Experimental Chemotherapy of Tuberculosis*

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At the beginning of this century epidemiologists (Wilbur, 1908) predicted that all the factors of which host resistance were compounded, both innate and acquired, which had been clearly influenced by social and economic change, were so gaining in ascendency over factors favourable to the tubercle bacillus—namely, virulence and number of organisms and hypersensitivity—that tuberculosis would naturally be eradicated by 1950, both here and in North America. However, it required the additional factor of chemotherapy to bring about the dramatic reduction in the disease which we have witnessed during the last 15 years or so. Chemotherapy is in our hands so powerful that for the most part other factors responsible for the outcome of infection can be disregarded in the treatment of tuberculosis.

Chemotherapy in its broadest sense comprises several components closely interrelated: (1) drug effectiveness—antibacterial potency and the required rhythm and duration of treatment; (2) drug administration—economics and cost of treatment and the available medical facilities; and (3) drug acceptance—tolerance of the drug and psychosocial attitudes.

In developed countries antituberculosis drugs have been discovered, and effective ways have been found of giving them through a highly organized and advanced medical service. This has resulted in a remarkable fall in morbidity and mortality of tuberculosis.

The obvious deficiency concerns the inability of patients to accept conventional treatment, and a large core of residual patients comprises precisely those not amenable to treatment for psychological reasons—less apparent is the fact that second-line or reserve drugs are so much less potent than isoniazid and streptomycin that factors influencing host resistance, such as bed rest, need to be considered once again in the management of patients.

In developing countries, however, the value of chemotherapy is much reduced for reasons of cost, lack of administrative and medical facilities, and rejection by the patient. In fact, our orthodox methods of antituberculosis chemotherapy are totally impractical for these vast areas of the world, which are teeming with tuberculosis. Of the 15 million cases of infectious tuberculosis, which was the approximate world prevalence in 1964, at least one-third, or 5 million, were thought to be in India and South America (ReVelle, Lynn, and Feldman, 1967). What can be done to reduce this great human burden which is such a hazard to health and to life itself? Economic and social advance will surely make a definite but slow impact. However, attention to those factors influencing drug effectiveness would probably yield the most rapid benefit. Firstly, new ways have to be found of using those drugs we have—ways hitherto considered unorthodox. Secondly, new compounds must be more actively sought—new compounds which ideally would be cheap and safe, easy to give, and able to eradicate the tubercle bacillus from the host.

It is to these ends that experimental chemotherapy should be directed at the present time. We should be greatly concerned by the diversion to other activity of the vast resources that were once devoted to the study and control of the host and parasite in tuberculosis. The very success of chemotherapy in developed and wealthy countries was the brake to this research and this poses a very real threat to the world-wide control of this very common disease.

Historical Background

Much of the early work in experimental chemotherapy was concerned with permeability of tuberculous lesions; permeability to a number of simple agents, such as arsenic, copper, gold, silver, and various dyestuffs.

Birkhaug (1940) quoted Calmette, who criticized the hap-hazard use of all these substances and insisted that “experiment alone, methodically conducted in animals sensitive to tuberculosis, would enable us to explore with profit the immense perspectives which chemotherapy had to offer.” Many ignored his advice and believed the failure of these and many other substances resulted from their inability to penetrate fibrotic and caseous tissue. This red herring has pursued chemotherapy ever since. In fact, most drugs not only penetrate avascular fibrotic lesions but also penetrate into host cells.

A scientific approach to experimental chemotherapy had been demonstrated already by Ambersen, McMahan, and Pinner (1931) when they carried out a clinical trial of sanocrynin in pulmonary tuberculosis in man. They randomly allocated patients with pulmonary tuberculosis into two groups and showed that this treatment had no effect whatsoever on the course of the disease.

One of the earliest experiments in animals with the use of such an approach was made by Rich and Follis (1938), when they showed that sulphonamides inhibited tuberculosis in guinea-pigs and rabbits. The effect was definite and was said to depend on an adequate daily dose of drug, maintenance of an effective blood concentration by divided doses, and continuing the drug throughout the experiment. Therefore a scientific approach to the investigation and treatment of tuberculosis in animals and man had been developed in the 'thirties, with an important understanding of the pharmacology of chemotherapeutic agents, factors which were unrecognized by several workers who could not confirm Rich and Follis's results.

At a time when tuberculosis was posing such great problems everywhere, Rich and Follis wisely drew no conclusions about the treatment of human tuberculosis and stressed the importance of controlled trials like Dr. Ambersen's and the search for modification of sulphonamides, which might then be more effective in tuberculosis, as had sulphapyridine in pneumococcal pneumonia.

The sulphones, however, were the first agents which not only inhibited tuberculosis and prolonged survival in vivo but...
reversed an established infection. Feldman, Mann, and Hinshaw (1942) demonstrated this by sequential liver biopsy in guinea-pigs. The stage was therefore set for the arrival of streptomycin, for the importance of pharmacological and biological observations were realized, together with the relevance of statistics and controlled trials to its assessment. Feldman and Hinshaw (1944) were given a small quantity of streptomycin, enough to treat four guinea-pigs for 54 days. Two control animals died and the remainder were grossly diseased, whereas those treated had minimal or no disease. In only one out of four treated animals could they recover tubercle bacilli from the spleen, but tissue extracts in all produced disease in previously uninfected guinea-pigs. They concluded that "streptomycin exerted a suppressive effect on the pathogenic propensities in the guinea-pig of the human form of Mycobacterium tuberculosis and the results were comparable with certain sulphones." This led to the use of streptomycin in human disease, in which impressive results were obtained, particularly in meningeval and miliary tuberculosis, but in most cases the arrest was only temporary and the additional bogy of toxicity and drug resistance were to temper optimism and prompt the same authors to write that "streptomycin was clearly not the answer to the problem of therapy in tuberculosis, but with the sulphones a great impetus to the search for the long-sought, specific cure."

Succeeding events, culminating in the clinical trial by the Medical Research Council and the Veterans' Administration in the United States of streptomycin and P.A.S., are well known. Since that time all potential antituberculosis agents, some of which have come into our hands for the treatment of the disease, notably isoniazid, have been subjected to controlled trial in animals rigorous enough to satisfy the earlier strictures of Calmette.

Search for New Antituberculosis Agents

Measurement of drug effect in vivo has largely been based on survival times and scoring methods in small mammals heavily infected with tubercle bacilli. Mice and guinea-pigs are chiefly used in the screening of organic compounds which have in-vitro activity before being judged suitable for clinical trials. Most compounds are rejected because they are relatively impotent or toxic.

As an example, in Fig. 1 the effects of ethionamide, prothionamide, and a relatively impotent drug "A" on the survival of white mice heavily infected with a virulent strain of tubercle bacilli are compared with those of isoniazid. Isoniazid is the most powerful and convenient agent with which to compare untreated drugs in vivo. Treatment was given for three weeks.

The control untreated animals all died within 60 days. Ethionamide at 200 mg/kg is clearly a potent antituberculosis agent, for it prolongs survival of 10 out of 12 animals, whereas isoniazid at 10 mg/kg prolongs survival of all animals for the 112 days of the experiment. Drug A, on the other hand, enabled only 4 out of 12 animals to survive 112 days. As ethionamide has certain troublesome side-effects in man, a search was made for an equally effective derivative without those side-effects. This led to the development and trial of prothionamide, which has a propyl group substituted for the ethyl group of ethionamide (see Fig. 2) (Noufflard-Guy-Loo and Berteaux, 1963). Prothionamide prolongs survival of white mice as effectively as ethionamide and is probably as potent in man, with less troublesome side-effects.

In screening drugs interest is chiefly focused on chemicals with antituberculosis activity of the same or greater order than isoniazid. It is remarkable that isoniazid and streptomycin, which were isolated so early on, have remained by far the most effective antituberculosis drugs available.

Limitation of Screening Procedures

Two series of compounds exist which exert interesting chemotherapeutic activity in animals, and serve to illustrate the value and the limitations of these screening procedures. Macrocyclon.—In 1951 Cornforth, D'Arcy Hart, Rees, and Stock reported the remarkable antituberculosis effect of certain high molecular weight, water-soluble, surface-active agents which they had encountered while searching for methods to alter blood lipids. One of these compounds of macrocyclic structure (Fig. 3), with long polyoxyethylene side-chains and called macrocyclon, suppressed tuberculosis in mice when given parenterally (Rees, 1958). However, it possessed no conventional in-vitro activity, little effect on tuberculosis in guinea-pigs, and none in monkey or man. Substances exist, therefore, which may escape notice by their lack of in-vitro activity in preliminary testing, but which might be of great value in vivo. Riminophenazines.—Barry (1946) described certain orange red phenazines derived from certain lichens. He synthesized a large number of these compounds, to which he gave the name "rimino" and whose outline structure is shown in Fig. 4. The riminophenazines vary in their solubility in water and fat.
and many possess in-vitro activity at very low concentrations against tubercle bacilli which may be resistant to isoniazid, streptomycin, and conventional drugs.

B663, one of the most active derivatives, in very small doses not only prolongs survival of tuberculous mice but is capable of preventing the disease in a remarkable fashion. Barry and Conalty (1958) gave 10 mg./kg. for two weeks to mice and then left the uninfected mice untreated for four weeks. These were afforded a considerable degree of protection on challenge with a usually lethal intravenous infection of a bovine strain. A similar effect was obtained in hamsters, but B663 exerted little antituberculosis effect in guinea-pigs or monkeys. The explanation for this great species difference probably lies, at least in part, in the reduction of B663 by macrophages in tissues, particularly lungs, spleen, and liver. High concentrations persist in, and colour, fat, and the skin itself becomes pink. These tissues are so avid for the compound that extracellular concentrations are very low. This intracellular deposition might account for the superior activity of riminophenazines in mice and hamsters, in which the tubercle bacilli are largely intracellular, and the retention of the dye therein may explain the chemoprophylaxis.

B663 and other riminophenazines are not altogether inactive in man, though little has been reported in straightforward tuberculosis. Mycobacterial skin ulcers have been treated successfully by systemic administration (Lunn and Rees, 1964), and Schonell, Crofton, Stuart, and Wallace (1968) have reported a striking remission with oral B663 and another riminophenazine B749 in a rare disseminated infection with M. avium which later proved fatal. These compounds may also be of value in human leprosy (Barry and Conalty, 1965).

Experience with macrocycin has shown us, therefore, that agents exist with little or no in-vitro activity but with considerable antituberculosis activity in the mouse and none in higher mammals. Riminophenazines, on the other hand, possess a degree of activity of the same order as isoniazid in vitro, of a greater order than isoniazid in the mouse, had yet little or no activity in monkey and man. These two, in many and probably all, and the same time have revealed new aspects of chemotherapy and have underlined the strict limitations not only of these methods of assessing new drugs but of the preliminary in-vitro testing.

**Drug Activity in Mice and Sterilization of Infection**

Special studies of tuberculosis in mice have revealed certain microbiological phenomena which are of great intrinsic interest, but which are not fundamentally understood, and, though having little or no direct relevance to man, may be of some significance. While the mouse has been used since the days of Koch for survival studies, it was Fenner, Martin, and Pierce (1949) who devised a method of counting organisms in tissue homogenates which allowed an accurate measure of an infection to be made at any given time. This technique has been developed for the study of chemotherapy (McCune and Tompsett, 1956).

When 20-g. white mice are infected intravenously with about a million virulent tubercle bacilli of the human (H37RV) strain and the animals are killed subsequently at regular intervals, tubercle bacilli, or more accurately viable units of tubercle bacilli, can be cultivated from the tissue homogenates and then counted. Remarkably consistent trends in the numbers of populations of organisms are found which are characteristic for any tissue studied.

In Fig. 5 changes in populations of tubercle bacilli in the lungs and spleen of mice are compared during 56 days. Each symbol represents the finding in one animal. In the spleen there is a high population, which rises and falls in the first three weeks and then remains constant or stable for as long as the animal lives—usually six months or so. In the lung, on the other hand, the population is lower at the start of the infection but rises to reach much higher numbers than in the spleen, after which once again the population stabilizes.

**Figure 5—Populations of M. tuberculosis (H37RV) in lungs and spleens of untreated mice. Each symbol represents the number of viable units in one animal.**

Populations of tubercle bacilli in this model remain stable despite progressive tissue changes, especially in the lung, where necrosis develops and accompanies the cellular reaction, and some organisms lie extracellularly. In the spleen, on the other hand, the tissue reaction remains cellular and the organisms are chiefly intracellular. Why the populations are so different in the lung and in the spleen and why they stabilize in this consistent way is quite unknown. The effects of external agents on the infection can therefore be measured.

**Measurement of Chemotherapeutic Effect**

The changes in populations of tubercle bacilli in the lung during 130 days of treatment with isoniazid and streptomycin, given from the day of infection, are shown in Fig. 6A. Each symbol refers to mean values obtained from at least three animals. The top line shows the persistence of the untreated populations at a very high level, the second is that obtained with streptomycin 200 mg./kg. intramuscularly, and the third is with isoniazid 25 mg./kg. by mouth. After a rapid fall in population during the first three weeks, where the line becomes dotted, no organisms may be recovered from some animals. This method permits detection of no fewer than 90 viable units per lung. The reduction in populations with isoniazid and streptomycin together is marginally and consistently greater than with isoniazid alone. The simultaneous changes in the spleen (Fig. 6B) are in striking contrast. Streptomycin is unable to prevent the initial rise, but reduces the population fiftyfold where it persists. Isoniazid and isoniazid with streptomycin can sharply reduce the numbers, but stability is achieved at about 10⁴ viable units per ml. Little if any further reduction occurs with triple drug treatment—isoniazid, streptomycin, and P.A.S.

There are three points here worthy of mention. (1) These changes are reproducible and the trends obtained are characteristic for each antituberculosis drug. (2) The magnitude of change produced by drugs is dependent on the tissue studied—for example, the spleen has a particular damping effect, partly due no doubt to the mainly intracellular site of the microbes,
but other factors must be operative. (3) There is an extraordinary persistence of the populations at a level characteristic for a given drug or drug combination; this is not due to the emergence of drug resistance, for in almost all cases the organisms are fully sensitive in vitro.

An exception to this is pyrazinamide, which with isoniazid is apparently able to cause organisms to vanish from the lungs and spleens of mice uniformly, and it is this phenomenon which Dr. McDermott and his colleagues have studied intensively (McCune, Feldmann, and McDermott, 1966).

Sterilization of Tissues

Pyrazinamide is closely related to nicotinamide (Fig. 2) and is active at pH 5.5 against human but not bovine strains of tubercle bacilli in vitro. Bovine strains lack an amidase which human strains lose when they develop resistance to pyrazinamide (Konno, Feldmann, and McDermott, 1967).

The effect in the lung of isoniazid 25 mg./kg. and pyrazinamide 2 g./kg. over three months alone and together is shown in Fig 7A. No organisms could be recovered from any animal treated with this drug combination after one month. Pyrazinamide drug resistance accounts for growth after two months' treatment with pyrazinamide alone. In the spleen (Fig 7B) pyrazinamide antagonizes isoniazid action in the first three weeks, but thereafter populations fall, and after two months no organisms could be grown in either this or very many other experiments with this drug combination except as an extreme rarity.

It might be supposed, therefore, that tubercle bacilli had been eradicated from these tissues. That this was not the case was simply shown by leaving animals untreated for a further three months, and from one-third of them bacilli could be grown from the spleen. It was then assumed that after three months' treatment with isoniazid and pyrazinamide the infection had been rendered latent. Every conceivable method for revealing organisms that depended on their growth consistently failed and these methods included special culture techniques, tissue culture, guinea-pig inoculation, etc. Search for bacilli by microscopy was unrewarding. More than $10^5$ bacilli are required per ml. of tissue for any to be seen in a section under the microscope. However, special preparations of homogenates revealed tubercle bacilli of normal morphology and staining characteristics (McCune, Feldmann, Lambert, and McDermott, 1966). It was therefore more appropriate to call organisms viable after this treatment sterile in the strict sense that they were unable to reproduce. Further studies went on to show that persistence of tubercle bacilli in a sterile state was not confined to one-third of animals (McCune, Feldmann, Lambert, and McDermott, 1966).

The percentage of animals from which tubercle bacilli could be recovered over a period of nine months is shown in Fig. 8. The sterile state had been produced by isoniazid and pyrazinamide. In untreated animals the revival rate rose from 30% at three months to 70% at nine months. Compare this revival rate with that achieved with high doses of cortisone—1 mg. per mouse. When cortisone was given daily in the first month no effect was observed. In the second month 30% revival resulted, in the third and fourth months the rate was so enhanced as to give 100% revival.

McDermott (1968) has made several observations on this sterile state:

1. It may be stable for long periods, for organisms remain unaffected by high-dose steroids for one month.

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**Fig. 6.—Changes in populations of M. tuberculosis (H37RV) in (A) lungs and (B) spleens of mice treated with streptomycin and isoniazid given singly and together.**

**Fig. 7.—Changes in populations of M. tuberculosis (H37RV) in (A) lungs and (B) spleens of mice treated with isoniazid and pyrazinamide given by mouth singly and together. The trends in populations are indicated by a dotted line at times when no organism could be cultivated from some or all animals.**
At nine months after treatment there remain in the tissues viable tubercle bacilli whose fate is uncertain.

(2) These revived bacilli are susceptible to drug action.

(3) Sterility is still achieved with isoniazid and pyrazinamide when treatment is delayed until populations have reached a very high level.

(4) Prolonging treatment for a total of six months abolished revival during six months of subsequent observation, which might imply that sterile organisms were susceptible to drug action, or that they reverted to the sterile state while reviving owing to drug action in the environment. This point is not yet clear.

(5) Tubercle bacilli in mice are also sterilized by isoniazid alone very occasionally, and by pyrazinamide alone in an unpredictable way. If bacilli are not sterilized by pyrazinamide alone they become resistant to it. Isoniazid given just for one month, and then pyrazinamide subsequently alone for two months, will uniformly and consistently render all bacilli sterile. No other sequence of the drugs or time relationship will achieve the same result.

It appears that at one month isoniazid persists (not drug-resistant) may be uniquely susceptible to the sterilizing action of pyrazinamide.

This is a remarkably specific action of isoniazid and pyrazinamide. In fact it is so specific that it could not uniformly be achieved in another strain of white mice when pyrazinamide was given at half the total dose in one dose a day for three months with isoniazid (Batten, 1968).

Canetti (1968) has produced a similar sterile state in mice treated with daily isoniazid and streptomycin. Treatment was delayed for two weeks until populations of tubercle bacilli in the lung were of the same order as those found in human cavities (10⁶). After 18 months' treatment 25% of animals had viable organisms in the lung or spleen; three months later, after treatment had stopped, the proportion rose to 75% and this revival could be accelerated by cortisone.

We have seen, therefore, that persistence of tubercle bacilli produced by chemotherapy in mice is of two sorts: one in which, despite continued presence of the drug, organisms survive in the tissues, often in quite large numbers, able to resume active growth when treatment is withheld while retaining in-vitro drug susceptibility throughout; the other in which a sterile state is assumed by the microbe which may continue for months. This may be either a random phenomenon or a reaction to the drug, but, whether it is a physiological adaptation or not, it is reversible. It is interesting in this context to note Chabbert's (1968) recent report in cases of human endocarditis of the persistence of unusual forms of bacteria recovered at operation or necropsy after chemotherapy, and to contrast this with such latent infections as described by Fauve, Pierce-Chase, and Dubos (1964). They found a natural latent corynebacterial infection in certain strains of mice which could be activated by a single large dose of cortisone and which accounted for variations between strains of mice in their susceptibility to corynebacterial challenge.

The tissue environment in man is certainly more hostile to the tubercle bacillus than in the mouse, and this may explain the rarity of revival of persisters as manifest in relapse after prolonged treatment in man. An easy acceptance of 100% prevention of relapse after so-called proper treatment should be resisted. The following is a case in point.

An antimony smelter aged 50 presented with the chest radiograph shown in Fig. 9: far advanced disease with cavitation and a positive sputum. Treatment with streptomycin and P.A.S. was supervised all the time. The sputum became negative after three months and the cavities closed by nine months. He completed two years' treatment with the residual reticulation of his occupational disease and tuberculosis (Fig. 10). Within four months there was extensive relapse, no cavitation (Fig. 11), but positive sputum and sensitive organisms. With further chemotherapy for more than two years he has remained well to the present (see Fig. 12), 12 years later.

One explanation for this relapse is that tubercle bacilli persisted in a less hostile environment provided by the antimony pneumonia, and it is of interest that we still do not know why chemotherapy is apparently less effective in pulmonary tuberculosis complicating simple pneumonia of coal workers than in tuberculosis of otherwise normal people (Medical Research Council, 1967). Persistence may be the explanation, rather than a simple failure on the part of patients to take their drugs.

In summary, we may say that persistence of tubercle bacilli and relapse of tuberculosis after chemotherapy are not comparable in man and mouse. This experimental model, however, may well provide evidence of a superior sterilizing potency on the part of new agents or combination of agents which may be forthcoming and which are needed to simplify the treatment of tuberculosis.
Rifampicin might prove to be just such an agent. This is a semisynthetic antibiotic of the rifamycin group produced from *Streptomyces mediterranei*, highly active not only against tubercle bacilli resistant to other drugs but also against many other bacteria (Pallanza, Ariolo, Furesz, and Bolzoni, 1967).

The effects of isoniazid and rifampicin were compared in the lung in the mouse model experiment. Both drugs were given by mouth from the day of infection (Fig. 13). Rifampicin at 20 mg./kg. causes a considerable fall in populations compared with the control, but not as great as isoniazid, also at 20 mg./kg., which after nine weeks causes organisms to disappear in some animals. At 40 mg./kg., however, rifampicin exerts a very powerful effect in this tissue, for no organisms could be recovered after three weeks. At 25 mg./kg. Grumbach and Rist (1967) have reported rifampicin to be superior to isoniazid in mice at the same dose in an experiment lasting four months. Given together they apparently sterilized the lungs and spleens at the end of this time. Rifampicin is clearly a highly active antituberculosis drug. Its potential remains to be assessed.

Activity of Agents Favourable to *M. tuberculosis*

This experimental model is useful for the study of external agents which favour the tubercle bacillus, and the effect that cortisone had on the revival of tubercle bacilli from the sterile state will be recalled.

Cortisone and corticotrophin cause striking growth of *M. tuberculosis* in tissues of mice (Batten and McCune, 1957). For example, populations of tubercle bacilli reached $10^7$/ml. in the lung after only 28 days' treatment with intramuscular corticotrophin. All the animals died about this time with overwhelming disease (Fig. 14). Cortisone 0.1 mg./day exerted a less powerful effect, and all animals survived the eight-week experiment.

What influence would these hormones have on antituberculosis drug activity? When isoniazid was added, drug activity was unimpaired by the steroid (Fig. 15). Thus those factors that allow tubercle bacilli to persist in the presence of isoniazid, whatever they may be, are not upset by these hormones. This was a consistent finding with all antituberculosis drugs used both in the lung and in the spleen.

Fig. 16 shows this to hold true also for the sterilizing action of isoniazid and pyrazinamide: in the lung at two months no organisms were recovered, either from those animals on the drug combination or when cortisone was added. No interference was found in the spleen. It would be of interest to see what effect adrenocortical steroids would have when given before or during treatment with isoniazid and pyrazinamide on revival from the sterile state.

Modification of Orthodox Methods of Chemotherapy

If new drugs or new combinations of drugs are not available at present to allow cheaper and simpler treatment of tuberculosis, can we modify chemotherapy appropriate to wealthier countries so that it retains its effectiveness and becomes a practical proposition for poorer countries with a high prevalence? The use of isoniazid, which is cheap and safe, alone is a controversial example, and its indiscriminate use in the treatment of tuberculosis is generally deprecated. However, isoniazid alone may be of considerable value, for example, if...
it follows a period of combined chemotherapy or in chemotherapy. In areas of high prevalence chemoprophylaxis may bring great benefit, as was to be found in Alaska (Comstock, Ferebee, and Hammes, 1967), where tuberculosis was rife among the Eskimos. In a controlled trial involving 85% of the population one-half were given 5 mg./kg. for one year in 1957. During the following six years of observation there was a 60% reduction in tuberculosis in the Eskimos treated with isoniazid. Clearly such a method has to be considered where prevalence is high, or in special groups in which the risk of developing overt tuberculosis is considerable.

### Intermittent Regimens

An increase in the interval between doses of drugs would reduce cost and ease the administration and supervision of treatment of a large population. Clinical experience with twice-weekly streptomycin with either daily isoniazid or daily P.A.S. was not really satisfactory, but isoniazid and streptomycin given on alternate days to Gorkhas produced good results (Eade, Harrison, Large, Mackay-Dick, Reid, and Riddell, 1959). Intermittent chemotherapy of experimental combinations in animals had not been studied to act as a yardstick. That the Tuberculosis Chemotherapy Centre in Madras (1964) should have turned to this method was therefore an inspired move, and the results of what has now become a classical study in the field are well known. With the organization and supervision at their disposal isoniazid in high dose (14 mg./kg.) and streptomycin (27 mg./kg.) given together twice a week were about as effective as daily isoniazid and P.A.S.—93% of patients had negative sputum cultures after nine months. Results of similar treatment elsewhere have been less satisfactory. In Morocco, Burzoni and Durante (1967) found that only 46% of patients had negative sputum cultures after eight months' treatment. The differences may have been due to the relatively smaller dose of streptomycin given to the Moroccan patients or to undisclosed previous treatment or perhaps to other factors yet to be discovered.

Evidence is now forthcoming from experimental chemotherapy which may prove helpful in deciding how intermittent treatment might best be applied to treatment in man.

Dickinson and Mitchison (1966) showed in vitro that antituberculosis drugs differed in their ability to delay growth of tubercle bacilli after exposure to drugs for periods of from 6 to 96 hours. They predicted that streptomycin in vivo would be unaffected by spacing out doses from one to eight days apart, whereas treatment with ethionamide and thiactzone would be impaired. Their predictions were borne out in the guinea-pig, when the mean total daily was kept constant.

In vitro they showed that less than 12 hours' exposure to 5 μg. of streptomycin per ml. would reduce viable counts and delay subsequent growth for more than eight days. On the other hand, at least 24 hours' exposure to 1 μg. of isoniazid per ml. was needed to reduce viable counts, and even then growth was delayed for less than seven days. Therefore as intervals between doses of isoniazid were increased in vivo the results would be impaired. The effect of spacing out doses of isoniazid and streptomycin one, two, four, and eight days apart were compared in mice, the same infection described above and the same techniques for measuring the infection in the lungs and spleen being used. The drugs were given from the day of infection for nine weeks.

The number of viable units of M. tuberculosis in lungs of mice receiving 50, 100, and 200 mg. of streptomycin per kg. as the mean total daily dose given at intervals of one, two, four, and eight days are found in Table I. Each single value represents the mean obtained from at least three mice.

With streptomycin viable units remain constant at each mean dose level as the interval between each dose is increased from one to eight days. At 200 mg./kg./day every eighth day each mouse would have had to receive 32 mg., which is a toxic dose. If a fall consistent with the general pattern is assumed a value of about 5.5 units can be calculated for this group. The means for each total daily dose are recorded in the right-hand column of Table I, and just show greater chemotherapeutic effect with doubling doses of drugs. Along the bottom line are the means obtained for each interval in days; the variation is seen to be negligible. So as the interval between doses of streptomycin is increased from one to eight days there was no reduction in drug effect.

Compare this with isoniazid (Table II) given in doses of 6.25, 12.5, and 25 mg./kg. at intervals of one, two, four, and eight days. As the mean daily dose was increased from 6.25 to 25 mg./kg. there was a striking reduction in the means of the viable units (right-hand column). In fact isoniazid in higher doses reduces populations of M. tuberculosis in the lung below detectable levels (about 90). Note, however, the rise in the mean populations as the drugs are spaced out from undetectable levels with daily use to a mean of over 10⁴ when given every eight days. Similar trends were observed in the spleen.

### Table I: Log₁₀ Viable Units of M. tuberculosis (H37RV) in Lungs of Mice Treated with Intermittent Streptomycin. Each Value Represents the Mean Obtained from at Least Three Animals.

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### Table II: Log₁₀ Viable Units of M. tuberculosis (H37RV) in Lungs of Mice Treated with Intermittent Isoniazid. Each Value Represents the Mean Obtained from at Least Three Animals.

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* Result from one animal only.

In murine tuberculosis, therefore, as doses are spaced out from one to eight days the influence of streptomycin on tissue populations is preserved, whereas that of isoniazid is reduced. The effects obtained with streptomycin are in general terms similar to those reported by Dickinson and Mitchison (1966) in the guinea-pig. Those observed with isoniazid are as predicted by their results in vitro. These results with isoniazid in mice are, however, at variance with those found in guinea-pigs (Dickinson, 1968), which showed no impairment of drug effect until doses were spaced out more than four days apart. The difference may be explained, however, by slower excretion of the drug in guinea-pigs with higher and longer sustained blood levels.

Canetti (1968) carried out extensive research into long-term intermittent chemotheraphy of murine tuberculosis. He has correlated clinical evidence in man and experimental evidence in mice and found that results obtained during treatment are comparable in mouse and man. Three aspects he considers of fundamental importance in intermittent therapy: (1) The total dose given in intermittent regimens should be governed by the known effective daily dose, so that the total dose given during the period of treatment is not reduced. (2) There must be a minimal interval between doses; that this varies from drug to drug is deduced from the experimental work reported above. (3) An initial period of daily treatment for at least one month renders subsequent intermittent treatment more effective.
Conclusion

It is unsatisfactory that the discovery of a new chemotherapy drug has never been based on a rational interference with mechanisms known to operate in the host or the parasite. Ironically some of the more toxic of antibiotics have been used to elucidate membrane and transport functions in the cell and protein synthesis. Progress in molecular biology relating to cell-wall structure and protein synthesis has resulted in a remarkable increase in our understanding of how bacteria are impaired or killed by chemotherapy. Further knowledge in this field may in fact lead to the discovery of an agent based on a rational interference with the function and structure of the tubercle bacillus. In the meantime the empirical search for better agents must be continued.

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References


Smoking Habits of Men Employed in Industry, and Mortality

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Summary: A study of the relation between smoking habits and lung cancer in male industrial workers over a period of three years has confirmed the earlier findings in doctors that the death-rate from lung cancer correlates closely with the number of cigarettes smoked. Of 54,460 men studied 68.7% were current cigarette smokers. The annual mortality rate from lung cancer was 0.33 per thousand in non-smokers and ex-smokers, and 1.2 per thousand for all cigarette smokers, and higher in heavy smokers.

Heavy cigarette smokers who retained the cigarette in the mouth between puffs ("drooping" cigarette habit) had an annual mortality rate of 4.1 per thousand.

The mortality from coronary thrombosis in smokers was nearly three times that in non-smokers. A mortality gradient with rising consumption of cigarettes was observed.

Some correlation between smoking and cancer of other sites and from non-neoplastic lung disease was observed in older men, but no correlation was found with other cardiovascular diseases and cerebrovascular diseases.

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