Centennial Review

The Diagnosis and Therapy of Tuberculosis During the Past 100 Years

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Methods for the radiographic diagnosis of tuberculosis have improved from simple fluoroscopy to computerized tomography. Although direct smear examination is still the most widely used bacteriological method of diagnosis, cultural methods with selective liquid media are sensitive and rapid. The use of antituberculosis drugs has changed tuberculosis from a disease with about a 50% mortality, treated by measures to collapse the affected lung lesions and by rest for the patient, to a condition successfully curable by chemotherapy. Key steps in the development of modern chemotherapy regimens were the demonstrations in clinical trials that (*1*) streptomycin was effective; (*2*) combination of drugs prevented the emergence of drug-resistant *Mycobacterium tuberculosis*; (*3*) chemotherapy under domiciliary conditions was effective and did not put family members at risk of infection; (*4*) patient compliance could be assisted by fully supervised intermittent regimens, or more effectively, by (*5*) shortening treatment by the introduction of rifampin and pyrazinamide, the two most potent sterilizing drugs, into the regimens. Regimens were divided into an initial intensive phase, while bacterial populations were high, and a longer continuation phase to complete sterilization. Pyrazinamide was shown to sterilize only in the intensive phase. The treatment of nonpulmonary tuberculosis followed the same plan, but when bacterial populations are low, fewer drugs are required in combination.

DIAGNOSIS

Radiography

During the first 30 to 40 years of the twentieth century, diagnosis was usually achieved with fluoroscopy, a dangerous procedure in which the patient stood so that an X-ray image of his chest appeared on a fluorescent screen without a film. Images on film were introduced later with the possible use of tomography to show slices of cavities or other lesions. Toward the end of the century, computerized axial tomography (1) and nuclear magnetic resonance tomography were also valuable, particularly when distinguishing between tuberculous and cancerous lesions. These tomographic techniques were of great value in diagnosing lesions outside the lungs, such as tuberculomas in the brain. Mass miniature radiography was introduced in the 1940s with the hope that it would increase the efficiency of screening for pulmonary disease (2), and could even be used in developing countries as an effective control measure. Unfortunately, because pulmonary lesions developed more rapidly than initially envisaged, radiographic screening for control purposes needed to be done at time intervals too small to be practical. The mass radiography equipment became sessile in hospitals in developing countries.

Bacteriology

Up to the 1950s, bacteriologic diagnosis was mainly by bright field examination of direct smears stained by the Ziehl-Neelsen method (3). Direct smear examination is rapid and highly specific but has low sensitivity. From then onwards, except in small developing country laboratories, fluorescence microscopy gradually replaced bright field microscopy because it is five times more rapid and is more specific (4). Cultures, which are more sensitive than smears but provide results more slowly, were rarely done up to the 1950s, but were particularly necessary for tissue specimens. Solid slopes of egg yolk media, particularly Lowenstein-Jensen medium, were widely adopted from about 1950 onwards. Culture in a liquid medium detoxified with ascitic fluid was described (5), but was plagued by high rates of contamination. Culture using liquid medium containing antibacterials selective for *Mycobacterium tuberculosis* (6) greatly improved results. Eventually, in 1977, an automated method incorporating a liquid medium containing radioactive palmitic acid and similar antibacterials was described (7). This method, which depended on measuring the radioactivity of CO_2, was widely adopted although it was too expensive for developing countries. Alternative methods that do not contain radioactive compounds but also depend on CO_2 production were then described and are now more widely used. These liquid medium methods increase the sensitivity of culture and speed up diagnosis substantially. Diagnosis by inoculation of guinea pigs was widely used between 1950 and 1970, particularly in Scandinavian countries, but was replaced by more efficient culture methods (8).

Drug susceptibility testing started in the 1950s with tests in liquid medium, but was later performed on solid medium slopes. Rapid molecular methods have been introduced for identifying mycobacteria in specimens, for identifying the mycobacterial species, and for drug susceptibility testing. These methods are not yet in wide use because of their high cost and uncertainty about their specificity. High-burden countries with few resources still depend almost entirely on direct smear examination.

Immunology

Serologic diagnostic methods work well when patients are smear positive but are much less effective in patients who have minimal disease or where the disease process cannot be sampled for conventional culture. These tests also do not perform well in human immunodeficiency virus–infected subjects. Childhood tuberculosis is an example of disease that is still difficult to diagnose with certainty. In this condition, the skin tuberculin reaction in its various forms is useful but has low specificity. The enzyme-linked immunosorbent spot assay for IFN-γ–producing cells (9) is highly

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TABLE 1. RESULTS OF BRITISH MEDICAL RESEARCH COUNCIL STREPTOMYCIN TRIAL

Regimen	No. of Patients	Deaths	X-ray Improvement (%)	Culture Negative 3 mo	Culture Negative 6 mo
SM	54	4	69	10*	8
Control	50	14	33	1	2

Definition of abbreviation: SM = streptomycin.
Data from Reference 13.
* Forty-one patients with resistant strains; two with sensitive strains (assessments are on the basis of modern criteria of drug resistance).

sensitive and specific, and appears to be able to detect any individual who harbors tubercle bacilli whether or not the disease is active, but this method is too expensive for widespread use.

TREATMENT

The Prestreptomycin Era

As the introduction of streptomycin (SM) was the first important advance in the therapy of tuberculosis, it is pertinent to consider what forms of treatment existed before its introduction in 1945. Since Ehrlich's original concept of chemotherapy derived from selective staining by dyes, attempts had been made to find chemotherapeutic agents for tuberculosis. These attempts were extensively reviewed by Hart (10). He indicated that sanocrysin, a gold salt, was widely used in treatment between 1925 and 1935. A number of different sulphones that had activity in experimental animals were also investigated but were never widely used in treatment. Vitamin D was also explored in early work, as was nicotinamide, from which several current antituberculosis drugs, including isoniazid (INH) and ethionamide, were subsequently developed as analogs. The basis of treatment was, however, rest for the patient in sanatorium and rest for the affected portion of the lung by collapse therapy through operative procedures on the chest wall (thoracoplasty) and the injection of air into the pleural cavity (artificial pneumothorax) (11). Pulmonary tuberculosis was reputed to have a 50% mortality, with tuberculous meningitis and miliary tuberculosis uniformly fatal.

SM

After the discovery of SM (12) and the proof of its antituberculosis activity in the guinea pig, small uncontrolled studies were undertaken in the United States, but the first clinical trial with a randomized intake in the history of medicine was started in 1946 by the British Medical Research Council (13). Because only a limited amount of SM was available in the United Kingdom, patients with advanced pulmonary disease could ethically be randomized to treatment with bed rest alone or bed rest plus 2 g SM daily. The results (Table 1) showed a substantial immediate advantage to the SM arm, but most patients developed SM-resistant strains, and the results of a 5-year follow-up indicated that they had little eventual benefit compared with the control arm. This study focused the aim of development during the next 20 years on preventing the emergence of drug resistance. For further complete references to the studies undertaken by the British Medical Research Council from 1946 onwards *see* Reference 14. In contrast to the results in pulmonary tuberculosis, a parallel study showed that SM was able to cure about 44% of patients with tuberculous meningitis (15). Drug resistance did not emerge in these patients because the bacterial population was too small to contain resistant mutants.

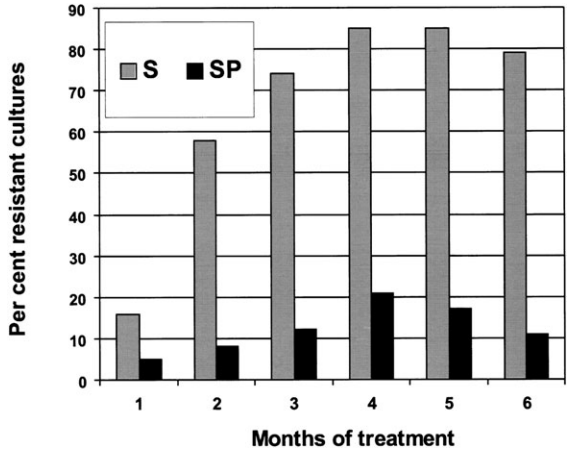

Figure 1. p-aminosalicylic acid (PAS) given with streptomycin (SM) reduces the emergence of SM resistance. The percentage of strains that is SM resistant in the SM series (indicated by S) and the SM plus PAS series (indicated by SP) during 6 months of treatment. Data from Reference 16.

Prevention of Drug Resistance

The next step was to conduct a randomized controlled trial (RCT) comparing treatment of acute pulmonary tuberculosis with either SM or p-aminosalicylic acid (PAS) or with both SM and PAS (16). The aim was to inhibit SM-resistant mutant bacilli with PAS, a very weak drug on its own. The results of this study showed that SM and PAS induced far fewer SM-resistant strains than SM alone (Figure 1).

Isoniazid and Effective Standard Treatment

Isoniazid (INH), introduced in 1952, was a more potent drug than SM or PAS, probably because it can be given safely at a dose size substantially above the minimal effective dose. Between 1952 and the mid 1960s, a series of RCTs on combinations of INH, SM and PAS were performed by the British Medical Research Council, the U.S. Veterans Administration, the U.S. Public Health Service, and elsewhere. In 1955, the British Medical Research Council performed the first national drug resistance survey, which showed that almost all strains with primary resistance were resistant to only one drug (17). Because of this finding, treatment with an initial three-drug phase lasting 2 to 3 months, followed by a continuation phase with two drugs was explored first in Scotland and then internationally (18). Whereas the regimen was highly successful and was adopted as standard in the Western world, it had to be given for at least 12 months with resulting frequent failures to complete treatment. Furthermore, it was too expensive in drug costs (particularly for PAS) to be widely used in developing countries. The need to reduce costs in developing countries led to a series of RCTs in East Africa on thiacetazone (TB1) as a cheaper alternative to PAS (14). The possible use of INH alone was also explored (19) on drug cost grounds and because the low guinea pig virulence of highly resistant strains suggested that they might not cause progressive human disease (20). Unfortunately, this thesis proved not to be true (21), and because initial INH resistance carried a poor prognosis, the use of INH in monotherapy has been abandoned. During the course of these and later studies, it was possible to assess the relative merits of other antituberculosis drugs by their ability to prevent the emergence of INH resistance when used

TABLE 2. EMERGENCE OF FAILURES DUE TO DRUG RESISTANCE DURING DOUBLE-DRUG THERAPY

Study Place	Isoniazid with	No. of Patients	Failure of Treatment (%)
East Africa	Rifampin	183	0.5
	Streptomycin	96	2
Madras	Ethambutol*	105	4
	PAS	309	12
East Africa	Thiacetazone	423	16

Definition of abbreviation: PAS = p-aminosalicylic acid.
Data are taken from Reference 22.
* With initial supplement of streptomycin daily for 2 weeks.

in a double-drug regimen with INH (Table 2). Whereas rifampin (RMP) was the most effective, SM and ethambutol were a little less effective, and PAS and TB1 much less effective. Estimation of SM (23) and INH (24) concentrations, and later also RMP (25) were made in several different types of tuberculous tissue obtained at resection soon after a drug dose was given. They showed that these drugs penetrated throughout lesions and caseous matter in concentrations adequate for bacteriostasis. Thus, there were no compartments, such as thick-walled cavities, into which these drugs failed to penetrate. Indeed, if this were not true, it would be difficult for combined therapy to be effective because there would always be compartments in which only one drug would be active and would therefore create resistant strains.

Domiciliary Treatment

In 1956, the Tuberculosis Chemotherapy Center (now the Tuberculosis Research Center) was established in Madras, India, to study whether older, standard treatment in hospital or sanatorium improved the results of chemotherapy (26). Patients all received INH/PAS for 1 year and were randomly allocated to treatment in sanatorium or at home. The treatment results were similar in the two arms of the study. Furthermore, family contacts were no more liable to develop tuberculosis than those treated at home (14). This study and similar experience elsewhere led to the widespread closure of tuberculosis beds and the spread of domiciliary treatment that could now be afforded throughout the world. Nevertheless, the problem of how to assure regular drug taking during a year of domiciliary treatment was immediately evident (27). The problem was investigated in two ways: (*1*) by the use of regimens with drugs given fully supervised in widely intermittent frequencies to make supervision easier, and (*2*) by shortening the duration of treatment.

Intermittent Regimens

The development of intermittent regimens was performed *in vitro* and in experimental tuberculosis of the guinea pig by Jean Dickinson (28) and partly in RCTs at the Tuberculosis Chemotherapy Center, Madras, starting with regimens of SM/INH, given once or twice weekly (29). Later on, other centers explored intermittent regimens. INH could be given efficiently twice weekly, but when given once weekly rapid acetylators fared much less well than slow acetylators (30). SM was of limited efficacy when given intermittently as the dose size could not be increased in intermittent regimens. Ethambutol was effective in preventing failure in intermittent regimens but was unfortunately followed by high relapse rates (31). RMP could be given intermittently with excellent effect (32), but the higher dose sizes required produced an immunologic "flu" reaction (33).

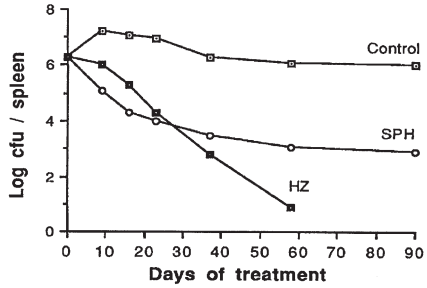

Figure 2. Cfu counts from the spleens of mice, either untreated (control), treated with SM, PAS and isoniazid (INH), indicated by SPH, or with INH and high dosage pyrazinamide (PZA), indicated by HZ, showing sterilization by HZ. Redrawn from Reference 36.

Laboratory Studies on Shortening Chemotherapy

From early on in the development of chemotherapy, the rationale for the slow fall in counts of viable bacilli during treatment presented a problem. The existence of persisting bacilli was recognized early and thought likely to occur when bacilli were in a stationary phase of growth or under anaerobic conditions (34, 35). However, perhaps the most important advance in the chemotherapy of tuberculosis was the series of long-term studies of experimental tuberculosis in mice performed in Walsh McDermott's Department at Cornell University on pyrazinamide (PZA) (36). A model system was established in which treatment with drugs was given to infected mice for periods of 3 months or even longer, and counts of viable bacilli in the organs were done throughout treatment. With slight modifications, this model is still being used today.

PZA, reviewed recently (37), was discovered in 1952. It is a remarkable drug that does not appear to have a genetic site of action but accumulates within the bacterial cell, where it acidifies its content and damages membranes. Unlike any other drug, as bacterial metabolism slows down PZA becomes more bactericidal. In the murine model, therapy with standard drugs—INH, SM, PAS—produced an initial fall in viable counts, but these then leveled out and it was difficult to sterilize the organs (Figure 2). When PZA was added, the counts continued downwards and eventually a state was reached in which all organ cultures were negative. PZA is thus a good sterilizing drug. However, because bacilli in a nonculturable form were still present, relapses eventually occurred. Similar experiments were performed later at the Pasteur Institute, Paris, showing the high sterilizing activity of RMP (38). *In vitro* experiments showed that the reason for the high sterilizing activity of RMP probably lay in the speed with which it started to kill bacilli as they recovered from dormancy and not to a particularly rapid kill of slowly growing bacilli (39). A hypothesis (Figure 3) was put forward to explain the activities of different drugs, on the basis of the presence of widely different growth rates within the bacterial population at the start of treatment.

Short-course Chemotherapy

As a direct result of the two sets of experiments on the treatment of murine tuberculosis with PZA and RMP, the multicenter RCT that established short-course chemotherapy was performed in East Africa in 1970 (40). All of the patients were in hospital throughout treatment to be sure that their prescribed treatment was actually taken. They were allocated at random 6-month regimens of (*1*) daily SM and INH (SH); (*2*) SH with the addition of RMP; (*3*) SH with the addition of PZA; and (*4*) SH with

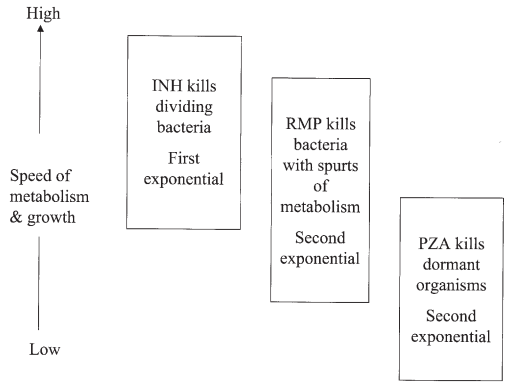

Figure 3. The hypothesis on the action of drugs according to their speed of growth at the start of treatment. INH kills multiplying bacilli. Rifampin (RMP) kills bacilli when there are spurts of metabolism. PZA is the only drug to kill dormant bacilli.

TABLE 4. THE EFFECT OF ADDING RIFAMPIN TO A REGIMEN ON SPUTUM CONVERSION AT 2 mo AND THE RELAPSE RATE CALCULATED OVER 24 mo AFTER THERAPY

				Percent of Patients	
Study	Place	Regimen*	No. of Patients	Cultures Pos. at 2 mo	Relapse
1	East Africa	6SH	154	51	29
		6SHR	148	31	2
2	Madras	2SHZ/5SHZ$_2$	129	28	6.2
		2SHRZ/5SHZ$_2$	261	8	2.3
3	Madras	3SHZ/2SHZ$_2$	236	26	13
		3SHRZ/2SHZ$_2$			4
		3SHRZ			20

Definition of abbreviations: $_2$ = twice-weekly dosage; H = isoniazid; Mos. = months; No. = number; Pos. = positive; R = rifampin; S = streptomycin; Z = pyrazinamide.

Data from Reference 41.

* The combination of H, R, S, or Z administered. The numbers preceeding drugs indicate month of administration.

the addition of TB1, given for 12 months as a control. After completion of treatment the patients were followed up with monthly bacteriology for 24 months. The primary endpoint was the rate of relapse during follow-up and the secondary endpoint was the proportion of patients who had a positive sputum culture at 8 weeks. This was the first RCT in which these end-points were used, which are the same as those currently in operation for modern RCTs. The results, summarized in Table 3, show the great reduction in relapse rates in the regimens containing RMP and PZA with a slight superiority of the regimen with RMP. These are the results that led to a burst of RCTs under the auspices of British Medical Research Council in East Africa, Hong Kong, Singapore, Madras, Algeria, and Prague (14). A few years later they were followed by the licensing of RMP in the United States and subsequent RCTs under the auspices of the U.S. Public Health Service.

Synergistic Sterilizing Activity of RMP and PZA

When either RMP (Table 4), or PZA (Table 5) was added to a regimen, there was a decrease in the proportion of patients with a positive 2-month sputum culture and a decrease in the relapse rate after treatment, indicating improved sterilizing activity. Furthermore, the addition of RMP to a regimen containing PZA or PZA to a regimen containing RMP, in Studies 2, 3, 4, 6, and 7 (Tables 4 and 5), also increased sterilizing activity and demonstrated the synergistic sterilizing activity of these two drugs.

TABLE 3. FIRST STUDY OF 6-mo REGIMENS INCLUDING RIRAMPIN AND PYRAZINAMIDE IN EAST AFRICA SHOWING EFFECT ON SPUTUM CONVERSION AT 2 mo AND RELAPSE RATES DURING 24 mo AFTER THERAPY

			Relapse	
Regimen*	No. of Patients	Culture Neg. at 2 mo (%)	(*No.*)	(%)
SHR	152	69	4	3
SHZ	153	31	13	8
SHT	104	28	23	22
SH	112	8	33	29

Definition of abbreviations: H = isoniazid; Neg. = negative; R = rifampin; S = streptomycin; Z = pyrazinamide.

Data are taken from Reference 40.

* The combination of H, R, S, or Z administered.

Division of Regimens into an Initial Intensive Phase Followed by a Continuation Phase

It is reasonable to suppose that viable bacterial populations are largest at the start of treatment and are therefore most likely to contain resistant mutants. Thus, to prevent resistant strains emerging, chemotherapy should be with the greatest number of drugs during an initial phase. The continuation phase could be based on fewer drugs, as the risk or drug resistance would be smaller. For this reason and because both RMP and PZA were expensive, much development in East Africa was with regimens in which use of RMP and PZA was confined to the first 1 or 2 months of therapy. Regimens with a 2-month intensive phase had lower relapse rates than those lasting only 1 month.

PZA is Only Active During the Intensive Phase

Three RCTs were done to investigate in which part of treatment PZA and RMP acted (Figure 4). In the Singapore and East African studies, the same 4-drug, 2-month intensive phase was followed by a 2-month continuation phase of INH or INH plus RMP, with or without PZA (14). The follow-up indicated that the relapse rates were substantially reduced by the presence of RMP in continuation but were not altered by PZA. In the Hong Kong study, similar relapse rates followed the addition of PZA for the initial 2, 4, or 6 months of the regimen (14). These studies provide convincing evidence that PZA sterilizes only during the intensive phase. A study of early bactericidal activity with PZA given in monotherapy for 14 days established that although it is bactericidal during the first 14 days, its main sterilizing activity must start afterwards (42). Despite these findings, there is evidence that PZA can help to prevent the emergence of drug resistance during the continuation phase (43). These findings were explained as due to the presence of active inflammation producing an acid microenvironment during the intensive phase (44). Acidity would be necessary to allow PZA to be bactericidal against the extracellular bacilli in cavities. In the continuation phase, inflammation would subside (except when a resistant strain emerged) allowing a return to a normal pH and to inactivity of PZA.

Steroids in Chemotherapy

A study was done in Madras in which the addition of high dosage steroids during the start of treatment did not alter the speed of bacterial clearance during therapy nor relapse rates afterwards

TABLE 5. THE EFFECT OF ADDING PYRAZINAMIDE TO A REGIMEN ON SPUTUM CONVERSION AT 2 mo AND THE RELAPSE RATE CALCULATED OVER 24 mo AFTER THERAPY

Study	Place	Regimen*	No. of Patients	Percent of Patients: Cultures Pos. at 2 mo	Percent of Patients: Relapse Rate
1	East Africa	6SH	154	51	29
		6SHZ	150	24	11
4	East Africa	2SHR/4TH	194	25	13
		2SHRZ/4TH	179	13	6
5	Hong Kong	2SHRE/4SHE$_2$	84	16	23
		2SHRZ/4SHZ$_2$	71	6	7
6	East Africa	6SHR	169	30	†
		2SHRZ/4TH	347	18	†
7	UK	2EHR/7HR	157	36	†
		2EHRZ/4HR	141	23	†
		2SHRZ/4HR	146	23	†

Definition of abbreviations: $_2$ = twice-weekly dosage; E = ethambutol; H = isoniazid; mo. = months; No. = number; Pos. = positive; R = rifampin; S = streptomycin; T = thiacetazone (TB1); Z = pyrazinamide.

Data from Reference 41.

* The combination of E, H, R, S, T, or Z administered. The numbers preceeding drugs indicate month of administration.

† = not relevant because of different continuation phase regimens.

(45). This finding confirmed the mouse experiments, which failed to demonstrate any increase in bactericidal activity when steroids, that are highly protuberculous in the mouse, are given with chemotherapy (46). It suggests that the concept of waking up dormant bacilli so that they can be killed more efficiently, as is often surmised, is unlikely to work. Steroids may however be used to suppress inflammation.

Modern Regimen of Chemotherapy

Two regimens emerged from the numerous RCTs. The first was a 6-month regimen in which RMP is given throughout, starting with 2 months of SM, INH, RMP, and PZA and is followed by 4 months of INH and RMP (2SHRZ/4HR). This regimen was pioneered in Singapore (14) and its efficacy and low toxicity confirmed by later studies in other countries. It is now widely used with ethambutol substituted for SM (2EHRZ/4RH) to avoid transmission of human immunodeficiency virus. The second was an 8-month regimen starting with the same 4-drug initial phase but continuing with 6 months of TB1 and INH (2SHRZ/6 TH). This regimen used a minimum of RMP and PZA because of their original high cost. However, in patients with an human immunodeficiency virus infection, all side effects were worse and the occurrence of serious Stevens-Johnson syndrome due to TB1 (47) in these patients led to the substitution of ethambutol for TB1 (2EHRZ/6EH), as well as for SM, without an RCT to justify the switch of drugs. A recent RCT has established that the 8-month regimen is distinctly inferior to the 6-month regimen with RMP throughout (48).

Tuberculosis in Organs Other Than the Lungs

The chemotherapy of nonpulmonary tuberculosis disease is similar to that of pulmonary tuberculosis except in two respects. In diseases where lesions do not communicate with the air, such as spinal tuberculosis and glandular disease, their bacterial content is much lower than in cavitary pulmonary tuberculosis. As a result they do not need as many drugs to be given to prevent the emergence of resistance. Thus, excellent results have been obtained in spinal disease with a 6-month, 2-drug regimen of INH and RMP (14). In tuberculous meningitis, the blood–brain barrier prevents penetration into the cerebral spinal fluid by SM and ethambutol once initial inflammation has subsided (49). Although these drugs are still given, they are not really necessary because of a small bacterial population. A simpler regimen of

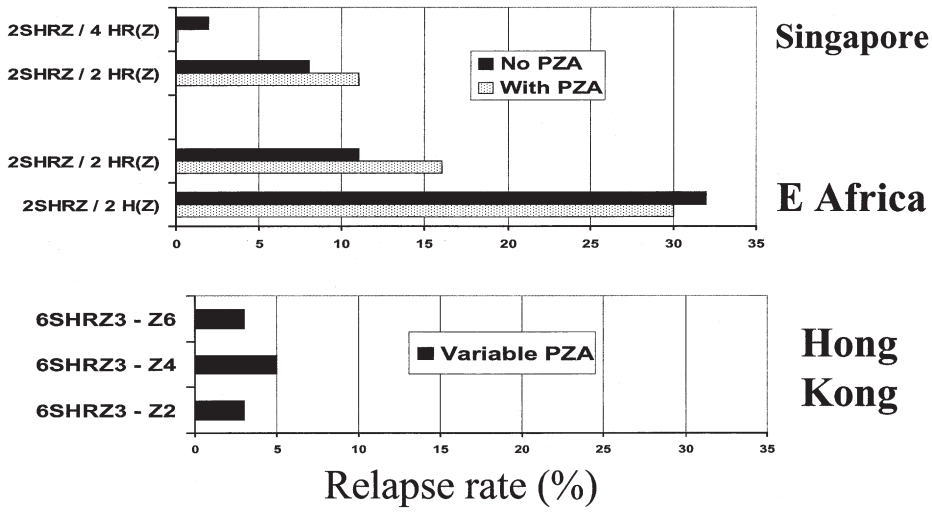

Figure 4. PZA given in the continuation phase does not reduce the relapse rate. In the Singapore and East African studies, the same 4-drug regimen (SHRZ) was given to all patients for 2 months. In each study, INH alone (H) or INH plus RMP (HR) was given in the continuation phase with or without PZA. In the Hong Kong study, all patients were given the 4-drug regimen (SHRZ), and they received SM, INH, and RMP 3 times per week for 6 months. To this was added PZA for 2, 4, or 6 months. Data from Reference 14.

TABLE 6. EFFECT OF INITIAL ISONIAZID RESISTANCE ON RELAPSE RATES: RIFAMPIN/ISONIAZID IN THE CONTINUATION PHASE

Regimen and No. of Clinical Trials	Initial Susceptibility to Isoniazid	Patients Assessed (*No.*)	Relapse (%)
SHRZ/RH, 6	Sensitive	1,225	5.2
	Resistant	61	8.2
EHRZ/EH, 1	Sensitive	190	3.7
	Resistant	23	4.0

Definition of abbreviations: E = ethambutol; H = isoniazid; R = rifampin; S = streptomycin; Z = pyrazinamide.

Data from References 48 and 51.

TABLE 7. SURROGATE MARKERS USED TO ASSESS NEW DRUGS

Preclinical
Bactericidal activity of drug combinations
Mouse studies
Clinical
Early bactericidal activity
2-mo sputum conversion
Serial sputum cfu counts
Speed of sputum conversion

INH, RMP, and PZA should be effective, except in infections with strains resistant to INH and RMP (50).

Role of Individual Drugs in Chemotherapy

An important advance in chemotherapy during the past two decades has been a better understanding of the role of the individual drugs in current regimens (51). The results of the original East African study (Table 3) have indicated that RMP and PZA have a major role in shortening the duration of chemotherapy and therefore in the sterilizing activity of the current 6-month regimen (2EHRZ/4RH). RMP is slightly more effective overall, but because PZA only acts for a limited period during the intensive phase, it is probably the most effective sterilizing drug during its period of action. In the absence of RMP in the continuation phase of the regimen, INH has slow sterilizing activity, but if RMP is included in the continuation phase then INH has no sterilizing action. This is shown by the similarity in relapse rates of patients with strains that are resistant just to INH and those that are sensitive (Table 6).

New Antituberculosis Drugs

In the absence of new drugs, little change has taken place in current regimens during the past 30 years. However, there is current interest in the fluoroquinolones (52) and at least three other potentially valuable drugs (including PA 824 and the diarylquinoline R207910) (53, 54) are near or at early clinical development. New drugs would only benefit standard therapy if they could shorten the treatment period from the current 6 months to 4 months or less. Otherwise their role would be confined to the treatment of patients with multi–drug-resistant tuberculosis caused by strains resistant to at least INH and RMP. Every new drug should be tested for activity in human disease by the early bactericidal activity method (55). A titration of the dose size used in the early bactericidal activity study allows determination of the "therapeutic margin," the ratio between the usual dose size and the minimal effective dose (Figure 5) (56, 57).

It would be unethical to shorten treatment with a regimen containing a new drug without evidence that the shortening would be successful. Hence, there is a need to explore surrogate markers that are assessments of drug action that correlate with the ultimate relapse rate in an RCT, and, therefore, provide evidence on whether the new drug is actively sterilizing and could shorten treatment. Surrogate markers can either be preclinical or clinical (Table 7). Preclinical assessments can be obtained from studies of the bactericidal activity of drugs in models of *in vitro* bacterial persistence, such as old, stationary, microaerophilically adapted cultures (58) or cultures containing RMP-tolerant bacilli in Models 2 and 3 of the Hu/Coates system (59). They can also be obtained from long-term experiments in the mouse (60). Whereas the preclinical assessments are of great value, it is becoming increasingly necessary to carry out a clinical study during the first 2 months of treatment in which one arm contains the new drug X. During the 2 months, the possible assessments are (*1*) the proportion of patients with positive sputum cultures at 2 months, a rather crude assessment, but well validated in several RCTs (41); (*2*) the speed of sputum conversion measured as a survival analysis using Kaplan-Meier graphs and the log-rank test; (*3*) the speed with which the count of viable bacilli is reduced during the biexponential phase of bacillary killing, as measured by serial sputum cfu counts during the period of the slow sterilizing phase starting at about 4 days and continuing at frequent intervals thereafter (61). Serial sputum cfu counting is probably the most efficient method of measuring speed of killing but it is only in an exploratory stage. If the new drug is not good

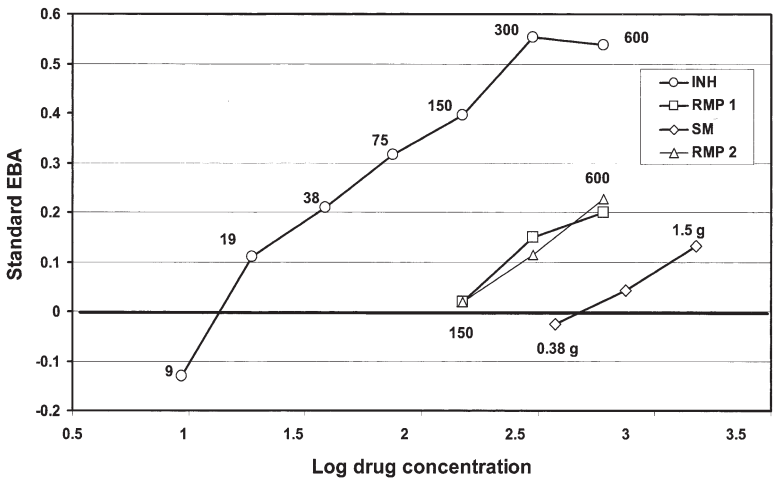

Figure 5. Titrations of dose sizes of INH, RMP, and SM against their early bactericidal activity (EBA) during the first 2 days of monotherapy with each drug. Each set of points is derived from one study. There are two available studies for RMP. The therapeutic margin is the ratio between the usual dose size (300 mg INH) and the dose size when the EBA is at zero (~ 15 mg INH). These ratios are 300/15 = 20 for INH, 600/150 = 4 for RMP, and 1.5/0.5 = 3 for SM. Data from references 55–57 and D.A. Mitchison (unpublished data), for the second EBA titration of RMP.

at sterilizing, for instance by being a cell wall inhibitor, its activity can be shown by a study of early bactericidal activity, but the only way to test it further is in the treatment of multidrug-resistant disease.

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