Papers and Originals

Chemotherapy of Tuberculosis: a Bacteriologist's Viewpoint*

D. A. MITCHISON,† M.B.

Brit. med. J., 1965, 1, 1331-1338

One of the most fruitful scientific methods for investigating the treatment of many conditions is the controlled clinical trial. Marc Daniels was a pioneer in the application of this procedure to the chemotherapy of pulmonary tuberculosis. He was responsible for the co-ordination of the first Medical Research Council co-operative studies in Britain. His scientific stature is demonstrated by the great care with which he collected his data, by his accuracy in the analysis of the results, and by his far-sighted judgment in their interpretation. It is fitting that many of the findings described in the present communication were obtained during the course of controlled clinical trials, carried out under the auspices of the Medical Research Council in the East African countries, and at the Tuberculosis Chemotherapy Centre, Madras.¹

Before the discovery of antibacterial drugs for the treatment of tuberculosis therapeutic measures were mainly aimed at increasing the patient's resistance to the disease. These measures included rest for the patient and for the affected organ, good food, and an absence of emotional strain. However, with the increasing use of antibacterial drugs the aim in treatment has changed. The host and his immune response have become less important, while the reaction between the tubercle bacillus and the drug has come to dominate the scene. If we now wish to improve the results of treatment we attempt to use better drugs and to give them in the most efficient manner. It is my purpose to illustrate this change in attitude and to try to explain some of the more important bacteriological concepts involved.

Action of Drugs on Sensitive Bacilli

When patients with pulmonary tuberculosis are given antibacterial drugs, successive cultures during the first months of treatment yield organisms which are identical in their drug sensitivity to those isolated before the start of treatment. The numbers of bacilli in these cultures become progressively smaller as treatment continues (Fig. 1). An explanation for the excretion of sensitive bacilli during treatment is important in understanding many of the phenomena occurring during chemotherapy. The occurrence of these sensitive organisms may be explained by two alternative hypotheses. One hypothesis assumes that the bacilli continue to multiply during the period that the sensitive organisms are isolated from the patient. Such multiplication might occur in particular areas in the lungs, or in parts of lesions, such as the interior of thick-walled cavities, which are often considered not to have a good blood supply. Again, multiplication might take place in the intervals between separate doses of the drug when concentrations in the lesions

fall below the bacteriostatic level. The second hypothesis assumes that the lesions are flooded with a fairly uniform concentration of the drug early in the treatment, and that the organisms then cease to multiply. On this hypothesis the bacilli coughed up during treatment would be the residue of the original population that has persisted in the lesions in a dormant state (see McDermott, 1958). As can be demonstrated in the test-tube (Schaefer, 1954; Mitchison and Selkon, 1956), dormant bacilli are not killed by isoniazid in concentrations that rapidly kill actively growing bacilli. The continued liberation of these persisting bacilli would be due to mechanical factors, such as the breakdown of caseous material in cavity walls.

FIG. 1.—Bacterial content of sputum during treatment with isoniazid plus P.A.S. (patients with isoniazid-resistant strains excluded). Data from Tuberculosis Chemotherapy Centre, Madras (1959).

The evidence discriminating between these two hypotheses will now be examined for isoniazid, the principal drug used in the treatment of tuberculosis. Isoniazid has a small molecule and diffuses rapidly (Dettli, 1961), even into cells (Mackness and Smith, 1952). It is not bound to protein (Claunch *et al.*, 1963), and its activity is not affected by variation in pH (Mitchison, 1952). Measurements of isoniazid content by labelling with radioactive carbon (Barclay *et al.*, 1953) or by microbiological assay (Bartmann and Freise, 1963) in normal lung tissue and in pulmonary lesions have indicated that it is present, at least for a period, following administration of the drug, in adequate amounts for bacteriostasis (Fig. 2). Can we identify particular parts of lesions where multiplication of bacilli could occur without the penetration of isoniazid? Tubercle bacilli require oxygen for growth. In some parts of a lesion oxygen may be supplied by diffusion from the blood, in

* Marc Daniels Lecture delivered to the Royal College of Physicians of London on 13 January 1965.

† M.R.C. Unit for Research on Drug Sensitivity in Tuberculosis, Postgraduate Medical School of London.

¹ Under the joint auspices of the Indian Council of Medical Research, the Madras State Government, the World Health Organization, and the Medical Research Council of Great Britain.

which case isoniazid would also diffuse to the bacilli. However, in the walls of open cavities oxygen may diffuse direct to the bacilli from the air, so that we cannot assume the penetration of isoniazid. Yet it is in such cavities that isoniazid-resistant bacilli are most frequently found (Canetti, 1954; Canetti and Grosset, 1958). The occurrence of resistant bacilli is good evidence that isoniazid must sometimes be present in bacteriostatic concentrations. These considerations make it appear unlikely that there are particular lesions, or parts of lesions, where isoniazid fails to penetrate completely.

FIG. 2.—Isoniazid content of serum, normal lung tissue, cavity wall, and caseous material in resected human material after an intramuscular dose of isoniazid 5 mg./kg. body weight. Data redrawn from Bartmann and Freise (1963).

We must now examine the possibility that isoniazid reaches bacteriostatic concentrations in the lesions for only limited periods after individual doses of the drug, and that the bacilli may multiply in the intervals when drug concentrations are sub-bacteriostatic. Fig. 3 shows the results of viable counts on a culture of tubercle bacilli which had first been exposed to isoniazid 1 μg./ml. for one or four days, and from which the isoniazid was then removed by washing (Dickinson and Mitchison, 1965). It is evident that the surviving bacilli, which have been washed free of the drug, do not start to multiply again for several days. Further evidence that bacilli do not multiply in the intervals between doses of isoniazid can be obtained from the results of treatment in patients who metabolize isoniazid slowly or rapidly. Patients can be classified into two genetically distinct groups of slow and rapid inactivators (Evans *et al.*, 1960; Gangadharam *et al.*, 1961a). In slow inactivators the serum concentrations of the active drug decrease only gradually after a dose, but in rapid inactivators the drug concentrations fall more quickly as a result of conversion of free isoniazid to biologically inactive compounds (Fig. 4).

FIG. 3.—Viable counts on *Mycobacterium tuberculosis*, strain H37Rv, in Tween-albumin liquid medium after exposure for one or four days to isoniazid 1 μg./ml. ↑=Addition of isoniazid; ↓=Removal of isoniazid by repeated washing on a cellulose membrane filter.

Table I shows the progress of patients treated with isoniazid alone in various dosages and with isoniazid plus *p*-aminosalicylic acid (P.A.S.) at the Tuberculosis Chemotherapy Centre, Madras (Gangadharam *et al.*, 1961b); the results of a co-operative study by the Veterans Administration of the United States (Harris, 1961) are also illustrated. The patients are classified as rapid or slow inactivators, and the length of time in each day that a bacteriostatic concentration of isoniazid (0.2 μg./ml. or more) was present in the serum is also indicated as the period of coverage. For the patients in Madras this period was estimated from isoniazid serum curves on a sample of the patients. For the patients in the Veterans study approximate estimates of the coverage period were obtained by application of the Madras data. It is evident that the period of coverage is often much shorter for the rapid than for the slow inactivators. If multiplication of the bacilli occurred between doses, patients whose coverage was incomplete would fare less well than those in which it was complete. Yet the progress of the patients was not related to the period of coverage, nor did the slow inactivators fare appreciably better than the rapid inactivators. Thus it seems probable that, even if isoniazid is present intermittently in the lesions, the bacilli do not multiply in the intervening period.

FIG. 4.—Serum concentration of isoniazid in slow and rapid inactivators after an oral dose of isoniazid 100 mg. plus sodium P.A.S. 5 g. Data from Gangadharam *et al.* (1961b).

TABLE I.—Response to Treatment in Rapid and Slow Inactivators of Isoniazid Related to Period that Minimal Concentrations of Isoniazid were Maintained in Serum

Regimen of Treatment	Rate of Inactivation of Isoniazid	Period of Coverage (hrs.)*	Total Patients	Percentage Patients with Favourable Response
Isoniazid 100 mg.† twice daily	{ Rapid Slow	4–12 26 or more	36 46	44 48
Isoniazid 200 mg.† twice daily	{ Rapid Slow	24–26 26 or more	27 39	56 59
Isoniazid 400 mg.† once daily	{ Rapid Slow	12–18 26 or more	32 36	66 72
Isoniazid 100 mg. + sod. P.A.S. 5 g.† twice daily	{ Rapid Slow	12–20 26 or more	26 57	85 95
Isoniazid 100 mg. + sod. P.A.S. 4 g. thrice daily	{ Rapid Slow	18–26 26 or more	99 126	95 94

* Period that 0.2 μg. or more of isoniazid per ml. was present in the serum after one full day's dosage.

† Dose for a 100-lb. (45-kg.) patient. Dosage adjusted to weight of patient.

Top four regimens from Gangadharam *et al.* (1961b): favourable response = bacteriologically quiescent disease at 12 months.

Bottom regimen from Harris (1961): favourable response = negative cultures at five and six months.

Finally, if sensitive bacilli continue to multiply during treatment some patients would be expected to remain consistently positive and to fail to respond to treatment while having drug-sensitive organisms in their sputum. In practice, experience from several studies (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960) shows that patients who remain consistently sputum-positive almost always have isoniazid-resistant organisms; the only exception to this rule is the very occasional patient who has in all probability been taking his medication in a grossly irregular manner, and may therefore have had quite long periods without the drug being present in the lesions (Medical Research Council, 1962).

These findings thus strongly suggest that appreciable multiplication of sensitive bacilli does not occur during treatment with isoniazid. Similar though slightly less convincing evidence for streptomycin has been reviewed elsewhere (Canetti, 1954; Mitchison, 1954, 1962a). A more recent experiment (Fig. 5) illustrates the inhibition of growth after exposure to streptomycin (Dickinson and Mitchison, 1965). As in Fig. 3, showing the action of isoniazid, the cultures were exposed to

FIG. 5

FIG. 5.—Viable counts on *M. tuberculosis*, strain H37Rv, in Tween-albumin liquid medium after exposure for one or four days to streptomycin 5 µg./ml. = Addition of isoniazid. ↓ = Removal of isoniazid by repeated washing on a cellulose membrane filter. FIG. 6.—Viable counts on *M. tuberculosis*, strain H37Rv, in Tween-albumin liquid medium during exposure to sodium P.A.S. 100 µg./ml.

ments P.A.S. sometimes completely prevented growth, though it never appeared to be truly bactericidal.

The mode of action of the second-line drugs is more uncertain. Ethionamide has many similarities to isoniazid *in vitro* and in animal experiments (Rist, 1960). From considerations of the blood concentrations attained in man, it seems likely that it prevents multiplication of sensitive organisms in the great majority of patients when the dose is 0.75 to 1 g./day. How-

FIG. 6

the drug for one or four days, and were then washed free of it. Growth was inhibited for several days after exposure.

The action of P.A.S. is, however, different from the action of isoniazid and streptomycin. Fig. 6 shows the results of viable counts done on cultures during exposure to P.A.S., which was not removed by washing and retained its microbiological activity throughout the experiment (Singh, 1954). Even though a high concentration of P.A.S. was present, the growth of sensitive bacilli was greatly delayed, but not completely prevented as with isoniazid or streptomycin. The action of P.A.S. is, however, rather variable in the test-tube; in other experi-

ever, thiocetazone, pyrazinamide, cycloserine, and viomycin may not be capable of full suppression of growth when given in their usual dosage to patients.

Emergence of Drug-resistant Bacilli

Strains of tubercle bacilli may be obtained during chemotherapy which are inhibited by higher concentrations of the drug than were the strains isolated before treatment. These resistant strains have been shown to emerge during treatment with every drug used in the chemotherapy of tuberculosis. According to genetic theory they originate from mutant bacilli present in very small numbers in the strain before it has come into contact with the drug (see Mitchison, 1962a). During treatment with a single drug the mutant bacilli multiply and replace the sensitive organisms (Fig. 7). The very fact that they multiply separates them in their behaviour from the sensitive organisms, which, as we have seen, probably do not multiply, and demonstrates that further treatment with the drug is unlikely to hinder their growth. Furthermore, the existence of any degree of resistance implies growth of the resistant bacilli in the lesions, so that the same failure to respond is to be expected during subsequent treatment whether the degree of resistance is high or low. This theoretical prediction has been confirmed in patients treated with isoniazid alone at the Tuberculosis Chemotherapy Centre, Madras (1960). Cultures were made from the sputum of these patients at monthly intervals during chemotherapy. Of the total of 305 patients, 185 yielded only sensitive organisms, and all had a favourable response (Table II). The remaining 120 patients produced at least one resistant culture during treatment. The degree of resistance of the first resistant culture isolated from each of these patients is shown in Table II, related to

FIG. 7.—Diagrammatic representation of changes in the bacterial population in pulmonary lesions during chemotherapy and the findings in sensitivity tests. Top left: The emergence of isoniazid resistance during treatment with isoniazid alone. Top right: Successful treatment with isoniazid plus streptomycin: the bacterial action of streptomycin on isoniazid-resistant mutant bacilli. Bottom left: Transitional resistance preceding sputum conversion: the bacteriostatic action of P.A.S. on isoniazid-resistant mutant bacilli. Bottom right: Relapse during treatment occurring as a result of slow growth of isoniazid-resistant bacilli. ----: Number of bacilli in lungs below which bacilli are not detectable in the sputum.

the progress of the patient during further chemotherapy with isoniazid. No association is evident between the degree of resistance of the culture and the response of the patients.

TABLE II.—Degree of Resistance to Isoniazid of First Resistant Culture Related to Progress During Treatment with Isoniazid Alone

M.I.C.* of First Resistant Culture ($\mu\text{g./ml.}$)	No. of Patients	Patients with Favourable Response	
		No.	%
1	23	5	22
5	40	8	20
50	28	4	14
> 50	29	7	24
Total with resistant culture	120	24	20
Total with only sensitive cultures	185	185	100

* The minimal concentration of isoniazid inhibiting growth of 20 or more colonies. Tests were done on slopes of Löwenstein-Jensen medium containing 0, 0.2, 1, 5, and 50 $\mu\text{g.}$ isoniazid/ml. Data from Selkon *et al.* (1964).

The administration of two drugs in treatment is usually successful in preventing the emergency of resistance because each drug prevents the growth of mutants resistant to the other drug (Fig. 7). There is, however, one circumstance in which resistant strains may be isolated during treatment without previous multiplication of the bacilli that composed them. When the initial bacterial population is large there may be appreciable numbers of resistant mutants at the start of treatment. These mutants are killed less rapidly than the sensitive organisms, since they are acted on by only one of the two drugs. If the second drug is purely bacteriostatic, like P.A.S., killing is likely to be particularly slow, as it is due only to the patient's defence mechanism (Fig. 7). Thus, as treatment continues, the proportion of resistant to sensitive organisms increases until the strain may appear resistant in a sensitivity test. Nevertheless, the resistant mutants are prevented from growing or are killed by the second drug, and the sputum cultures eventually become negative. This type of "transitional" resistance immediately precedes sputum conversion, and does not carry a bad prognosis.

Studies at Madras have shown that when patients are treated with isoniazid plus P.A.S., about half of those who yield a resistant culture at any time during treatment eventually attain bacteriologically quiescent disease, and the emergence of resistance in these patients is of the transitional type (Selkon *et al.*, 1964). If more efficient chemotherapy is given, transitional resistance becomes increasingly important. Thus, in a recent co-operative study under the auspices of the International Union Against Tuberculosis (Bignall, 1964), the patients were treated with triple chemotherapy (isoniazid, streptomycin, and P.A.S.) for the first six months, and all of the patients with resistant cultures at six months eventually became sputum-negative.

Various Regimens of Treatment

Table III summarizes the therapeutic efficacy of various chemotherapy regimens in newly diagnosed patients with organisms sensitive to isoniazid at the start of treatment. Patients with a favourable response to treatment have been defined as those with bacteriologically quiescent disease at 12 months, but they also include a few with a single positive culture in the last few months of the year of treatment.

Treatment with isoniazid alone was least successful; the proportion of patients with a favourable result ranged from 44% of 86 treated with a low dosage to 68% of 211 treated with a moderate or high dosage of isoniazid. A double-drug regimen of isoniazid plus P.A.S. or isoniazid plus thiacetazone was considerably more successful, the proportion of patients showing favourable results ranging from 85% to 89%. The regimen in which an initial period of triple-drug chemotherapy (streptomycin, P.A.S., and isoniazid) was given before a change

to double-drug treatment was the most successful, 99% of the 581 patients responding favourably.

TABLE III.—Efficacy of Various Regimens of Treatment

Regimen of Treatment		Total Patients	Patients with Favourable Response*		Reference
Dose of Drugs	No. of Doses/Day		No.	%	
Isoniazid 100 mg.†	2	86	38	44	Tuberculosis Chemotherapy Centre, Madras (1960)
Isoniazid 200 mg.†	2	66	38	58	" " "
Isoniazid 400-650 mg.†	1	211	144	68	Tuberculosis Chemotherapy Centre, Madras (1960, 1963a, 1963b)
Isoniazid 100 mg.† + sod. P.A.S. 5 g.	2	314	274	87	Tuberculosis Chemotherapy Centre, Madras (1959, 1960, 1965) E. African/British Medical Research Council (1963a)
	2	176	150	85	
Isoniazid 300 mg. + thiacetazone 150 mg.	1	64	57	89	" " " "
Isoniazid 100 mg. + streptomycin 1 g. + P.A.S. 4 g. for 6 mths, then isoniazid + P.A.S.‡	2	581	574	99	Bignall (1964)
	1				
	3				

* Including patients with a doubtful response.

† Dose for 100-lb. (45-kg.) patient. Dose adjusted to weight of patient.

‡ Minimum requirement.

The reason for the low percentage of patients with a successful result in the regimens with isoniazid alone is clear. All patients who failed to attain bacteriologically quiescent disease yielded isoniazid-resistant organisms during treatment. However, the reason why the efficacy of the regimen increased with the size of the dose of isoniazid is less obvious. The poor results with the low dosage do not seem to be due to failure of inhibition of sensitive organisms at the start of treatment, since resistant organisms were obtained from all of the patients, and since further analyses (not tabulated here) indicated that the sensitive organisms were eliminated at the same rate, irrespective of the isoniazid dosage. An explanation is perhaps provided by examining the degree of resistance of the first resistant culture obtained from the patients (Table IV). It is

TABLE IV.—Degree of Resistance of the First Resistant Culture from Patients Treated with Various Dosages of Isoniazid Alone

Size of Single Dose of Isoniazid* ($\mu\text{g.}$)	Total Patients	Patients With Only Sensitive Cultures		Patients with Resistant Cultures					
				Minimal Concentration of Isoniazid Inhibiting First Resistant Culture ($\mu\text{g./ml.}$)†					
		No.	%	1		5		50 or More	
400	69	39	57	3	4	13	19	14	20
200	67	34	51	5	7	9	13	19	28
100	84	27	32	15	18	18	21	24	29

* For a 100-lb. (45-kg.) patient. Dosage adjusted to weight of patient.

† Sensitivity tested on Löwenstein-Jensen slopes containing 0.2, 1, 5, and 50 $\mu\text{g.}$ isoniazid/ml. Data from Selkon *et al.* (1965).

evident that the proportion of patients yielding strains with high degrees of resistance (minimum inhibitory concentration of 50 $\mu\text{g./ml.}$ or more of isoniazid) is similar in the two treatment regimens with the lower isoniazid doses, and is only slightly lower with the highest dose. However, as the size of the dose of isoniazid increased, the proportion of patients who never yielded a resistant culture also became larger, and, correspondingly, the proportion of patients who yielded strains with low degrees of resistance (M.I.C. of 1 $\mu\text{g./ml.}$ isoniazid) decreased. Thus it seems probable that an increased dose of isoniazid is more efficacious because it suppresses the growth of mutants with relatively low degrees of resistance.

The finding that isoniazid plus P.A.S. or isoniazid plus thiacetazone is more effective than isoniazid alone may be explained by the inhibitory action of P.A.S. or thiacetazone on isoniazid-resistant mutants. However, some patients treated with the double-drug regimen failed to attain bacteriologically

quiescent disease, and their organisms became resistant to isoniazid. Failure of these regimens is probably due to multiplication of isoniazid-resistant mutants occasionally occurring in the presence of P.A.S. or thiacetazone. As previously noted, P.A.S. is probably not as successful as isoniazid or streptomycin in completely inhibiting growth. When the dose of thiacetazone was reduced from 150 mg. to 100 mg., given in each case with the same dose of isoniazid, the proportion of patients with a favourable response decreased from 89% to 59% (East African/British Medical Research Council, 1963a). It is therefore reasonable to suppose that even the 150-mg. dose is not completely effective in suppressing growth of isoniazid-resistant mutants.

The regimen which started with triple-drug chemotherapy yielded the best results. Five of the seven patients who had an unfavourable response died in the first eight weeks of treatment before chemotherapy could have its full effect; in only two patients was there a suggestion that the regimen itself had failed. Improved results with a regimen starting with triple-drug treatment may be due to several mechanisms. First, the number of resistant mutants may be so greatly reduced by the bactericidal action of the drugs to which they are sensitive that there is little chance of these organisms growing again during the subsequent phase of less efficient double-drug treatment. Secondly, even though the strains concerned are regarded as drug-sensitive by conventional criteria, a minor tendency for strains to be slightly resistant to one or other of the drugs at the start of treatment might result in a failure to respond if two drugs only were used, whereas the administration of a third drug would tend to prevent the growth of organisms. Thirdly, there is the interesting possibility that inhibition of the growth of sensitive organisms does not occur immediately at the start of treatment. Even a few bacterial divisions at this time would substantially increase the number of resistant mutants present, and would therefore increase the chance of a failure to respond. The addition of a third drug might decrease the extent of this initial growth. If this factor is of importance the addition of a third drug to the regimen might prove of value even if it were given only for a few days.

At this point it should be noted that, although the addition of streptomycin to a regimen of isoniazid plus P.A.S. improves the response (Medical Research Council, 1962), there is no evidence that treatment with the triple-drug regimen is superior to treatment with streptomycin plus isoniazid. It may be that the simple substitution of streptomycin for P.A.S. or thiacetazone might be as effective as the triple-drug combination.

Intermittent Chemotherapy

Antibacterial drugs are usually prescribed in several doses during the day, with the exception that streptomycin is given in a single daily dose. However, the study at Madras on patients treated with isoniazid alone (Tuberculosis Chemotherapy Centre, Madras, 1960) showed that a total daily dose of 400 mg. of isoniazid was more effective when given in a single dose each day than when given as two doses of 200 mg. in the day. The results of this study, as well as other related evidence from animal experiments (Corper and Cohn, 1947; Palmer *et al.*, 1956; Bloch, 1961; Canetti *et al.*, 1963; Grumbach *et al.*, 1964), suggested that it would be of value to explore intermittent chemotherapy, in which the interval between the doses was greater than one day. Several advantages accrue from intermittent dosage. The drugs can be given fully supervised, thus eliminating irregularity in drug-taking among patients who otherwise co-operate in their treatment. The total amount of drug administered is usually smaller, thus decreasing the cost of the regimen. In the developing countries the widespread use of chemotherapy is often limited by the cost of the drugs (Fox, 1964). Furthermore, a smaller total dose of the

drug might diminish the risk of certain types of toxicity, such as vestibular damage due to streptomycin and peripheral neuropathy due to isoniazid (Mitchison, 1962b).

A study has been carried out at Madras comparing a standard regimen of isoniazid plus P.A.S. with a regimen in which isoniazid in a large dose and streptomycin were given twice weekly (Tuberculosis Chemotherapy Centre, Madras, 1963c). The results are summarized in Table V. As measured by radiographic improvement, attainment of bacteriologically quiescent disease at 12 months and by the emergence of isoniazid-resistant strains, the intermittent regimen appears at least as good as the standard regimen of isoniazid plus P.A.S. Further studies are in progress in which the interval between the doses has been extended to one week.

TABLE V.—Comparison of Treatment with Twice-daily Isoniazid Plus P.A.S. and Twice-weekly Isoniazid Plus Streptomycin

Assessment of Progress at 12 Months	Treatment Series			
	Isoniazid 100 mg. + sod. P.A.S. 5 g.* Twice Daily		Isoniazid 650 mg. + streptomycin 1 g.* Twice Weekly	
	No. of Patients	%	No. of Patients	%
Considerable or greater radiographic improvement	39	59	44	61
Bacteriologically quiescent disease	56	85	68	94
Isoniazid-resistant culture	5	8	2	3
Total patients	66	100	72	100

* Dose for a 100-lb. (45-kg.) patient. Dosage adjusted to weight of patient.

As we are in the process of exploring the use of intermittent chemotherapy in man it is perhaps worth commenting on one matter of relevant theory. As with normal "continuous" chemotherapy, the key question still seems to be whether the intermittent regimen will completely suppress the multiplication of the organisms. If growth begins soon after the drug concentrations in the lesions fall below those necessary for bacteriostasis we would expect at least two bacterial divisions to have occurred between each dose of the twice-weekly regimen. The number of resistant mutants would then increase at a rapid rate, partly because they would have appeared afresh from the population of dividing sensitive bacilli, and partly because the mutants present at the beginning of treatment would also have multiplied. Looked at from another aspect, one of the most efficient ways of producing resistant strains *in vitro* is to alternate periods of growth with periods of exposure to the drug. Since drug-resistant strains only rarely emerged in the patients treated with the intermittent regimen (Table V), it seems highly probable that no appreciable growth of sensitive organisms occurred during treatment in the majority of patients. Clearly there must be a limit to the extent that the interval between doses can be stretched; this limit is presumably the length of time during which the organisms remain inhibited after exposure to the drug.

Initial Drug Resistance

Some factors affecting the response to chemotherapy, such as the virulence of the bacilli and the influence of corticosteroids, are concerned with the operation of the patient's defence mechanisms during chemotherapy. If potent chemotherapy prevents the multiplication of organisms in the lesions, factors such as these which affect the growth in *untreated* patients should become less important as the efficacy of the drug regimen is increased. Other factors, such as the initial drug-sensitivity of the bacilli or their numbers in the lesions, concern the reaction between the drugs and the bacilli and are likely to be of greater importance even when the regimen of chemotherapy is fairly efficient. Table VI summarizes the response to treatment of patients whose organisms were initially either

sensitive or resistant to the drug used. When the patients were treated with a single drug, in this case isoniazid alone, the response was much less satisfactory in patients with initially resistant strains than in those with sensitive strains. When the patients were treated with two drugs, isoniazid plus P.A.S. or isoniazid plus thiacetazone, the progress of those with initial isoniazid resistance was still much less satisfactory than the progress of those with initially sensitive organisms. These findings are to be expected, since neither P.A.S. nor thiacetazone is, by itself, capable of prolonged inhibition of isoniazid-resistant organisms present in large numbers at the start of treatment. Furthermore, resistance to P.A.S. and thiacetazone emerged rapidly in the majority of patients with initial isoniazid resistance (East African/British Medical Research Council, 1963b).

TABLE VI.—Initial Drug Sensitivity of Organisms Related to Progress of Patients During Chemotherapy

Treatment Regimen	Pretreatment Sensitivity of Organisms	Total Patients	Patients With Favourable Response		Reference
			No.	%	
INH alone	INH-sensitive	359	219	61	Tuberculosis Chemotherapy Centre, Madras (1960, 1963a, 1963b)
	INH-resistant	26	7	27	
INH + P.A.S.	INH-sensitive	314	273	87	Tuberculosis Chemotherapy Centre, Madras (1959, 1960, 1965)
	INH-resistant	21	5	24	
INH + thiacetazone (TBI)	INH-sensitive	176	146	83	E. African/British Medical Research Council (1963b)
	INH-resistant	21	11	52	
	INH-sensitive	304	220	72	
	INH-resistant	39	15	38	
	TBI-sensitive	162	124	77	
	TBI-doubtful	53	36	68	
	TBI-resistant	10	5	50	

INH = Isoniazid.

When we examine the influence of initial sensitivity to thiacetazone on the response of the patients, a small but definite association is found, indicating that patients with the more resistant strains fare less well. The association between thiacetazone sensitivity and response should be less than the association between isoniazid sensitivity and response, because treatment with isoniazid alone is much more potent than treatment with thiacetazone alone.

There is unfortunately little reliable evidence on the influence of initial resistance during triple-drug chemotherapy. In the study of the International Union Against Tuberculosis (Bignall, 1964) there was some suggestion that resistance to one of the three drugs used did not result in any substantial reduction in the efficacy of the regimen, whereas resistance to two or three drugs carried a less satisfactory prognosis. Such a finding is to be expected, since there would still have been two drugs to suppress growth of organisms resistant to only one of the three drugs used.

Severity of the Disease on Admission to Treatment

Table VII shows examples of the associations found between the assessments and the progress of the patient, as measured by the attainment of bacteriologically quiescent disease at one year. When treatment with isoniazid alone was given at Madras, patients with extensive cavitation at the start of treatment fared less well than those with smaller degrees of cavitation. In patients treated with isoniazid plus P.A.S. the association is less evident.

In the International Union study, where triple-drug chemotherapy was given for six months, the methods of assessment of the pretreatment x-ray films were different. An unfavourable response was shown by 1 (2%) of 66 patients with no initial cavitation, and by 6 (3%) of 237 patients with cavitation. Thus, as the efficacy of the regimen increases, the association between pretreatment cavitation and the progress of the patients becomes weaker. With regard to the bacterial content of the sputum, patients with large numbers of organisms before treatment more often have an unfavourable response than patients with smaller numbers of organisms (Table VII). The association is also weaker in patients treated with isoniazid plus P.A.S. than with isoniazid alone.

There are two possible explanations for these associations. First, large cavities tend to contain enormous numbers of tubercle bacilli growing in the cavity walls. The larger the population of bacilli the greater the number of resistant mutants to be expected. Since the mutant bacilli are the source of drug-resistant strains that later may emerge and be responsible for a failure to respond to chemotherapy, the chance of an unfavourable response would be greater in those with large bacterial populations at the start of treatment. Similarly, the bacterial content of the sputum probably measures the size of the bacterial population within the lungs. Secondly, the growth of resistant organisms may be less inhibited by the patient's defence mechanisms in a large cavity than in a smaller cavity or in non-cavitary exudate. In this explanation, but not the first, the operation of the host's defence mechanisms may play a part. It is not possible to say which of these two mechanisms is more important. However, there is no need to postulate that the drugs fail to penetrate into cavities.

Virulence of Tubercle Bacilli

The virulence of the organisms appears to be a factor affecting the response to treatment that is entirely concerned with the reaction between the host and the bacillus. Strains of tubercle bacilli from Indian patients were found to vary in their virulence in the guinea-pig to a much greater extent than British strains (Mitchison *et al.*, 1960; Bhatia *et al.*, 1961). Virulence tests in guinea-pigs were done on pretreatment strains from patients in the Madras study, who were then given

TABLE VII.—Response to Treatment Related to Various Assessments on Admission

Pretreatment Assessment				Isoniazid Alone*			Isoniazid + P.A.S.†			Isoniazid + Streptomycin + P.A.S. (6 mths.), then Isoniazid + P.A.S.‡		
				No. of Patients	Unfavourable Response		No. of Patients	Unfavourable Response		No. of Patients	Unfavourable Response	
					No.	%		No.	%		No.	%
Extent of cavitation	Extensive	..	..	34	27	79	73	15	21	237§ 57¶	6	3
	Moderate	..	..	179	75	42	99	8	8		1	2
	Slight or nil	..	..	146	36	25	73	4	5		—	—
Bacterial content of sputum	3+ or 2+	..	..	252	116	46	170	24	14	—	—	—
	1+ or 0	..	..	105	22	21	75	3	4	—	—	—
Guinea-pig virulence of bacilli	High	..	..	65	34	52	21	0	0	—	—	—
	Moderate	..	..	79	34	43	36	6	17	—	—	—
	Low	..	..	44	17	38	22	2	9	—	—	—

* Tuberculosis Chemotherapy Centre, Madras, (1960, 1963a, 1963b).

† Tuberculosis Chemotherapy Centre, Madras (1959, 1960).

‡ Bignall (1964).

§ Cavitation present

¶ No cavitation.

isoniazid alone or isoniazid plus P.A.S. (Ramakrishnan *et al.*, 1961). In the patients treated with isoniazid alone an association was found between the virulence of the strains isolated at the start of treatment and the patients' progress during chemotherapy (Table VII). An unfavourable response was obtained in 52% of 65 patients with strains of high virulence and in 38% of 44 patients with strains of low virulence. This association, though statistically significant, is much weaker than the associations between the extent of initial cavitation and response or between the bacterial content of the sputum and response. No association was found between virulence and response when treatment with isoniazid plus P.A.S. was given (Table VII). These associations were not affected by the condition of the patient on admission to the study, since no relation was found between virulence and the severity of the disease before treatment (Ramakrishnan *et al.*, 1961).

Corticosteroids

Corticosteroids depress the immune response in experimental tuberculosis of mice and rabbits (Hart and Rees, 1950; Lurie *et al.*, 1951). In man their administration in the absence of chemotherapy may result in the lighting up of fresh tuberculosis or deterioration of established disease (American Trudeau Society, 1952). For instance, Des Autels *et al.* (1956) found that deterioration occurred in 12 of 13 patients given A.C.T.H. or cortisone and little or no effective chemotherapy. However, a study conducted by the Tuberculosis Society of Scotland (1960) showed that prednisolone was of some benefit when efficient triple-drug chemotherapy was given at the same time. During the first few months of treatment radiographic improvement and sputum conversion occurred more rapidly in the patients given prednisolone and chemotherapy than in those given chemotherapy alone (Fig. 8). However, the eventual results at 12 months were similar in the two series.

FIG. 8.—Radiographic improvement and results of sputum cultures during treatment with prednisolone and triple-drug chemotherapy. P: Series of 87 patients given prednisolone 20 mg. (for first three months) and isoniazid 200 mg. streptomycin 1 g., plus P.A.S. 10 g. daily. C: Series of 91 patients given chemotherapy only. (Data from Tuberculosis Society of Scotland Research Committee, 1960.)

These favourable results in the prednisolone series might be due to a greater reduction in inflammatory exudate under the influence of the corticosteroid, leading to more rapid clearing of x-ray opacities and to a more rapid elimination of persisting sensitive bacilli from the cavities. It is also possible that the persisting sensitive bacilli might have started to grow, making them more susceptible to the bactericidal action of isoniazid and streptomycin. Whatever the explanation, it is evident that an alteration in the immune response of the patients which is harmful in the absence of effective chemotherapy seems to be slightly beneficial in its presence.

When we consider the influence of these various factors that may affect the outcome of chemotherapy it is evident that as

the potency of the drug regimens increases so the influence of all of the factors becomes less. This is particularly clear when we look at the virulence of the bacillus or the concomitant administration of corticosteroids, both of which involve the effect of the host's defence mechanisms on the bacillus during treatment. Factors concerning the reaction between the drug and the bacillus, particularly drug sensitivity, are of greater importance even when moderately efficient chemotherapy is given. However, with the triple-drug regimen used in the International Union study the only factor which definitely resulted in a less satisfactory result was the presence of pre-treatment resistance to more than one drug. Similar highly efficient regimens are of course in general use in Great Britain, and they are also to be recommended for other countries which can afford the high cost of the drugs. Yet tuberculosis is predominantly a disease of poverty, and poor countries are often forced to use less efficient methods of treatment on grounds of economy. For such countries the best policy may be to allocate regimens of varying grades of efficacy according to the type of disease encountered in the patient. In these circumstances assessments of the extent of cavitation in the pretreatment radiographs and the bacterial content of the sputum continue to be of great value. For instance, regimens starting with initial triple-drug therapy could be reserved for patients with considerable cavitation and heavily positive sputum, while double-drug treatment or even treatment with isoniazid alone in a dosage of 400 mg. a day might be used for patients with less severe disease.

Conclusions

The phenomena encountered during the chemotherapy of tuberculosis may be explained on the basis of a fairly simple hypothesis, that multiplication of sensitive tubercle bacilli stops soon after the start of chemotherapy with potent drugs such as isoniazid or streptomycin, and that the emergence of drug-resistant strains, when this occurs, is due to the continued multiplication of mutant organisms present at the start of treatment. Unsuccessful treatment results from the emergence of drug-resistance, and this may be prevented by the use of a second drug in the treatment. However, some of the drugs used to accompany isoniazid or streptomycin are not completely effective in suppressing multiplication, and some varieties of double-drug treatment therefore fail in a small proportion of cases. The use of triple-drug treatment, including isoniazid and streptomycin, appears to produce almost uniformly satisfactory results, with the principal exception of a few patients who die in the early weeks as a consequence of the disease present before the drugs were started. The remarkable success of this type of treatment is one of the strongest arguments that bacterial multiplication does in fact stop very soon after the beginning of chemotherapy.

If we accept these hypotheses then certain conclusions follow. Host factors, such as the type of disease when treatment is started and the patient's immune response, become of decreasing importance as the potency of drug treatment increases. The killing of the bacilli is being done by the drugs and not by the patient. I have attempted to demonstrate that this conclusion is in fact supported by the findings in clinical studies. We must therefore conclude that many accepted measures in the treatment of tuberculosis are becoming increasingly irrelevant in an age when potent chemotherapy can be given. In particular, treatment by surgery, by prolonged rest in bed, and by dietary supplements is usually unnecessary and serves only to prolong the patient's incapacity and to increase the cost of his treatment. Furthermore, much of the familiar apparatus for the control of treatment also loses its original purpose. Simple bacteriological methods of smear and culture examination are adequate for assessing the response to treatment. The addition of complex sensitivity tests during treatment may only confuse

the assessment, because resistant strains are often of the transitional type and are followed by sputum conversion. Although radiography plays a part in the diagnosis of tuberculosis, it is rarely necessary as an adjunct to treatment. Simplification in treatment allows the emphasis to be put where it belongs, on the administration of an effective drug combination for a sufficient length of time and on seeing that it has been taken by the patient.

One of the merits of theoretical explanation of the mechanisms occurring during chemotherapy is the light that it throws on further developments in the field. Clearly, a need still exists for a new drug that approaches isoniazid in high potency, low toxicity, and low cost. The discovery of such a drug would mean that we could stop much of the present use of P.A.S. and streptomycin. Both of these drugs are toxic, are expensive, and have particular disadvantages; P.A.S. does not always suppress bacterial growth, while streptomycin has to be given by injection. Of more immediate importance, we need to explore the balance between the initial phase of chemotherapy, in which the growth of bacilli is halted and the majority of the organisms are rapidly killed, and the later phase in which the residual persisting bacilli are only gradually eliminated. Already there is evidence (Grumbach, 1962; Medical Research Council, 1962; Bignall, 1965) that chemotherapy needs to be more intensive in the early phase than in the later phase, but we do not know how long the early intensive phase should be. There is some suggestion from the theoretical mechanisms postulated and from experimental studies in animals (Grumbach *et al.*, 1964) that it might be much shorter than is customary.

Finally, we need to examine the use of intermittent chemotherapy. Here again the theoretical model suggests that the use of an initial short period of continuous high-dosage chemotherapy might stop bacterial multiplication abruptly, and allow a subsequent long period of intermittent chemotherapy to be extremely successful in maintaining bacteriostasis. Furthermore, drugs other than isoniazid and streptomycin might be given intermittently. In selecting a drug for clinical trial the decisive factor may well be the length of time during which the bacilli continue to be inhibited after a temporary exposure to the drug (Dickinson and Mitchison, 1965).

Summary

In pulmonary tuberculosis chemotherapy with isoniazid, and probably streptomycin, completely prevents further growth of sensitive tubercle bacilli early in treatment. Viable sensitive bacilli obtained from the sputum during treatment appear to be the remnants of the original population which have persisted in a dormant state. Neither *p*-aminosalicylic acid nor thiacetazone inhibits growth so completely, and the occasional failure of a double-drug regimen of isoniazid plus P.A.S. or isoniazid plus thiacetazone is due to the growth of isoniazid-resistant mutants in the presence of these drugs. Triple-drug therapy (or possibly isoniazid plus streptomycin) is highly successful in preventing growth of sensitive bacilli and resistant mutants; the occasional resistant strain isolated during treatment is not due to growth of mutants and does not carry a poor prognosis. Factors such as initially severe disease and the concomitant

administration of corticosteroids, which result in a poor response when inefficient chemotherapy is given, are of little prognostic importance with potent drug treatment; it is the drugs that kill the bacilli and not the patient's immune response. In consequence, measures of treatment other than efficient chemotherapy are largely unnecessary, and the control of treatment should be based on smear and culture examinations of sputum.

REFERENCES

- American Trudeau Society (1952). *Amer. Rev. Tuberc.*, **66**, 254.
 Barclay, W. R., Ebert, R. H., LeRoy, G. V., Manthei, R. W., and Roth, L. J. (1953). *J. Amer. med. Ass.*, **151**, 1384.
 Bartmann, K., and Freise, G. (1963). *Beitr. Klin. Tuberk.*, **127**, 546.
 Bhatia, A. L., Cillag, A., Mitchison, D. A., Selkon, J. B., Somasundaram, P. R., and Subbaiah, T. V. (1961). *Bull. Wld Hlth Org.*, **25**, 313.
 Bignall, R. (1964). *Bull. int. Un. Tuberc.*, **74**, 79.
 Bloch, H. (1961). *Amer. Rev. resp. Dis.*, **84**, 824.
 Canetti, G. (1954). *Bull. int. Un. Tuberc.*, **24**, 146.
 — Grumbach, F., and Grosset, J. (1963). *Tubercle (Lond.)*, **44**, 236.
 — and Grosset, J. (1958). *Rev. Tuberc. (Paris)*, **22**, 778.
 Claunich, B. C., Castro, V., and Barnes, W. T. (1963). *Transactions of the 22nd Research Conference in Pulmonary Diseases*, p. 37. Veterans Administration, Washington, D.C.
 Corper, H. J., and Cohn, M. L. (1947). *Science*, **106**, 446.
 Des Autels, E. J., Zvetina, J. R., Berg, G. S., Fershing, J., and Freeman, S. (1956). *Dis. Chest*, **30**, 486.
 Dettli, L. (1961). *Schweiz. med. Wschr.*, **91**, 921.
 Dickinson, J. M., and Mitchison, D. A. (1965). In preparation.
 East African/British Medical Research Council (1963a). *Tubercle (Lond.)*, **44**, 301.
 — (1963b). *Ibid.*, **44**, 393.
 Evans, D. A. P., Manley, K. A., and McKusick, V. A. (1960). *Brit. med. J.*, **2**, 485.
 Fox, W. (1964). *Ibid.*, **1**, 135.
 Gangadharan, P. R. J., Bhatia, A. L., Radhakrishna, S., and Selkon, J. B. (1961a). *Bull. Wld Hlth Org.*, **25**, 765.
 — Devadatta, S., Fox, W., Nair, C. N., and Selkon, J. B. (1961b). *Ibid.*, **25**, 793.
 Grumbach, F. (1962). *Amer. Rev. resp. Dis.*, **86**, 211.
 — Ganetti, G., and Grosset, J. (1964). *Tubercle (Lond.)*, **45**, 125.
 Harris, H. W. (1961). *Transactions of the 20th Research Conference in Pulmonary Diseases*, p. 39. Veterans Administration, Washington, D.C.
 Hart, P. D., and Rees, R. J. W. (1950). *Lancet*, **2**, 391.
 Lurie, M. B., Zappasodi, P., Dannenberg, A. M., and Swartz, I. B. (1951). *Science*, **113**, 234.
 McDermott, W. (1958). *Yale J. Biol. Med.*, **30**, 257.
 Mackaness, G. B., and Smith, N. (1952). *Amer. Rev. Tuberc.*, **66**, 125.
 Medical Research Council (1962). *Tubercle (Lond.)*, **43**, 201.
 Mitchison, D. A. (1952). *Lancet*, **2**, 858.
 — (1954). *Brit. med. Bull.*, **10**, 115.
 — (1962a). *Ibid.*, **18**, 74.
 — (1962b). In *Drugs, Parasites and Hosts*, edited by L. G. Goodwin and R. H. Nimmo-Smith, p. 267. Churchill, London.
 — and Selkon, J. B. (1956). *Amer. Rev. Tuberc.*, **74**, Pt. 2, p. 109.
 — Wallace, J. G., Bhatia, A. L., Selkon, J. B., Subbaiah, T. V., and Lancaster, M. C. (1960). *Tubercle (Lond.)*, **41**, 1.
 Palmer, C. E., Ferebee, S. H., and Hopwood, L. (1956). *Amer. Rev. Tuberc.*, **74**, 917.
 Ramakrishnan, C. V., Bhatia, A. L., Fox, W., Mitchison, D. A., Radhakrishna, S., Selkon, J. B., Subbaiah, T. V., Velu, S., and Wallace, J. G. (1961). *Bull. Wld Hlth Org.*, **25**, 323.
 Rist, N. (1960). *Advanc. Tuberc. Res.*, **10**, 69.
 Selkon, J. B., Devadatta, S., Kulkarni, K. G., Mitchison, D. A., Narayana, A. S. L., Nair, C. N., and Ramchandran, K. (1964). *Bull. Wld Hlth Org.*, **31**, 273.
 Schaefer, W. B. (1954). *Amer. Rev. Tuberc.*, **69**, 125.
 Singh, B. (1954). *The Synergistic and Antagonistic Action of Chemotherapeutic Agents on the Tubercle Bacillus in vitro*, p. 91. Ph.D. Thesis, Univ. London.
 Tuberculosis Chemotherapy Centre, Madras (1959). *Bull. Wld Hlth Org.*, **21**, 51.
 — (1960). *Ibid.*, **23**, 535.
 — (1963a). *Ibid.*, **28**, 455.
 — (1963b). *Ibid.*, **29**, 457.
 — (1963c). *Lancet*, **1**, 1078.
 — (1965). *Bull. Wld Hlth Org.* In press.
 Tuberculosis Society of Scotland Research Committee (1960). *Brit. med. J.*, **2**, 1751.