt to assess the activity of new antituberculosis drugs. The intention is not to review exhaustively all the tests that have been used, but merely to discuss a few of them to assess their value and limitations. Such tests have of course the aim of determining whether the drug would be valuable therapeutically in man, and to give answers to at least the following questions. 1) How active is the new drug in comparison with known antituberculosis drugs? Only an approximate answer to this question is possible at best, since any figure obtained may be greatly modified by the pharmacological properties of the two drugs under comparison. In general, it is difficult to do a biological standardisation, *i.e.*, a direct comparison of two pharmacologically active preparations, when the active principles involved are different chemical substances, probably with different modes of action. 2) Is the drug capable consistently of eradicating the disease? This is largely an academic question at present since no drug is known which will consistently eradicate tuberculous infections in animals, leaving the lesions free from any living tubercle bacilli. But extensive investigations of this type have been performed, and will be done again with new drugs (and new combinations of drugs). The question is of course to a large extent bound up with a knowledge of the extent to which drugs can produce a bactericidal effect *in vivo*. 3) What is the

value of the new drug in combination with other drugs? There are in fact two aspects to this, which are, however, closely bound together when it comes to treatment of human disease (Section V). (a) Does the combination of two or more drugs produce an increase in the chemotherapeutic efficiency or not, or are the actions of the two drugs antagonistic so that the combination is less effective than either drug alone? (b) Does the combination of drugs prevent or delay the emergence of resistant forms of the bacilli? This is one of the greatest dangers in the treatment of human disease and was a frequent occurrence before the principles of adequate treatment had been worked out. It can be said that no single and completely reliable method has yet been worked out to answer this question.

These are some of the main data we require on the chemotherapeutic activity of a new substance, and they have of course to be evaluated in relation to information about its toxicity. Other specialised aspects of the chemotherapeutic action of drugs have been investigated, *e.g.*, the possible value of a drug when lung cavities are present. The possible anti-inflammatory action of the drug, which may contribute to its effectiveness in arresting the disease, also needs consideration. The experimental investigation of the actions of anti-inflammatory drugs, *e.g.*, cortisone and prednisone, in tuberculosis will not be discussed here.

A. Tests in vitro

Testing *in vitro* has been used very extensively as a screening method. It suffers from such serious disadvantages that it is now questionable whether any significance should be attached to the results so obtained. The fact that it may give positive results with compounds which have in fact no appreciable effect *in vivo*, *e.g.*, with certain oestrogens, is not particularly important in a preliminary screening test. Similarly, the finding that the activity of a compound *in vitro* gives little indication of its value *in vivo*—for example, rifomycin (Section VII, N) is even more active than isoniazid in the test tube, but is a good deal less active in animals—is no great drawback in a first test. Much more disturbing is the finding that substances may be totally inactive *in vitro* and yet be of value in the treatment of the disease in animals and man. Such a finding is not unexpected when the substance, *e.g.*, Macrocyclon (Section VII, O), is believed not to act on the *Mycobacteria* but to enhance the resistance of the body to the mycobacterial infection. The test *in vitro* may, however, be negative with substances which are in fact chemotherapeutically active *in vivo*. One explanation of this is that the substance itself may be inactive, but may undergo metabolic changes in the body which liberate the active material. The classical example of this in chemotherapy is the conversion of prontosil to sulphanilamide, and similarly there are derivatives of isoniazid which owe their activity to the liberation of the hydrazide in the body. But a substance may be inactive *in vitro*, under the usual conditions of testing, and yet be active *in vivo*, even though there is no evidence that it undergoes any changes which lead to the liberation of an active factor. Good examples of this are pyrazinamide

and nicotinamide. These substances are quite active *in vitro* at an acid pH, and yet quite inactive in a neutral medium. It has been reported (344) that the tuberculous lesion has at least in part a pH on the acid side and it is possible that this accounts for the activity of pyrazinamide and nicotinamide *in vivo*. These findings and their implications have been discussed by McDermott and Tompsett (216). It seems more likely, however, that the conditions in which drugs act *in vivo* have been imperfectly reproduced *in vitro*, perhaps due to differences in bacterial metabolism in the synthetic medium, and that this explains the discrepancies observed. The environmental factors which affect inflammation and may modify the response of bacteria to drugs have been discussed by Dubos (100). The selection of the medium to be used for cultivating the organisms is of importance since the results obtained may be markedly affected by it. The decision whether to use a complex nutrient medium containing proteins, or to use a synthetic, chemically defined medium may depend on the characteristics of the drug being investigated, for example, with regard to protein-binding. The report of the American Trudeau Society (7) is well worth consulting with regard to recommendations for testing both *in vitro* and *in vivo*. The choice of the test organism is probably more important, and may have more influence on the results obtained than the choice of medium. Because of the slow growth and dangers associated with the use of virulent human or bovine strains of tubercle bacilli, many laboratories have used avirulent or attenuated strains of *Mycobacteria* for routine screening. This is definitely unwise since very misleading results can be obtained. For example, as long ago as 1950, the American Trudeau Society warned against the use of the avirulent strain ATCC 607. When Logemann *et al.* (196) used this strain in a drug screening procedure, they found that 250 μg/ml of a certain piperazine derivative was required to inhibit its growth, whereas 0.4 μg/ml of the same drug inhibited the human strain H37Rv.

B. Tests in vivo

1. Species differences. In tuberculosis there are certain special difficulties which are encountered in assessing the results of animal investigations. The disease is not the same in all animal species and this must first be considered. The main animal species used experimentally are the mouse and the guinea pig and these have been used increasingly in the evaluation of new drugs. Rabbits and monkeys have also been used.

The *mouse* is on the whole comparatively resistant to tuberculosis and large infecting inocula have to be used to produce a progressive disease. On the other hand, smaller inocula given intravenously will produce a chronic type of disease which is suitable for some kinds of studies, *e.g.*, of the effect of corticosteroids (255). Moreover, very small inocula (about 10 organisms) injected into the cornea will produce a disease which is progressive for some time and is suitable for the evaluation of antituberculosis drugs. Further characteristics of the infection in the mouse are that the disease is essentially intracellular, that allergy is at a minimum, and that necrosis is comparatively slight and caseation does

not occur. The drug has therefore to act on bacilli usually localised within cells and subject to the intracellular environment. The literature concerning the use of the mouse in experimental chemotherapy and the pathogenesis of mouse tuberculosis has been reviewed by Raleigh and Youmans (252). In addition, as Fong and his co-workers (118) have pointed out (in several papers of which only the most recent is quoted), the effect of the bacilli on the cells is also important, and the extent to which they are capable of destroying the cells may become an important factor in the spread of the disease. This emphasizes that the progress of the disease is not merely a function of the total number of bacilli present, but also of the action of the bacilli on the tissues. In fact, McCune and Tompsett (213) have shown that once a population of tubercle bacilli has attained a sufficiently high level, the resulting pulmonary lesions show a steady progression in size even though the size of the bacterial population remains stable.

The *guinea pig* is much less resistant to tuberculosis than the mouse, and even a mild infection tends to be progressive and ultimately fatal. The disease is not essentially intracellular as in the mouse. Allergy is very striking and necrosis extensive. Tuberculosis in the guinea pig is thus in many respects different from that seen in the mouse. Chemotherapeutic agents have to produce an action on bacilli not only in and immediately outside of cells, but also located in necrotic tissue.

The *rabbit* infected with bovine tubercle bacilli develops a progressive and usually fatal type of tuberculosis similar in many respects to that in the guinea pig. Rabbits are, however, very resistant to infection with the human type of tubercle bacillus, though the extent of this resistance varies with the genetic make-up of the rabbit (200). As in the guinea pig, allergy is important, but the sensitivity to tuberculin is slightly less in the rabbit. While rabbits are not generally used as a screening test for new drugs, pulmonary tuberculosis in the rabbit infected by the airborne route is very similar morphologically to that in patients with chronic cavitary lesions and is useful for the intimate study of the mode of action of drugs in special circumstances (125, 199, 308).

In *man*, resistance to the disease is higher than in guinea pigs, and the majority of infections are controlled by the natural defenses of the body without any chemotherapy, though the extent to which the disease progresses before it is brought to a halt varies considerably. The organisms may be in cells, but particularly in acute infections many of the *Mycobacteria* are extracellular. Allergic phenomena are important in man as are necrosis and caseation, in fact more so than in the guinea pig, and caseous material may harbour enormous numbers of bacilli. The development of cavities in the lungs adds another factor not normally seen in the other species discussed, though it can occur in rabbits and monkeys. A particularly important factor in the persistence of human tuberculosis during prolonged treatment with drugs is the development of resistant organisms, and though this can also occur in animal experiments, usually under special circumstances, in this case these resistant organisms are not usually the reason for the persistence of infection. Thus, for example, Robson

and Sullivan (274) treated mice with isoniazid for eleven months, and eight months later recovered from such animals tubercle bacilli which were still highly sensitive to isoniazid, in great contrast to what usually happens in man (see below, Sections V, VI).

2. Mouse survival test. The most widely used screening test *in vivo* is the mouse survival method, in which treatment is started on the day of infection and a very acute disease is produced which usually kills most of the control animals within less than 30, and sometimes less than 20, days. The various stages of this infection were reviewed by Walter *et al.* (336), who pointed out that at first most of the bacilli are intracellular, though later on there is extensive extracellular multiplication with some necrosis due to allergy. This is thus a type of tuberculosis rather akin to miliary tuberculosis in man, and very different from the chronic lung tuberculosis which is the most common manifestation of the disease. Nevertheless, the mouse survival method has proved very valuable and foretold with reasonable accuracy the therapeutic value of the three main drugs used clinically. Since then, there have been unexpected findings, of which perhaps the most important is that on the phenazines (Section VII, L) which are extremely active by this test and by several other animal tests. It is now several years since these compounds were described by Barry and yet the clinical reports available show that they have no activity in man, quite apart from the question of their toxicity. This is very puzzling, particularly since there is evidence that one of them (B663) can be taken up by tuberculous lesions (2, 287). The question still remains, however, whether there are at present unknown differences between the tuberculous lesions in man and in various animals which might account for the discrepancy between the experimental and clinical effects of phenazines. The same applies to the results with thiourea derivatives (Section VII, H), where negative clinical results have been authenticated, though some of these compounds are highly active in animal experiments. The findings with Macrocyclon (Section VII, O), a compound which in all probability acts by increasing the resistance to infection in animals and man, further strengthen the belief that there may be differences between tuberculosis in mice and man which account for differences in the effectiveness of some drugs.

More important, however, would be the finding that a drug is inactive in the mouse test and yet of value in human tuberculosis. This appears to be the case with cycloserine (Section VII, E), which is apparently quite inactive by itself in systemic mouse tuberculosis though of some value clinically. It might be said that since cycloserine has a comparatively low activity it would not appreciably affect the acute infection in mice resulting from the intravenous injection of a large inoculum of *Mycobacteria*, but in fact such a test does reveal the value of PAS, which by itself has only a very moderate action in human disease. The failure of cycloserine to control mouse tuberculosis may be due to its rapid elimination from this species (78).

Another mouse test which is sometimes used is the delayed treatment test, in which mice are infected with a large number of bacilli and the disease allowed to progress for one or two weeks before treatment is started. As opposed to the

first test, which measures the ability of a drug to prevent the development of disease, this test shows whether the drug can cure an established infection. In general this is a much severer test of a drug, but whether it is really qualitatively different from the immediate treatment test is very doubtful, though Grumbach and Rist (130) quoted experimental results which suggest that this may be so.

Perhaps the value of one more experimental tuberculous lesion is worth discussing, bearing in mind the general approach developed above, *i.e.*, tuberculosis of the cornea in mice. This is essentially a strictly localised lesion which can be studied from day to day and presents all the aspects described and reviewed by Walter *et al.* (336) for generalised tuberculosis in the mouse, particularly with regard to changes in total mycobacterial populations, though the corneal lesion is self-limiting (270), unlike the overwhelming intravenous infection. With the corneal technique a good assessment can be made of the three main drugs used clinically and it also clearly shows up the possible advantages of large doses, for example, of isoniazid, as compared with smaller doses (274). As with the other *in vivo* methods discussed, it has also given fallacious results for phenazines and for thioureas, though it has shown up the value of cycloserine. There is, moreover, no evidence that the simple and safe corneal method does not give as reliable results for screening purposes as the mouse survival method or the guinea pig method.

3. Guinea pig tests. The guinea pig has always been considered as more satisfactory than the mouse for chemotherapeutic work on tuberculosis, though it suffers from the disadvantage that more drug, space, and time are required, and there is a greater element of risk. Thus, it is not always suitable for routine screening. A small inoculum, even a single organism, is sufficient to initiate the disease, which then progresses slowly until the animal dies with extensive tuberculosis, usually after several months, though as a rule the animals are killed at a predetermined time and the extent of the disease is assessed in a semi-quantitative manner. Allergy is marked and there is much necrosis. The disease in this animal is thus more akin to human tuberculosis than that commonly studied in the mouse. The disease in the guinea pig responds to the main tuberculostatic drugs, as indeed it does in the mouse. For example, Karlson and Feldman (171) studied the effect of streptomycin and of isoniazid, and also of various combinations of these drugs, in guinea pigs. By using the criterion of extent of macroscopic disease, after some two months of treatment they were able to assess the value of the various treatments. The question is whether study of the disease in the guinea pig offers any advantages over a similar study in the mouse. There is no doubt that very careful observations in the guinea pig will reveal certain effects which are not necessarily apparent in the mouse. Thus, in the study of Karlson and Feldman (171) microscopic examination of the lesions revealed certain aspects of the lesion which were not apparent on macroscopic scrutiny; these were of value in differentiating between certain treatments which, on a merely macroscopic basis, appeared to be equally effective. Likewise, in the study of allergic aspects of the disease, the guinea pig offers obvious advantages over the mouse. For the actual screening of drugs in

relation to their possible effectiveness in man, no such advantage is at present obvious, and the guinea pig shows no advantage over the mouse. For example, in the evaluation of the phenazines, the results in guinea pigs were highly promising, as in the mouse, and equally irrelevant, it would appear, to the clinical usefulness of these compounds. Similarly, the information elicited by experiments on guinea pigs with cycloserine was not more informative than that in mice, since only after careful microscopic examination in the former species were suggestive effects attributable to cycloserine observed. The results with thiourea derivatives are even more relevant to our conclusion, since the results in guinea pigs were even better than in mice; the guinea pig results lead to the expectation that some of these compounds would be valuable in man, and yet they have proved most disappointing in human tuberculosis.

A localised cutaneous lesion in the guinea pig similar to the corneal test in mice, which can be studied daily without killing the animals, has been described as a method for drug screening (261, 277). This involves the production of a cutaneous ulcer by the intradermal injection of a small number of organisms. The small variation in response permits reliable results to be obtained using only a few animals, and the test can be completed in 30 days. Hence this method seems to have most of the advantages of the mouse screening tests.

In general, however, there is little to suggest that any real advantage is obtained by screening compounds in guinea pig tuberculosis instead of mouse tuberculosis, and the greater expense and risk involved in handling guinea pigs seem hardly worthwhile.

4. Tests on monkeys. Schmidt (285) has done extensive work on tuberculosis in rhesus monkeys, on the basis of which he has suggested that serious consideration should be given to doing controlled simian studies regularly before the introduction of new agents for the treatment of human tuberculosis; he has expressed the belief that adequate information cannot be obtained from experimental rodent tuberculosis, which differs in important respects from human disease. The tests on monkeys are of course not suitable for screening new compounds, but only for comparing selected ones accurately with those already in clinical use.

When tubercle bacilli (10^1 to 10^4 viable units) are introduced into the bronchi, an acute infection develops which is similar to that seen in the disease which frequently occurs naturally in these animals. By 3 to 5 weeks after inoculation there is involvement of more than one lobe, and this is followed by a rapid aggravation of the disease and usually by death of the animal. This form of the disease in monkeys is similar to acute tuberculosis seen in human infants, and differs in important respects from the subacute, comparatively localised, cavitary disease usually seen in the human adult. It is important to realise that most of the chemotherapeutic studies reported up to the present by Schmidt and his co-workers have been carried out on monkeys with the acute disease.

Schmidt has also made attempts to induce a more chronic type of disease in monkeys, *i.e.*, of a type usually seen in man and therefore more suitable as a model for chemotherapeutic studies. He has produced such a disease by two

different methods, namely: 1) Intratracheal vaccination with BCG and subsequent challenge (6 to 10 weeks later) with H37Rv (1 to 5 $\times$ 10^3 viable units into a bronchus); 90% of animals so challenged developed well-circumscribed, slowly progressive, cavitary lesions, strikingly similar to the subacute lesions found in man. 2) Animals inoculated in the usual way (to produce an acute disease) and treated with PAS (250 to 500 mg/kg) or thiocarbanidin (Section VII, H) (50 to 250 mg/kg); animals so treated uniformly developed slowly progressive, thick-walled, ragged cavitary lesions. A large group of such animals (treated at first with thiocarbanidin) was then used in an experiment to compare the values of isoniazid, streptomycin, and PAS, alone and in combination. Schmidt stated that there was a remarkable parallelism between the results of this study and those of controlled clinical investigations. "As in man, the subacute cavitary disease in the monkey presents a severe challenge to therapy. Eradication of tubercle bacilli from residual lesions is difficult; if bacilli are not eradicated promptly, high proportions of final isolates are drug resistant." Schmidt expressed the intention to use this method extensively in further studies on the biology and therapy of pulmonary tuberculosis.

Acute tuberculosis in monkeys has so far been used in the study of three new drugs, cycloserine, thiocarbanidin, and phenazine (B663). It is interesting briefly to compare the results so obtained with those found in rodents, on the one hand, and in man on the other.

Schmidt has shown that *cycloserine* protects animals against an otherwise fatal infection, causes regression of extensive and severe lung lesions, and effects limited bacteriological control. At a dosage of 100 mg/kg, which has quite an appreciable effect on the disease and produces no toxic actions, blood levels of 20 to 60 μg/ml are produced. The findings in monkeys also suggest that a combination of isoniazid with cycloserine deserves trial in human tuberculosis in cases where the usual combinations cannot be used. These results in the monkey are more in agreement with clinical findings than with those obtained in rodents. This may be due to the fact that following its administration the blood level of the drug is well maintained in man and in the monkey but falls off rapidly in other species, *e.g.*, the mouse and the guinea pig (77, 78, 79). This would not explain, however, the striking effect of cycloserine in the mouse when tested by the corneal method, unless the corneal level falls more slowly than the blood level.

Thiocarbanidin was used in doses up to 500 mg/kg per day and was found to be less effective than PAS (288). When administered in combination with suboptimal doses of isoniazid, thiocarbanidin produced no beneficial effect. These results in the monkey are more in keeping with clinical experience than with experiments in rodents. The relative ineffectiveness of the drug both in man and in the monkey is attributed to low blood levels, due either to poor absorption or rapid degradation, but this requires further investigation.

Phenazine (B663) is a particularly puzzling drug since it is highly effective in mice and guinea pigs, ineffective in human tuberculosis and, in preliminary experiments, appears to be active in human leprosy. Schmidt (287) found no

significant effects in the monkey with doses of 10 mg/kg given orally in aqueous suspension (with 2% Tween 80). There was quite a marked effect if administration of 100 mg/kg was started at the time of inoculation, though very little activity if the treatment was delayed for one month. In such animals resistance to the drug developed rapidly and this was followed by fatal tuberculosis. With 100 mg/kg given in aqueous suspension, the maximal plasma concentration ranged from 1.75 to 3.3 μg/ml. This could be increased appreciably when the drug was given in oil (also orally) but such levels produced severe convulsions and death in a high proportion of the animals. Schmidt found also that large amounts of the phenazine were stored in the lungs, both in normal and in tuberculous tissue. On the whole, therefore, the results in monkeys are much nearer to the clinical findings in tuberculosis than are those in rodents.

V. COMBINATION OF DRUGS: THE RELEVANCE OF ANIMAL TESTS TO ACTIVITY IN HUMAN DISEASE

It would be very valuable to be able to forecast from animal experiments how effective various combinations of drugs would be in the treatment of human disease. There are two aspects to the question. First, there is the capacity to demonstrate experimentally that a combination of two drugs produces an effect on the disease which is more marked than that achieved by either drug alone. All the methods so far discussed (and others not mentioned) can do this. Generally speaking, when two drugs produce an additive effect in animals, be it an increase in survival time, a decrease in the severity of lesions, or an effect on the total number of bacilli present in the animal or one of its organs, then such an additive effect also occurs in human disease. For example, Karlson and Feldman (171) demonstrated the additive effect of streptomycin and isoniazid in their early experiments on guinea pigs, and the same result was shown by the mouse intracorneal method (127). The studies of Schmidt quoted above show a similar additive effect for a number of other drugs.

The second aspect, however, is that one of the main problems in the therapy of human tuberculosis is the emergence of drug-resistant organisms, and this resistance is much more difficult to elucidate experimentally than clinically. It should be added that the problem has been attacked only sporadically and that it deserves much more systematic study, though there are some very interesting published data. In man, treatment with one drug rapidly leads to the emergence of resistant *Mycobacteria*. In animals this is not so, with some curious exceptions. Thus, Karlson and Feldman (171) treated guinea pigs for 61 days with isoniazid, streptomycin, or a combination of the two drugs and failed to find any resistant organisms at the end of that period. They did, however, state that three out of eight animals treated with streptomycin for 144 days and five out of six treated for 215 days had streptomycin-resistant bacilli. Similarly, Youmans *et al.* (356) found highly resistant bacilli in mice treated with streptomycin for some five months, particularly in those treated with high doses. Isoniazid treatment of mice for six months led to the appearance of organisms highly resistant to isoniazid (128), though we have reported exactly the opposite

result (274). Even in monkeys, therapeutic (but not prophylactic) administration of isoniazid for a year led only to the emergence of fairly resistant (1 to 10 μg/ml) bacilli (285).

In contrast to this, Grumbach (128) has described a method by which the development of resistance in mice can be regularly demonstrated. She found that if mice are infected intravenously and treatment with streptomycin or isoniazid is delayed for 15 days (at which time macroscopic lesions are already present in the lungs), resistant bacilli (to either drug) can be already recovered from such animals after only six weeks of treatment. It is, moreover, of interest that combined treatment with both drugs prevents the appearance of resistant *Mycobacteria*. This has been investigated further by Rist (262) and Canetti *et al.* (68), who found that under these conditions resistant organisms appeared earlier with large than with small doses of isoniazid (a finding similar to that obtained with streptomycin, *v.s.*). Similar results with delayed treatment in mice have been found by Grumbach (129) using pyrazinamide, isoniazid, and a combination of these drugs. Canetti (67) discussed the factors necessary for the emergence of resistant mutants as the main bacillary population, and considered three essentials, *i.e.*, a large initial population, a sufficiently high concentration of the chemotherapeutic substance acting on these bacilli, and the presence of general or local factors favourable to the proliferation of the bacilli. These seem quite reasonable in relation to the effect of the main antituberculosis drugs, but do not explain why, with some drugs tested under the same conditions in animals, resistance is nevertheless found to develop quite quickly. A striking example of this phenomenon is the development of resistance to ditophal (diethyl-dithio-isophthalate) within 10 days in mice inoculated intravenously and treated from the beginning of infection (86). Resistance to pyrazinamide has also been observed early. For example, Steenken *et al.* (309) found that resistant *Mycobacteria* could be isolated from the spleen of guinea pigs after 46 to 69 days of treatment; early development of resistance to cycloserine was also encountered in some of these experiments. Early development of resistance to two propylene sulphide derivatives has also been observed in mice (4).

VI. THE PERSISTENCE OF *MYCOBACTERIA* IN TISSUES

Lastly, the continued persistence of bacilli in the tissues of animals and man, even after very prolonged chemotherapy, needs discussion. This is not necessarily due to the development of resistance, since fully susceptible bacilli are sometimes present and can subsequently start to multiply again after cessation of treatment. These findings are well exemplified in the results of McCune *et al.* (214) and those of Canetti (66) on the recovery of bacilli from human lungs resected at operation after prolonged chemotherapy, as well as in the results of Robson and Sullivan (274) on the cornea of mice. The usual explanation given is that non-multiplying bacilli with a low metabolic activity are no longer susceptible to the bactericidal effect of drugs nor to the normal defense mechanisms of the body.

VII. PHARMACOLOGY OF INDIVIDUAL DRUGS

A. Isoniazid

It seems rather a remarkable coincidence that the most potent of all the antituberculosis drugs, isoniazid (INH, Rimifon, Pycazide, Nydrazid, isonicotinic acid hydrazide) should have been discovered independently and simultaneously at three separate centres. However, this would seem to be the case, since Lott and co-workers at the Squibb Laboratories (42) and Fox at Hoffmann-La Roche (120) both published their results in 1952, having arrived at the drug by different pathways, while Domagk in a communication at the congress on internal medicine in Wiesbaden, Germany, in April 1952 claimed that isonicotinic acid hydrazide had first been synthesized by Offe and tested by Domagk as early as 1950 at the laboratories of Farbenfabriken Bayer in Germany.

The first reports on its activity in mice (42, 133) and in guinea pigs and rabbits (307) showed it to be a remarkably potent drug, considerably more so than any other known at that time. Even at this early stage, however, it was shown that resistance to it could develop very rapidly and to a very marked extent.

The first reports of its effectiveness in human patients (269) showed that clinically it was also a most powerful drug against both pulmonary and non-pulmonary tuberculosis. It also produced a very rapid change in the well-being of the patient, such as was never before experienced with other drugs. Subsequent experience has shown that isoniazid is indeed a remarkably effective drug, though it is unfortunately not completely effective in all types of tuberculosis.

Absorption and distribution. The absorption, distribution, and excretion of isoniazid was first studied by Rubin *et al.* (279) and Benson *et al.* (38) in animals and by Elmendorf *et al.* (106) in man. The drug is well absorbed after oral administration, the level in the plasma reaching a peak after about one to two hours and falling to a half or less in six hours. In the dog, Rubin and Burke (278) showed that after oral administration a plasma peak is usually reached after about 30 minutes and thereafter the clearance from the plasma is about 19% per hour. In man, between 50% and 70% is excreted in the urine in 24 hours and by this time the plasma level is usually below the limits of detection (about 0.5 μg/ml). In patients receiving a dose of 1.5 mg/kg twice daily for several weeks, there was no evidence of cumulation.

The drug is distributed throughout the body water, and substantial concentrations are found in the cerebrospinal fluid of both normal subjects and patients with tuberculous meningitis, and in pleural effusions, saliva, and faeces (106). The drug also passes through the placental barrier and into the milk in concentrations comparable to those in the plasma (340). Using radioactive isoniazid labelled with C^{14} in the carbonyl position, it has been shown in mice that the drug is localised mainly in the liver, skin, lungs, brain, and kidneys, the concentration remaining high in the lungs and skin even after the plasma level has fallen to trace amounts (276). The distribution of isoniazid in tuberculous guinea pigs (178) and man (15, 93) has also been studied using C^{14}-labelled isoniazid injected intramuscularly. This has shown that the drug penetrates

well into caseous material, and although initially the concentration in plasma and muscle is higher than in the infected tissue, the caseous material retains the isoniazid for a longer period. Thus, even after 96 hours in the guinea pig, when the amount in the plasma and muscle was negligible, the amount in a caseous lymph node was still well above the bacteriostatic level. Similarly in man, the capsule of a resected pulmonary lesion contained more isoniazid than did the blood or skin, and a similar result was obtained in a resected tuberculous cervical lymph node. These results demonstrate that isoniazid penetrates and remains for long periods in caseous lesions in amounts well above the bacteriostatic level. The skin of both mice and man contains significant amounts and may act as a storage depot.

Metabolism and excretion. The first report of the isolation and identification of the metabolites of isoniazid was by Hughes (157) who showed that in rhesus monkeys and also in one human patient, the main excretion product was 1-isonicotinyl-2-acetyl hydrazine. Of the dose administered to monkeys, 60 to 100% was excreted in the urine in 15 hours and 74 to 94% of this was in the acetylated form. Further studies by Hughes *et al.* (159) have shown that all species studied eliminate part of the isoniazid unchanged in the urine, the percentage unchanged varying with the species, and in man with the individual. In man the two major excretion products are acetyl isoniazid and isonicotinic acid, but other products are excreted as well. These include an isonicotinic acid conjugate, probably isonicotinyl glycine, one or more isonicotinoyl hydrazones, and traces of N-methyl isoniazid. The various excretion products of the monkey, dog, guinea pig, rat, and mouse have also been described (159). The dog is rather unusual, the main excretion product being isonicotinic acid as well as free isoniazid. In one series of experiments (278), for example, a total of 67% of the dose was excreted, 28% as isoniazid and 40% as isonicotinic acid. Since the method of analysis used did not distinguish between isoniazid and the acetyl isoniazid, some of the drug may be acetylated in the dog, though it is known that this species fails to acetylate sulphonamides.

Bernstein *et al.* (42) showed that 1-isonicotinyl-2-acetyl hydrazine is about one-tenth as toxic as isoniazid. However, the acetyl derivative has less than one-hundredth the antituberculosis activity of isoniazid *in vivo*. Hughes (157) suggested that the probable failure of dogs to acetylate isoniazid accounted for the higher toxicity of isoniazid in this species than in man or the monkey. She also suggested that variation in completeness of acetylation may account for the differences in toxicity of isoniazid in different patients. Urine analysis in patients suggested that they fell into two groups from the point of view of metabolism (159). In one group, all the isoniazid given could be recovered from the urine as free isoniazid and its hydrazones, acetyl isoniazid, and isonicotinic acid. In the other group, the sum of these fractions accounted for 38 to 84% of the dose administered, and these patients had more free isoniazid and less acetyl isoniazid than the first group of patients. The pattern of metabolism for any one patient was relatively constant over several months, and irrespective of the dose between 5 and 20 mg/kg. The incidence of peripheral neuritis was

greater in the group with less urinary acetyl isoniazid and high free isoniazid plasma levels than in the first group. Later work by Biehl (44, 45) supported this suggestion by showing that the percentage of administered isoniazid excreted by patients in the free form was bimodally distributed throughout the population, suggesting that individuals belong to one of two classes—slow inactivators or rapid inactivators. Further work (12, 108, 109, 140, 174, 223) confirmed this view, and Knight *et al.* (174) and Evans *et al.* (108) have shown that the metabolism of isoniazid is genetically controlled. The "slow inactivators" (*i.e.*, those who have high blood levels of the free compound and who excrete a high proportion of the free drug in the urine) are autosomal homozygous recessive, and the "rapid inactivators" are of the two genotypes—heterozygous and homozygous dominants. Furthermore, there is a definite "dosage" effect of the gene controlling the dominant character, so that the heterozygotes have significantly higher plasma levels of isoniazid than the homozygous dominant subjects.

Evans *et al.* (108) considered the implications that this observation may have in the clinical use of isoniazid in three areas: 1) the development of polyneuritis with long term treatment, 2) the response of the tuberculous disease to the treatment, and 3) the development of isoniazid-resistant bacilli. With regard to the first point, they quoted the results of Hughes *et al.* (158) and the subsequent analysis of Biehl (45) that in one series of patients, four out of five slow inactivators developed polyneuritis, while only two out of ten rapid inactivators developed this complication. The number of subjects thus examined, however, is too small for valid analysis. The results of Mitchell *et al.* (223) are quoted to examine the second point. The number of patients studied was again small, but there seemed to be a trend for patients with higher serum levels of isoniazid to have a quicker reversal of infectiousness and also a higher frequency of reversal. With regard to the third point, there seemed to be no correlation between the isoniazid inactivator phenotype and the development of isoniazid-resistant tubercle bacilli (45).

Toxicity. The original reports on isoniazid described some of the toxic manifestations in animals and man (38, 42, 106, 269, 279, 307). The LD50 in mice by the oral route is about 140 to 190 mg/kg, and is practically the same by intravenous, intraperitoneal, intramuscular, or subcutaneous injection, thus showing that the drug is well absorbed by all these routes. Animals receiving toxic doses show evidence of C.N.S. stimulation with apprehension, excitement, and tremor followed by clonic and tonic convulsions, with death resulting from respiratory failure. The sensitivity of different animal species to isoniazid varies considerably, the LD50 orally in the rat, for example, being over 1400 mg/kg, and in the dog about 50 mg/kg. The monkey shows the same type of toxic effects but is less sensitive to the drug, being able to tolerate up to 320 mg/kg orally (289), and even after chronic administration of high doses this animal shows little evidence of toxicity. Fatal doses of isoniazid can be rendered innocuous by the simultaneous administration of either phenobarbitone or pentobarbitone, which prevents the C.N.S. stimulation (238).

The principal form of toxicity seen clinically is peripheral neuritis, as first reported by Biehl and Skavlem (47) and Biehl and Nimitz (46), who found a 17% incidence among patients receiving doses of about 6 mg/kg and a 44% incidence among patients receiving 16 to 24 mg/kg. One of their patients died from toxic encephalopathy due to isoniazid. The only other common toxic effect was gastric intolerance, seen in 10% of the patients, which occurred mostly among those receiving 20 mg/kg daily. No toxic effects were reported on the peripheral blood or on kidneys, though mild transient liver dysfunction was observed. The incidence of peripheral neuropathy is highest among the patients with the highest blood levels of isoniazid, so that, as mentioned previously, the "slow inactivators" have the highest incidence of this toxic effect. Epileptic patients would seem to be particularly susceptible to the convulsant effects of isoniazid and a fatality in such a patient has been reported (113).

The symptoms of isoniazid toxicity resemble those of vitamin B_6 deficiency, and Biehl and Vilter (48) showed that patients receiving large doses of isoniazid excreted large amounts of pyridoxine, and that the peripheral neuritis could be prevented by administering pyridoxine along with isoniazid. The pyridoxine, however, does not seem to interfere with the antibacterial action of isoniazid (81, 132, 326). In contrast to adults, children receiving doses of 5 to 20 mg isoniazid/kg for long periods do not show evidence either of toxicity or of pyridoxine deficiency (226). The mode of action of isoniazid in producing the peripheral neuropathy is not fully understood, but it is probable that the isoniazid competes with pyridoxal phosphate for the enzyme, apotryptophanase (51, 192), and so disturbs the metabolism of the neurone.

Conditions resembling this clinical peripheral neuropathy have been produced in rats (359) and guinea pigs (210). Other substances which antagonise the toxic effects of isoniazid without interfering with its bacteriostatic effect include sodium glucuronate; a large number of amino-acids, particularly glycine and L-cysteine (245, 248); calcium pantothenate (210); cycloserine (246); glycerine (247); and a number of vitamins (249). Allergy to isoniazid has also been reported (218) and where it is necessary to continue treatment, the patients may be desensitised to the drug (59). This is, however, a rare complication of isoniazid therapy (168).

Action on the tubercle bacillus. The activity of isoniazid in inhibiting the multiplication of *M. tuberculosis in vitro* is considerably greater than that of most other antituberculosis agents, concentrations in the range of 0.01 to 0.25 μg/ml being effective (13, 42, 133, 307), depending on the medium used for the cultivation of the organisms and the duration of contact of the drug with the organisms (11). Furthermore, the activity is very selective to the *Mycobacteria* since other organisms may require more than 1000 μg/ml before inhibition is seen (176, 239). It has also been shown that the activity is exerted on growing and not resting bacilli (150) and that at least one further multiplication of the bacilli occurs before the bacteriostatic effect is achieved (14, 204). In media which support good growth of tubercle bacilli, isoniazid may have a bactericidal effect (220, 281) and also cause loss of acid-fastness. Unlike streptomycin, which is

relatively inactive against bacilli within macrophages, isoniazid is active in the same concentration against both intra- and extracellular organisms (204, 312).

Bacterial resistance to isoniazid develops rapidly both *in vitro* (220, 239) and *in vivo*, so that for example in the clinical trials of isoniazid given alone, resistant organisms were observed in 11% of patients after one month, rising to 21% after three months treatment (323). The combination of isoniazid with streptomycin markedly reduced the incidence of resistance to isoniazid (5, 324, 328). The development of resistance *in vitro* to five hundred times the normal inhibitory concentration of isoniazid has been shown to follow a "one-step" pattern with a mutation rate of about 1 to 3 $\times$ 10^{-6} per bacterium per generation, about one thousand times the rate for streptomycin (313).

The mode of action of isoniazid. This has been extensively investigated but the problem has not yet been solved. In a short review of the literature, Maher *et al.* (207) concluded that three facts are fairly well established, namely: 1) There is a direct relationship between isoniazid susceptibility, pathogenicity, and catalase activity of *M. tuberculosis*. This is also clearly brought out by the experiments of Tirunarayanan and Vischer (320), who considered, however, that isoniazid sensitivity or resistance is more closely related to peroxidase than to catalase. 2) Isoniazid must form a metal chelate *in vitro* and *in vivo* before its antituberculosis potential can be realised. 3) Isoniazid-metal complexes somehow interfere with oxidative enzyme reactions involving pyridine nucleotides. The data of Fisher (115, 116), Middlebrook (221), and Knox (175) should be consulted for some of the earlier work on the subject.

There seems to be a consensus that the bactericidal effect of isoniazid is ultimately produced by some breakdown product, the liberation of which is related to the presence of catalase or peroxidase. Thus, Winder (349) suggested that free radicals produced by the autoxidation of isoniazid are responsible for its action, catalase or peroxidase possibly being the autoxidation catalyst. Krüger-Thiemer (183) believed that isoniazid is oxidised within the tubercle bacillus to isonicotinic acid; this would lead to a disturbance of coenzyme I (or II) and a compensatory enhanced oxidation of substrate hydrogen by the autoxidisable flavin enzymes; thus, more hydrogen peroxide would be formed, which was suggested to be the real cause of the inhibition of bacterial multiplication. The view that isoniazid is broken down by peroxidase, possibly at the surface of, or within, the cell, and that the breakdown product exerts the inhibitory effect by blocking an essential function of the cell was also developed by Tirunarayanan and Vischer (321).

Willett (346) has found that the tubercle bacillus contains diaminopimelic acid decarboxylase (DAP), an enzyme which requires pyridoxal phosphate as coenzyme, and which is inhibited by isoniazid. She believed that important points of attack of isoniazid on *M. tuberculosis* are those enzyme systems requiring pyridoxal phosphate as coenzyme. Schaeffer (282) produced evidence that isoniazid affects the dehydrogenase activity of the tubercle bacillus and suggested that there is consequent inhibition of the synthesis of a lipid component in the bacillary membrane leading to irreversible injury to the bacterial cell.

Several studies with radioactive isoniazid have been performed, but these have not added to our understanding of the mode of action of the drug (16, 50).

Thus, in spite of the very interesting work that has been done, there is no satisfactory explanation of the mode of action of isoniazid. The highly specific activity of this drug should be a challenge to further investigation of this question.

B. Streptomycin

The first really satisfactory drug for use in the treatment of tuberculosis was streptomycin (N-methyl-L-glucosaminidostreptosidostreptidine). This was isolated by Waksman and his colleagues (283, 284) from the actinomycete, *Streptomyces griseus*. Confirmation of the effectiveness of the drug against experimental infection in animals and human tuberculosis soon followed (111, 112, 147). A vast amount of work on streptomycin, mainly on its antituberculosis effect, soon accumulated and about six thousand papers on the drug were published during the eight years following its discovery. These have been listed by Waksman (334) and the work up to 1953 summarised by him in his Nobel Prize address (335). A later review of the drug covering the period up to 1957 was published by Long (197). Full details of the chemistry have been given by Waksman (335), Birkinshaw (49), Wolfrom *et al.* (350), and Regna (257). Because of the large number of papers already published on streptomycin, it is proposed in this present review to mention only briefly, with key references, the work up to 1958.

Absorption, distribution, and excretion. The absorption, distribution and excretion of streptomycin in various species have been studied by numerous workers (52, 144, 211, 305, 361) and are similar for dihydrostreptomycin (152, 189, 251). The drug is not absorbed when administered orally, but following intramuscular injection it passes rapidly into the blood, reaching a peak concentration about one hour later. The volume of distribution is equivalent to about 30% of the total body weight, suggesting that the drug is distributed in the extracellular space. Following injection of the usual therapeutic dose of 0.25 to 1.0 g, a peak plasma level of about 40 to 60 μg/ml is achieved, which falls to a half in about 4 or 5 hours, and effective amounts may still be present after 24 hours. In the blood it is loosely bound to γ-globulin. The drug penetrates poorly into the cerebrospinal fluid, though effective concentrations can be achieved following usual doses given intramuscularly (189). In patients with meningitis, the inflammation of the meninges allows much greater diffusion into the cerebrospinal fluid (189, 361). Streptomycin (not more than 100 mg), but not dihydrostreptomycin, may also be given intrathecally and high levels will be maintained in the cerebrospinal fluid for more than 24 hours (144, 179).

The drug passes through the placenta into the foetal circulation, though the foetal blood level is considerably below that in the maternal blood, *i.e.*, usually a half or less (189, 352). It also diffuses into pleural fluid (189), saliva (37), ocular fluid (361), and milk (104). The fact that the drug does not penetrate

well into cells is of importance since tuberculosis tends to be an intracellular infection. This has been demonstrated *in vitro* by Suter (312) who showed that in a liquid medium, extracellular tubercle bacilli were inhibited by a concentration of 0.5 μg of streptomycin/ml, but if the bacilli were intracellular in phagocytes, then a concentration of 80 to 100 μg/ml was required for inhibition. This can be contrasted with isoniazid, which penetrates well into cells, and under whose action the bacilli were inhibited by the same concentration of isoniazid whether they were extra- or intracellular.

In dogs, over 90% of the drug is excreted within 24 hours in the urine, and in man the majority of the drug is similarly excreted, mainly by glomerular filtration. Occasionally in man, the percentage excreted in the urine may be less than this (211). Some of the drug is concentrated by the liver and excreted in the bile (144).

Toxicity. Much of the toxicity ascribed in the early days to streptomycin was in fact due to impurities, some of which had histamine-like activity; these are not present in the crystalline substances available today. The toxicity of streptomycin and dihydrostreptomycin has been studied in a variety of species by Molitor *et al.* (224), Rake *et al.* (251), and Edison *et al.* (102). Actual values for the LD50's are not easy to assign due to the relative impurity of the early samples when most of the work was done. However, the subcutaneous LD50 in mice has been reported (251) as 1.44 g/kg for streptomycin and 1.60 g/kg for dihydrostreptomycin. Following administration of large doses, one observes restlessness, respiratory distress, loss of consciousness, coma, and death from respiratory failure, the heart continuing to beat for several minutes after cessation of respiration. It is probable that the respiratory failure is in fact due to paralysis of the respiratory muscles, since it has been shown that streptomycin has a curariform action, and the acute toxic effects can be antagonised by calcium and by neostigmine (54). Chronic administration may produce hepatic and renal failure and central nervous system damage.

The commonest toxic effect seen in man is eighth nerve damage. Two-thirds of patients receiving 2 g per day showed evidence of labyrinthine damage, and if the dose was reduced to 1 g per day, this occurred in one-sixth of the patients (236). Symptoms usually occurred after 20 to 30 g of streptomycin had been administered and were manifested either as vertigo or nystagmus, or, more usually, both. If streptomycin was given in large doses, *i.e.*, 3 g per day for three months, then deafness could also occur. Dihydrostreptomycin was introduced in the hope of being less toxic, and early work did in fact show that vertigo did not occur following dihydrostreptomycin. Further experience, however, showed that this drug caused a progressive deafness which was irreversible in all severe cases and occurred in as many as 31% of patients (126). Thus, streptomycin is the drug of choice since the labyrinthine disturbances can usually be overcome by the patient whereas the deafness cannot. The site of action of the neurotoxic effect has been studied in animals by Berg (39) and Christensen *et al.* (71) and the experimental work reviewed by Jongkees and Hulk (167). Glorig (126), in a survey of the work on the site of action, stated

that the lesions in both the vestibular and cochlear systems are definitely in the end organ. There is marked degeneration of the hair cells in both the semicircular canals and the organ of Corti. The central lesions are probably secondary to the end organ changes.

The other common toxic effect of streptomycin is the development of allergic sensitivity, both by the patient and by the person handling the drug. Agranulocytosis has also been reported (110).

As mentioned previously, streptomycin has a curariform effect (54). It will potentiate the action of curare and will reintroduce the paralysis caused by decamethonium if given a short time after the paralysis has worn off (43). This paralysing effect of streptomycin occurs also in isolated human intercostal muscle (280) and has led to respiratory failure in patients when streptomycin has been given following an operation during which curare was used (117).

Actions on the tubercle bacillus. In the original reports (283, 284) it was shown that streptomycin was effective *in vitro* against both gram-positive and gram-negative bacteria and in particular against *M. tuberculosis* and also against related organisms like *Erysipelothrix* and *Actinomycetes*. The concentration which inhibits the growth of tubercle bacilli varies between about 0.1 μg/ml and 10 μg/ml, depending both on the medium used for cultivation and on the strain of organism. The majority of tubercle bacilli are inhibited by 2 μg/ml or less (355), at which concentration the drug is usually bacteriostatic in action, though at higher concentrations the drug may be bactericidal (243).

Resistance to streptomycin can develop fairly easily *in vitro* by repeated subculture in medium containing streptomycin, and the resistance once developed is permanent (348). The resistant strains probably develop by selective growth of naturally occurring resistant mutants, which are present in cultures in a ratio of 1 in 10^6 bacilli for streptomycin, compared with about 1 in 10^5 bacilli for isoniazid resistance (75, 156).

Streptomycin-dependent organisms may also develop, and such bacilli have been recovered from animals after treatment with streptomycin (188) and after cultivation *in vitro* in streptomycin-containing media (161, 354). Streptomycin-dependent organisms may be either pathogenic or non-pathogenic.

Mode of action. Like many other antimicrobials, streptomycin is most active against organisms which are undergoing active multiplication and is relatively inactive against organisms in the lag or resting phase. Thus, tubercle bacilli are protected against the action of streptomycin if they are suspended in a non-nutrient medium (222), and, in a nutrient medium, the activity of streptomycin decreases as the generation time of the organisms increases (150). The pH of the environment is also important since the effectiveness of streptomycin is reduced in acid media (1). This may be of importance in the clinical use of streptomycin since tuberculous lesions may often have a pH as low as 4 (344). Streptomycin becomes fixed to the bacterial cell (40, 194), though resistant organisms do not take up streptomycin (314). As a result of its action, morphological changes occur in the bacteria (333). Exactly how the drug acts at a biochemical level is still unknown, although many possible actions of streptomycin have been sug-

gested. Cohen (74) has suggested that the drug acts by combination with deoxyribonucleic acid at the cell surface, and Prina (250) has demonstrated the release of nucleic acid from bacteria following streptomycin action. Donovick *et al.* (98), however, doubted that there was any correlation between the precipitation of nucleic acid by streptomycin and its antibacterial effect. Henry *et al.* (146) and Stacey (303) have shown that streptomycin may interfere with the carbohydrate metabolism of the tubercle bacillus, and the hypothesis has been put forward (235) that the drug interferes with the mechanism for oxaloacetate-pyruvate condensation in the terminal respiratory metabolism of the bacillus, and that 2-phospho-4-hydroxy-4-carboxy-adipic acid is involved (325).

The drug is certainly more effective against aerobic organisms than against anaerobic ones, and the rate of carbohydrate metabolism both *via* the Embden-Meyerhof pathway and *via* others is reduced (137, 347). When these are fully inhibited, the Q_{O_2} values for oxidation of glucose, glycerol, lactate, and formate are reduced by 40% to 80%. Thus, it seems possible, as suggested by Hancock (137), that the primary site of action of streptomycin in *Staph. aureus* is in the chain of respiratory enzymes. This could result in a decreased activity of the tricarboxylic acid cycle and so decrease the amount of high energy phosphate available. As a secondary effect this could reduce the rate of carbohydrate metabolism *via* the Embden-Meyerhof and other pathways, and would also inhibit nucleic acid synthesis at an early stage. This is supported by the observations of Hancock (137) that there is a reduction in the rate of incorporation of glutamic acid, alanine, and proline into the protein fractions and that these changes in protein synthesis are not seen until some time after the inhibition of glucose metabolism is observed. There is some indirect evidence that streptomycin may interfere with cytochrome activity (193). An interesting alternative hypothesis, that streptomycin acts by inactivating, or even destroying, ribosomes and so producing a disorder of protein synthesis within the cell, has been proposed by Spotts and Stanier (302).

C. p-Aminosalicylic acid

The discovery of *para*-aminosalicylate (Aminacyl, Paramisan, PAS, Pasade) was made as a direct result of fundamental work on the metabolism of the tubercle bacillus. Bernheim (41) found that benzoic acid and salicylic acid increased the oxygen uptake of this organism in a more or less specific manner, thus suggesting that they, or similar substances, might be important metabolites of the tubercle bacillus. This led Lehmann to study the antituberculosis action of a large series of structurally related compounds in the hope of finding some which would inhibit the bacterial metabolism by a mechanism of substrate competition. He found that of all the compounds studied, PAS was the most active *in vitro* against a bovine strain of tubercle bacilli (187). These results have been substantially confirmed. Early work on the antibacterial activity of PAS was reviewed by Robson and Keele (271). Some aspects of the pharmacology of PAS, including toxicity studies, were described by Bavin (30) and Bavin and James (31), while

Bavin *et al.* (33) gave the tuberculostatic activity, both *in vitro* and *in vivo*, of a number of related compounds.

Absorption and fate. *p*-Aminosalicylic acid is well and rapidly absorbed from the intestine. Its metabolism in various species was studied by Way *et al.* (338) and by Venkataraman *et al.* (331). After an oral dose of 4 g in man, a maximum plasma level of the order of 7.5 mg per 100 ml is reached in 1 to 2 hours, and this falls to less than 1 mg within 6 hours. Similar results have been obtained in children (296). The drug is excreted in the urine, both unchanged and as various degradation products, *i.e.*, unchanged PAS (14 to 33 % of the total dose administered); acetyl *p*-aminosalicylic acid (28 to 63 %); *p*-aminosalicyluric acid (0 to 26 %) and various other unidentified amines (337). There is evidence that in man a high proportion of PAS is excreted by the tubules and that this can be blocked by probenecid, but this does not apply to the dog (63). In the guinea pig, probenecid increases the concentration of PAS in the serum and also increases its therapeutic effectiveness in experimental tuberculosis (69). According to Hollander (154) a combination of PAS with a polyamine resin produces a PAS concentration in the serum equivalent to that produced by its PAS content; it is well tolerated in man and therapeutically effective.

Alin and Helander (6) investigated the distribution of PAS in the tissues, taking advantage of the fact that it gives a strong green fluorescence in ultraviolet light. They showed that the substance is accumulated in elastic fibres. In and around fresh lung tubercles in mice there was less PAS than in the rest of the pulmonary tissue; they attributed this to the destruction of the elastic connective tissue. Using C^{14}-labelled PAS, Heller *et al.* (145) found that the drug penetrates readily into caseous tissue in the guinea pig, reaching concentrations comparable to those in the liver and lung, though the caseous material has no affinity for PAS as it has for isoniazid.

Relation to isoniazid blood levels. One of the major pathways of isoniazid metabolism is by acetylation, and it has been shown that this is competitively inhibited by PAS *in vitro* (164). It would thus be expected that higher blood levels of isoniazid would result from the administration of the drug together with PAS. This has been shown to be so in man by Mandel *et al.* (209) and others (36, 228), who concluded that the therapeutic superiority of treatment with isoniazid plus PAS over isoniazid alone may be attributable not only to the antimicrobial activity of PAS itself, but also to the higher concentration of isoniazid acting on the tubercle bacilli. These results have, however, been seriously criticised by Peters (241, 242).

Related compounds. Derivatives of PAS have been studied, particularly in the hope of obtaining a compound which would be better tolerated in the gastrointestinal tract, thus allowing therapeutic doses to be taken reliably. Of these the calcium salt of *benzamidosalicylic acid* (calcium B-PAS, Therapas) is probably the most interesting, though its introduction has led to a good deal of controversy. It is an insoluble salt, almost tasteless, and there is no question that it is better tolerated than PAS in man; but the controversy has been about its therapeutic effectiveness. It has been assumed that its activity is due to the

liberation of PAS in the body. However, since the blood levels so produced are low, as compared with those achieved by the administration of PAS, there has been scepticism about the value of B-PAS as an alternative to PAS in combination with other drugs.

The pharmacological properties of B-PAS have been described by Bavin and James (32) who found, in studies on mice, that the compound has a tuberculostatic activity about half that of PAS. They found that following administration of B-PAS, the blood level of PAS was much lower but much more sustained than after the administration of an equivalent dose of PAS. Appreciable amounts of B-PAS itself were recovered from the blood and from other tissues (liver, lung, spleen, and kidney) after the administration of the drug. It is decomposed to PAS by homogenates of various tissues. This action is probably enzymatic and accounts for the PAS present in the body and excreted in the urine. Similar low blood levels were obtained by Citron and Kuper (72) who reviewed the literature on the subject. An interesting finding is that of Drain *et al.* (99) who measured the blood levels of PAS chemically and microbiologically (on *M. tuberculosis* H37Rv) in subjects taking B-PAS and found that these were similar; by contrast, sodium PAS produced microbiological levels much lower than those determined chemically. Moreover, after 15 days of administration of B-PAS, the blood PAS levels (measured both chemically and biologically) had risen appreciably and were not much different from the microbiological level in subjects taking PAS for the same period.

In view of the fact that appreciable amounts of B-PAS are present in the blood following the administration of the drug, the results of Zeyer *et al.* (360) become significant. Using two forms of C^{14}-labelled B-PAS, they found that *in vitro* the drug is concentrated in tubercle bacilli; 90% of the radioactivity present in the bacilli is in the form of B-PAS and none as free PAS. The authors believed that the observed concentration of the B-PAS by the bacilli may be relevant to the action of the drug in tuberculous infections.

Clinically, the value of B-PAS could be best tested by its efficacy in preventing the development of resistance, when given together with isoniazid or streptomycin. The evidence, reviewed by Citron and Kuper (72), is unfortunately scanty and not convincing.

Phenyl PAS (Tebanyl, Tebamin, phenyl-4-amino-salicylate) is well tolerated by patients and has therefore been investigated as an alternative to PAS. The few data were reviewed by Citron and Kuper (72). The drug gives low levels of serum PAS which are, however, maintained for a prolonged period. There is also some evidence that with prolonged administration the blood level rises progressively. Clinical data are scanty and do not allow any conclusion as to the value of the drug.

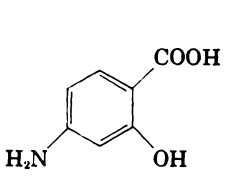
The thiosemicarbazone of 4-(4′-aldehydo)phenylthiocarbamidosalicylic acid has been shown to be more active than PAS *in vivo*, though less active than streptomycin. The toxicity of the new compound is lower than that of the thiosemicarbazones (Section VII, G) (139). 5-Bromo-salicylhydroxamic acid (BSH) is another substance which although not a derivative of PAS is related

to it (330). This substance has only low antituberculosis activity by itself *in vitro* (217) and has not been investigated extensively. However, Hornung *et al.* (155) recently reported that 3 g daily of BSH is as effective as 10 g daily of PAS in delaying the emergence of resistance to isoniazid, and Johnson *et al.* (163) have shown that it is four times as effective as PAS in increasing the blood level of isoniazid in patients.

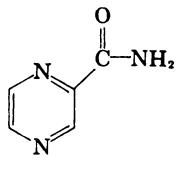
Antithyroid action of PAS. It is now well known that occasionally prolonged administration of PAS produces a goitre with or without myxoedema. Thus, MacGregor and Somner (201) found that of 83 tuberculous patients treated for five months or more, 20 developed a goitre, often with hypothyroidism. Studies with radioactive iodine showed changes in thyroid function in all patients tested while receiving PAS. Many patients showed a hypothyroid pattern of radio-iodine excretion and an avidity of the thyroid gland for radio-iodine on withdrawal of PAS. There was also some suggestion that irreversible changes may be induced by the prolonged use of PAS; the histological appearance of the thyroid gland of a patient who had received more than 10 kg of PAS was that of extreme degeneration and disorganisation. It is of interest that Beattie and Chambers (34) found that after prolonged administration of PAS to rats there were signs of exhaustion of the thyroid gland, with complete degeneration of the follicular epithelium in some animals.

The mechanism of action of PAS is to inhibit organic binding of iodine in the synthesis of thyroid hormone, and the drug has no effect on the iodine-concentrating mechanism of the thyroid gland (103). It has been suggested that thyroxine should be given to all patients in whom a goitre is noted, or to whom PAS is to be given for more than six months.

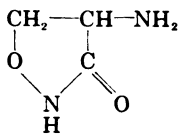
Effect on prothrombin time. It is well known that salicylates cause a lengthening of the prothrombin time and this has also been found with PAS. This effect is partly reversed by streptomycin (315). The action of PAS is more marked in patients who are in poor condition and especially in cases of liver damage, and it is suggested that vitamin K should then be administered.



p-Aminosalicylic Acid (PAS)



Pyrazinamide



Cycloserine

D. Pyrazinamide

Following the finding that nicotinamide had antituberculosis activity in mice and guinea pigs, pyrazinamide (Aldinamide, pyrazine-2-carboxamide) was synthesized and shown to be more active, by Kushner and by Solotorovsky and their associates in 1952 (see Robinson *et al.*, 268). Its activity is greater than that

of PAS, cycloserine, or viomycin, but less than that of isoniazid or streptomycin. It is the pyrazine analogue of nicotinamide. Its comparatively high activity is unfortunately balanced by a liability to produce toxic effects on the liver. The drug has no significant activity *in vitro* against *M. tuberculosis* at a pH around neutrality, and blood withdrawn from human subjects after administration of pyrazinamide also exerted no antituberculosis activity *in vitro* (215). Moreover, the tissues of mice to which the drug had been administered had no effect on tubercle bacilli *in vitro*. Nevertheless, at an acid pH (5.0 to 5.5) pyrazinamide, as well as nicotinamide, is highly active *in vitro* against human tubercle bacilli. This was discussed by McDermott and Tompsett (216) with reference to the possibility that tuberculous lesions and the interior of macrophages may have an acid pH, though this cannot be the full explanation for the discrepancy of the activities of pyrazinamide *in vitro* and *in vivo*. The activity *in vivo* has been investigated with human strains of bacilli, and according to McCune *et al.* (214) it is not effective against bovine strains. Mackaness (203) has shown that the growth of tubercle bacilli of human origin, cultured intracellularly in monocytes *in vitro*, is completely inhibited by pyrazinamide concentrations of 12.5 μg/ml, whereas that of a bovine strain, under similar circumstances, was not inhibited by much higher concentrations of the drug. Basilico (27) found this drug to be more effective against intracellular organisms than extracellular ones.

The drug is effective when given orally, and its activity in experimental tuberculosis in mice and guinea pigs has been demonstrated by Malone *et al.* (208) and by Dessau *et al.* (95). Grumbach (129) showed that when mice were treated with pyrazinamide alone the disease was at first controlled, but very resistant bacilli then appeared and the course of the disease started again; this did not occur when pyrazinamide was used in conjunction with isoniazid, in which case the lesions became sterile at the end of four months. This was in keeping with the observations of McCune and Tompsett (213). Further studies, however, showed that the combination of pyrazinamide with isoniazid (and other drugs) did not consistently sterilise the tissues but produced a latent infection from which bacilli could subsequently still be recovered. Incidentally, the capacity of tubercle bacilli to remain latent for long periods in tissues, with retention of the capacity to start multiplying again at some future period represents an interesting problem for which no explanation has yet been obtained. It is discussed by Canetti (66).

Fate in the body. There is little information about this aspect. Caccia (65) has described a colorimetric method for the determination of pyrazinamide in blood and urine. He has found in experiments in man that following oral administration, the maximum blood concentration is reached in about two hours and that much is excreted in the urine. It is possible that study of the metabolism of this drug might give some indication as to why some subjects are prone to its selective toxic action on the liver.

Toxicity. This has been studied in animals by Robinson *et al.* (268). The oral LD50 in mice is approximately 3.5 g/kg, and the value is somewhat higher in rats. The drug is well tolerated in large doses over long periods in rats, but in

dogs hepatotoxic effects may appear. The hepatic toxicity in man has been reviewed by United States Public Health Service Tuberculosis Therapy Trial (329). Depending on the dose and duration of treatment, 2 to 6% of patients show evidence of hepatic damage; in some series the incidence is as high as 20% (227). The results of liver function tests suggest that the drug is not hepatotoxic for most patients, but that its action is highly selective. It has been suggested that routine serum glutamic-oxalacetic or glutamic-pyruvic transaminase test should be performed every two weeks to give warning of liver damage (227). Other toxic effects, which seldom require discontinuance of therapy, include gastrointestinal disturbances, malaise, joint pains, nervousness, dysuria, mild febrile reactions, and retention of uric acid which may lead to an attack of acute gout. Pyrazinamide causes a rise in serum uric acid and should thus be used with care in patients with a history of gout (291).

Clinical use. There have now been several investigations on the value of pyrazinamide, mostly given in combination with other drugs, usually isoniazid, and there is no doubt that pyrazinamide can produce a beneficial effect in tuberculosis in man. However, bacterial resistance tends to develop rapidly when the drug is given by itself. McDermott and his co-workers in 1954 concluded that the high incidence of hepatitis makes the use of a dose of 50 mg pyrazinamide/kg with 5 mg isoniazid/kg inadvisable in man. Since then other workers have thought that the drug could be used if given under strict supervision, preferably in a hospital, and with the understanding that it involves a calculated risk. This would be reasonable in selected cases for short periods in preparation for operation, or where the main drugs are for some reason not satisfactory. There is no question that pyrazinamide should not be used as part of the treatment of primary tuberculosis. Some of the clinical results have been discussed as part of the toxicity report (329), and the clinical indications are well set out in New and Non-official Drugs (231).

E. Cycloserine

Cycloserine (Oxamycin, Seromycin, D-4-aminoiso-oxazolidin-3-one) is a broad spectrum antibiotic produced by *Streptomyces orchidaceus* which was first isolated by Harned *et al.* (138). It is a drug which on the whole produces better results clinically than could be anticipated from the experimental results. The substance is quite soluble in aqueous media, but is very unstable in neutral or acid solutions, though stable in alkali. It has been synthesized by Stammer *et al.* (304) of the Merck Laboratories. Cycloserine has low activity against a wide variety of gram-positive and gram-negative organisms and inhibits the growth of *M. tuberculosis in vitro* in concentrations of 5 to 10 μg/ml, including strains resistant to isoniazid, pyrazinamide, PAS, streptomycin, and viomycin (17). It is, however, usually inactive against experimental tuberculosis in mice, though effective in infections in mice with a number of other organisms (82, 83, 339). Cycloserine has comparatively little effect in experimental tuberculosis in guinea pigs, even in doses up to 150 mg/kg (240), though there is evidence of healing, particularly at the highest dose. Wolinsky and Steenken (351), however,

found that 270 mg/kg was ineffective in guinea pig tuberculosis. In monkeys the drug has produced some beneficial effects (9, 286), and similarly some effect is seen in rabbits (311).

Fate in the body. This has been studied in a number of species including man. The drug is well absorbed when given orally, from both the stomach and intestine; the levels in the blood and excretion in the urine are approximately similar following either oral or parenteral administration in the dog and man. Excretion is by glomerular filtration and is not affected by probenecid. The drug diffuses to an appreciable extent into the cerebrospinal fluid and into the amniotic fluid and is probably distributed in the total body water. The metabolism and excretion of the drug in monkeys have been studied by Conzelman (77), following its administration both orally and by intramuscular injection. About 25% less of the drug was excreted after oral than after intramuscular administration possibly due to decomposition in the acid of the stomach; about 43% of the dose administered intramuscularly was excreted in the urine over a period of three days but 50% of this was excreted in the first twelve hours. The excretion in the urine in man (78) followed a very similar pattern; 64% was excreted within three days, of which one-half appeared within the first twelve hours. About 35% was metabolised to unknown substances. Excretion in common laboratory animals was much more rapid. The serum half-life values, *i.e.*, the time required for the serum level to reach half of that previously recorded, for the monkey was seven and three-quarter hours but for the mouse was only 23 minutes; for the guinea pig it was one hour, and the rabbit, two and one-half hours (79). This more rapid excretion probably accounts for the poor therapeutic activity of cycloserine in these animals. The blood level in monkeys during chronic toxicity studies was determined by Anderson *et al.* (9). The animals received 100 to 300 mg/kg orally and each dose produced a large rise in blood concentration, *e.g.*, from 20 to 100 μg/ml, which was maintained for more than seven hours. In man, the blood level was determined following the oral administration of 250 to 1000 mg, and again it was found that the concentration in the blood was maintained for over twelve hours. With repeated doses of 750 mg at six hourly intervals, the blood levels gradually rose to over 50 μg/ml. Up to 20% of the drug was excreted in the urine (339).

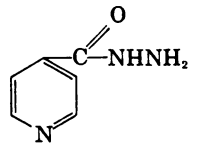
Toxicity. The acute toxicity of cycloserine is low, the subcutaneous LD50 in the mouse being 2.8 g/kg. Toxic doses in mice produce ataxia followed by prostration and death. Chronic studies in rats, cats, dogs and monkeys showed no toxic effects except for a temporary anaemia and reticulocytosis in dogs and monkeys (9).

Clinical use. Cycloserine has been used extensively in cases where other chemotherapeutic substances have failed, usually because of the development of bacterial resistance, and the drug has proved to be of value in such patients. Preliminary results were described as impressive by Epstein *et al.* (107). There have been several clinical trials since then, mostly of cycloserine given in combination with other drugs; in such cases, it is difficult to attribute the success obtained to any particular drug used in the combination (see, for example, 135).

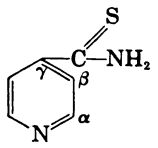
The general impression is that the drug has been more effective in man than in mice or guinea pigs. Toxic effects are by no means uncommon and include convulsions and other effects on the central nervous system. These were discussed by Storey and McLean (310).

F. Thioisonicotinamides: Ethionamide

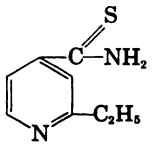
Soon after the discovery of isoniazid in 1952, three groups of workers discovered the antituberculosis activity of thioisonicotinamide (123, 131, 219). Like isoniazid, this is a derivative of isonicotinic acid, but it is not a derivative of isoniazid. The similarity in structure can be seen in the figure below:



Isonicotinic acid hydrazide (Isoniazid)



Thioisonicotinamide



α-Ethyl thioisonicotinamide (Ethionamide)

In vitro, thioisonicotinamide was inhibitory to the growth of tubercle bacilli only at a concentration of 20 μg/ml, or greater, a concentration about four hundred times greater than that necessary with isoniazid. Tests in mice *in vivo*, however, showed the drug to be more active, with a potency one-fortieth of that of isoniazid and twice that of streptomycin (123, 261). Clinical trial of thioisonicotinamide, however, was disappointing in that the maximum tolerated dose of 1 g per day was ineffective (332).

From the discrepancy between the results *in vitro* and *in vivo*, it seemed likely that some more active metabolite of thioisonicotinamide was in fact responsible for its activity *in vivo*. Meltzer *et al.* (219) studied derivatives of thionicotinamide prepared by oxidation and methylation of the amido nitrogen but did not find compounds of any greater activity. Libermann, Rist and colleagues prepared derivatives substituted on the nucleus in the *alpha* position. Starting with the α-methyl thioamide, which was twice as active *in vivo*, they prepared a higher homologous series many of which were considerably more active (131, 190, 191).

The compound chosen by them for detailed study and clinical trial was the α-ethyl thioamide (261, 262, 263, 264, 266), later called ethionamide (Trescatyl, 1314Th, Trecator, α-ethyl thioisonicotinamide).

Absorption, distribution and excretion. The drug is well absorbed following oral, rectal or subcutaneous administration. Practically nothing is known of its fate in the body, though Rist (261) found less than 1 % of the drug in an active form in the urine, suggesting that most of it is metabolised. This is supported by the observation that the serum levels as measured microbiologically are usually higher and reach a peak later than when measured polarographically, suggesting also that the metabolite is more active than the parent drug.

In man, following the oral administration of 1 g, a peak serum concentration of about 20 μg/ml is reached after three hours, falling to 3 μg/ml in nine hours and to 0.6 μg/ml (*i.e.*, the minimal inhibitory concentration) after twenty hours (261). Slightly higher and more prolonged blood levels have been recorded following rectal administration of the same dose (29), although absorption tended to be more irregular by this route. Owing to gastric intolerance, which is a common side-effect, the drug has been administered to man as enteric coated tablets, and the blood levels reached following use of these tablets have been reported to be about one-third lower than those following uncoated tablets (55). Occasionally there was a failure to absorb the drug when it was given as enteric coated tablets. Significant levels in the cerebrospinal fluid have also been reported following oral administration in normal subjects and in tuberculous patients (160, 264).

Toxicity. The acute LD50 of ethionamide given orally to mice is 1000 mg/kg (261), and doses of 250 mg/kg daily for 40 days were tolerated without changes in growth or histological appearance. No effects were seen following administration of 40 mg/kg per day to guinea pigs for 36 days. Following the oral administration of a lethal dose to mice, the animals die after 24 to 48 hours while in a deep coma. This is in marked contrast to the toxic effect of isoniazid, where administration of a lethal dose (200 mg/kg) is followed by convulsions and death in about one hour.

Numerous reports of the toxicity in man have been published (55, 57, 58, 300). The commonest effects observed are on the gastrointestinal tract, *i.e.*, anorexia, nausea, and vomiting. With a daily dose of 0.5 g per day these symptoms are only occasionally seen (*i.e.*, in about 10 % of patients) but with 1 g per day effects have been observed in about half of the patients, and these are often severe enough to cause withdrawal of treatment. There may be a racial difference in the susceptibility to these toxic effects since they were not observed in two small trials on Africans (162, 185). Effects resembling ganglionic blockade, with severe postural hypotension, have been reported. Drowsiness has also been described (134), and a case of peripheral neuropathy, presumably due to ethionamide, has also been reported (244).

Antibacterial action. In vitro, ethionamide will inhibit the multiplication of human strains of tubercle bacilli and photochromogenic bacteria in a concentration of 0.6 to 1.2 μg/ml. Bovine strains of tubercle bacilli, including BCG, are more resistant to the drug and are inhibited only by a concentration of 5.0 μg/ml (262). The scotochromogenic mycobacteria are inhibited by 20 to 40 μg/ml. The drug is equally effective *in vitro* against bacilli whether these are extracellular or intracellular in monocytes (28).

Like isoniazid but unlike thiocarbanilide and thiacetazone, which also contain a $-CSNH_2$ group, ethionamide causes rapid loss of acid-fastness if added to a culture of mycobacteria. It is as effective as PAS in inhibiting the acetylation of isoniazid by liver tissue *in vitro* (163).

In both the mouse and guinea pig, ethionamide is active orally and has about one-tenth the activity of isoniazid, being effective in a dose of 12 mg/kg. In the

mouse it is about eight times more active than streptomycin, and about twice as active as streptomycin in the guinea pig. When treatment is delayed until two weeks after infection, then a rapid bactericidal effect, similar to that of isoniazid, is seen (267).

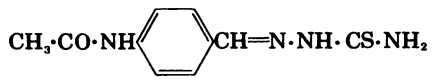
Development of resistance. Bacterial resistance to ethionamide develops rapidly *in vitro* and *in vivo* if the drug is used alone. In mice ethionamide, streptomycin, or isoniazid if used alone will produce a well-established resistance after four months' treatment. Combination of ethionamide with either streptomycin or isoniazid can completely prevent this but combination with either thiocarbanilide or thiacetazone fails to do so (267). Bacilli resistant to isoniazid, streptomycin, PAS, viomycin, cycloserine, or thiacetazone are all sensitive to ethionamide, but bacilli resistant to ethionamide may be resistant to thiacetazone and thiocarbanilide (266).

Clinical use. In man, good clinical results have been reported in cases of pulmonary tuberculosis with bacteria resistant to other drugs (55, 57, 58). The drug has also been used in tuberculous meningitis due to bacilli resistant to isoniazid and streptomycin (119), and it has also been used locally by direct application to tuberculous wounds, fistulae, and cavities, with good results (165).

G. Thiosemicarbazones

The thiosemicarbazones were discovered by Domagk and his co-workers some 15 years ago (96), and were extensively tested clinically in Germany. Although these drugs were obviously quite active in the treatment of tuberculosis, they were also frequently found to be toxic. The German results were reviewed by Hinshaw and McDermott (148) who stated that thiacetazone (Conteben, TB1, Berculon A, Amithiozone, Thiosemicarbazone, *p*-acetamidobenzaldehyde-thiosemicarbazone) had a "potential toxicity somewhat like that of the arsenicals used in the treatment of syphilis." Nevertheless, they recommended experimental clinical work with this drug in the United States. The introduction of isoniazid soon afterwards discouraged temporarily further work on the thiosemicarbazones. More recently, the need for drugs other than PAS for use in combination with isoniazid has led to a re-evaluation of thiacetazone. In a preliminary trial in East Africa in patients with acute extensive pulmonary tuberculosis (101), it was found that thiacetazone (150 mg per day, *i.e.*, a lower dose than was previously used) was about as effective as sodium PAS (10 g per day) as a companion drug for isoniazid (200 mg per day) in producing sputum conversion and preventing emergence of isoniazid-resistant strains. It is also noteworthy that in this trial the frequency of toxic effects was about the same with the two combinations (*i.e.*, isoniazid plus PAS, and isoniazid plus thiacetazone). The advantage of thiacetazone, particularly in underdeveloped countries, is that it is cheap and that the small quantity needed can easily be mixed with isoniazid in a single oral preparation. It is interesting in this connection that some years ago Domagk (personal communication) stated that a mixture of nine parts of isoniazid and one part of thiacetazone was highly effective experi-

mentally, and that such a mixture was being used clinically in doses of 10 mg/kg with apparently good results and without development of resistance to isoniazid. The thiosemicarbazones thus require further consideration as potential drugs in the treatment of tuberculosis (and leprosy).



Thiacetazone

Experimental results. Two main compounds have been investigated, thiacetazone and the derivative in which SO_2—C_2H_5— replaces CH_3—CO—NH— (TB3; Berculon B), also originally described by Domagk and subsequently subjected to further experiments by Hoggarth and Martin (153) and by Spinks (301). Methods for estimating these compounds in the body were described by the latter author and by Behnisch *et al.* (35). These compounds are well absorbed from the intestine and large proportions are excreted in the urine. According to Spinks (301) the ethyl sulphonyl derivative attains a higher concentration in the blood and is more slowly eliminated from the body than thiacetazone.

Chemotherapeutic data on these compounds were discussed by Domagk in his original publications, as well as by Karlson *et al.* (173), Francis *et al.* (121), Rees and Robson (256), and Barry *et al.* (24). Their potency lies between that of PAS and that of streptomycin. Cymerman-Craig *et al.* (84) have described diphenyl derivatives which, though highly active *in vitro*, are unfortunately inactive *in vivo*. Other derivatives active *in vitro* were described by Tisler (322).

The toxicity of thiosemicarbazones has been investigated in dogs and monkeys by Francis *et al.* (121). The crucial question at present, however, is whether these drugs can be used in man in doses which will be effective and not unduly toxic.

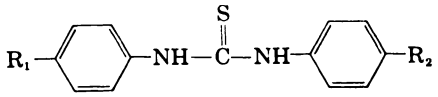
It is of interest that among this group of compounds are substances active against vaccinia virus, both in embryonated eggs and in mice (136). This presumably implies some effect on the synthesis of nucleoprotein, and in keeping with this is the finding of some effect on experimental leukaemia (56). It would appear that this group of compounds deserves wider investigation.

H. Derivatives of thiourea

Mayer and his co-workers started from the hypothesis that antifungal agents might be effective against mycobacteria because of morphological similarities between these organisms. They found that a number of recognised antifungal substances exerted a specific antimycobacterial activity; amongst these the most interesting were substituted thioureas. This observation led to considerable work on these compounds (212), and although no important chemotherapeutic substances have been discovered, the data deserve discussion. One of the striking findings is poor correlation between the activities of these compounds *in vitro* and *in vivo*, thus emphasizing the importance of the latter type of test in the

search for new substances. It is also obvious that the studies on these compounds *in vitro*, *e.g.*, those of Welsch *et al.* (341), are of very limited predictive value.

Mayer and his co-workers examined many diphenyl thiourea derivatives (thiocarbanilides) of the general structure



Thiocarbanilides

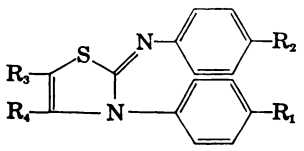
Several of these were found to be quite effective in experimental infections in mice and in guinea pigs (180). The following are among the most active compounds:

	R_1	R_2
Su 1795	C_2H_5O	$OCH_2CH(CH_3)_2$
Su 1906	$CH_3(CH_2)_3O$	$N(CH_3)_2$
Su 2079	$CH_3(CH_2)_3O$	$OCH_2CH_2N(C_2H_5)_2$
Su 2358	$(CH_3)_2CHCH_2O$	$OCH_2CH_2N(C_2H_5)_2$

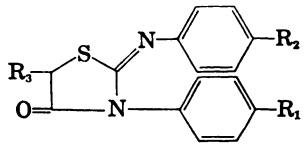
To this list may be added thiocarbanidin (THC, Thioban, 4-(α-pyridyl)-4′-isobutoxy-thiocarbanilide), described by Youmans *et al.* (358), who also investigated a series of substituted thioureas. In mouse tests, the compounds were much less active than isoniazid, with an activity of the same order as that of PAS. Youmans indeed emphasized that increasing the dose produces little increase in effect. In the guinea pig, however, compounds such as Su 1795 and Su 1906, given at optimal doses, were capable of eliminating all evidence of active tuberculosis, even by microscopic examination (180).

Another point of interest is that these substances did not show cross-resistance with isoniazid, with PAS, or with streptomycin, but did show such cross-resistance with a thiosemicarbazone (thiacetazone), suggesting a common mode of action. Resistance to the thioureas develops only slowly *in vitro* (181).

Studies have also been made on a number of related compounds synthesised from thiocarbanilides, namely thiazolines and thiazolidones (105).



Thiazolines



Thiazolidones

Some of these compounds were found to be highly active *in vivo*, the most interesting being a thiazoline, Su 3068 (3-(*p*-isoamyloxyphenyl)-2-(*p*-isoamyloxyphenylimino)-4-methyl-4-thiazoline HCl), which increased the survival time of mice even when given in quite low doses. It also exerted a marked suppressive effect on the development of tuberculosis in guinea pigs.

At present very little is known about the fate in the body of these substances or about their mechanism of action. Kradolfer and Schmidt (182), who reviewed some previous work, have shown that after an S^{35}-labelled diphenylthiourea (Thiambutosine, DPT, Ciba 1906, Su 1906) was given subcutaneously in oily suspension to mice, it was slowly eliminated from the body, and was stored to some extent in the lungs and liver. Youmans *et al.* (358) administered several active thiocarbanilides to monkeys and, using microbiological determination, found that significant blood concentrations were attained only with thiocarbanidin.

The clinical investigations with these compounds have so far proved disappointing. It was clearly shown in the East African study (101) that a combination of Su 1906 (2 g/day) with isoniazid did not produce sputum conversion, or prevent isoniazid resistance. Similar results with thiocarbanidin (2 g/day) given in combination either with isoniazid or with streptomycin, have been reported by Donohoe *et al.* (97).

I. Viomycin

Viomycin (Vinactane, Viocin) is an antibiotic produced by a species of *Streptomyces* which was discovered and developed independently in the laboratories of Pfizer and of Parke, Davis (26, 114); the latter investigators acknowledge that the cultures were isolated and screened for antimicrobial activity in the laboratory of Burkholder at Yale. It is a strongly basic polypeptide; the sulphate and hydrochloride are very soluble in water but virtually insoluble in organic solvents (142). *In vitro* it is active against a variety of strains of *M. tuberculosis*, though 2 to 4 times less active than streptomycin. It is fully effective against streptomycin-resistant organisms, both *in vitro* and in murine and guinea pig tuberculosis (151, 172, 306, 357). Viomycin was found to be active against tubercle bacilli resistant to PAS and to neomycin. Rapid development of resistance to viomycin *in vitro* was demonstrated by Steenken and Wolinsky (306).

The studies of P'an *et al.* (237) showed viomycin to have low toxicity in mice; the LD50 of the sulphate given subcutaneously was 1.4 g/kg. No ill effects were observed in rats given 100 mg/kg for six weeks, or in dogs given the same dose for five months. In cats, however, disturbances of posture and gait were seen quite early (five weeks) at half this dose. Moyer and Handley (229) confirmed the absence of nephrotoxicity in dogs.

The absorption and excretion of viomycin in man have been studied by Werner *et al.* (342) and found to be similar to those of streptomycin. When the drug is given intramuscularly (25 to 50 mg/kg), the maximum serum concentration is reached in about two hours, and a high proportion of the dose given is recovered in the urine; the penetration into the cerebrospinal fluid is poor. The clinical use of viomycin has been reviewed (10). It can produce a variety of toxic effects, including albuminuria, damage to the eighth nerve, urticaria, eosinophilia, and disturbance of electrolyte balance due apparently to abnormal loss of calcium and potassium in the urine. Hence the drug should be used only in the absence of any reasonable alternative, when the patient's organisms have become re-

sistant to the major antituberculosis drugs. Intermittent administration, *e.g.*, two doses per week, has appreciably reduced the risk of toxic effects.

J. Streptovaricin

Streptovaricin (Dalacin) is an antibiotic produced by *Streptomyces spectabilis* which was discovered in the Upjohn Laboratories. It is a complex consisting of at least five microbiologically active substances which can be differentiated by chromatography. The antibiotic is active against a variety of gram-positive and gram-negative organisms and against *Mycobacterium tuberculosis* in concentrations of the order of 0.1 to 0.3 μg/ml. Streptovaricin does not show cross resistance with streptomycin or with any other antimicrobial drug in common use. Resistance to the drug develops *in vitro* (294). The drug is effective in experimental mouse tuberculosis when given either orally or subcutaneously in doses of the order of 50 to 100 mg/kg. Of the various fractions isolated, the one labelled "C" appears to be the most active in the mouse test. Strains of *M. tuberculosis* made resistant to the drug *in vitro* have a reduced virulence in mice (258).

The clinical results obtained with streptovaricin have not been encouraging and toxic effects have occurred in some of the trials (94, 230, 260, 327). It is possible that purer preparations may give better results.

K. Ethyl mercaptans

The antituberculosis activity of mercaptan compounds was first studied by Anderson and Chin (8), who showed that 2,3-dimercaptopropanol (BAL) in a concentration of 100 μg/ml inhibited the growth of tubercle bacilli *in vitro*. Del Pianto (91) investigated the antituberculosis activity of mercaptan compounds and found that a mixture of sodium 2-mercaptobenzothiazole-5-sulphonate and sodium ethylthiosulphate was highly effective both in experimental animals and in man, though either drug alone was relatively ineffective. Later work (92) showed that sodium ethylthiosulphate was more active than PAS in experimental infections in mice when incorporated in the diet, but not when given either subcutaneously or intraperitoneally; it was inactive against *M. tuberculosis in vitro*.

Of over 350 compounds related to ethyl mercaptan studied by Brown *et al.* (60), over 50 were active if given in the diet at a concentration of 0.2% or less. Antituberculosis activity resided in the series of compounds based on the formula C_2H_5—S—R, and, in general, structural modifications which decreased the tendency for cleavage of the C_2H_5S—linkage decreased the antituberculosis activity. The most interesting compound with regard to efficacy and low toxicity was S-ethyl-L-cysteine; further study (298) showed this compound to be more active than either PAS or pyrazinamide, but less active than isoniazid or streptomycin. Again, the compound was more active in the diet than by injection, and was inactive *in vitro*. Other workers failed to confirm the activity of S-ethyl-L-cysteine. Solotorovsky *et al.* (297, 299) showed that the activity was in fact due to decomposition of the drug in the diet, with the release of ethyl mercaptan vapour, which produced a good antituberculosis effect if the animals were kept in closed containers as was the practice at the Merck laboratories.

Kushner *et al.* (184) reported on a number of mercaptans and esters, many of them ethylthiol esters with activity comparable to that of streptomycin. They believed that the activity of the compounds was due to the release of ethyl mercaptan. One compound studied in more detail was ethyl thiol-*p*-acetamidobenzoate, an odourless solid which is more acceptable than ethyl mercaptan. However, toxicity studies in mice revealed enlarged and cyanotic spleens. Since they found that ethyl mercaptan also caused this condition, none of the compounds was tried clinically.

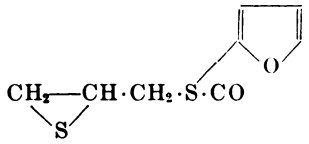
Davies *et al.* (90) tested a number of compounds similar to those described by Del Pianto (91) and found that of a series of sodium thiosulphate derivatives tested, the only active compound was sodium S-ethyl thiosulphate. This decomposed on storage to give diethyl disulphide, which was also very active, as was a series of other disulphides; but activity was found only if one of the S-substituted radicles was an ethyl group. Since all the active compounds could be decomposed *in vivo* to give ethyl mercaptan, this substance was also tested for antituberculosis activity, and was found to be active against intracellular, but not extracellular, bacilli (87); among simple mercaptans, activity was restricted to the ethyl homologue.

A large number of derivatives of ethyl mercaptan was studied, and the most active members *in vivo* were a series of ethylthiol esters which could release ethyl mercaptan after absorption. These drugs were invariably more active following parenteral administration than by the oral route, and effective concentrations could be achieved by percutaneous absorption. The compound most suitable for human use was ditophal (Etisul, ETIP, diethyl-dithio-isophthalate), since this is virtually odourless (86). It had definite activity in mouse tuberculosis if given in high doses, but the activity fell off very rapidly at lower doses; in guinea pigs, which do not tolerate the drug well, little antituberculosis effect was obtained (88). One of the most serious disadvantages of the compound, however, was the very rapid and permanent development of resistant organisms. It is normally very difficult to produce resistance to a drug in mice, but only ten days treatment with ditophal caused development of complete resistance (86). The antituberculosis effect of the ethyl thiol compounds could be antagonised *in vivo* by the methyl analogues, suggesting that ethyl mercaptan interferes with an essential methyl- or methylthiol-containing system in the bacteria.

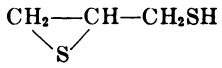
There have been no reports on the absorption, *etc.*, of ditophal, but the metabolism of compounds related to ethyl mercaptan has been studied by Snow (295) and Lowe (198). With both S^{35}-labelled and C^{14}-labelled diethyl disulphide and ethyl thiolbenzoate, it was shown that the substances are fairly evenly distributed throughout the body, with no specific cumulation in any tissue. They are excreted mainly in the urine as sulphate and also as ethyl-methyl-sulphone and ethyl-methyl-sulphoxide, which have no antituberculosis activity *in vivo*. A substantial amount (3 to 7 %) is also excreted in the breath as ethyl mercaptan.

Not all thiols, however, act by conversion to ethyl mercaptan. A series of thiols, dithiolans, thiol esters, dimercaptopropyl esters, and episulphides studied by Acred and Brown (3) included some compounds with high antituberculosis

activity *in vitro* and *in vivo*. These workers were unable to detect any ethyl mercaptan in the expired air of the mice receiving any of the compounds, and they suggested that the active agent was not ethyl mercaptan but was 3-mercaptopropylene sulphide. The most active compound found by them was 3-(2-furoylthio-)propylene sulphide, which was as active in mice as streptomycin if treatment was started at the time of infection. It was, however, completely inactive against an established infection (4).



(3-(2 Furoylthio)propylene sulphide)



3-Mercaptopropylene sulphide

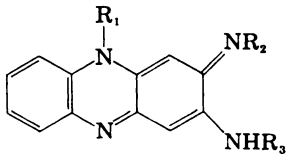
Bacterial resistance developed rapidly to these compounds both *in vivo* and *in vitro*. In addition, they were inactive orally and produced ulcers on injection, and were odorous, oily, and unstable; hence, they were considered to be unsuitable for clinical use.

Clinical use. Of all these thiol compounds, the only one which has had any extensive clinical use has been diethyl dithiolisophthalate (ditophal, ETIP, Etisul), which has been tried with quite reasonable success in the treatment of leprosy. It is applied as a liquid which is rubbed into the skin to provide a systemic effect (61) but produces an unpleasant odour in the breath due to the formation of ethyl mercaptan. Resistance develops readily to the drug if used alone, but this is prevented if it is used in combination with other antileprosy drugs such as diaminodiphenyl sulphone or 4-butoxy-4′-dimethylamino-diphenylthiourea (Ciba 1906) (Section VII H) (85). Although the latter drug is a thiourea which contains the C—S linkage, it probably has a different mode of action from the thiols like ditophal (89). Ditophal has also been used in the treatment of cutaneous tuberculosis (lupus vulgaris) (73) with encouraging results.

Little information is available on the toxicity of ditophal, but in one series of 133 patients treated for periods up to three months, no toxic effects were observed (85); one case of contact sensitisation producing an eczema has been reported (73).

L. Phenazines

For many years, Barry and his co-workers have synthesised and studied the antituberculosis activity of a large number of compounds based on the phenazine nucleus.



Phenazines

When $R_1 = R_3 =$ phenyl and $R_2 = H$, the structure is anilinoaposafranine, which has antituberculosis activity both *in vitro* and *in vivo* when tested in mice and guinea pigs (18, 19, 76). Other compounds were studied with constituents at R_1 and R_2, and $R_3 = H$. Of 30 derivatives of these 2 types studied by Barry and co-workers (25), seven of eleven derivatives of the former had significant activity when measured by the increased survival time of mice infected intravenously with the bovine strain of the tubercle bacillus. The most active compound was the $R_1 = R_3 =$ *p*-chlorophenyl derivative; a dose of 100 mg/kg daily in the diet for 14 days, starting on the day of infection, increased the median survival time from about 15 days to 150 days. Of 19 derivatives with $R_3 = H$, 12 showed significant activity, and again the most active compound was the $R_1 = R_2 =$ *p*-chlorophenyl derivative. In general, the derivatives with $R_3 = H$ were slightly more active than derivatives with $R_2 = H$, but the difference was not usually striking; the most active of all the compounds studied was the *p*-chlorophenyl derivative of the latter series. The activity of this compound and its isomer with $R_3 = H$ was greater than that obtained by these authors with PAS, streptomycin, or thiosemicarbazones, and was similar to that obtained with isoniazid. These phenazine derivatives were also shown to be effective in tuberculosis in guinea pigs.

The absorption and fate of these compounds has not been investigated. They are coloured pigments and tend to accumulate in the tissues and persist there for some time after cessation of treatment (25). This is very similar to what is seen with the rimino compound B663, and since this is also a phenazine derivative, the pharmacology of these compounds may be similar to that of B663 described below.

Further synthesis led to the discovery of a new group of compounds, the rimino-compounds (20, 23) with substituents other than H on R_1, R_2, and R_3. Among 67 compounds of this type studied, 65 had antituberculosis activity in murine tuberculosis. About 20 of these showed high activity, a dose of 5 mg/kg per day in the diet producing an increase to at least double the median survival time of the control mice. Some were considerably more active than this; the most extensively studied compound, B663 ($R_1 = R_3 = C_6H_4Cl(p)$; $R_2 = -CH(CH_3)_2$) showed significant activity at a dose of 1 mg/kg per day. On a molar basis this is equivalent to about 0.3 mg of isoniazid/kg, at which level isoniazid is inactive. This high activity has also been confirmed by other authors (2, 149, 233, 234). In monkeys, however, Schmidt (287) reported that while a dose of 100 mg/kg daily was effective if started four days before infection, this dose was practically ineffective in treating an established infection; lower doses were without effect in either test. It is difficult to explain this species difference in activity, since although the plasma levels in monkeys is low (about 2 μg/ml following 100 mg/kg), the drug is concentrated in the tissues to a level as high as 5,000 μg/g. If the drug was given in oil to increase the plasma level, then severe convulsions were produced, which were fatal in a high percentage of cases.

In vitro, B663 has about the same activity, or may be even slightly less active

than isoniazid, and no cross resistance is seen with other antituberculosis agents. In fact "catalase-negative" isoniazid-resistant organisms are more susceptible to phenazines (20). Furthermore, seventeen subcultures of the strain of tubercle bacillus H37Rv in the phenazine B663 failed to produce resistance to the drug (23), though in monkeys resistance develops fairly rapidly and the resistant organisms are fully pathogenic (287). It is probable that the apparent high activity *in vivo* is due to the unusual distribution of B663. This drug accumulates in the tissues, and after a short period of treatment crystals of the unchanged drug can be seen in various organs of the animals (23) probably including the tuberculosis lesions (2). At low dose levels, probably some cumulation of the drug has to occur before an effective tissue concentration is produced, so that an erroneous impression of its activity is obtained. This is supported by the experiments of Noufflard and Berteaux (233) who showed that if treatment is delayed until an established infection is present, then B663 in low doses takes longer to act than isoniazid in the same dose (10 mg/kg), but that the effect of the B663 lasts longer.

Furthermore, most of these workers have shown (2, 20, 233) that treatment with B663 before infection, or one single dose during infection, will afford much more marked protection than isoniazid under the same conditions. However, when B663 is compared with isoniazid under conditions in which the latter is normally effective (2, 149) isoniazid is generally more effective than B663.

The relation of chemical structure to antituberculosis activity among the rimino compounds has been studied by Barry and Conalty (23), but no very general conclusions can be reached; those interested should consult the original papers. Other phenazines studied by Barry and co-workers (23) included eleven glyoxalinophenazines of which about half showed mild antituberculosis activity, and four aposafranones none of which showed antituberculosis activity. These latter two groups of compounds have not been studied further.

The absorption and distribution of two phenazine compounds have been studied in various animal species (22). These are B663 (R_1 = R_3 = *p*-chlorophenyl; R_2 = *iso*propyl) which is insoluble in water but freely soluble in fat, and B720 (R_1 = R_3 = phenyl; R_2 = diethyl-amino-ethyl) which is slightly soluble in water (50 μg/ml) and freely soluble in fat. These substances are well absorbed after oral administration to mice, rats, hamsters, guinea pigs, and rabbits. When administered to man, however, about 90% of the drug was recovered unchanged from the faeces.

The most striking feature of these compounds is the marked cumulation which occurs in all tissues of the body. This may be so marked that actual crystals of B663 may be seen in the tissues and globules of the drug appear in macrophages. The tissue concentration of the drug increases so rapidly that in the spleen, where it is most marked, the concentration after feeding the drug for 28 days is one thousand times that after feeding for only three days.

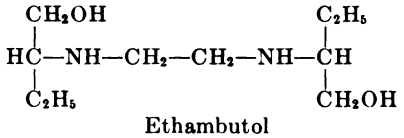
In man it has been found that a fine micronised preparation of B663 is better absorbed and that the absorption is aided by dissolving in olive oil (21). The same persistence may occur in man as in animals, since quite marked levels

have been found in the plasma 18 days after stopping the administration of B663 orally in olive oil. Also, four days after the last dose of B663 the level in body fat (biopsy of fat from the thigh) was 50 times greater than the level in the plasma. Clinical trials have been conducted using the micronised preparation of B663, which is well absorbed in man, as well as in the crystalline form. In 16 patients treated with doses of B663 up to 600 mg/day in the "micronised" form for about two months, no therapeutic effect was observed either radiologically or bacteriologically. The drug was well tolerated, though some restlessness and insomnia were seen. The patients also developed a red coloration of the skin after about one week's treatment; this was not considered a serious obstacle (124). Encouraging results have, however, been obtained using the drug in lepromatous leprosy (62).

Conclusions. Of the large number of phenazine compounds studied, a high percentage show antituberculosis activity. In some cases this is very striking, and good results have been obtained in experimental infections using doses of phenazines considerably below those which would be effective using any other therapeutic agents. These drugs show marked cumulative properties and persist in the body for several weeks, even following one dose. This could be an advantage clinically in the chemoprophylaxis and treatment of tuberculosis in backward countries where effective treatment could be obtained by administration once a week or even at longer intervals. However, the fact that these compounds have been available for the past four years or so and that as yet no reports of clinical trials have been published suggests that the compounds are not sufficiently active in man to warrant extensive trials.

M. Ethambutol

While screening randomly selected compounds at the Lederle research laboratories, Thomas *et al.* (316) found that N,N′-diisopropylethylene diamine had antituberculosis activity when tested in mice. After studying numerous related substances, the most promising one found by them was 2,2′-(ethylenediimino)-di-1-butanol dihydrochloride; the results of testing both *in vivo* and *in vitro* showed that the activity resided in the *dextro*-isomer (ethambutol), which was more than 200 times more active than the *levo*-isomer (345). It is a white, odourless, crystalline compound, heat stable and highly water soluble.



Ethambutol

The compound is well absorbed after oral administration to mice and guinea pigs, a peak serum concentration being reached after about one hour, after which it is excreted within about six hours (169).

Toxicity. The *dextro*-, *levo*- and *meso*-isomers have all the same remarkably low acute toxicity in mice, the LD50 of the racemate being greater than 12.8 g/kg orally, 1.6 g/kg subcutaneously, between 0.8 and 1.6 g/kg intraperitoneally,

and between 200 and 400 mg/kg intravenously. In a preliminary report in which no histological results were given, Schmidt and co-workers (290) found no evidence of toxicity in monkeys with doses of ethambutol of up to 200 mg/kg daily given by stomach tube for a period of 26 weeks. Higher doses of 1200 to 1600 mg/kg daily produced signs of CNS damage, namely, loss of the grasp reflex, incoordination of muscle movements, and laboured breathing. They found the racemic mixture to be about twice as toxic as the *d*-isomer (ethambutol), so that doses of 1200 mg/kg of the racemate were fatal, producing signs of CNS damage similar to those seen with ethambutol, and impairment of vision leading to complete blindness. Toxic effects with ethambutol were seen when the blood level exceeded 20 μg/ml, and these workers suggested that the maximum permissible blood level might be in the order of 10 μg/ml.

Activity. Ethambutol inhibits the growth of tubercle bacilli (H37Rv) in a concentration of 1 to 4 μg/ml *in vitro* and is effective against isoniazid- and streptomycin-resistant organisms. It seems to be specific for *Mycobacteria* since it is inactive both *in vivo* and *in vitro* against other bacteria, fungi, and viruses (170, 316). After oral administration to mice infected with various strains of *M. tuberculosis*, the efficacy (tolerancy/potency) ratio is similar to that of isoniazid and parenterally it is superior to that of streptomycin. For example, the dose required to protect 50% of mice (ED50) for 60 days after infection, in tests where all the controls died within 26 days, was 90 mg/kg orally with the racemic mixture which contains 50% ethambutol. Under the same conditions, the ED50 for isoniazid was 1.2 mg/kg but the maximum tolerated dose (MTD) of the racemate was 6.4 g/kg compared with 100 mg/kg for isoniazid, so that the ratio of MTD/ED50 was almost the same (70 for the racemate and 80 for isoniazid). In monkeys, definite therapeutic effects have been demonstrated against pulmonary tuberculosis (290) using single oral doses of 50 to 100 mg/kg daily. The effect of the ethambutol developed slowly during the first few weeks in a manner similar to that of streptomycin and some evidence was obtained that resistance to the drug could develop fairly quickly *in vivo* if it is given alone.

Preliminary tests *in vitro* suggest that resistance does not develop easily to ethambutol. Experiments using a delayed treatment type of test in guinea pigs (169) showed that a dose equivalent to 50 mg/kg subcutaneously was very effective and the same dose orally was somewhat less active. Furthermore, after two months' treatment there was no evidence of toxicity or of the development of resistance.

In preliminary clinical trials, patients with organisms resistant to other antituberculosis drugs have been treated for periods up to three months with a dosage of 25 mg/kg with encouraging clinical response and sputum conversion. A serious toxic effect, reversible loss of vision, has been reported during these trials, however; hence the future status of the drug is doubtful (195).

N. Rifomycin

This is a complex of substances first isolated from the fermentation broth of a new species of *Streptomyces, St. mediterranei* (292, 293) and two of these, Rifomycin B and Rifomycin SV have been studied fairly extensively; the latter

antibiotic has the empirical formula $C_{37}H_{49}NO_{12}$. These antibiotics are highly active *in vitro* against a number of gram-positive organisms, including *Staphylococcus aureus* and *Streptococcus pyogenes* and also against *M. tuberculosis* (317, 319).

The absorption and fate of Rifomycin SV has been studied by Maffii *et al.* (205, 206) and Fürész and Scotti (122). After oral administration, absorption is poor and irregular but following intramuscular injection, therapeutic blood levels persist for eight hours or more. Large amounts are eliminated in the bile and only small amounts in the urine; none was found in the cerebrospinal fluid. The acute toxicity is low, some preparations having an intravenous LD50 in mice of 2.0 g/kg, and limited studies showed little chronic toxicity, though there was marked local irritation at the site of injection. *In vitro*, Rifomycin SV is very active against *M. tuberculosis*, the minimal inhibitory concentration being as low as 0.005 μg/ml against some strains. Rifomycin is effective against organisms resistant to the other antituberculosis drugs.

The value of Rifomycin in experimental infections in mice (318) and guinea pigs (225) has been demonstrated, and encouraging results in experimental tuberculosis have been found. When tested by the intracorneal method, Rifomycin SV has about one-half the activity of isoniazid on a molar basis (275).

There are still insufficient clinical results available to assess its probable value in the chemotherapy of tuberculosis.

O. Macrocyclon

Attempts have been made to produce therapeutic effects in tuberculosis by the use of drugs which would increase the natural defenses of the body, rather than produce a specific action on the *Mycobacteria*. Indeed, much work has been done, by Westphal (343) particularly, to increase resistance to infection by the administration of lipopolysaccharide (or lipoid) obtained from gram-negative organisms, and it has even been claimed that these substances are of value in the treatment of tuberculosis (see 273 for review). At the present time this line of work is mainly of theoretical interest. A rather similar approach arose from the chance observation by Cornforth *et al.* (80) that the commercial surface-active agent, "Triton A20" (WR1339), produced a striking suppressive effect on the course of an acute tuberculous infection in mice. The commercial preparation is, however, rather toxic and produces lipaemia and liver damage. The results obtained with it led to the synthesis of a number of compounds which would have similar activity, but sufficiently low toxicity to be suitable for clinical use. The best of the compounds used was called Macrocyclon. It has an average of 12½ ethylene oxide units per phenolic nucleus, giving a molecular weight of about 4000. The drug and its congeners are extremely stable (253). Compounds with 25 to 30 ethylene oxide units per phenolic nucleus are inactive, and those with still more ethylene oxide units (45 to 75) tend to enhance a tuberculous infection ("protuberculous action"). A similar spectrum of activity, depending apparently on the physical properties of the compound, has also been found in another mycobacterial infection, rat leprosy.

Chemotherapeutic activity. Macrocyclon is inactive *in vitro* against *M. tuberculosis*, even at a concentration of 2%, and none of the "protuberculous" compounds enhances growth of the bacilli *in vitro*. No tuberculostatic substance has been detected in the blood and tissue fluids of animals given large doses of the drug. It therefore seems unlikely that the drug produces a toxic effect on the organisms, and it probably acts by enhancing the effectiveness of the natural defenses of the host or by making the organisms more susceptible to the natural defensive mechanisms. This is in agreement with the finding of Mackaness (202) that tubercle bacilli grow slowly or not at all in monocytes derived from animals treated with the surface active agent, although the bacilli grow freely in monocytes from untreated animals.

Macrocyclon is effective in acute tuberculous infections in mice and guinea pigs, and in experimental rat leprosy, the activity being of the order of that of streptomycin. The effect of the drug has been investigated in more detail by doing bacterial counts in infected mice. It has been found that Macrocyclon will decrease the multiplication of the bacilli in the lungs in an acute infection, but has no effect in a chronic infection in which the bacilli do not appear to be multiplying (141). In a parallel investigation, Niffeneger and Youmans (232) found that the effect of Macrocyclon is similar to that of previous immunisation with BCG, suggesting that the drug may act by enhancing the power of acquired immunity. In another experiment, however, Bloch and his co-workers (see 336) found that Macrocyclon had a marked therapeutic effect in mice injected intravenously with *M. tuberculosis* without any effect on the viable counts, as compared with untreated controls; they concluded that the therapeutic activity of the compound cannot be explained by the antibacterial action of the macrophages of the treated animals.

Fate in the body. Macrocyclon is inactive when given orally and has to be given by subcutaneous or intravenous injection. Studies with the compound labelled with C^{14} show that no radioactivity can be detected in the tissues following its administration orally. When given parenterally in mice, the blood level drops in two to three days, during which time a small amount is excreted in the urine. The highest levels are found in the liver, and relatively high concentrations are also found in monocytes, spleen, and lungs. The level in these tissues falls slowly, and Macrocyclon can still be detected eight weeks after a single injection. About 50% of the dose given is excreted in the faeces in four to six weeks, presumably because of excretion in the bile (253).

Toxicity. The LD50 of Macrocyclon in mice, rats, and rabbits is about 2.5 g/kg. Prolonged administration to mice produced only some vacuolation of the Kupffer cells of the liver and the reticuloendothelial cells of the spleen and lymph nodes. Prolonged administration to monkeys also produced some vacuolation of the reticuloendothelial cells, which disappeared after cessation of treatment, and also some rise in the blood cholesterol (253).

Clinical use. A pilot trial in patients with advanced pulmonary tuberculosis showed that even large doses of the drug produced no beneficial effect. This may be due to the fact that in such cases most of the bacilli are extracellular

and therefore not exposed to the enhanced activity of the macrophages, if this is indeed the mechanism of action of Macrocyclon. There is in fact some evidence that the drug is active in human leprosy, in which disease the bacilli are intracellular.

Administration of the drug to man has so far produced no serious toxic effects. There was an increase in the serum cholesterol, and pruritus with a maculopapular rash, which disappeared after cessation of treatment (53).

Finally, it is worth pointing out that increased resistance to tuberculosis has been produced by two types of substances, namely 1) surface active agents (the Macrocyclon type of substance) and 2) bacterial lipopolysaccharides, both the type isolated by Westphal (343) and also the lipopolysaccharide obtained from tubercle bacilli by Choucroun (70). This latter substance was claimed to represent the immunogenic fraction of tubercle bacilli. However, this is not supported by the results of Robson and Smith (272), though it can produce some effect on a tuberculous infection in mice and a more marked effect on infection with *M. leprae murium* (254). The possibility should be considered that all these substances have a similar mechanism of action and one which resembles that involved in acquired immunity. Further investigation of these questions seems desirable.

VIII. CONCLUSION

Great progress has been made in the development of antituberculosis drugs, and at present two potent substances and one less potent substance are in common use (isoniazid, streptomycin, and PAS). In addition, several other substances of lower potency and higher toxicity are available, which are used when the organisms have become resistant to the main drugs. There has thus been no major advance in this problem since 1952.

What is needed, in order of increasing importance, is 1) an alternative to PAS, *i.e.*, an orally active and well-tolerated drug which can be combined with isoniazid, 2) an additional drug of the order of potency of isoniazid and preferably active by oral administration, and 3) a drug which will eradicate the disease much more rapidly than any so far discovered, so that early cases of the disease can be cured as rapidly as penicillin cures syphilis.

These achievements would not seem impossible in the light of modern chemotherapeutic research, and it is thus rather deplorable that the problem of the drug treatment of tuberculosis is in many quarters considered as essentially solved.

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