

The Indian Journal of Tuberculosis

Vol. XIV

New Delhi, March 1967

No. 2

DRUG RESISTANCE

Dr. P.R.J. Gangadharam of the Tuberculosis Chemotherapy Centre, Madras, was the recipient of the First Junior Award for the best technical and scientific paper at the recent Hyderabad Conference. The subject of the paper "Drug Resistance" should make every tuberculosis worker as also the planners and officials think seriously, for drug resistance has many disquieting features.

Studies undertaken by the Indian Council of Medical Research at nine Urban centres revealed the very disturbing fact that incidence of Primary Drug Resistance, that is, resistant strains in patients denying history of having taken anti-tubercular drugs for more than 10 days is quite high. Against Isoniazide the average rate of resistance was 22.2%, against streptomycin 14.8% and against PAS 4.1%.

Incidence of Secondary Drug Resistance i.e. resistance showed in patients who had received anti-tubercular drugs was much higher, the average of 9 centres for Isoniazide and Streptomycin being well over 40%.

In plain language this means that in nearly half the treated cases and a little less than one quarter of fresh cases, vital anti-tubercular drugs like Isoniazide and Streptomycin may be completely ineffective.

Primary and Secondary resistance in interrupted are mostly due to inefficient drug therapy. It has been conclusively proved that in fresh cases any combination of two primary drugs taken in correct doses and without interruption for a period of 12 to 18 months give 90% recovery rate. This can be improved and made almost 100% by an intensive application of three drugs in the first month or two. So, there should be really no opportunity for the development of resistance. A badly treated case or a patient who through his own neglect fails to take correctly given treatment, has 50% chances of developing resistant strains (Secondary Resistance). When he passes on his disease to others, they will inherit these resistant strains (Primary Resistance).

So, the remedy to prevent development of resistance is entirely in our hands namely—good, efficient treatment to all infectious cases. Dealing with

these already having resistant strains is a cause of higher headache. Here, we will have to use newer antibiotics like Pyrazine, Ethionamide and Cycloserine. It is worth remembering that to treat resistant cases with newer drugs and to get results comparable to those with primary drugs in first case, three and not two newer drugs must be used.

It is superfluous to say that every Tuberculosis worker, Official and Planners must take the problem of drug resistance seriously and deal with it with vigour and determination.

DRUG RESISTANCE IN TUBERCLE BACILLI AND ITS IMPACT ON THE CHEMOTHERAPY AND EPIDEMIOLOGY OF TUBERCULOSIS*

P.R.J. GANGADHAUAM

(Central Laboratory, ICMR Drug Resistance Survey, Tuberculosis
Chemotherapy Centre, Madras)

At the outset, I thank the Tuberculosis Association of India for selecting me for this Award. I am accepting this honour with the blessings of all the veterans and learned scholars in the field of tuberculosis in India for my future guidance.

The subject I have chosen for the review is "Drug Resistance" which as you all know, is of most contemporary interest. The review covered all aspects of drug resistance in tubercle bacilli, particularly the genetic, biochemical and bacteriological aspects and also dealt briefly on the epidemiological control of tuberculosis. Time does not permit me to deal with all the aspects in detail. Hence, I am constrained to limit to a very brief presentation of all the salient aspects of my review.

Before one goes further, one should define what he means by the various terms he has been using. By *sensitive strains* one means those strains of tubercle bacilli which normally respond to low concentrations of the drugs in a uniform manner. In contrast, *resistant strains* are those which can grow in higher concentrations of the drug and they are, therefore, definitely different from sensitive strains. According to Mitchison (1961), resistance can be defined as a decrease of sensitivity to the drug of sufficient degree to be reasonably certain that the strain obtained is different from a sample of wild strains of tubercle bacilli that have never come in contact with the drug. *Primary drug resistance* is that which is caused by infection from an outside source of drug resistant tubercle bacilli. In other words, patients who are supposed to have primary drug resistance should, from the beginning, have resistant bacilli without treatment. In contrast, *acquired drug resistance* is the one which has resulted due to faulty management of treatment of the patients, who had originally sensitive tubercle bacilli. However, in practice, it is not easy to decide whether a patient is having primary or acquired drug resistance because it takes a fantastic amount of checking on the possibility of previous, but unreported treatment, to really come to a fairly pure untreated group of patients. It is, therefore, necessary to view the results of many of the so-called primary

drugs resistance Surveys in the world as reporting not true primary drug resistance, but a mixture of it with some unknown amount of undisclosed acquired drug resistance. Such a type of drug resistance is tentatively termed as "initial drug resistance".

Let us now go into some genetic aspects of drug resistance. Two mechanisms have been suggested for the development of resistance. The first, a process of genetic selection of natural resistant variants from a population that is predominantly susceptible. These variants arising from genetic mutation become more numerous as susceptible cells are inhibited by the drug. The second, a process of adaptation or adjustment by which susceptible cells, in response to a noxious environment, alter their metabolic processes in a manner that enables them to survive in its presence. There has been a great controversy in the past two decades as to which of these two theories can explain the origin of drug resistance. Fairly convincing evidence is forthcoming in recent years to favour the genetic mutation theory of the origin of drug resistance, though the possibility of other mechanisms has never been completely excluded. Bryson and Demerec (1950, 1955) and Bryson and Sybalski (1955) after prolonged study of streptomycin and isoniazid resistance of tubercle bacilli, came to the conclusion that genetic variation or mutation plays a very important part in the emergence of resistant strains than phenotypic adaptation. They have distinguished two major types of drug resistance (1) the so-called "penicillin-type" in which resistance appears in a series of multiple genetic steps and (2) single step mutation to a high level of resistance. Resistance to streptomycin and isoniazid is believed to develop by single step mutation. There are other findings in literature which also support the mutation theory. These include the occurrence of resistant organisms before exposure to the drug as shown by Schaeffer (1963) using Lederberg's replica planting technique, the occurrence of spontaneous mutants, at the rate of 1 in 10^5 for isoniazid and 1 in 10^6 for streptomycin resistance; by the occurrence of permanent and irreversible changes and the experimental findings of transfer of genetic material by transformation and transduction. (Tsukamura 1960, Watanabe and Fukasawa 1961a, 1961b).

* Paper presented at the Tuberculosis and Chest Diseases Workers Conference at Hyderabad.

Let us now proceed to the mechanisms of drug resistance. There are several mechanisms postulated, but for our discussion, we are confining to the 3 most important ones, namely (1) difference in uptake of the drugs (2) insusceptible mechanisms and (3) destruction of the drug. Considering the first one, the evidence obtained by Barclay and colleagues (1953, 1954) and by Youaat (1958a, 1958b) using C^{14} labelled isoniazid shows that there is a difference in the uptake of the drug between the sensitive and resistant tubercle bacilli, the sensitive bacilli taking much more radio-active drug than the resistant bacilli. These authors even suggested that this difference was the result of the alteration of the cell permeability. On the other hand, studies with C^{14} labelled PAS did not support the same finding. For instance, PAS resistant-tubercle bacilli have not only taken the C^{14} labelled drug to a much higher degree than the sensitive bacilli, but also retained the radio-active material unchanged after weeks of washing; the sensitive strains under these conditions lost 50% of the radioactivity. These observations on the uptake of the drugs, therefore, make it difficult to interpret the phenomenon of drug resistance by a process of adsorption, absorption or penetration. Similarly, the available evidence on the mechanism of the action of these drugs particularly from my own work carried out at the pharmacology laboratory, in the Indian Institute of Science, Bangalore and also at the National Jewish Hospital at Denver Colorado does not warrant an explanation by means of insusceptible mechanism. Evidence of the destruction of the drug by the resistant tubercle bacilli on the basis of penicillinase activity in 'penicillin-resistant bacteria' is not found in tubercle bacilli though there is some suggestion by Youmans (1960) and by Toida (1962) of the occurrence of isoniazid destroying substances in tubercle bacilli.

There are certain biological variations consequent to the development of drug resistance. Considering first the differences in growth rates, it was found that certain media are more suitable for the development of drug resistant bacilli than others. Contrary to the experience with other bacteria, the nutritional requirements of drug resistant tubercle bacilli, particularly those resistant to isoniazid, are more exacting than their parent sensitive strains. Thus, Middlebrook and his colleagues and Fisher found that biotin, bovine albumin fraction V and hemin or whole serum were essential for the growth of drug-resistant tubercle bacilli. Later studies by Knox and co-workers have established that catalase had a growth-promoting effect of isoniazid-resistant strains of tubercle bacilli which was 100,000 times that of hemin.

Coming to the differences in some enzymes, you all know the classical finding of Middlebrook that isoniazid-resistant tubercle bacilli have diminished catalase activity. Extensive biochemical work carried out by Andrejew and co-workers (1957) in Paris has shown that the deficiency in the catalase in isoniazid-resistant bacilli is due to the deficiency in the capacity to synthesise the protein portion of the catalase molecule and not due to the hemin or cytochrome components.

However, a number of workers found that the correlation between catalase activity and isoniazid-resistance is not so good always; furthermore, the atypical mycobacteria which are naturally highly resistant to isoniazid, possess a very high degree of catalase activity. Studies carried out by Andrejew and co-workers (1956, 1957) and Thirunarayanan and Visher (1957) have established the loss of peroxidase activity in isoniazid resistance and that better agreement has been found between the loss of peroxidase activity and isoniazid resistance, both in typical and atypical mycobacteria, than between catalase activity and Isoniazid resistance. Besides these, deficiencies in dehydrogenase and urease activities also were observed. Such deficiencies in enzymes were not noted with other drug-resistant bacilli; *in fact*, streptomycin-resistant tubercle bacilli were found to show a greater salicylate effect than the sensitive strains.

Coming to the third point, several workers have established that isoniazid-resistant, catalase deficient tubercle bacilli are naturally attenuated to the guinea-pig. It was also mentioned that the ability to infect such animals was not changed; the aspect which had changed was the *ability to initiate a progressive disease*. On the other hand, these organisms are not attenuated to the mouse. The question naturally arises, whether man is similar to the guinea-pig or to the mouse in this respect. Some workers suggest that these organisms are not dangerous to man, where progressive disease attributed to them has not been shown to develop. According to the results obtained in the WHO Tuberculosis Project in Kenya, their infectivity to humans also is remarkably less than that of the sensitive organisms. On the other hand, the consensus of the opinion of the world experts in tuberculosis seems to be that these organisms cannot be considered to be attenuated to humans. In contrast to the isoniazid-resistant bacilli, no attenuation in virulence of streptomycin or PAS resistant tubercle bacilli is observed; *in fact*, Zakariadze (1956) found an increase in virulence of streptomycin-resistant tubercle bacilli.

Having discussed the general biological aspects, let me briefly turn on to the techniques available for testing drug resistance. Sensitivity tests for tubercle bacilli can be classified as *direct* or *indirect*. In the *direct test*, the sputum concentrate is directly inoculated on the drug-containing, as well as on to the drug-free control slopes; the *indirect* method consists of first isolating or culturing the organisms from the sputum with subsequent subcultures onto the control and drug-containing medium. The direct sensitivity tests can further be classified as follows: (1) Middlebrook and Cohn's method using 7H-10 medium in quadruple Felson plates; this method is being used in several American laboratories; (2) the gradient plates using Sybalski's method using 7H-10 medium; this method is being used by Hobby and her colleagues and the Veterans Administration of U.S.A.; (3) the other direct sensitivity test procedures using Lowenstein-Jensen medium in universal containers (Mackey, 1964) and by us at the Central Laboratory of the ICMR Drug Resistance Survey (Devaki et al, 1967).

The indirect sensitivity tests may also be classified into 3 main categories (i) the absolute concentration method developed by Meissner and her co-workers and used by the U.S. Veterans Administration Services; (ii) the resistance ratio method introduced by Mitchison and used in all the investigations carried out by the Medical Research Council of Great Britain and its units in various countries, the controlled clinical trials in the Tuberculosis Chemotherapy Centre, Madras, and in the series of co-operative investigations on the prevalence of drug resistance in India conducted by the ICMR, about which you listened yesterday and in last year's conference; (iii) the proportion method developed by Canetti and co-workers.

The relative merits of these techniques, especially the indirect tests were discussed in several conferences including the one at Ahmedabad and I would only briefly discuss some of them.

Direct sensitivity tests are preferred to indirect sensitivity tests on the following grounds (a) they deal with a more representative cross-section of the population present in the patient, since all the bacilli in the biological specimens are obtained in the sample used for inoculation (b) preliminary culturing of biological specimens in drug-free medium which may permit the overgrowth of colonies that vary in their susceptibilities is avoided. Subculturing may also result in the production of a non-representative population with properties of drug susceptibility entirely different from those present in the original specimen. This altered composition of sensitive and resistant bacilli would give an erroneous impression of original bacte-

rial sensitivity and (c) the time required for reading the sensitivity test is reduced by about 4 weeks. On the other hand, the direct sensitivity tests suffer from the criticism in that they are useful only when the sputum contains adequate number of bacilli as shown by direct-smear positivity, since the results may not be reliable when their number in the inoculum is scanty. However, recent evidence from the Central Laboratory of the ICMR Drug Resistance Survey (Devaki et al, 1967) indicates that the direct sensitivity test may still be valuable even if the direct smear is negative. According to Mitchel and Bell, (1958) the choice between the direct and indirect sensitivity test is primarily a matter of individual preference.

Even without the availability of the techniques, we can predict drug resistance by other means. For instance, we can predict drug resistance pretreatment by a thorough questioning; a patient who had extensive chemotherapy and still positive bacteriologically has most certainly, drug resistant organisms. However, I may hasten to let you know that this is very difficult in practice to assess the previous history. During treatment, if a patient has had regular treatment for some time, say, 6 months, and is still positive on smear, he has most probably drug resistant tubercle bacilli. Studies carried out at the Tuberculosis Chemotherapy Centre, have revealed that smears can also be used in predicting drug resistance even in controlled clinical trials.

Having discussed the techniques of drug resistance, let me briefly touch upon certain aspects which are closely related to it. First, the microbial persistence where the bacteria are not killed by the drugs even though they are sensitive to them as demonstrated by the *in vitro* tests. This is supposed to be due either to the dominant nature of the organisms or the inability of the drugs to reach the sites of bacterial proliferation. In man, microbial persistence is of great importance because it is probably responsible for (a) the fact that bacteriocidal activity of drugs on organisms in lesions is lower than in actively growing cultures *in vitro*, resulting in the need for a lengthy period of treatment, (b) the occurrence of relapse (usually due to drug-sensitive organisms) even after a prolonged course of chemotherapy, such as a year in the treatment of pulmonary tuberculosis. Coming to natural resistance, it has been mentioned that bovine strains of tubercle bacilli are naturally resistant to PAS and that anonymous mycobacteria are resistant to the standard drug. Even among typical human bacilli, those obtained from our country have shown some degree

of natural resistance to PAS and thiacetazone in some parts of India. Coming to cross resistance, it has been found that once a bacillary population has become resistant to a drug, it may happen that this population is also resistant to another drug which has some chemical resemblance to the first. Cross resistance was shown to exist between thiacetazone and ethionamide, thiacetazone and isoxyl and streptomycin and kanomycin etc.

Let me now proceed to discuss briefly the most important and interesting aspect in drug resistance, that is, the clinical significance. There is sufficient evidence available in literature to show that *in vitro* drug resistance correlates with clinical response in that patients with drug sensitive organisms fare better with chemotherapy than the patients with drug resistant organisms; this is more pronounced in the case of isoniazid resistance. Evidence is also forthcoming in recent times that even low degrees of drug resistance have clinical significance. Let us now discuss the various factors responsible for the development of drug resistance. These can be classified as biological, clinical and sociological. The biological factors include (a) initial bacillary population (b) local factors inside the host favourable for the multiplication of resistant bacilli (c) presence of the drug in insufficient concentration and (d) patient's inactivation status. The clinical factors include (a) treatment with single drugs (b) inadequate dosage of the drug (c) insufficient duration (d) interference by occult medicine (e) interference by other indigenous systems of medicine, and the sociological factors which are most important in my opinion, are (e) irregularity in drug-taking (b) not following treatment for the entire period (c) avoidance of other exogenous infections. Having perused the list of some of the causes of drug resistance, let us ponder over how best we could avoid them.

It has been suggested that (1) double drug therapy should be given to all patients suffering from pulmonary tuberculosis in adequate doses of the drugs to which the bacteria are susceptible; (2) the duration of chemotherapy should be at least for one year and possibly more (3) every effort should be bestowed to see that the drugs are properly taken in the prescribed manner for the prescribed time and (4) there must be no chance of exogenous infection with resistant bacilli during the course of chemotherapy. The mere fact that 2 drugs were prescribed does not, however, guarantee that they are both taken by patients unless they are given in a single catchet or tablet. If they are dispensed separately,

preferential omission of the less acceptable drug may occur leading into the development of resistance to the drug taken regularly.

This brings us to the question how drug resistance emerges in clinical practice. If daily streptomycin and isoniazid in adequate doses are given very little emergence of resistance occurs as this treatment ensures about 99% success. On the other hand, if PAS substituted either of these drugs, about 10% of the patients develop drug resistance, mainly due to the failure of the PAS to prevent emergence of the resistant bacilli to the other companion drug, streptomycin or isoniazid.

Studies carried out at our Centre have indicated that when isoniazid alone is prescribed, drug resistance developed in 2 stages. In the first stage, occurring very early in the treatment, highly resistant mutants of bacilli grew freely whatever the isoniazid dosage but mutants of low resistance were prevented from growing to an extent depending upon the peak isoniazid concentrations in the serum. In the second stage, organisms with relatively lower resistance continue to multiply though still partially inhibited by isoniazid and become more resistant, particularly in slow inactivators.

Let us now briefly touch upon the treatment of drug resistant cases for pulmonary tuberculosis. Treatment for such cases although not hopeless, leaves much to be desired. Depending upon the situation, several of the second line drugs are recommended along with the initial treatment drugs to which the bacilli are sensitive. There are 7 or 8 acceptable second line drugs, but their cost and instability in tropical conditions pose serious limiting factors for their use. Treatment of these failure cases can be considered separately under 3 headings as follows:

(1) If isoniazid-resistance has not developed, even though the bacilli are resistant to streptomycin and PAS, a combination of pyrazinamide and isoniazid is acclaimed to be the best. Recently, following the studies carried out in East Africa and in our Centre and several other places in India, thiacetazone and isoniazid combinations may also be recommended:

(2) When the bacilli are resistant to isoniazid, it is generally believed that any combination of drugs will not be satisfactory. Streptomycin and PAS combinations are normally prescribed in such cases. Studies carried out at the Tuberculosis Chemotherapy Centre, Madras have established the value of the combination of pyrazinamide and streptomycin in such cases;

(3) when the tubercle bacilli are resistant to all 3 drugs or at least both to isoniazid and streptomycin, the situation is highly deplorable, especially in a large part of the developing world. The drugs to be administered under these conditions are the 2nd line drugs which the patient can tolerate and to which he is not likely to be resistant. Studies carried out at the Tuberculosis Chemotherapy Centre have indicated that a combination of ethionamide and cycloserine is better than thiacetazone and cycloserine. It is also believed that the combination of pyrazinamide and ethionamide is probably the best one under these circumstances, with a supplement of thiacetazone and viomycin, if possible. Furthermore, it is also suggested that inclusion of isoniazid as one of the drugs in these regimens even though the bacilli are resistant to this drug shows some more additional benefit.

While these recommendations are perhaps feasible, and therefore can be followed in developed countries, it is a matter of serious consideration whether these can be applied in a developing country like India for the following reasons:

(a) On economic grounds, it is definitely impossible to offer such a costly treatment to every patient with resistant organisms; the crippled health budget is perhaps not even sufficient to offer a standard double drug combination of initial chemotherapy for every patient for one year;

(b) on grounds of economy of foreign exchange, these drugs are not yet imported, and, therefore, are mostly not available in India;

(c) many institutions are not yet well-equipped to follow the patients very carefully with the series of tests necessary to guard against the onset of toxic manifestations usually associated with these drugs.

Having discussed the various aspects of drug resistant, let us touch upon the prevalence of infection by drug resistant tubercle bacilli in the community. One of the most important recommendations of the experts in tuberculosis is that every country which is contemplating mass chemotherapy programme, should assess the prevalence of drug resistance in their community. Such information naturally would enable the authorities in the country to plan the correct chemotherapy programme, as well as arrive at a useful information regarding the success of these programme. For want of time I am not going to discuss the results of the 2 drug resistance surveys which the ICMR has been conducting in our country. The results were presented by me in last year's and this year's

conferences and are too well-known to you. On the other hand, I would like to briefly highlight some of essential requirements of the primary drug resistance surveys if the results are to be meaningful. These criteria are: (1) that reliable bacteriological techniques have been used in the investigation (2) the levels of drug resistance chosen have been found to be clinically meaningful (3) all drug sensitivity tests are performed in a single laboratory under carefully defined conditions allowing precise control of inoculum size, medium, incubation period and other environmental factors (4) the naturally resistant mycobacteria like the unclassified or atypical mycobacteria have been excluded and (5) perhaps most important, the patients from whom the resistant bacilli were isolated are those, who never had taken any previous antituberculosis chemotherapy. I may mention that the ICMR Drug Resistance Surveys in the country are in our considered opinion fulfilling these criteria to a satisfactory level.

Before I conclude, I would like to briefly touch upon some epidemiological aspects of drug resistance. While some authorities (Canetti 1964) indicate the usefulness of the incidence of primary drug resistance as a good epidemiological yard stick, prevalence of a large component of drug resistant tubercle bacilli will complicate the control programme and other antituberculosis measures in the community. Unlike the situation in U.S.A. and other countries, tuberculosis in India may be spreading both by exogenous infections as well as by endogenous break-downs. While the exogenous infection by these drug-resistant bacilli is a danger of the present, endogenous breakdowns with these organisms is a danger for many years to come. Fridodt-Moller (1962) rightly expresses considerable concern regarding the possible future by saying "This may only be the beginning, being the result of infections which took place several years ago, How many shall we find in 10 to 20 years when those infected today develop their tuberculosis disease". Of course, this sort of unfortunate development may be taking place in parts of the developing world.

Some authorities (Canetti 1962) consider the epidemiological aspects of drug resistance separately for developing nations. Though this bifurcation of the problem is justified on economic grounds, it becomes erroneous when we consider the fact that countries become closer and closer every day, thanks to the efficient international communication and co-operation we have today. As examples, we can refer to the recent finding of Thomas (1963) of Miller and co-workers (1966) of the high

incidence of drug resistance in immigrants. It is, therefore, necessary to view this problem as a threat to the whole world, even though temporarily certain areas are hit harder than others. Of course, if we aim at eradication of tuberculosis in the world at least in the next century (certainly it is not even in sight in this century!) an all round global attack should be launched in all seriousness, right now.

Finally, considering the actual situation as it is existing in India and other developing countries, about a quarter to one third of the tuberculosis patients when they first report to the chest clinics cannot be treated with standard initial chemotherapy, even if the chemotherapy is made available. To this, is to be added the influence of poor hygiene, poor nutrition, and overcrowding, in enhancing the exogenous and endogenous spread of tuberculosis. All these factors show us the shocking and threatening picture, whether the situation degrades to that of the pre-chemotherapeutic days. Though some authorities like Dr. Meyers (1963) think that the situation does not deteriorate to such a level in the developed nations, it is worth our serious concern whether it does in the poorer nations.

It should also be our most serious endeavour as to how best we can correct the weakest point in the development of drug resistance, that is, the patient and his co-operation to treatment. May be the phthisiologists will do well to invite the help and advice of sociologists and psychologists in this endeavour.

REFERENCES

- Andrejew, A., Gernez-Riewx, C. and Tacquet, A. (1956) *Ann. inst. Pasteur*, 91, 586
- Andrejew, A., Gernez-Riewx, C. and Tacquet, A. (1957) *Ann. inst. Pasteur*, 93, 281
- Barclay, W.R., Ebert, R.H. and Koch-Weser, D. (1953) *Amer. Rev. Tuberc.*, 67, 490
- Barclay, W.R., Koch-Weser, D., and Ebert, R.H. (1954) *Amer. Rev. Tuberc.*, 70, 784
- Bryson, V. and Demerec, M. (1950) *Ann. N.Y. Acad. Sci.*, 53, 783
- Bryson, V. and Demerec, M. (1955) *Amer. J. Med.* 18 723
- Bryson, V. and Sylabski, W., (1955) *Advance. Genetics*, 7, 1
- Canetti, G. (1964) in host factors in Chemotherapy-Chemotherapy of Tuberculosis Barry Ed. Butterworth.
- Devaki, V., Mohan, K. and Gangadharam, P.R.J. (1967) under publication
- Frimodt-Moller, J. (1962) *Tubercle*, 43, 88
- Miller, A.B., Tall, R., Fox, W., Lafford, M.J. and Mitchison, D.A., (1966) *Tubercle*, 47, 92
- Mitchell, R.S. and Bell, J.C., (1958) *Modern Chemotherapy of Tuberculosis Antibiotics Monograph. No. 11*, Medical Encyclopedia, New York
- Myers, A., (1963) *Dis. Chest*, 43, 327 Schaeffer, W.B. (1963) Personal communication
- Thirunarayanan, M.O., and Visher, W.A., (1957) *Amer. Rev. Tuberc.*, 75, 62
- Thomas, H.E., (1963) *Tubercle.*, 44, 27
- Toida, I. (1952) *Amer. Rev. Resp. Dis.*, 85, 720
- Tsukamura, M (1960) *Amer. Rev. Resp. Dis.* 81, 403
- Watanabe, T., and Fukasawa, T. (1961a) *J. Bad.* 81, 669
- Watanabe, T., and Fukasawa, T. (1961b) *J. Bad.* 81, 679
- Youatt, J. (1958a) *Aust. J. Expt. Biol. Med.*, 36, 223
- Youatt, J. (1958b) *Amer. Rev. Tuberc.* 78, 806
- Youmans A.S., and Youmans G.P., (1960) *Amer. Rev. Tuberc.*, 81,929
- Zakariadze, T.V., (1956) quoted by *Biol. Abst.* 35, 701, 1960)

AN INVESTIGATION INTO THE CLINICAL RESPONSE AND DEVELOPMENT OF DRUG RESISTANCE IN THE TREATMENT OF PULMONARY TUBERCULOSIS WITH THIA CETAZONE AND ISONIAZID

NARAYANA SETTY, R. M. BARTON AND F. I. TOVEY,
(*P. K. Sanatorium and Holdsworth Memorial Hospital, Mysore*)

The standard treatment of the ordinary case of tuberculosis has come to be recognised as one of the following:

(i) Streptomycin, 1 gram daily + Isoniazid, 300 mg. daily, or

(ii) Isoniazid, 300 mg.+ Para amino-salicylic acid, 12 grams daily. A combination of two drugs to which the tubercle bacilli were sensitive was found necessary because of the additive effect of two drugs, but even more important because the combination delayed the development of drug resistant strains of the bacilli.

In developing countries like India and Africa where domiciliary treatment has largely to be relied on, the combination of INH and PAS has obvious advantages as no injections are required necessitating daily visits of the patient to hospital or dispensary or daily visits of trained personnel to the home of the patient. This combination of the two drugs is, however, not without certain difficulties and drawbacks, and our own experience is in line with that of others. PAS may cause gastrointestinal disturbances with vomiting and diarrhoea. Further, for some patients the bulkiness of PAS is a disadvantage. Most important is the cost. At present market rates for one month's treatment with PAS costs Rs. 25/-. These reasons may either lead patients to stop taking the drugs or to take smaller and inadequate doses. This has caused a search to be made for a more suitable companion drug for INH to replace PAS.

Thiosemicarbazones have been in use for a considerable time mostly in Continental Europe, one of the most well known being Bayer's 'COTABEN'. But it had the disadvantage of being toxic mostly to the liver, kidney and nervous system. Various derivatives have been tried in the effort to find a less toxic form. Among these has been Thiacetazone. Reports, including two from East Africa, have shown its value specially in combination with INH, but only a few of these—at the time of writing, none in India—have been associated with a concurrent bacteriological investigation specially with regard to the development of resistance of the bacilli to the drugs.

The present project was to try this combination of INH and Thiacetazone and to assess the clinical, radiological and bacteriological response in Indian patients.

Material and Methods

The patients were selected from the general outpatient department of the P.K. Sanatorium, Mysore. All of them were sputum positive for acid-fast bacilli with the exception of three with pleural effusion, and had not been treated with any anti-tuberculous drugs. The patients came from villages around Mysore. Apart from this, it was an unselected group. Most of the patients belonged to the poorer classes with a diet of low nutritive value. All the patients were admitted initially to the wards of the Sanatorium for the early part of their treatment.

On admission, a skiagram of the chest and sputum examination by direct smear and culture were done. When a positive culture was obtained, sensitivity studies were done for INH and Thiacetazone. In most patients, this was repeated every month while the patient was in the Sanatorium and as regularly as possible when the patients attended as outpatients. The average stay of each patient was six months.

After the initial investigations, the patients were put on INH 300 mg. daily in one dose and Thiacetazone 150 mg. daily in two doses. When the sputum became negative for TB or there was marked improvement, the patient was discharged and further treated on a domiciliary basis.

It was originally intended to have a control group treated with INH and PAS but so few patients were found who had had no previous treatment whatever, that the control group had to be omitted and comparison had to be made with groups of other workers in India and elsewhere who had used INH and PAS.

Bacteriological

After the collection of specimens of sputum in the Sanatorium in sterile McCartney bottles, the specimens were sent to the laboratory of

the Holdsworth Memorial Hospital, Mysore. If received in time, they were processed the same day; otherwise they were kept in the refrigerator overnight. When the patients were in the Sanatorium, specimens were collected on three consecutive days and processed separately. When patients attended as out-patients, it was usually possible to collect only one specimen.

A direct smear was made from the specimen and then the sputum was treated with an equal volume of 4 per cent NaOH, being shaken occasionally, for 20 minutes, centrifuged at approximately 3000 rpm for 20 minutes, the supernatant fluid poured off, the bottles filled with sterile distilled water and again centrifuged for 20 minutes, and the supernatant fluid discarded. The sediment was sown by a loop on two bottles of Lowenstein Jensen medium (without potato flour). Incubation was at 37°C. and the cultures were examined weekly for eight weeks.

If growth typical for the tubercle bacillus was observed, it was taken for sensitivity testing. If specimens from 3 consecutive days were all positive only one was taken. If growth was scanty, a sub-culture was made to obtain more growth.

For sensitivity testing, an emulsion was made by taking two loopfuls of growth and emulsifying them in 0.5 ml. of sterile distilled water in small McCartney bottle; as no shaking machine was available, this was done by means of the loop and shaking by hand. One loopful (4 mm. diam.) was taken for each tube of medium.

Lowenstein Jensen medium was used in 1 oz. McCartney bottles with the following concentrations of the drugs:

INH-0, 0.1, 0.2, 1.0 and 5.0 micrograms/ml.

Thiacetazone-0, 0.5, 1, 2, 4, 8, 16, 32 micrograms/ml.

The cultures were examined at 2 weeks and a final reading taken at 4 weeks. 20 or more colonies were counted as a growth, less than 20 were ignored. A growth was noted as +, ++, +++ (+ denoting from 20 up to 100 colonies, ++ growth between 100 and 200 colonies, +++ as confluent growth).

With INH growth in tube containing 1 microgram was considered as resistant to INH; if growth was found in the 0.2 microgram bottle and not in the 1 microgram bottle

it was repeated; growth again in 0.2 or higher was counted as resistant.

With thiacetazone, the culture was considered resistant to Thiacetazone if growth was found in the bottle containing 16 or more micrograms/ml.

Results

The total number of patients originally admitted under the investigation was 100, but 10 of these have been omitted either as staying under treatment less than 3 months, or as being finally diagnosed as suffering from non-tuberculous lung disease. Of the 90 remaining patients, the cultures showed 8 or 8.9 per cent to have initial or primary resistance to INH; these 8 are dealt with separately. 82 or 91.1 per cent were sensitive. Of the same number of patients, 20 or 22.2 per cent were resistant to Thiacetazone and 70 or 77.8 per cent were sensitive. Resistance to both drugs was shown by 5 or 5.5 per cent of the patients.

Bacteriological Studies with regard to development of resistance: Sensitivity studies during treatment

The analysis of results during treatment is not easy for several reasons. Many of the patients went to continue treatment at home after a period of three months in the Sanatorium, and although they were supposed to report monthly, this was not done and so specimens were not received regularly. Others either in the Sanatorium or after discharge said they had no sputum and no specimens could be sent to the laboratory. This meant that varying numbers of specimens were received at different stages of the treatment. This is shown in the following table:

On admission, specimens were received from	all patients.
At three months after treatment from	66 patients
At four months after treatment from	48 patients
At five months after treatment from	46 patients
At six months after treatment from	23 patients

Specimens were received from a few patients up to 18 months after the beginning of treatment. These figures do not represent the number of patients under treatment as some patients produced a specimen one month and not the next. Of the specimens examined, 31 or 47 per cent were still positive either by

smear or culture at three months; 19 or 39.6 per cent at four months; 19 or 41.3 per cent at five months, and 11 or 48 percent at six months. Those still sputum positive tended to stay longer in the Sanatorium.

Of the patients' specimens received at 6 months after treatment, 75 per cent were resistant to INH and 62.5 per cent resistant to Thiacetazone.

With regard to the development of resistant cultures in patients, the position is as follows:

Of the 8 patients who were primarily resistant to INH, 5 were also resistant to Thiacetazone, and 2 developed resistance to Thiacetazone.

Of the 82 patients primarily sensitive to INK:

	Primary Thiacetazone Culture	Acquired resistance to INH
Sensitive	67	18
Resistant	15	4

These figures suggest that the development of resistance to INH is not affected by initial resistance to Thiacetazone.

Of the 82 patients, 7 acquired resistance to Thiacetazone while 15 were primarily resistant. Of the 7 patients, 4 also developed resistance to INH and 3 remained sensitive to INH. In addition, 3 patients developed a temporary resistance to Thiacetazone, but this was not found later.

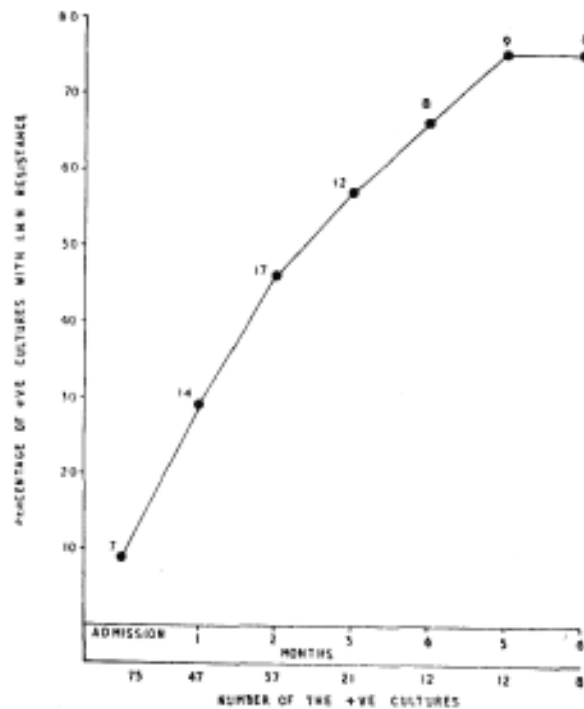
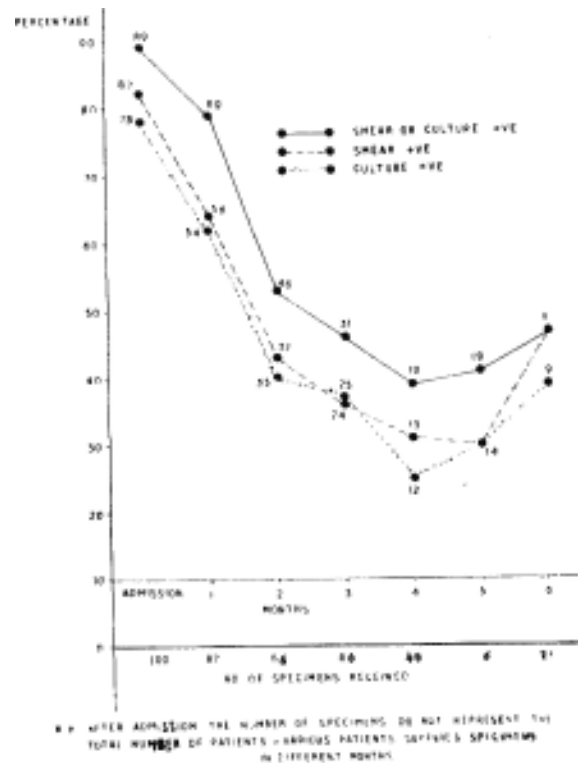
The percentage of bacteriological sputum conversion and the development of INH resistance and thiacetazone resistance is shown in the following graphs:

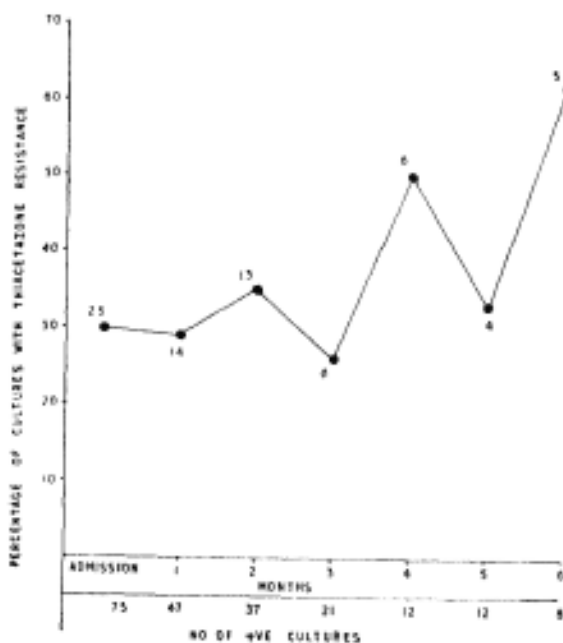
Bacteriological sputum conversion

Graph 1—On admission, out of 100 specimens, 89 proved positive. On instituting therapy, the conversion rate was maximum during the first two months. After 6 months, 23 patients still had sputum and of these 11 were still positive.

Graph 2—*INH Sensitivity:* There was progressive increase in resistance to INH rising from 9 per cent to 75 per cent at the end of 6 months.

Graph 3—*Thiacetazone Sensitivity:* There was no such consistent change with thiacetazone and in later months, the percentage of resistant bacteria varying widely from month to month.





Clinical Results

If the cases are classified according to their clinical and radiological condition on admission, we find the following:

- | | | |
|---------------------------------|---|----|
| (i) Early or localised lesions | — | 29 |
| (ii) Acute extensive lesions | — | 13 |
| (iii) Chronic extensive lesions | — | 40 |
| Total | — | 82 |

The general results of treatment, taking into consideration, clinical, radiological and bacteriological response, can be classified as follows:

- | |
|---|
| (i) Improved—response good or moderate, all showing sputum conversion |
| (ii) Not improved—response poor or relapse. |

The general results are shown in Table I. Of the 82 cases, 70 or 85 percent were improved, the period of observation being 18 months in the earlier cases and 6 months in the latest cases; 12 or 15 per cent showed no improvement. 7 of them relapsing. Two of these relapses were probably attributable to malnutrition as clinical response was good as long as they remained as in-patients with good food.

As could be expected, the response varied

TABLE 1 — CLINICAL RESULTS

(X-RAY ASSESSMENT AND SPUTUM CONVERSION)

	NUMBER	IMPROVEMENT		NO IMPROVEMENT	
		GOOD	MODERATE	POOR	RELAPSE
WHOLE SERIES EXCLUDING 8 WITH PRIMARY I.N.H. RESISTANCE	82	55 67%	15 18%	5 6%	7 9%
		85%		15%	
EARLY OR LOCALISED LESIONS	29	26 88%	3 12%	0	0
		100%			
EXTENSIVE ACUTE LESIONS	13	10 76%	1 8%	0	2 16%
		84%		16%	
EXTENSIVE CHRONIC LESIONS	40	10 47.5%	11 27.5%	5 12.5%	3 12.5%
		75%		25%	

greatly with the nature and extent of the disease, the early or localised lesions and the acute extensive lesions responding well.

Of the 29 patients with early or localised lesions, all showed a good response, clinical radiological and bacteriological, 26 of them or about 90 per cent showing good improvement and 3 moderate. Of the 13 patients with extensive acute lesions, 10 showed a good response, 1 moderate, and 2 relapsed after an initial good response. Of the 40 patients with chronic extensive lesions, 19 or 47.5 per cent showed good improvement, 11 or 27.5 per cent moderate improvement, and 5 or 12.5 per cent a poor response and 5 or 12.5 per cent relapsed.

Cases with initial INH resistance

Of the 8 cases with initial INH resistance,

TABLE 2 CASES WITH INITIAL I.N.H. RESISTANCE

	NUMBER	PROGRESS			
		GOOD	MODERATE	POOR	RELAPSE
WITHOUT THIACTAZONE RESISTANCE					
LOCALISED	1				1
EXTENSIVE ACUTE CHRONIC	1				1
TOTAL					
WITH INITIAL THIACTAZONE RESISTANCE					
LOCALISED	1				
EXTENSIVE ACUTE	1				1
EXTENSIVE CHRONIC	3	1	1		2
TOTAL	5	1	1		3
ACQUIRING THIACTAZONE RESISTANCE					
LOCALISED					
EXTENSIVE ACUTE & CHRONIC	2				2
TOTAL	2				2
GRAND TOTAL	8	1	1		6

only 2 showed any improvement; 5 of this group had also primary thiacetazone resistance and 2 later acquired it. Of the 8 cases, 1 had a localised lesion, 1 an extensive acute lesion and 6 had chronic extensive lesions.

Patients with initial thiacetazone resistance

Twenty patients had primary thiacetazone resistance of whom 15 cases (75 per cent) showed improvement and the remaining 5 cases no improvement.

TABLE 3. CASES WITH INITIAL THIA CETAZONE RESISTANCE

	NUMBER	PROGRESS			REMARKS
		GOOD	MODERATE POOR	RELAPSE	
WITH I.N.H. RESISTANCE					
LOCALISED	5	5			(9) R I S O (2) S I R 2
EXTENSIVE ACUTE	2	2	1		
EXTENSIVE CHRONIC	4	3			
TOTAL	11	10	1		
WITH INITIAL INH RESISTANCE					
LOCALISE 0	1			1	(4) 5 3 8 5 6 5 7 (BS) 5 4
EXTENSIVE ACUTE	1	1			
EXTENSIVE CHRONIC	3		1	2	
TOTAL	5	1	1	3	
ACQUIRING INH RESISTANCE					
LOCALISED 1	2	2			(4) 5 2 3
EXTENSIVE ACUTE					
EXTENSIVE CHRONIC	2	1		1	
TOTAL	4	2		1	
GRAND TOTAL	20	15	2	4	

Among these 20 patients, the eleven patients who were sensitive to INH showed improvement. Conversely, among the 5 patients who had initial resistance to INH, three showed poor improvement. The remaining 4 patients developed secondary or acquired INH resistance and two showed poor improvement or relapsed.

Patients without thiacetazone resistance

Seventy patients were initially sensitive to thiacetazone and of these, 56 cases (80 per cent) showed improvement. In the remaining 14 cases, there were equal numbers with poor responses or relapses.

Patients with acquired INH resistance

In the whole series, 22 patients acquired

TABLE 4. CASES WITH ACQUIRED INH RESISTANCE

	NUMBER	MONTHS WHEN ACQUIRED	PROGRESS			
			GOOD	MODERATE	POOR	RELAPSE
WITHOUT THIA CETAZONE RESISTANCE						
LOCALISED	2	2 2				
EXTENSIVE CHRONIC	7	(2)(2)(2)				2
TOTAL	9					2
WITH INITIAL THIA CETAZONE RESISTANCE						
LOCALISED	1	1	1			
EXTENSIVE CHRONIC	3	1 1 2				1
TOTAL	4		1			1
ACQUIRING THIA CETAZONE RESISTANCE						
LOCALISED	2	4 2	2			
EXTENSIVE ACUTE	2	1 1				2
EXTENSIVE CHRONIC	5	2 2 2 1			1	1
TOTAL	9	2 2 2 1			1	1
GRAND TOTAL	22		2		1	2

INH resistance which was permanent in 20 and temporary in 2 (one of these becoming resistant again). Eleven of these cases showed improvement and the remaining cases showed either poor response or relapse. Eight of the latter had initial or acquired thiacetazone resistance.

Among the 22 cases, 9 cases were sensitive to thiacetazone, 6 of these cases improved. 4 cases had initial permanent thiacetazone resistance and 2 of these cases improved. The remaining 9 cases developed acquired thiacetazone resistance (5 permanent and 4 temporary) and of these, 6 cases showed no improvement (or relapse including 2 from the temporary group). Another interesting feature is that 15 cases out of the 22 cases who developed acquired INH resistance had the chronic extensive type of pulmonary tuberculosis.

Patients with acquired permanent thiacetazone resistance

There were 9 cases who developed permanent thiacetazone resistance during therapy of whom 4 showed good improvement and the remaining 5 cases no improvement or relapse

TABLE 5. CASES MTH ACQUIRED PERMANENT THIA CETAZONE RESISTANCE

	NUMBER	MONTHS WHEN ACQUIRED	PROGRESS		
			GOOD	POOR	RELAPSE
WITHOUT INH RESISTANCE LOCALISED EXTENSIVE CHRONIC	1	2 2 7	3		
TOTAL	3		3		
MTH INITIAL INH RESISTANCE EXTENSIVE CHRONIC	2	3 2		2	
TOTAL	2			2	
ACQUIRING INH RESISTANCE LOCALISED EXTENSIVE CHRONIC	1 3	THIA C 4 4 10 INH 4 2 2 2	1	2	1
TOTAL	4		1	2	1
GRAND TOTAL	9		4	4	1

3 cases with bacilli which remained sensitive to INH responded well. Among the 6 cases with primary or acquired resistance to INH, the failure rate of treatment was high—only one case with a localised lesion showing any improvement and in whom the resistance was acquired at 4 months when the disease was already well controlled.

Patients with temporary thiacetazone resistance

There were 10 patients which belonged to this category and the progress of the disease was more encouraging than in the first group who had permanent thiacetazone resistance. In three, the temporary resistance was initial and in seven acquired. 6 cases (60 per cent)

TABLE 6 **CASES WITH TEMPORARY THIA CETAZONE RESISTANCE**

WITHOUT INH RESISTANCE	INITIAL		ACQUIRED				PROGRESS			
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	GOOD	PROGATE	PROG	PROG
LOCALISED	1	4	2	3.1	5	6.1	1	1		1
EXTENSIVE ACUTE	1									
EXTENSIVE CHRONIC										
TOTAL	2		2		5		2	2		1
ACQUIRING INH RESISTANCE										
MONTH WHEN ACQUIRED										
LOCALISED	2		1	1	2					
EXTENSIVE ACUTE	1		1	1	4					1
EXTENSIVE CHRONIC 2-3-1	1	3	2	2.1	2		1	2		1
TOTAL	1		4				1	1	2	1
GRAND TOTAL	3		7				3	3	2	2

showed improvement. The remaining 4 showed no improvement, two of these having a relapse. Even here, the cases which responded well were those who were sensitive to INH. The poor results were obtained in the group of cases which developed INH resistance during therapy.

The ratio of temporary thiacetazone resistance to permanent resistance was 1: 2.6 much higher than with INH with which temporary resistance occurred in only 2 out of 22 cases, one of these becoming resistant again afterwards.

Side effects

Ten patients complained of possible side effects which were severe in seven.

6 patients had a rash—one having exfoliative dermatitis.

2 patients had albuminuria.

2 patients had jaundice—one was probably infective hepatitis.

In all the patients who had rashes, the thiacetazone was eventually stopped and they were changed over to another combination of drugs. In the serious cases of drug reaction, the patients were put on corticosteroids. Two cases had prolonged corticosteroid therapy in order to continue the drugs, but eventually the thiacetazone had to be stopped.

Of the 2 patients who developed jaundice, one after investigation was thought to have infective hepatitis. The second patient had a haemolytic jaundice. This patient developed anaemia and jaundice at the tenth month of therapy and when the thiacetazone was stopped, she made an uneventful recovery.

The rash was mostly of the maculopapular type; it left behind a black pigmented area which persisted for many months. The case of exfoliative dermatitis was of a very

severe type. The rash appeared usually about the end of the third week. It did not regress quickly with the cessation of the drug. There was some relief of severe skin irritation when treated with anti histamine drugs and corticosteroids. It is now thought advisable to stop the drug immediately on the appearance of any type of rash. Most of the patients who developed the rash were later treated with streptomycin and INH and still later with INH and PAS without recurrence of the rash.

DISCUSSION

Efficacy of treatment

In the trials conducted in East Africa, the regime of thiacetazone 150 mg. plus isoniazid 300 mg. administered in a single dose was shown to be similar to the standard regime of PAS plus INH, both in therapeutic efficacy and toxicity (E. African BMRC, Second Thiacetazone Investigation, 1963). 89 per cent of the patients on thiacetazone and INH showed favourable results.

Likewise in Delhi, Sikand, Goyal and Mathur (1963) found that domiciliary chemotherapy with the combination of thiacetazone and INH given in 2 doses was as effective as the regime of streptomycin 1 g. plus 150 mg. INH twice daily. The rate of sputum conversion was 85-90 per cent.

In our present series, out of the 82 cases who were sensitive to INH when put on this combination, radiological improvement and bacteriological conversion of sputum was found in 70 patients at the end of 6 months (85 per cent). The remaining 12 cases showed no improvement, half of this number relapsing after an initial good response. To evaluate the results further, it is necessary to compare the data of other series of patients on INH alone, with patients on INH plus PAS. There have been many such investigations done in the past showing the superiority of INH plus PAS over INH alone. The findings of the Chemotherapy Centre, Madras (1959-65) showed that with a low dosage of INH, only 44 per cent of patients responded favourably and in those treated with a moderate to high dosage, only 68 per cent responded well. In comparison, in patients treated with INH and PAS, favourable results were seen in 87 per cent. In a similar group in the East African series treated with INH and PAS, good results were seen in 85 per cent.

Drug Resistance

Table 8 shows some interesting observations concerning INH resistance. At the Chemotherapy Centre, Madras (1959-65), 385

TABLE 7

Efficacy of various regimens of therapy

(Modified from Mitchison, 1965)

Regimen of Treatment Total		Total patients	Patients with favourable response		Reference
Dose of Drugs	No. of doses per day		No.	Per cent.	
Isoniazid 100mg.	2	86	38	44	Tuberculosis Chemotherapy Centre, Madras (1960)
Isoniazid 200 mg.	2	66	38	58	-do-
Isoniazid 400-650 mg.	1	211	144	68	Tuberculosis Chemotherapy Centre, Madras (1960, 1963a, 1963b).
Isoniazid 100mg.	2	314	274	87	Tuberculosis Chemotherapy Centre, Madras (1959 1960, 1965).
+sod PAS 5 g. Isoniazid 300mg. + Thiacetazone 150 mg.	2	176	150	85	E. Af./ B.M.R.C (1963a).
	1	64	57	89	-do-
Isoniazid 150mg. Thiacetazone 75mg.	2dose 2	82	70	85	The present series.

patients were treated with INH alone (26 had initial resistance to INH) and 335 patients were treated with INH and PAS (21 had initial resistance to INH). The resistant cases in both groups showed a less favourable response whereas the sensitive ones showed a favourable response in 61 per cent with INH alone, and in 87 per cent with INH and PAS. The remaining cases developed acquired INH resistance (i.e. 13 per cent for INH and PAS and 39 per cent for INH alone).

A small series done at Madanapalli showed a 36 per cent emergence of resistance at the end of one year when INH alone was administered.

The work of Selkon *et al.* (1964) from Madras showed a 50-76 per cent clinical improvement with varying doses of INH alone with a 24-50 per cent emergence of acquired INH resistance. The group which had INH plus PAS showed improvement in 90 per cent, the remaining 10 per cent developing INH resistance.

Our present series of patient on INH and thiacetazone showed a 24 per cent incidence of acquired INH resistance, but 50 percent of these still showed a

good response to treatment. This may be because in the eleven patients showing a good clinical response, INH resistance often seemed to develop just before bacteriological sputum conversion and did not adversely affect the clinical result. This phenomenon has also been noted by Mitchison (1965).

Our figure of 24 percent for the emergence of acquired INH resistance with INH and thiacetazone is high when compared with the 15 per cent reported by Menon (1965), and the 5 per cent reported by the East African BMRC trials on the same dosage. If, however, we take the figures for emergence of INH resistance in persistently positive sputum cultures, our figure falls to 14 per cent. It appeared in 11 out of 12 such cases.

In the East African series, the presence of initial resistance to thiacetazone was less serious prognostically than initial resistance to INH and our own series shows similar results.

Summary

(1) A combination of thiacetazone 150 mg. with INH 300 mg. gives results comparable with a combination of PAS and INH, showing clinical and bacteriological improvement in 85

TABLE 8

*Initial drug sensitivity of organisms related to progress of patient during chemotherapy**(Modified from Mitchison, 1965)*

Regimen of treatment	Pretreatment of Sensitivity of organisms	Total patients	Patients with favourable response		Reference
			No.	Percent	
INH alone	INH sensitive	359	219	61	Tuberculosis Chemotherapy Centre, Madras, 1960, 1963 a & b.
	INH resistant	26	7	27	
INH plus PAS	INH sensitive	314	273	87	Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1965
	INH resistant	21	5	24	
INH and thiacetazone TBL	INH sensitive	176	146	83	E. African/ B.M.R.C 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	
The present series	INH sensitive	82	70	85	
INH and thiacetazone TBL.	INH resistant	8	2	25	
	TBI sensitive	70	56	80	
	TBI resistant	20	15	75	

per cent. INH resistance developed in 24 per cent.

(2) The presence of primary INH resistance carries a bad prognosis especially in chronic lesions and if found indicates a change of drugs as early as possible.

(3) The incidence of acquired INH resistance (24 per cent) in the presence of thiacetazone is less than in other series in which INH alone is given.

(4) The acquisition of INH resistance was less serious in early or localised lesions than in chronic extensive lesions. Acquisition of INH resistance was more serious if there was also an acquired thiacetazone resistance.

Ind. J. Tub., Vol. XIV, No. 2

(5) Initial INH resistance was often accompanied by initial (62 per cent) or acquired (25 per cent) thiacetazone resistance. 45 per cent of cases with acquired INH resistance also acquired thiacetazone resistance.

(6) Thiacetazone resistance, initial or acquired, was temporary in 66 per cent and the outcome was better than in those cases where it was permanent. Initial thiacetazone resistance did not result in an increased development of INH resistance.

Acknowledgements

This research was made possible by a grant from Unichem Limited and by the kind per-

mission of the Government of Mysore to admit the selected cases on a priority basis. We are grateful to the Director of Medical Services in Mysore, Bangalore, for his encouragement. We thank the members of the nursing staff and laboratory staff of the P.K. Sanatorium and the Holdsworth Memorial Hospital for all their help. We are grateful to Dr. Ramalingam, M.D. and Dr. Kamalaksharappa, M.B.B.S. for their care of the patients. We also thank Dr. Bhaskar, M.D. for his help in preparing this paper.

BIBLIOGRAPHY

East African British Medical Research Council
(1963a), *Tubercle* (Lond.) 44, 301.

(1963b), *Ibid.*, 44, 393.

Menon (1965), *Tubercle* (Lond.), 46, 19.

Mitchison (1965), *Brit. Med. J.*, 1, 1333

Selkon, J.B. *et. al.* (1964), *Bull. Wld. Hlth. Org.*, 31, 273.

Sikand, B.K., Goyal, S.S. and Mathur, G.P. (1963), *Ind. J. Tuberculosis*, 11, 38,

Tuberculosis Chemotherapy Centre, Madras

(1959), *Bull. Wld. Hlth. Org.*, 21, 51.

(1960), *Ibid.*, 23, 535.

(1962a), *Ibid.*, 28, 455.

(1963b), *Ibid.*, 29, 457.

(1963c), *Lancet*, 1, 1078.

(1965), (Quoted by Mitchison).

COMPARATIVE VALUE OF SPUTUM SMEAR EXAMINATION AND CULTURE EXAMINATION IN ASSESSING THE PROGRESS OF TUBERCULOUS PATIENTS RECEIVING CHEMOTHERAPY*

S. DEVADATTA, S. RADHAKRISHNA, WALLACE FOX, D. A. MITCHISON,
S. RAJAGOPALAN, S. SIVASUBRAMANJAN & H. STOTT

In studies of the chemotherapy of pulmonary tuberculosis undertaken at the Tuberculosis Chemotherapy Centre, Madras, the progress of patients has been assessed mainly on the results of sputum cultures for tubercle bacilli. However, culture facilities are either very limited or unavailable in most tuberculosis clinics in developing countries. The present paper therefore attempts to study the efficacy of the simple method of sputum smear examination for tubercle bacilli, relative to that of culture examination (and isoniazid-sensitivity tests), in assessing the progress of patients receiving chemotherapy and in classifying their response to treatment. The comparisons are based on the ability, during treatment, of these methods to predict the outcome at the end of one year of chemotherapy. The patients for these comparisons have been drawn from the first 3 chemotherapy studies undertaken at the Centre (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1963). In these studies, five regimens, ranging in therapeutic efficacy (as measured by the proportion of patients showing a favourable response, assessed mainly by strict bacteriological criteria) from about 45% to 90%, had been investigated.

Patients

In this report, 532 patients, who were included in the main analysis of three chemotherapy studies (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1963), have been considered. All were aged 12 years or more, had pulmonary tuberculosis confirmed by culture examination, had had no previous chemotherapy (except 18 (3%) who had received up to two weeks of chemotherapy), and were excreting *isoniazid-sensitive* tubercle bacilli on admission to treatment. They were prescribed isoniazid plus *p*-aminosalicylic acid (sodium PAS) or one of four regimens of isoniazid alone for 12 months (see footnote to Table 5).

Seventeen patients have been excluded from all the analyses in the present report because

* From the Tuberculosis Chemotherapy Centre, Madras 31, India. The Centre is 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.

This paper is also published in the *Bulletin of the World Health Organization*.

their response to the initially prescribed chemotherapy could not be assessed, eight because they died of non-tuberculous conditions and nine because their chemotherapy was changed on account of drug toxicity. Of the remaining 515, who form the subject of this report, 9 died of tuberculosis and 40 had the initially prescribed chemotherapy changed on account of serious radiographic or clinical deterioration. These 49 patients have been classified as having an unfavourable response to treatment and included in all the analyses up to the time of death or change of chemotherapy.

Investigation during treatment

All the patients had routine monthly examinations at the Centre which included a chest radiograph and sputum examination. At the end of each month of treatment, they were asked to produce two collection specimens of sputum. In addition, from three months onwards, when it was anticipated that some of the patients would have difficulty in expectorating, a pair of laryngeal swabs was taken from all patients at each monthly examination. The sputum specimens were examined for the presence of tubercle bacilli both by smear and by culture, and the laryngeal swabs by culture only. An isoniazid-sensitivity test was set up on one positive culture, if available, at each monthly examination.

Methods of obtaining specimens

Collection specimens of sputum. The patient was given a sterile screw-capped McCartney bottle (1 oz¹) to take home and was instructed to expectorate into it until it was nearly full. On the following morning, the bottle was handed in at the laboratory; the specimen was thus material collected overnight and sometimes over 24 hours.

Laryngeal swab specimens. A pair of swabs was obtained at the Centre from each patient as described by Velu, Narayana & Subbaiah (1961).

Bacteriological procedures

Direct smear examination of sputum specimens. A new glass slide was used for the preparation of each smear. Purulent or, failing

¹ 1 fluid ounce=28 ml.

this, mucoid material or any deposit in the sputum was selected for examination. The whole smear was examined by fluorescence microscopy (Hoist, Mitchison & Radhakrishna, 1959) and was reported as positive if it contained a minimum of four acid-fast bacilli of typical morphology.

Culture of sputum specimens- and laryngeal swab specimens. From each sputum specimen and from each pair of laryngeal swabs (processed as one specimen), cultures were set up on Lowenstein-Jensen medium without potato starch (Tuberculosis Chemotherapy Centre, Madras, 1959), and examined weekly for 8-9 weeks; they were reported as negative if no growth was present by that time.

Sensitivity tests. Tests of sensitivity to isoniazid were set up on Lowenstein-Jensen medium slopes containing concentrations of isoniazid of 0.2, 1, 5 and 50 µg/ml, as well as on a drug-free control slope, by inoculating approximately— 0.01 mg. (moist weight) of tubercle bacilli (Tuberculosis Chemotherapy Centre, Madras, 1959). A culture isolated during treatment was classified as isoniazid-resistant if it yielded a growth of 20 colonies or more on the slope containing 0.2 µg/ml.

Definitions of favourable and unfavourable response to treatment

Patients with a *favourable* response to treatment are defined as those who yielded only negative cultures (usually 7-9) at least at the last three monthly examinations, that is, at 10, 11 and 12 months. In addition, 21 (4%) patients who yielded only one positive culture at 10, 11 or 12 months following at least three consecutive monthly examinations at which all cultures were negative have also been regarded as having had a favourable response for reasons reported earlier (Velu et al., 1960, 1961; Devadatta et al., 1961a; Dawson et al., 1966).

Patients with an *unfavourable* response to treatment are defined as (a) those who never had all cultures negative at three consecutive monthly examinations (b) those who had a total of two or more positive cultures at the last three monthly examinations following culture negativity at three or more consecutive monthly examinations or (c) those who had had their chemotherapy changed owing to serious radiographic or clinical deterioration or (d) those who died of tuberculosis.

It will be noted that these definitions do not depend on the results of smear examination of sputum specimens.

Results

Although five regimens with different levels of therapeutic effectiveness were investigated, only the amalgamated results have been presented in Tables 1 to 4; the findings in the individual treatment series are briefly discussed on page 17.

Prognostic value of smear, culture and isoniazid-sensitivity examinations of one sputum specimen during treatment

Table 1 compares the value of smear examination, culture examination and isoniazid-sensitivity tests in predicting favourable response to treatment. The analysis is based on the results obtained with one sputum specimen each month. In order that the comparisons between the three methods are based on the same population, only those patients who had both smear and culture results available on the same specimen, together with an isoniazid-sensitivity test result at that month if the culture was positive, have been included in the analysis.

Considering first the results of smear examination, 65% of 198 patients with a positive smear at month had a favourable response at one year, as compared with 82% of 282 patients with a negative smear. The corresponding proportions at two months were 54% of 125 and 82% of 365 patients. Thus, in the first 2 months, smear examination was not of much prognostic value. However, this value increased in subsequent months. Thus, 33% of 108 patients with a positive smear at 3 months as compared with 86% of 375 with a negative smear had a favourable response at one year; the corresponding proportions at 6 months were 3% of 70 and 88% of 415, and these remained fairly stationary for the rest of the year, the proportions at 12 months being 0% of 49 and 91% of 399, respectively. An indication of the gradual increase from month to month in the prognostic value of smear examination is provided by the trend observed in the percentage of correct predictions,¹ when the results of smear examination at each month are used to predict the outcome at the end of one year of treatment. This proportion was 62% at 1 month, 82% at 3 months, 90% at 6 months 89% at 9 months and 92% at 12 months.

Culture examination in the first 2 months of treatment was also not of much prognostic value. However, its value, like that of smear examination, increased subsequently. Thus 10% of 98 patients with a positive culture at 6 months had a favourable response at one year, as compared with 93% of 387 patients with a negative culture; the corresponding

TABLE I

Favourable response at one year related to results of smear, culture and isoniazid-sensitivity tests on one sputum specimen, at monthly intervals during treatment

Months after start of treatment		Smear		Culture		Isoniazid sensitivity		Total patients at analysis*
		Positive	Negative	Positive	Negative	Sensitive	Resistant or culture-negative	
1	Total patients	198	282	362	118	28	452	480
	Favourable response No.	128	230	248	110	5	353	
	%	65	82	69	93	18	78	
	% correctly predicted	62		47		78		
2	Total patients	125	365	241	249	65	425	490
	Favourable response No.	67	298	142	223	8	357	
	%	54	82	59	90	12	84	
	% correctly predicted	73		66		84		
3	Total patients	108	375	157	326	87	396	483
	Favourable response No.	36	323	69	290	13	346	
	%	33	86	44	89	75	87	
	% correctly predicted	82		78		87		
4	Total patients	79	405	111	373	95	389	484
	Favourable response No.	17	348	23	342	11	354	
	%	22	86	21	92	12	91	
	% correctly predicted	85		89		90		
5	Total patients	80	407	102	385	95	392	487
	Favourable response No.	14	360	14	360	9	365	
	%	18	88	14	94	9	93	
	% correctly response	87		92		93		
6	Total patients	70	415	98	387	95	390	485
	Favourable response No.	2	367	10	359	7	362	
	%	3	88	10	93	7	93	
	% correctly predicted	90		92		90		
7	Total patients	61	415	100	376	98	378	476
	Favourable response No.	5	360	10	355	8	357	
	%	8	87	10	94	8	94	
	% correctly predicted	87		93		94		

8	Total patients	66	404	92	378	90	380	470	
	Favourable response	No.	4	361	7	358	5		360
	%	6	89	8	95	6	95		
	% correctly predicted	90		94		95			
9	Total patients	60	405	78	387	75	390	465	
	Favourable response	No.	5	361	3	363	1		365
	%	8	89	4	94	1	94		
	% correctly predicted	89		94		94			
12	Total patients	49	399	69	379	65	383	448	
	Favourable respons;	No.	0	363	4	359	1		362
	%	0	91	6	95	2	95		
	% correctly predicted	92		95		95			

* See text on preceding page

proportions at 12 months were 6% of 69 and 95% of 379. The proportion of correct predictions¹ was 47% at 1 month, 78% at 3 months, 92% at 6 months, 94% at 9 months and 95% at 12 months.

Considering next the results of isoniazid-sensitivity tests, 18% of 28 patients with a resistant culture at one month had a favourable response at one year, as compared with 78% of 452 patients with an isoniazid-sensitive or a negative culture. The corresponding proportions at two months were 12% of 65 and 84% of 425 patients. Thus, even in the early months of treatment, the isoniazid-sensitivity test was of moderate prognostic value. Thereafter, its value increased further. Thus, 7% of 95 patients with a resistant culture at 6 months had a favourable response at one year, as compared with 93% of 390 with a sensitive or negative culture, and at 12 months the corresponding proportions were 2% of 65 and 95% of 383. The proportion of correct predictions² was 78% at 1 month, 84% at 2 months, 87% at 3 months, 93% at 6 months,

¹ This figure is derived for each month by adding the number of patients with a *negative* result at that month and a *favourable* response at one year to the number with a *positive* result at that month and an *unfavourable* response at one year, and expressing the sum as a proportion of the total patients assessed. For example, considering smear results at three months, the figures are 323 and 72, respectively, resulting in 395 correct predictions out of a total of 483, that is, 82% of correct predictions.

² This figure is derived for each month by adding the number of patients with a negative or an isoniazid-sensitive culture at that month and a favourable response at one year to the number with an isoniazid-resistant culture at that month and an unfavourable response at one year, and expressing the sum as a proportion of the total patients assessed.

94% at 9 months and 95% at 12 months.

On comparing the prognostic value of smear examination with that of culture examination, it is seen that neither examination is very satisfactory in the early months of treatment; thus, at three months, predictions based on smear results would have been correct in 82% of the patients, the corresponding figure for culture results being 78%. At subsequent months, both types of examination yielded very satisfactory results—for example, the proportion of correct predictions at six months was 90% with smear results and 92% with culture results; nevertheless, the prognostic value of culture was consistently better than that of smear. For assessing the statistical significance of this difference, it is convenient to present the results as in Table 2. This table shows that, at four months, correct predictions were made by both smear and culture results for 396 of 484 patients and incorrect predictions by both in 40, so that predictions based on smear and those based on culture were equally efficient or inefficient in 436 (90%) of the patients. Of the remaining 48 (10%) patients, 14 were correctly predicted by smear but incorrectly by culture and, conversely, 34 were correctly predicted by culture and incorrectly by smear; this difference is statistically significant ($P < 0.01$). The findings at subsequent months also showed a consistent and statistically significant benefit to the culture examination. However, this benefit was usually of the order of only 5% and is, to a certain extent, offset by the fact that cultures are likely to become contaminated; further, it always takes much longer to obtain culture results—according to the procedure used in this laboratory

TABLE 2

Comparison of the prognostic value of smear examination and culture examination of one sputum specimen, at monthly intervals during treatment

Prediction based on smear results	Prediction based on culture results	Months after start of treatment													
		4		5		6		7		8		9		12	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Correct	Correct	396	82	415	85	424	87	406	85	417	89	413	89	407	97
Incorrect	Incorrect	40	8	28	6	21	6	21	4	21	4	24	5	19	4
Correct	Incorrect	14	3	11	2	11	2	10	2	6	7	3	1	5	1
Incorrect	Correct	34	7	33	7	23	5	39	8	26	6	25	5	17	4
Total		485 100		48 100		485 100		476 99		470 100		465 100		448 100	
X ² a		8.33		11.00		4.24		17.16		12.50		17.29		6.55	
P		<0.01		<0.001		<0.05		<0.001		<0.001		<0.001		=0.01	

^a Based on the third and fourth rows of figures and having one degree of freedom.

up to nine weeks - as compared with smear results, which usually can be obtained the same day.

On comparing next the prognostic value of culture examination with that of the isoniazid-sensitivity test, the latter was found to be uniformly better in the early months of treatment; thus, the *difference* in the magnitude of correct predictions was 31% at 1 month, 18% at 2 months, 9% at 3 months, and 1% at most of the subsequent months (Table 1). (However, according to the procedure used in this laboratory, it takes four weeks after a culture has become positive for the sensitivity test result to become available.) An assessment of the statistical significance of this difference must of necessity be confined to patients who produced a positive culture. At one month, there were 362 patients with positive cultures; both types of examination yielded correct predictions in 23 patients and incorrect predictions in 5 (not tabulated here). In the remaining 334 patients, the outcome was correctly predicted by culture results in 91 and by isoniazid-sensitivity results in 243, the difference being highly significant statistically ($P < 0.001$). The corresponding differences at two months and three months were also highly significant ($P < 0.001$). However, at four months, discrepant predictions were obtained for only 16 patients; the prediction based on culture was correct in 4 cases and that based on isoniazid sensitivity in 12, the difference just attaining statistical signifi-

cance ($P = 0.05$). At subsequent months, none of the differences was statistically significant.

It may be concluded that the examination by smear of one overnight specimen of sputum at monthly intervals is of great prognostic value, it being possible to predict correctly from four months onwards the outcome of treatment in at least 85% of the patients. Examination by culture increases this proportion to at least 89%, the increase being statistically significant. No further benefit is derived after four months (in terms of correct predictions) by the performance of an isoniazid-sensitivity test. However, in the early months of treatment, the proportion of correct predictions can be significantly increased by considering the results of isoniazid-sensitivity tests.

Comparison of the prognostic value of smear examination of one and two sputum specimens during treatment

Since smear examination of one sputum specimen during treatment was found to be of great prognostic value from four months onwards, it was decided to investigate whether the smear examination of an additional specimen would enhance the value, particularly at 1, 2 and 3 months. Analyses were therefore undertaken, along the lines of those in Table 1, on all patients with smear results on two sputum specimens at the monthly examination,

irrespective of the availability of culture results.

Analyses, not tabulated here, showed that in the first 3 months of treatment, prognostication based on two smears was unsatisfactory, as was the case with one smear. Further, they also showed that from four months onwards, when the majority of sputum smears were negative, the prognosis of one positive smear plus one negative smear was invariably closer to that of two positive smears than to that of two negative smears; consequently, in Table 3, which presents the findings at 4-9 months and at 12 months, patients have been classified as having "at least one positive smear" or "both smears negative", when considering the prognostic significance of two smears. These findings show that, at four months, the proportion of patients whose response was correctly predicted by considering the smear result of one specimen was 84%, as compared with 86% by considering the smear results of two specimens; the corresponding figures at five months were 87% and 86%, respectively; neither of these differences attained statistical significance. Both at six months and at seven months, the proportion of correct predictions was significantly (although only slightly) greater when based on smear results of two sputum specimens instead of one; thus, the proportions were 92% and 90% at six months ($P=0.02$), and 90% and 87% at seven months ($P=0.001$). At eight months and subsequently, the differences were smaller and were statistically non-significant.

It may be concluded that, from six months onwards, the prognostic value of two smear examinations each month is slightly greater than that of one smear examination.

Comparison of the prognostic value of two smears, one culture and two cultures during treatment

It has been shown that the prognostic value of culture examination of one sputum specimen during treatment is slightly greater than that of smear examination of one specimen from four months onwards. It has also been shown that the prognostic value of smear examination of two specimens is slightly greater than that of one specimen from six months onwards. It was therefore decided to compare the prognostic value of smear examination of two specimens with that of culture examination of one specimen from six months onwards. This comparison is presented in Table 4, which also includes the results obtained by culture examination of two specimens. Only patients with both smear and culture results available on two specimens of sputum at the monthly

examinations have been included in the analysis. As with smears, the prognosis of one positive culture plus one negative culture was invariably closer to that of two positive cultures than to that of two negative cultures, so that patients have been classified in the table as having "at least one positive culture" or "both cultures negative", when considering the prognostic significance of two cultures.

At six months, the proportion of correct predictions was 92% whether two smears or one culture formed the basis of prediction. However, both at seven and at eight months, predictions based on two smears were correct in 90% of the patients, as compared with 93% in the case of one culture, a significant difference ($P<0.02$). The corresponding proportions were 91% and 94% at nine months ($P=0.02$), and 92% and 94% at 12 months ($P=0.05$). It may be concluded that, from six months onwards, the prognostic value of culture examination of one sputum specimen is slightly but consistently greater than that of smear examination of two specimens.

On comparing next the prognostic value of culture examination of one sputum specimen with that of two specimens, little difference was found in the proportions of correct predictions (Table 4).

Findings in the individual treatment series

The patients in the present report received isoniazid plus sodium PAS (PH) or one of four regimens of isoniazid alone, namely, HI-1, HI-2, H and H650 (for details, see footnote to Table 5). It was found that the HI-1 and H650 regimens were of the same order of therapeutic efficacy (Tuberculosis Chemotherapy Centre, Madras, 1963); this was also true of the HI-2 and H regimens which were, however, less effective (Tuberculosis Chemotherapy Centre, Madras, 1960). The PH regimen was the most efficacious.

Separate analyses were undertaken for patients in the PH, HI-1/H650 and HI-2/H series to determine the extent to which the main conclusions drawn so far may have to be modified by the differences between the regimens in therapeutic efficacy. Table 5 summarizes the findings of analyses based on one sputum specimen collected at monthly intervals during treatment, from four months onwards. First it is seen that both smear and culture are of less prognostic value in the HI-2/H series, the mean proportion of correct predictions being 83.6%, and 89.2%, respectively, as compared with 89.2% and 95.7%, in the HI-1/H-650 series and 92.1%, and 94.3% in the PH series, respectively. Secondly, the contrast in

TABLE 3

Favourable response at one year related to the results of smear examination of one sputum specimen and of two sputum specimens, at monthly intervals during treatment

Months after start of treatment		One smear		Two smears		Total patients in analysis ^a
		Positive	Negative	Atleast one Both positive negative		
4	Total patients	76	393	92	377	469
	Favourable response No.	16	336	21	331	
	%	21	85	23	88	
	% correctly predicted	84		86		
5	Total patients	85	397	102	380	482
	Favourable response No.	16	348	25	339	
	%	19	88	25	89	
	% correctly predicted	87		86		
6	Total patients	71	407	89	389	478
	Favourable response No.	2	360	6	356	
	%	3	88	7	92	
	% correctly predicted	90		92		
7	Total patients	64	404	80	383	468
	Favourable response No.	7	351	8	350	
	%	11	87	10	90	
	% correctly predicted	87		90		
8	Total patients	69	402	79	392	471
	Favourable response No.	4	350	9	355	
	%	6	90	11	91	
	% correctly predicted	90		90		
9	Total patients	58	394	68	384	452
	Favourable response No.	5	352	7	350	
	%	9	89	10	91	
	% correctly predicted	90		91		
12	Total patients	49	389	57	381	438
	Favourable response No.	0	364	2	352	
	%	0	91	4	92	
	% correctly predicted	92		93		

^a See text on opposite page.

TABLE 4

Favourable response at one year related to results of examination by smear of two sputum specimens and by culture of one and of two sputum specimens, at monthly intervals during treatment

Months after start of treatment		Two smears		One culture		Two cultures		Total patients in analysis ^a
		At least one positive	Both negative	Positive	Negative	At least one positive	Both negative	
6	Total patients	73	323	81	315	95	301	396
	Favourable response No.	6	296	9	293	14	288	
	%	8	92	11	93	15	96	
	% correctly predicted	92		92		93		
7	Total patients	69	324	88	305	98	295	393
	Favourable response No.	6	290	9	287	14	282	
	%	9	90	10	94	14	96	
	% correctly predicted	90		93		93		
8	Total patients	66	315	80	301	91	290	381
	Favourable response No.	8	284	8	284	10	292	
	%	12	90	10	94	11	97	
	% correctly predicted	90		93		95		
9	Total patients	60	303	65	298	79	284	363
	Favourable response No.	6	275	3	278	10	271	
	%	10	91	5	93	13	95	
	% correctly predicted	91		94		94		
12	Total patients	49	310	61	298	67	292	359
	Favourable response No.	2	283	4	281	7	278	
	%	4	91	7	94	10	95	
	% correctly predicted	92		94		94		

^aSee text, page 578.

prognostic value between smear and culture examinations is smallest in the PH series, the mean difference in the proportion of correct predictions being 2.2% as compared with 6.5% in the HI-1/H650 series and 5.6% in the HI-2/H series; the differences in these figures between the PH series and each of the other two series attain statistical significance ($P < 0.02$). These findings suggest that the value of both types of examination, particularly the smear examination, is adversely affected if the regimen is of efficacy and both are prognostically more

efficient and yield closely similar results if the regimen is of high efficacy.

Comparison of smear and culture examinations in assessing the therapeutic efficacies of antituberculosis regimens

At the Centre, the therapeutic efficacy of antituberculosis regimens is assessed mainly on the basis of culture results of sputum specimens collected at 10, 11 and 12 months. It is now proposed to study the value of similar

TABLE 5

Comparison of the prognostic value in individual treatment series of smear examination and culture examination of one sputum specimen at monthly intervals during treatment

Treatment series ^a	Assessment	Percentage of correct predictions									
		Months after start of treatment									
		4	5	6	7	8	9	10	11	12	Mean
PH	Smear	88	88	92	92	92	93	94	94	96	92.1
	Culture	89	93	94	95	96	95	95	97	95	94.3
HI/H650	Smear	89	88	90	85	92	88	90	91	90	89.2
	Culture	94	96	95	95	94	96	98	97	96	95.7
HI-2/H	Smear	76	87	85	81	85	85	85	82	86	83.6
	Culture	84	87	87	88	91	90	91	93	92	89.2

^a PH: 3.9-5.5 mg/kg body-weight (200 mg for 100-lb (45.4-kg) patient) of isoniazid plus 0.2-0.3 g/kg body-weight of sodium PAS (10 g for 100-lb patient), daily in two divided doses, for 12 months.

HI-1: 7.8-9.6 mg/kg body-weight (400 mg for 100-lb patient) of isoniazid alone, daily in one dose, for 12 months.

HI-2: 7.8-9.6 mg/kg body-weight (400 mg for 100-lb patient) of isoniazid alone, daily in two divided doses, for 12 months.

H: 3.9-5.5 mg/kg body-weight (200 mg for 100-lb patient) of isoniazid alone, daily in two divided doses, for 12 months.

H650: 12.5-15.2 mg/kg body-weight (650 mg for 103-lb patient) of isoniazid alone, daily in one dose, for 12 months.

TABLE 6

Therapeutic efficacies of antituberculosis regimens in three controlled studies, as assessed by smear results and culture results

Study	Treatment series ^a	Total patients	Patients with a favourable response			
			Classification based on culture results ^b		Classification based on smear results ^c	
			No.	%	No.	%
First	Sanatorium (PH)	81	78	90	12	89
	Home (PH)	81	67	83	69	85
Second	PH	86	78	91	77	90
	HI-1	64	47	73	48	75
	HI-2	66	38	58	43	65
	H	86	38	44	41	48
Third	H650	51	37	73	38	75

^a For details, see footnote to Table 5.

^b For details, see page 574.

^c For details, see — text.

assessments based solely on smear results. Such a study would be of special interest to centres in developing countries with limited or no culture facilities.

In the following analyses, the definition of favourable response, based on smear results only, is "all smears (usually 7-9) negative at 10, 11 and 12 months", and that of unfavourable response is "at least one positive smear at 10, 11 or 12 months, or change of chemotherapy owing to serious radiographic or clinical deterioration, or death from tuberculosis". The corresponding definitions based on culture results have been stated on page 81.

Both sets of definitions were applied to patients in three studies undertaken at the Centre and the findings are presented in Table 6. The first study was a comparison of the efficacy of treatment with isoniazid plus PAS at home and the same treatment in sanatorium, for 12 months (Tuberculosis Chemotherapy Centre, Madras, 1959). The second was a comparison of the efficacies of four antituberculosis regimens, three of isoniazid alone and one of isoniazid plus PAS, in the domiciliary treatment of pulmonary tuberculosis for 12 months (Tuberculosis Chemotherapy Centre, Madras, 1960). In the third, which was a controlled study of isoniazid toxicity, only one antituberculosis regimen, namely a high dosage of isoniazid, was used (Tuberculosis Chemotherapy Centre, Madras, 1963).

the first study, 90% of 81 patients treated in sanatorium were classified, on the basis of smear results, as having had a favourable response as compared with 89% on the basis of culture results. The corresponding proportions for 81 patients treated at home were 85%, respectively. In the second, the proportions classified as having had a favourable response on the basis of culture results were 91%, of 86 PH, 73%, of 64 HI-1, 58%, of 66 HI-2 and 44% of 86 H patients; the corresponding proportions based on smear results were 90%, 75%, 65% and 48%, respectively. In the third study, 73% of the 51 patients had a favourable response as assessed by culture results, as compared with 75% as assessed by smear results.

Thus, there is not much difference between the smear assessment and the culture assessment of the therapeutic efficacy of the regimens. Further analyses (not tabulated here) showed that, on the average, the two assessments yielded an identical classification in 95% of the patients, (range, 92%-99%). However, an inspection of the proportions of patients with a favourable response to the various regimens shows that the variation from regimen to

regimen is greater for the classification based on culture results than for that based on smear results. This is reflected by the range of these proportions, which is 44%-91% for the former and 48%-90% for the latter. This suggests that the ability to detect differences in efficacy between regimens is greater with cultures than with smears. In the Annex (page 93), this conclusion is confirmed on a larger number of regimens obtained by including three more studies undertaken at the Centre. It is also shown there that this shortcoming of a chemotherapy study based on smear examination alone can usually be offset by a 20% increase in the number of patients admitted to study.

Applicability of the findings in situations where smear examination alone is available for diagnostic purposes

All the analyses in this report are based on 515 patients who had at least one positive culture on admission to treatment and who were excreting isoniazid-sensitive bacilli (see page 81). However, not infrequently, unsatisfactory situations are encountered where facilities for culture examination (and sensitivity tests) are not available, and where the initial diagnosis of tuberculosis is based mainly on the results of smear examination. It is therefore of interest to know how the conclusions in the foregoing pages would be affected in such situations, since patients without a positive smear would not be included while those with isoniazid-resistant organisms (and at least one positive smear) would not be excluded. In the present instance, there were 43 patients in the former category and 28 in the latter; on excluding the 43 and including the 28, the modified population for analysis became 500.

Analyses were undertaken on the modified population to compare the prognostic value of smear examination and culture examination of one sputum specimen at monthly intervals during treatment. These showed that, apart from decreasing very slightly (1% to 3%) the proportion of correct predictions, the changes in the population did not have any important effect either on the prognostic value of smear or on that of culture. It therefore seems reasonable to conclude that the findings in the present paper are also applicable to situations where smear examination alone is available for diagnostic purposes; however, it must be emphasized that this conclusion is based on data from a clinic where excretors of acid-fast bacilli of non-tuberculous origin are uncommon.

Discussion

The progress during treatment of patients

with pulmonary tuberculosis is usually followed by periodic bacteriological, radiographic and clinical examinations. In economically favoured countries, it is possible to prescribe regimens of chemotherapy which produce a favourable response in virtually 100% of the patients (Crofton, 1960; Canetti, 1962). In such situations, despite current practice, it is obviously not important to follow closely the progress of patients receiving chemotherapy provided it is ensured that they are in fact receiving the prescribed drugs. On the other hand, in developing countries, regimens which *do not* produce a favourable response in 100% of the patients often have to be prescribed for economic reason (Lauckner, 1959; Canetti, 1962; *Tubercle (Land.)*, 1963; Fox, 1964). Under these circumstances, it is important to assess periodically the progress of patients so that those whose disease is not responding to chemotherapy may be detected as early as possible.

Of the various methods used to follow the progress of patients, bacteriological assessments are the most valuable (American Trudeau Society, 1959). It has been shown at the Centre that the erythrocyte sedimentation rate is an unsatisfactory measure of progress, as an elevated rate is compatible with bacteriologically quiescent disease at the end of a year of chemotherapy (Tuberculosis Chemotherapy Centre, Madras, 1959; or at the end of a 2-year (Devadatta et al., 1961a) or a 4-year (Dawson et al., 1966) period of follow-up. Also, serial radiography is of limited value in assessing the progress of patients since radiographic deterioration—for instance, an increase in the size of cavities—can occur in the presence of persistent bacteriologically quiescent disease (Devadatta et al., 1961a), and radiographic improvement is not necessarily associated with a favourable bacteriological response (Devadatta et al., 1961b).

The bacteriological methods most commonly used are examination of sputum smears for organisms morphologically resembling tubercle bacilli, culture examination for tubercle bacilli and tests of sensitivity to the drugs prescribed. Culture examination and drug-sensitivity tests take many weeks to yield results, require more skilled staff, and are economically not possible as routine methods in most tuberculosis clinics in developing countries at the present time. Moreover, cultures may become contaminated with other organisms; this occurred in 9.1% of the 11,445 cultures set up during treatment from sputum specimens obtained from the 515 patients who form the subject of this report. Smear examination has the disadvantage of being less sen-

sitive than culture examination in detecting tubercle bacilli (Corper & Cohn, 1933; Andrews & Radhakrishna, 1959). It is also more likely to produce false positive results since it is not usually possible to distinguish tubercle bacilli from other acid-fast organisms. However, this occurred very rarely in the present study; thus, of 2,098 sputum specimens examined during treatment which yielded a positive smear, only 0.1% were subsequently found to be due to acid-fast saprophytes. Another disadvantage is that a smear-positive culture-negative result would be regarded (often wrongly) as indicating the presence of viable tubercle bacilli if culture examination is not undertaken (Tuberculosis Chemotherapy Centre, Madras, 1959; Great Britain, Medical Research Council, 1962); in this study, of the 2,098 sputum specimens with a positive smear, 7.1% yielded a negative culture.

Smear examination, however, does not need complicated laboratory facilities, is easy to perform, and results can be obtained on the same day that specimens are submitted to the laboratory; further, under Indian conditions, it is approximately 10 times less expensive than culture examination and 20 times less expensive than a sensitivity test to a single drug (E.M. Mackay-Scollay, quoted by Fox, 1964). It was decided, therefore, to compare the value of smear examination with that of culture examination and isoniazid-sensitivity tests in assessing the progress of patients receiving chemotherapy. The smears were examined by fluorescence microscopy; this method yields at least as many positive results as the Ziehl Neelsen method, no more false positives, the advantage of a very great saving in the preparation and examination of (Hoist, Mitchison & Radhakrishna, 19

The findings in the present study are based on the results of smear and culture examination of sputum specimens collected at monthly intervals from 515 patients receiving isoniazid, either alone or in combination with PAS; all 515 patients had bacteriologically confirmed pulmonary tuberculosis with isoniazid-sensitive organisms on admission to treatment. The results revealed that, from four months onwards, the examination by smear of one sputum specimen per month is a very valuable method of assessing the progress of patients receiving chemotherapy. For example, 97% of 70 patients with a positive smear result at six months had an unfavourable response at one year and 88% of 415 with a negative smear result had a favourable response. (The response to treatment was classified as favourable or unfavourable, mainly on the basis of culture results of a total of 7-9 sputum speci-

mens, obtained at 10, 11 and 12 months). If the smear result at six months had been used to predict the response to treatment, correct predictions would have resulted for 90% of the patients, and this proportion remained fairly constant for the rest of the year. Examination by culture of one sputum specimen was of slightly more prognostic value. Thus, if the results of one culture examination at six months had been used to predict the outcome of treatment, correct predictions would have resulted for 92% of the patients. However, in situations where sputum conversion occurs unusually late—there is evidence that this happens in Hong Kong (British Tuberculosis Association/Hong Kong Tuberculosis Treatment Services, 1964)—the value of both methods of examination at six months in predicting the outcome at the end of one year of chemotherapy might be less.

The close agreement between predictions based on smear and culture examinations can be understood by considering the changes in the bacterial population in any patient receiving chemotherapy. Thus, if the disease is responding, the sputum first becomes negative on smear examination because of a decrease in the bacterial population and then negative on culture examination, the disease eventually attaining bacteriological quiescence. On the other hand, if the disease stops responding to the chemotherapy, the sputum remains positive, or eventually becomes positive, on smear examination because of an increase in the bacterial population. Furthermore, the frequency of smear-positive culture-negative results and of acid-fast saprophytes—the two main causes of false positive results on smear examination—was not high among the specimens in this study.

Culture examination of two sputum specimens at monthly intervals did not prove to be of greater prognostic value than that of one specimen. However, with a contamination rate of 9%, the examination of two specimens instead of one would substantially reduce the number of patients with *no information* available on account of contaminated cultures. Isoniazid-sensitivity tests increased the prognostic value of positive cultures during the first 4 months of treatment. However, this benefit is not of much practical value because it might take one month after isolation of a positive culture to obtain the isoniazid sensitivity result. After four months of treatment, no benefit was derived by considering the results of isoniazid-sensitivity tests, the explanation for this finding being that nearly all positive cultures obtained in later months from patients receiving chemotherapy containing isoniazid are resistant to that drug

(Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1963; East African/British Medical Research Council Isoniazid Investigation, 1960; Selkon et al, 1964).

This study has shown that it is possible to draw accurate conclusions regarding the efficacies of antituberculosis regimens from controlled trials, even if sputum specimens are examined by smear only instead of by culture. However, in such trials, the likelihood of detecting differences in efficacy between regimens would be slightly decreased, a disadvantage which can usually be offset by a 20% increase in the number of patients admitted to study. These findings suggest that it would be inappropriate not to *consider* controlled chemotherapy studies in developing countries merely because facilities for culturing tubercle bacilli are very limited. Such countries usually have large numbers of tuberculous patients and, provided an *efficient* smear service can be set up, accurate conclusions can be drawn from controlled studies in which smear examination only is undertaken during treatment, although a slightly larger number of patients would have to be admitted. Even in these circumstances, it would be *very desirable* to undertake culture and drug-sensitivity examinations on sputum specimens obtained from patients *before* admission to the study. If facilities for quick and efficient transportation of the sputum specimens are available, such tests could be arranged either at a central laboratory in the same country or in a more developed country. For instance, a central laboratory in Madras is successfully undertaking cultures and drug-sensitivity tests on sputum specimens collected from centres in various parts of India (P.R.J. Gangadharam, personal communication, 1965). Such tests have also been carried out successfully in London on sputum specimens collected from patients in Hong Kong (Hong Kong Government/British Medical Research Council Investigation, 1964), and from patients in Rhodesia (Joan F. Hefferman, personal communication, 1966).

It is concluded that the value of smear examination of overnight specimens of sputum at monthly intervals approaches closely that of culture examination and drug-sensitivity tests assessing the progress of tuberculous patients receiving chemotherapy, and in assessing the therapeutic efficacies of regimens.

Summary

1. This paper compares the value of smear examination for tubercle bacilli of overnight specimens of sputum, month by month, with that of culture examination and isoniazid-sensitivity tests in assessing the progress of patients

treated with isoniazid, either alone or with sodium PAS, in three chemotherapy studies. The comparisons are based on the ability of these bacteriological methods to predict, during treatment, the response at the end of 12 months, which was classified as favourable or unfavourable, mainly on the basis of culture results at 10, 11 and 12 months.

2. Smear examination, from four months onwards, of one sputum specimen was a very valuable method of assessing the progress of patients receiving chemotherapy. For example, 97% of 70 patients with a positive smear at six months had an unfavourable response at one year and 88% of 415 with a negative smear had a favourable response. If the results of one smear examination at six months had been used to predict the outcome of treatment, correct predictions would have been made in 90% of the patients.

3. Culture examination of one sputum specimen was of slightly greater prognostic value than smear examination from four months onwards. Thus, if the result of a culture examination at six months had been used to predict the outcome of treatment, correct predictions would have been made in 92% of the patients. However, the slight advantage, which is statistically significant, is partially offset by the delay in obtaining the results and loss of information due to contamination.

4. There was evidence suggesting that the value of smear and culture examinations, particularly the former, was adversely affected if the regimen was of low efficacy and that both were prognostically more efficient and yielded closely similar results if the regimen was of high efficacy.

5. The prognostic value of smear examination of two sputum specimens was slightly greater than that of one specimen, but less than that of culture examination of one specimen, for six months onwards.

6. Culture examination of two sputum specimens offered no further benefit over that of one specimen except in substantially reducing losses due to contamination.

7. Isoniazid-sensitivity tests were found to increase the prognostic value of positive cultures during the first 4 months of chemotherapy only; moreover, the test results might not become available until as long as one month after the culture had become positive.

8. Reliable conclusions could be drawn regarding the therapeutic efficacies of regimens by considering the results of smear examination, since the two types of assessment (smear and culture) yielded, on the average, identical

classification in 95% of the patients. However, smear examination was slightly less sensitive than culture examination in detecting differences in the therapeutic efficacies of various anti-tuberculosis regimens; this disadvantage can usually be offset by admitting about 20% more patients.

REFERENCES

- American Trudeau Society (1959) *Aincr. Rev. resp. Dis.*, 80, 118.
- Andrews, R. H. & Radhakrishna, S. (1959) *Tubercle (Land.)*, 40, 155.
- British Tuberculosis Association/Hong Kong Tuberculosis Treatment Services (1964) *Tubercle (Land.)*, 45, 299
- Canetti, G. (1962), *Tubercle (Land.)*, 43, 301
- Corper, H. J. & Cohn, M. L. (1933) *J. Lab. clin. Med.*, 18, 515
- Croft on, J. (1960) *Brit. med. Bull.*, 16, 55
- Dawson, J.J.Y., Devadatta, S., Fox, W., Radhakrishna, S., Ramakrishnan, C. V., Somasundaram, P.R., Stott, H. & Velu, S. (1966) *Bull. Wld Hlth Org.*, 34, 533
- Devadatta, S., Andrews, R. H., Angel, J. H., Bhatia, A. L., Fox, W., Janardhanam, B., Radhakrishna, S., Ramakrishnan, C. V., Subbaiah, T. V. & Velu, S. (1961 a) *Bull. Wld Hlth Org.*, 24, 149
- Devadatta, S., Bhatia, A. L., Andrews, R. H., Fox, W., Mitchison, D. A., Radhakrishna, S., Ramakrishnan, C.V., Selkon, J.B. & Vein, S. (1961b) *Bull. Wld Hlth Org.*, 25, 807
- East African/British Medical Research Council Isoniazid Investigation (1960) *Tubercle (Lond.)*, 41, 83
- Fox, W. (1961) *Brit. med. J.*, 1, 135
- Great Britain, Medical Research Council, Tuberculosis Chemotherapy Trials Committee (1962) *Tubercle (Lond.)*, 43, 201
- Holst, E., Mitchison, D. A. & Radhakrishna, S. (1959) *Indian J. med. Res.*, 47, 495
- Hong Kong Government/British Medical Research Council Investigation (1954) *Tubercle (Land.)*, 45, 77
- Lauckner, J. R. (1959) *J. fop. Mcd. Hyg.*, 62, 1
- Selkon, J.B., Devadatta, S., Kulkarni K. G., Mitchison, D. A., Narayana, A. S. L., Narayanan Nair, C. & Ramachandran, K. (1964) *Bull. Wld Hlth Org.*, 31, 273
- Snedecor, G. W. (1956) *Statistical methods*, 5th ed., Ames, Iowa State College Press, p. 259.
- Tubercle (Land.)*, 1963, 44, 183
- Tuberculosis Chemotherapy Centre, Madras (1959) *Bull. Wld Hlth Org.*, 21, 51
- Tuberculosis Chemotherapy Centre, Madras (1960) *Bull. Wld Hlth Org.*, 23, 535
- Velu, S., Andrews, R.H., Angel, J. H., Devadatta, S., Fox, W., Gangadharan, P. R. J., Narayana, A. S. L., Ramakrishnan, C.V., Selkon J. B. & Somasundaram, P. R. (1961) *Bull. Wld Hlth Org.*, 25, 409
- Velu, S., Andrews, R. H., Devadatta, S., Fox, W., Radhakrishna, S., Ramakrishnan, C.V., Selkon, J.B., Somasundaram, P. R. & Subbaiah, T. V. (1960) *Bull. Wld Hlth Org.*, 23, 511
- Velu, S., Narayana, A. S. L. & Subbaiah, T. V. (1961) *Indian J. Tuberc.*, 8, 128.

Annex

Relative value of smear and culture examination in assessing the therapeutic efficacies of antituberculosis regimens

The relative value of smear examination and culture examination in detecting differences in efficacy between antituberculosis regimens may be assessed from Table 7, in which the

TABLE 7

Therapeutic efficacies of eight antituberculosis Regimens, as assessed by smear results and culture results of sputum specimens

Regimen ^a	Number of patients	Percentage of patients with a favourable response	
		Classification based on culture results ^b (P _c)	Classification based on smear results ^c (P _s)
PH	380	86	88
HI-1	64	73	75
HI-2	66	58	65
H	86	44	48
H650	143	68	68
SHTW	72	94	90
TH	65	82	83
P ₆ H/H	63	67	68
Range		44-94	48-90
Variance		257.14	193.84
Standard deviation		16.0	13.9

^a PH was studied on four occasions, H650 on two and all the remainder on only one occasion.

^b For details, see page 574.

^c For details, see page 581-

results obtained with eight regimens used at the Centre since 1956 have been summarized. The details regarding the first five regimens—namely, PH, HI-1, HI-2, H and H650—have already been presented (see footnote to Table 5); those for the last three—namely, SHTW, TH and P₆H/H—are given below:

SHTW. 12.5-16 1 mg/kg body-weight (650 mg for 100-lb (45.4-kg) patient) of isoniazid in one dose and a uniform dosage of 1 g of strep-

tomyacin, administered together twice weekly, for 12 months.

TH. 4.6-8.0 mg/kg body-weight (300 mg for 100-lb patient) of isoniazid daily plus 2.3-4.0 mg/kg body-weight (150 mg for 100-lb patient) of thiacetazone daily, in one dose, for 12 months.

P₆H/H. A uniform dosage of 200 mg of isoniazid daily plus 6 g of sodium PAS daily in one dose for the first 6 months, followed by 6.2-7.7 mg/kg body-weight (300 mg for 100-lb patient) of isoniazid alone in one daily dose for the second 6 months.

The dispersion of the percentages of patients with a favourable response to these eight regimens, as measured by their variances, is larger for assessments based on cultures than for those based on smears (see columns headed P_c and P_s, in Table 7). For assessing the statistical significance of this difference, the usual variance ratio test of Fisher is not applicable since the variances, being based on the same set of patients, are not independent. However, it is evident that, whatever the correlation between the variances of P_c and P_s may be, the sum (P_c + P_s) and the difference (P_c—P_s) of the two assessments of efficacy will be uncorrelated if the variances of P_c and P_s are equal. From this, it follows that the statistical significance of the difference in variances may be assessed by computing the correlation coefficient, *r* between (P_c+P_s) and (P_c—P_s) and testing for its deviation from zero. In the present instance, *r*=0.67, *P*=0.07,

To visualize what this difference means in practice, it will now be expressed in terms of the number of patients required, if smear examination alone is undertaken, to attain the same amount of discrimination between regimens as is obtained with 100 patients assessed by culture examination. For this purpose, an unfavourable response was scored as 0, a favourable response as 1, and analyses of variance undertaken (Table 8).

The variance ratio measures the differences in mean efficacy between regimens, relative to variations in response between patients receiving the same regimen. Obviously, the larger the variance ratio, the greater is the discrimination between the regimens. It is seen from Table 8 that the variance ratio obtained from smear results (14.05) is smaller than that from culture results (16.62). Using component analysis (Snedecor, 1956), it is found that a 20% increase in the number of patients would result in an increase in the variance ratio for smear results from 14.05 to 16.62.

It may be concluded that the ability to detect differences in efficacy between various anti-tuberculosis regimens is less with smear examination than with culture examination and that this difference borders on statistical significance ($P=0.07$) but can usually be compensated for by an increase of about 20% in the number of patients admitted to study.

TABLE 8

Analyses of variance for data in table 7

Source	Degrees of freedom	Classification based on culture results			Classification based on smear results		
		Sum of squares	Mean square	Variance ratio	Sum of squares	Mean squares	Variance ratio
Between regimens	7	19.1747	2.7392	16.62	15.8354	2.2622	14.05
Between patients receiving the same regimen	931	153.4643	0.1648		149.9366	0.1610	
Total	938	172.6390			165.7720		

A TWO-YEAR FOLLOW UP OF PATIENTS WITH QUIESCENT PULMONARY TUBERCULOSIS FOLLOWING A YEAR OF CHEMOTHERAPY WITH AN INTERMITTENT (TWICE WEEKLY) REGIMEN OF ISONIAZID PLUS STREPTOMYCIN OR A DAILY REGIMEN OF ISONIAZID PLUS PAS†

BY O. NAZARETH, S. DEVADATTA, C. EVANS, WALLACE FOX, B. JANARDHANAM, N. K. MENON, S. RADHAKRISHNA, C. V. RAMAKRISHNAN, H. STOTT, S. P. TRIPATHY & S. VELU

(From the Tuberculosis Chemotherapy Centre*, Madras)

Introduction

An earlier publication from this Centre showed that an intermittent regimen of isoniazid plus streptomycin, given twice weekly under supervision, was at least as effective in the treatment of pulmonary tuberculosis over a one-year period as a standard unsupervised daily oral regimen of isoniazid plus PAS (Tuberculosis Chemotherapy Centre, Madras, 1964). This encouraging result offered prospects of a change in the orientation of drug administration from unsupervised daily self-administration to supervised intermittent administration. However, before such a change could be recommended, it was important to know whether the satisfactory results obtained in the first year would be maintained in subsequent years. This report presents the progress, over a two-year period of follow-up, of the patients who had bacteriologically quiescent disease at one year. It also provides information on the few patients who were classified as having disease of bacteriologically doubtful status at one year.

Plan and Conduct of Study

Chemotherapy During the First Year

The two regimens studied in the first year (and the mean and range of dosages on admission to treatment) were:

SHTW: Streptomycin sulphate by intramuscular injection in a uniform dosage equivalent to 1 g. of streptomycin base (mean 27.0 mg./kg body-weight, range 18.2-53.7 mg./kg.) plus a high dosage of isoniazid (mean 19.9 mg./kg. body-weight, range 12.5-16.1 mg /kg) in a single oral dose, *both drugs given at the same time twice-weekly*, at intervals of 3 and 4 days alternately. The dosage of isoniazid was graded according to the patient's weight, a patient weighing 45 kg. (100 lb.) receiving 650 mg.

† The Centre is under the joint auspices of the Indian Council of Medical Research, the Madras State Government, the World Health Organisation and the Medical Research Council of Great Britain.

* This paper is also published in the *Tubercle (London)*.

PH: Isoniazid (mean 4.4 mg./kg. body-weight, range 3.7-6.3 mg./kg.) plus sodium PAS (mean 0.22 g./kg. body-weight, range 0.18-0.32 g./kg.) *daily* in two divided doses; the two drugs were dispensed together in cachets, the patient being given a week's supply at a time. The dosages of isoniazid and PAS were graded according to the patient's weight, a patient weighing 45 kg. (100 lb.) receiving 200 mg. of isoniazid and 10 e. of sodium PAS.

Definition of Bacteriologically Quiescent Disease at One Year.

A patient's disease was classified as bacteriologically quiescent at one year if all the cultures (usually eight or nine) at 10, 11 and 12 months were negative.

Assessment of Cavitation at One year

At one year, the full radiographic series (including tomograms) were reviewed by any two of the Centre's physicians (and in case of disagreement, by a third); the patients were classified as having residual cavitation or as having no residual cavitation.

Allocation to Treatment in the Second Year

As the patients in this study formed part of another investigation (still in progress) to determine the effect of isoniazid alone in preventing bacteriological relapse, they were allocated at random at one year to treatment in the second year with isoniazid or a placebo, after stratification for residual cavitation. The first allocation was made in June 1962 and the last in January 1963.

Treatment in the Second Year

(a.) *For Patients with Residual Cavitation at One Year*—Either isoniazid 400 mg. plus 6 mg. pyridoxine in a single tablet daily for 12 months or a placebo, calcium gluconate* 500 mg., in a single tablet daily for 12 months (b) *For Patients with No Residual Cavitation*

* From December 1963, 500 mg. lactose was substituted for the calcium gluconate.

at One Year—Either isoniazid 300 mg. for the first six months and the placebo for the second six months, or the placebo for 12 months, both in a single tablet daily.

Treatment in the Third Year

No anti-tuberculosis drugs were prescribed in the third year unless the patient had a bacteriological relapse requiring treatment.

General Management

During the second year all patients were examined at monthly intervals; those receiving isoniazid attended the Centre twice a month to collect their tablets, and those receiving the placebo once a month. In the third year, all the patients attended the Centre at three-monthly intervals for routine examinations. The homes were visited once a month by a health visitor, in both the second and third years. These visits were made within a week of the routine monthly examinations in the second year and the routine three-monthly examinations in the third year to deliver a bottle for an overnight collection specimen of sputum and to remind the patient to attend the Centre on the appointed day. The other two visits in each quarter in the third year were made to maintain contact with the patients.

Treatment was changed only if a patient had a bacteriological relapse associated with a serious radiographic deterioration, the latter being confirmed by an independent assessor (Dr. K.S. Sanjivi).

Collapse Therapy and Resection

It was the policy to avoid, if possible, collapse therapy or resection; in the event, no patient in this study had such measures.

Assessments of Progress

The routine assessments included (a) a clinical examination, including weighing, at monthly intervals in the second year and at three-monthly intervals in the third year, (b) a postero-anterior chest radiograph at three-monthly intervals in the second year and at six monthly intervals in the third year and (c) bacteriological examination of sputum specimens, as detailed below.

Bacteriological Investigations

The standard procedure was to obtain 14

² This routine was started in February, 1963, that is, eight months after the start of the follow-up study; previously radiographs were taken at monthly intervals.

sputum specimens per patient in the second year and nine in the third year (for details, see Table 1). Occasionally, extra specimens were collected, especially if a positive result, whether on smear or on culture, had been obtained.

The sputum specimens were examined by direct smear and culture. If a positive culture was obtained, a sensitivity test for isoniazid was set up each month, and for streptomycin or PAS as well depending on which drugs the patient had received in the first year. The techniques employed for smear and culture examinations and sensitivity tests, and definitions of resistance, have been described earlier (Tuberculosis Chemotherapy Centre, Madras, 19-14). Identification tests (colonial morphology, catalase activity and niacin production) were undertaken on all cultures selected for sensitivity tests, using procedures described by Thomas and others (1961).

Definition of Bacteriological Relapse in the Two-year Period

A bacteriological relapse is defined as two or more positive cultures in any six month period—that is, in seven consecutive monthly examinations in the second year (when examinations were performed monthly) or in three consecutive three-monthly examinations in the third year (when examinations were performed every three months).

Definition of an Isolated Positive Culture

An isolated positive culture is defined as one positive culture in any six month period during the two years of follow-up.

Assessment of the Radiographs

An independent assessor (Dr. J. Fridodt-Moller), who was unaware of the treatment or other details of any individual patient, made the following assessments from postero anterior radiographs:

- (a) The number of lung zones (Daniels and others, 1948) involved in disease at 12 months.
- (b) The total extent of the radiographic lesion (Tuberculosis Chemotherapy Centre, Madras, 1960) at 12 months.
- (c) Radiographic changes from 12 to 24 and from 24 to 36 months.

Progress of patients with bacteriologically quiescent disease at one year

There were 119 patients (66 SHTW, 53 PH)

TABLE I

Planned intensity or bacteriological examinations during the second and third year

Year	Month	Type ¹ and number of specimens	Total number of specimens
Second	13 to 23	One collection specimen or a supervised spot specimen	
	14	Two collection specimens and a supervised spot specimen	14
Third	27.30 and 33	One collection specimen and a supervised spot specimen	9
	36	Two collection specimens and a supervised spot specimen	

* A collection specimen was one collected overnight in the home and a supervised spot specimen was one produced under supervision in the clinic.

with bacteriologically quiescent disease at one year of which 118 were allocated at random to treatment in the second year. The remaining patient (PH) was not allocated to treatment because he left Madras; his progress is described on page 33.

Condition on Original Admission to Treatment

Most of the 118 patients had had advanced disease at the time of their *original* admission to treatment at the Centre. Thus, 97% of the 66 SHTW and 94% of the 52 PH patients had cavitated disease initially: 27% and 23% respectively had gross or extensive radiographic lesions; and 89% and 88% respectively had a positive smear from a single overnight sputum specimen. The distributions of age were broadly similar for the two series. Thus, 26% of the patients in both series were below 25 years of age while 21% of the SHTW and 31% of the PH patients were 45 years or over. Of the SHTW patients 58% were males compared with 69% of the PH patients.

Condition at One Year

At one year, that is at the start of the present follow-up study, residual cavitation was present in 33% of the 66 SHTW and 29% of the 52 PH patients. Four or more lung zones were involved in disease in 20% and 22% respectively, and extensive or moderate residual lesions were present in 14% and 17% respectively.

It may be concluded that the two series of

patients were broadly similar in their condition at the time of the original admission to treatment and at one year.

Distribution of Patients According to Treatment During the Second Year

As stated earlier (page 27) the treatment policy for the second year was based on the cavitation status at one year. Considering first the patients with residual cavitation, 11 (50%) SHTW and nine (60%) PH patients were allocated isoniazid for 12 months and the remaining were allocated placebo for 12 months (Table 11). Considering next the non-cavitated patients, 25 (57%) of the SHTW and 19 (51%) of the PH patients were prescribed isoniazid for six months followed by placebo for the next six months, the remaining being prescribed the placebo for the full year. Thus the two series of patients were similar in respect to treatment received during the second year.

Deaths

There were five deaths (four SHTW, one PH), all in the second year and all from non-tuberculous causes. One patient (SHTW) died from cardiac failure and 1 (SHTW) from status asthmaticus, both in the fourteenth month; another (SHTW), who had an advanced carcinoma of the cervix, committed suicide in the fifteenth month; a fourth (SHTW) died in the nineteenth month as a result of the rupture of an amoebic abscess into the

TABLE II

Distribution of patients according to treatment during the second year

Cavitation status at one year	Treatment during the second year	SHTW patients		PH patients	
		No.	%	No.	%
Cavitated	Isoniazid (12 months)	11	50	9	60
	Placebo	11	50	6	40
	Total	22	100	15	700
Non-cavitated	Isoniazid (6 months)	25	57	19	51
	Placebo	19	43	18	49
	Total	44	700	37	100

pericardium while the fifth patient (PH) died in the twentieth month from a perforated gastric ulcer. All the cultures for tubercle bacilli had been negative for 10, 6, 13, 17 and 19 months respectively before death. No necropsies were performed.

Changes in Weight

Over the two-year period, the mean change in weight was an increase of 0.23 kg. (0.5 lb.) in the SHTW series and 0.36 kg. (0.8 lb.) in the PH series.

Changes in Radiographic Appearances

Over the second year, 64% of 66 SHTW and 71% of 52 PH patients showed no change in radiographic appearances (Table III); 27% and 23% respectively showed radiographic improvement, which was classified as slight in all except one patient (PH) in whom it was assessed as considerable. Radiographic deterioration was reported in two SHTW patients and one PH patient, while another patient (PH) had treatment changed on account of a serious radiographic deterioration associated with a bacteriological relapse.

Over the third year the large majority of patients in both series, namely 85% of the SHTW and 76% of the PH, showed no radiographic change while 8% and 14% respectively showed slight improvement (Table III). Three SHTW and four PH patients showed radiographic deterioration while one in each series had treatment changed for a serious radiographic deterioration associated with a bacteriological relapse.

In summary, the radiographic changes in the two series were similar, both in the second year and in the third year, the majority of patients in both series in each year showing no change.

Intensity of Culture Examination

In the second year, 77% of the SHTW and 78% of the PH patients had 13 to 15 cultures, the mean being 14.0 for both series. In the third year, 77% of the SHTW and 65% of the PH patients had eight to 10 cultures, the mean being 9.1 and 9.2 respectively.

Bacteriological Relapse

A bacteriological relapse occurred in five (8%) of the 66 SHTW and six (12%) of the 52 PH patients during the second and third years, three in each series occurring in the second year (Table IV). (Further analyses, not tabulated here as the numbers are small, indicated that the relapse rate was no higher in the SHTW series than in the PH series, whether the patients received isoniazid or placebo in the second year, and whether or not they had residual cavitation at one year.) In three (all PH) of these 11 patients, an isolated positive culture had been obtained prior to the relapse—16, 16 and seven months earlier.

Table V gives the details for the 11 patients who had a bacteriological relapse. One of the SHTW (CIII) and two of the PH patients (C60, C93) had a serious radiographic deterioration confirmed by an independent assessor (Dr. K. S. Sanjivi), and had chemotherapy restarted before the end of the two-year follow-up period. Two SHTW (C64, C83) and two PH patients (C123, C158) had a spontaneous

TABEL III

Changes in radiographic appearances

Changes in radiographic appearances	12-24 months				24-36 months			
	SHTW patients		PH patients		SHTW patients		PH patients	
	No.	%	No.	%	No.	%	No.	%
Improvement	18	27	12	23	5	8	7	14
No change	42	64	37	71	53	55	38	76
Deterioration	2	3	1	2	3	5	4	8
Change of treatment on account of a bacteriological relapse associated with a serious radiographic deterioration	0	0	1	2	1	2	1	2
Non-tuberculous death	4	6	1	2	0	0	0	0
Total	66	100	52	700	62	700	50	700

TABLE IV

Results of culture examinations

		SHTW patients		PH patients	
		No.	%	No.	%
Bacteriological	Second year	3		3	
	Third year	2		3	
	Total	5	8	6	12
Isolated positive culture		11	17	14	27
All cultures negative		46	70	31	60
Non-tuberculous death		4	6	1	2
Total		66	101	52	101

sputum conversion without retreatment and thereafter produced only negative cultures for 16, 12, 12 and nine months respectively. In the remaining two SHTW (C118, C112) and two PH patients (C43, C40), the relapse became manifest only towards the end of the third year.

The results of sensitivity tests -are also presented in Table V. It can be seen that most of the relapses occurred with drug-sensitive organisms, and in patients who did not receive any chemotherapy beyond one year.

Details for patients who had a bacteriological relapse

Serial number	Chemotherapy in the first year	Cavitation status at 1 year	Treatment in the second year	Months at which one or more positive cultures were obtained*	Results of sensitivity tests†		Associated with serious radiographic deterioration	Month retreatment started
					Isoniazid	Streptomycin		
C111 C64	SHTW SHTW	Cavitated Cavitated	Placebo Isoniazid (12 months)	23, 23(2), 24(4), 25	All S	All S	Yes	25
C83 C118 C112	SHTW SHTW SHTW	Non-cavitated Non-cavitated Non-cavitated	Placebo Placebo Placebo	19, 20 16, 21, 23, 24 27, 33 33, 36	Both S S R, R, R Both S Both S	Both S S, R, R, R Both S Both S	No No No No	— — — —
C60 C123	PH PH	Cavitated Cavitated	Placebo Isoniazid (12 months)	14, 15(3) 14, 21, 22, 24	Both S	—	Yes	15
C93	PH	Non-cavitated	Placebo	14, 30, 33, 34(3), 35(3)	All R All S	—	No Yes	35
C158 C43 C40	PH PH PH	Non-cavitated Non-cavitated Non-cavitated	Placebo Placebo Isoniazid (6 months)	22, 27 30, 36(3) 20, 36(3)	Both R C, S NG, S	— — —	No No No	— — —

* When more than one positive culture was obtained in a month, the number is indicated in brackets.

† S = sensitive ; R = resistant ; C = contaminated ; NG = no growth.

Isolated Positive Cultures

An isolated positive culture was produced by 11 (17%) of the SHTW and 14 (27%) of the PH patients (in addition to the three patients (all PH) who subsequently had a relapse (see above)). In the case of two patients (one SHTW, one PH), it was produced on two occasions separated by intervals of 10 and 15 months respectively.

Of the 27 isolated positive cultures produced, 19 (eight SHTW, 11 PH) were in the second year and eight (four SHTW, four PH) in the third. In only 6 (4 SHTW, 2 PH) were the sputum smears positive; the growth was less than five colonies in 14 (six SHTW, eight PH) of the 27 cultures and five to 19 colonies in three (all PH).

Twenty-three of the isolated positive cultures were produced by 11 SHTW and 12 PH patients whilst they were receiving no chemotherapy. Of those in the SHTW series, three sensitive to both streptomycin and isoniazid, four were resistant to both, and two each were sensitive to one drug but resistant to the other; in the PH series, seven were isoniazid-sensitive, four isoniazid-resistant and one was contaminated. Four isolated positive cultures were produced whilst the patient was receiving isoniazid, one (SHTW) with streptomycin- and isoniazid-sensitive organisms, two (both PH) with isoniazid-sensitive organisms and one (PH) with isoniazid-resistant organisms.

Progress of the Patients Not Allocated to Treatment in the Second Year

The PH patient who received no specific therapy (see page 28) had six cultures "examined in the second year and five in the third; all were negative.

Progress of patients with disease of bacteriologically doubtful status at one year

There were five patients (two SHTW, three PH) who had disease of bacteriologically doubtful status at one year, that is had one positive culture at 10, 11 or 12 months following at least three consecutive monthly examinations with all cultures negative. One (SHTW) was allocated at random to isoniazid for six months and three (one SHTW, two PH) to the placebo; the fifth patient (PH) continued with the initially prescribed chemotherapy up to 14 months when he was allocated to isoniazid alone until 18 months. Three (two SHTW, one PH) produced only negative cultures during the second and third years; one other patient (PH) produced only negative cultures until the thirty-third month when he died suddenly, possibly from a cardiac condi-

tion. The fifth patient (PH) produced one positive culture, which was isoniazid-sensitive, at 14 months and subsequently had a relapse with isoniazid-resistant organisms at 21 months whilst on the placebo, retreatment was started at 30 months.

Progress of all 124 patients in this follow-up

Considering the amalgamated results for the 119 patients with bacteriologically quiescent disease and the five patients with disease of bacteriologically doubtful status at one year, five (7%) of 68 SHTW and seven (12%) of 56 PH patients had a bacteriological relapse during the two-year period of follow-up, three and four respectively occurring in the first year of follow-up; the relapse was associated with a serious radiographic deterioration in one SHTW and two PH patients. Thus the experience in the SHTW series was at least as good as that of the PH series over the two-year period of follow-up.

Discussion

An earlier report from this Centre described a controlled comparison of a fully supervised twice-weekly regimen of high dosage isoniazid plus streptomycin (the SHTW regimen) and a standard unsupervised daily oral regimen of isoniazid plus sodium PAS (the PH regimen) in the treatment of pulmonary tuberculosis for one year (Tuberculosis Chemotherapy Centre, Madras, 1964). The great majority of patients came from the poorest sections of the community and had cavitated disease and positive sputum smears on admission to treatment; all were managed on an out-patient basis with clinic supervision. At the end of the year of treatment the proportion of patients with a favourable response, assessed by stringent bacteriological criteria, was 94% for the SHTW and 85% for the PH series. The encouraging results with the SHTW regimen in the first year of treatment have been confirmed by the interim findings of a current study at this Centre (Menon, 1965).

However, it is important to know whether the satisfactory results obtained at one year are maintained subsequently. The present report compares the progress, over a two-year period of follow-up, of the 66 SHTW and 52 PH patients from the earlier study who had bacteriologically quiescent disease at one year. (As these patients formed part of another investigation to determine the effect of isoniazid alone in preventing bacteriological relapse, half of them were allocated at random to isoniazid alone for six or for 12 months and half to a placebo.) The weight changes and radiographic changes during this period were

similar for the two series. The intensity of bacteriological examination was similar and high in both series (approximately 14 cultures per patient in the second year and nine in the third year). A bacteriological relapse, defined as two or more positive cultures in any six-month period, occurred in five (8%) of the SHTW and six (12%) of the PH patients, and was associated with a serious radiographic deterioration in one (2%) and two (4%) respectively. It is of interest to consider the consolidated experience from previous studies of 196 PH patients with bacteriologically quiescent disease at 1 year (Devadatta and others, 1961; Velu and others, 1961, and unpublished data). These patients had been admitted with similar criteria of eligibility and had broadly similar disease on admission and at one year as the patients in the present study. Over a two-year period of follow-up, 14 (7%) had a bacteriological relapse which was associated with a serious radiographic deterioration in 10 (5%). Thus, the progress of the SHTW patients in the present study was at least as satisfactory as that of the PH patients in the present study and in previous studies at this Centre.

Although based on small numbers, the relapse rates in patients who received no anti-tuberculosis chemotherapy in the two-year period of follow-up are of special interest since they measure the stability of bacteriological quiescence produced by a year of chemotherapy with the two regimens; these were similar, being four (13%) of 30 in the SHTW series and four (17%) of 24 in the PH series. Further, the finding that three of the above four bacteriological relapses in the SHTW series were with cultures that were sensitive to both isoniazid and streptomycin is also encouraging to the use of the SHTW regimen, since relapse with sensitive cultures is likely to present less serious problems in the subsequent management of the patients than relapse with resistant cultures.

In the present study 17% of 66 SHTW and 27% of 52 PH patients produced an isolated positive culture (identified as tubercle bacilli) during the period of follow-up. That such results should occur from time to time in patients who are otherwise consistently sputum-negative is not surprising, since drug-sensitive tubercle bacilli may persist in the lungs even after prolonged and appropriate chemotherapy (McDermott, 1959). The Medical Research Council (1962) has reported that of 86 patients who received two or three years of standard combined chemotherapy, five produced an isolated positive culture in the second or third year, while still receiving chemotherapy. Further, Stewart, Turnbull and MacGregor

(1956) have reported isolation of viable tubercle bacilli in resected lung specimens from six of 28 patients who had effective chemotherapy, and whose sputum had been negative for 6 to 17½ months before surgery.

As has previously been reported, isolated positive cultures are of no serious consequence (Raleigh, 1957) and do not presage a bacteriological relapse in the great majority of patients (Velu and others, 1961; Dawson and others, 1966*). This is confirmed in the present study for the PH regimen and demonstrated for the SHTW regimen, for only three (11%) of 28 patients (none of 11 SHTW and three of 17 PH patients) who produced an isolated positive culture had a bacteriological relapse subsequently.

In the present study, the SHTW regimen was at least as effective bacteriologically as the PH regimen in that 70% of 66 quiescent patients who had received it in the first year produced only negative results throughout the two-year period of follow-up as compared with 60% of 52 patients who had received the PH regimen; the corresponding proportions for patients who received no specific chemotherapy in the second year were 60% of 30 and 58% of 24 respectively. There do not appear to be any reports in the literature on whether more effective regimens of chemotherapy—for instance triple-drug therapy with streptomycin, PAS and isoniazid—have a greater bacteriocidal effect. However, Canetti, Grosset and Le Lirzin (personal communication, 1966), in unique data on resected lung specimens from approximately 2000 patients, have found that combined chemotherapy with three drugs for the first three months of treatment followed by two drugs is more sterilizing on the pulmonary lesions than are two-drug regimens throughout. Further, they found that viable tubercle bacilli can often be isolated from the lung even after very many months of chemotherapy, especially if, on pathological examination, there is evidence that one or more draining bronchi are still open.

In conclusion, the present study has shown that bacteriological quiescence following one year of an intermittent (twice-weekly) regimen of high-dose isoniazid plus streptomycin is at least as stable, over a two-year period of follow-up, as that attained following a year of a standard daily regimen of isoniazid plus PAS. In the past, the chemotherapy of tuberculosis had to be approached in terms of daily regimens, which for out-patient treatment usually implied self-administered chemotherapy for a major part and often for the entire duration of treatment. The findings of the

present study indicate that it is now possible to approach the chemotherapy of tuberculosis with an alternative method, namely supervised bi-weekly chemotherapy. This method has a major advantage over self-administered chemotherapy in that it permits a precise knowledge of, and therefore greater control over, the amount of chemotherapy the patient actually receives. The relative roles and application of these two methods of chemotherapy is clearly a subject of great practical importance, especially in developing countries.

Summary

In the main analysis of a year's study of twice-weekly high dosage isoniazid plus streptomycin (SHTW) in comparison with a standard daily regimen of isoniazid plus PAS (PH) under domiciliary conditions, 66 SHTW and 53 PH patients had attained bacteriologically quiescent disease at one year. All the patients have now been folio wed-up over a two-year period. Of these, 66 SHTW and 52 PH patients had been allocated at random to treatment in the second year with isoniazid alone or with placebo. No patient was prescribed anti-tuberculosis drugs for the third year.

The condition of the patients in the two series was broadly similar, both at the time of their original admission to treatment and also at the start of the period of follow-up.

There were five deaths (four SHTW, one PH) in the follow-up period, all in the second year and all from non-tuberculous causes; all five patients produced only negative cultures in the second year and for at least six months immediately before death.

The radiographic progress was similar for the two series in the second and third years, the majority of patients in both series showing little change.

The patients were under intensive bacteriological investigation, an average of 14 cultures being examined per patient in the second year and nine in the third year. A bacteriological relapse occurred in five (8%) SHTW and six (12%) PH patients. In one and two patients respectively, this was associated with a serious radiographic deterioration. An isolated positive culture was produced by 17% of the SHTW and 27% of the PH patients.

Four of the SHIW patients had a relapse with streptomycin-and isoniazid-sensitive cultures and four of the PH patients with isoniazid sensitive cultures.

It is concluded that bacteriological quiescence following a year of twice-weekly isoniazid plus streptomycin is at least as stable over a two-year period of follow-up, as that attained

following a year of a standard daily oral regimen of isoniazid plus PAS.

We wish to acknowledge the devoted work of the entire staff of the Centre, particularly the public health nurses, social workers and health visitors whose efforts have largely been responsible for the completeness of the data.

REFERENCES

- Daniels M., Ridehalgh, F., Springett, V. H., & Hall, I. M. (1948), *Tuberculosis in Young Adults*, p. 217, H. K. Lewis, London.
- Dawson, J. J. Y., Devadatta, S., Fox, W., Radhakrishna, S., Ramakrishnan, C. V., Somasundaram, P. R., Stott, H., Tripathy, S. P., & Velu, S. (1966). A year study of patients with pulmonary tuberculosis in a concurrent comparison of home and sanatorium treatment for one year with isoniazid plus PAS. *Indian J. Tuberc.*
- Devadatta, S., Andrews, R. H., Angel, J. H., Bhatia, A. L., Fox, W., Janardhanam, B., Radhakrishna, S., Ramakrishnan, C. V., Subbaiah, T. V., & Velu, S. (1961). Progress in the second and third years of patients with quiescent pulmonary tuberculosis after a year of chemotherapy at home or in sanatorium, and influence of further chemotherapy on the relapse rate. *Bull. Wld Hlth Org.*, 24, 149.
- McDermott, W. (1959). *Publ. Hlth Rep., Wash.*, 14, 485.
- Medical, Research Council (1962). Long-term chemotherapy in the treatment of chronic pulmonary tuberculosis with cavitation. *Tubercle Land.*, 43, 201.
- Menon, N. K. (1965). (Intermittent chemotherapy of pulmonary tuberculosis. XVIIIth International Tuberculosis Conference . . . Munich, October 5-9th, 1965.
- Raleigh, J. W. (1957). The late results of prolonged multiple-drug therapy for pulmonary tuberculosis. *Am. Rev. Tuberc.* 76, 540.
- Stewart, S. M., Turnbull, F. W. A., & MacGregor, A. R. (1965). The influence of chemotherapy on the bacterial content of tuberculous pulmonary lesions *Tubercle, Land.* 37, 388.
- Thomas, K. L., Joseph, S., Subbaiah, T. V., & Selkon, J. B. (1961). Identification of tubercle bacilli from Indian patients with pulmonary tuberculosis. *Bull Wld Hlth Org.* 25, 747.
- Tuberculosis Chemotherapy Centre, Madras (1960). A concurrent comparison of isoniazid plus PAS with three regimens of isoniazid alone in the domiciliary treatment of pulmonary tuberculosis in South India *Bull Wld Hlth Org.*, 23, 535.
- Tuberculosis Chemotherapy Centre, Madras (1964). A concurrent comparison of intermittent (twice-weekly) isoniazid plus streptomycin and daily isoniazid plus PAS in the domiciliary treatment of pulmonary tuberculosis. *Bull. Wld Hlth Org.*, 31, 247.
- Velu, S., Andrews, R. H., Angel, J. H., Devadatta, S., Fox, W., Gangadharam, P. R. J., Narayana, A. S. L., Ramakrishnan, C. V., Selkon, J. B., & Somasundaram, P. R. (1961). Progress in the second year of patients with quiescent pulmonary tuberculosis after a year of domiciliary chemotherapy, and influence of further chemotherapy on the relapse rate. *Bull. Wld Hlth Org.*, 25, 409.

AWARD OF T.A.I. GOLD MEDAL

The second award of the Tuberculosis Association of India's Gold Medal for outstanding work in the Tuberculosis field was conferred on Dr. R.B. Billimoria of Bombay at the time of 22nd Tuberculosis and Chest Diseases Workers' Conference held in Hyderabad in February, 1967.



Dr. R.B. Billimoria

Dr. Rustomji Bomanji Billimoria was born on 29th May, 1882 in Bombay. After a brilliant academic career he graduated from St. Xavier's College. He joined Grant Medical College and obtained general proficiency in surgery and hygiene and took his L.M.S. in 1907. In 1910, he took M.D. in medicine with distinction. He won medals in Anatomy and Surgery. He was the first Doctor to get this distinction. He held various positions in the medical field. He was in the beginning of his career Lord Reay Lecturer on medicine and was the Honorary Pathologist and Bacteriologist in the Parsee General Hospital. Later he was Honorary Consulting Physician in the G.T. and Masina Hospitals. He served the Indian Military Hospital during the World War II with the rank of lieutenant-Colonel. Dr. Billimoria was the founder-Director of the Bel-Air Sanatorium for TB in Panchgani which is today one of the finest Sanatoria in Maharashtra. He contributed several authoritative papers on tuberculous and allied subjects in various Journals in India and abroad. He was the first Chairman of the Standing Technical Committee of the Tuberculosis Association of India, and also the first President of the TB Workers' Conference held in Calcutta in 1948. In 1936 he won the Kaiser-i-Hind Gold Medal, in 1945 he was honoured as C.B.E. Recently he was given the award of Padmabushan from the President of India in recognition of his valuable contribution in the Tuberculosis field. For many years Dr. Billimoria was on the Advisory Committee set up by the Government of Maharashtra for Tuberculosis, and was until recently on the Executive Committee of the Tuberculosis Association of Maharashtra.

XXII TB CHEST DISESES WORKERS' CONFERENCE

The Twentysecond Conference of Tuberculosis and Chest Diseases Workers in India was held in Hyderabad from 3rd to 6th February, 1967. The Tuberculosis Association of Andhra Pradesh played host. The inauguration and scientific sessions were held in the Osmania Medical College, Residency, Hyderabad. About 500 delegates from all over India attended the conference. Sri Lai, Secretary to Government of Andhra Pradesh, on behalf of Sri S.R.Y. Sivarama Prasad, Minister for Health, Government of Andhra Pradesh and Chairman of the Reception Committee, welcomed the delegates.

The Conference was inaugurated by Sri Pattom Thanu Pillai, Governor of Andhra Pradesh before a large and distinguished gathering. In the course of his address the Governor gave a birds eye survey of the tuberculosis problem in India and gave constructive suggestions to meet its challenge. The Governor presented the "Tuberculosis Association of India Gold Medal" to Dr. R.B. Billimoria of Bombay. As Dr. Billimoria was not able to be present, the award was received by Dr. M.D. Deshmukh, Honorary Secretary of the Maharashtra State Anti-TB Association to be formally given to Dr. Billimoria at a special function in Bombay. The Governor also presented the cash award of Rs. 300/- to Dr. P.R.J. Gangadharam for the best technical paper submitted by competitors. Dr. Gangadharam's paper was on "Drug Resistance in Tubercle Bacilli and its impact on the chemotherapy and Epidemiology of Tuberculosis" a summary of which was read by him at the Conference.

Messages wishing the Conference success were received from numerous distinguished persons including the President of India, Vice-President of India, Prime Minister, Governor of Andhra Pradesh, Chief Minister, Andhra Pradesh, Union Health Minister, etc.

The Conference was presided over by Dr. Khushdeva Singh of Patiala. In his Presidential address Dr. Khushdeva Singh touched upon some salient aspects of the tuberculosis problem in India and suggested steps to be taken to combat this disease. Dr. N.L. Bordia, Adviser in Tuberculosis to the Government of India and Honorary Technical Adviser of the Tuberculosis Association of India gave a detailed review of the progress of anti-tuberculosis work in India after the last Conference.

The Scientific sessions included three symposia on "Prevalence of Drug Resistance and Clinical Significance of Drug Resistance", "Tuberculosis in Industry" and "Emergencies of Chest Practice", a Panel discussion on "Treatment Default—Administrative, Organisational and Sociological consideration", two sessions on "B.C.G. Vaccination (Direct Vaccination) and Type of Tuberculosis Developing among BCG Vaccinated persons and the course of Disease among them" and "Economics of Health" and other important papers. As Dr. K.N. Rao, Director General of Health Services and Chairman of the Association was unable to be back from Geneva in time, the public lecture scheduled to be given by him was given to Dr. Toman of the World Health Organisation. Dr. Toman gave an illuminating talk in the afternoon of the first day of the conference on "Case-finding".

Drs. K. Somayya, M.K. Pandit and D. Umopathy Rao assisted by a number of volunteers were in charge of the arrangements connected with the conference. Social engagements included a Reception by the Mayor of Hyderabad in the Public Gardens and complementary lunches by some commercial firms. A delightful cultural show was organised. Messrs. Pfizer Limited, Unichem Laboratories and Wander Pharmed Limited gave attractive gifts to the delegates.

Dr. N.K. Naik, Principal of the Osmania Medical College, Hyderabad placed the College premises including equipment, furniture, etc. at the disposal of the Andhra Pradesh Association. The business session of the Conference elected Dr. L.R. Dongrey and Dr. J.L. Bhatia as the representatives of the conference on the Central Committee of the Tuberculosis Association of India.

The Tuberculosis Association of India is deeply grateful to Sri Pattom Thanu Pillai, Governor of Andhra Pradesh and all officials of the Andhra Pradesh Government for all the assistance they gave in connection with the Conference. It is also grateful to the Mayor of Hyderabad for his help. We are grateful to Drs. Somayya, Pandit, Umopathy Rao and to the volunteers and associates for making arrangements for the Conference.

The Andhra Pradesh Association brought out an attractive Souvenir on the occasion of the Conference. Detailed proceedings of the conference will be published by the Tuberculosis Association of India separately.

NEWS & NOTES

Annual Meetings of the Association

The twenty-eighth Annual General Meeting of the Tuberculosis Association of India will be held on 27th April at 11.45A.M. in the Conference Hall of the Association. The President of the Association will preside. The Chairman of the Association will present the report on the working of the Association during 1966. The Accounts for the 1966 will be presented by the Honorary Treasurer. The meeting will also elect members to the Central Committee as provided for in the rules.

A meeting of the Central Committee of the Association will be held immediately after the Annual General Meeting.

The Conference of Secretaries of the State TB Associations and Seal Sale Organisations in India will be held in the conference hall of the Association at 2.30 P.M. on the 27th April.

The Standing Technical Committee of the Association will meet on the 28th April.

A meeting of the Editorial Board of the Text Book on Tuberculosis as also of the Indian Journal of Tuberculosis will be held on the 26th April.

Chest and Heart Association Fellowship

The Tuberculosis Association of India has selected Dr. D. Umaphy Rao, Officer-in-charge, TB Demonstration and Training Centre, Irrumnuma, Hyderabad, for the award of the Chest and Heart Association—India Fellowship for 1966-67. The Fellowship is of the value of £.500 and the selected candidate is required to undergo a study of about three months in the United Kingdom in the Spring this year. Dr. Umaphy Rao is expected to arrive in London in the first week of April to begin his training there.

Seal Sale Award 1967

The Association's Trophy for the highest Seal Sale collections will be awarded to Delhi TB Association. This Trophy will be presented at the Annual General Meeting of the Association in April.

Delhi Seminar

Under the joint auspices of the Delhi TB Association and Delhi Medical Association, a

Seminar on Tuberculosis Control in Delhi was held on 10th and 11th March in the auditorium of Delhi Medical Association. Dr. Sushila Nayar, the then Minister for Health, inaugurated the Seminar. Dr. K.N. Rao, Director-General of Health Services, and Dr. N.L. Bordia, Adviser-in-Tuberculosis, Government of India, presented papers on Tuberculosis, as an international and national problem respectively. Dr. B.K. Sikand reviewed the problem from the point of view of a Specialist. Drs H.B. Dingley, S.P. Pamra, K.G. Mahajan, N.K. Chuttani, P.N. Taneja who participated in second day's discussions presented papers on extra-pulmonary tuberculosis, diagnosis and diagnostic procedures in pulmonary TB, treatment and related problems. Dr. N L. Bordia made the concluding remarks on the discussion.

Mysore Workers' Conference

The Second Mysore State Tuberculosis and Chest Diseases Workers' Conference was held in Mangalore, South Kanara on 14th and 15th January, 1967. Dr. K. Nagappa Alva, Minister for Health, Government of Mysore inaugurated the Conference. His Eminence valerian cardinal gracies, Archbishop of Bombay, graced the occasion with his presence and delivered his valedictory address on the 15th. Dr. M.D. Deshmukh and Dr. Ugrankar, Bombay participated in the proceedings. Shri B.M.Cariappa, Secretary-General, Tuberculosis Association of India, presented a paper on the "Voluntary Effort in the Control of Tuberculosis" at the Conference. The Conference unanimously recommended that top priority be given to introducing comprehensive clinic-based home treatment programmes for tuberculosis in all the districts. It also suggested that a panel system of general practitioners on the lines of E S.I. scheme making adequate financial compensation to doctors should be instituted.

Junior Award

The Tuberculosis Association of India gives a cash prize of Rs. 300/- to a tuberculosis worker, preferably below 45 years of age, for an original article not exceeding 30 double-spaced foolscap typed pages (approximately 6,000 words) excluding charts and diagrams, on a subject relating to tuberculosis in which he or she is specialising or has worked on and adjudged best by a special committee of the Association. A summary of the article selected for the prize will be presented by the author at the next TB and Chest Diseases Workers Conference to be held early next year. Papers

should be sent in quadruplicate to reach this office on or before 30th November, 1967.

Chest Diseases Prize Award

The Indian Association for Chest Diseases has initiated a prize of Rs. 200/- to be given to the author of the best article published during the previous year either in Indian or foreign Journal on any subject in the speciality of Chest disease. Those who desire to be considered for this prize may send 6 copies of their article to the Secretary, Indian Association for Chest Diseases, C/o Silver Jubilee Tuberculosis Hospital, Kingsway Camp, Delhi-9 by not later than 31st July, 1967.

Chest and Heart Conference: Eastbourne

The Chest and Heart Association, London, will be holding a International Chest and

Heart Conference in Eastbourne, England, from 4th to 8th April, 1967. The programme of the conference will include lectures, clinical and scientific meetings. There will also be visits to hospitals, clinics and rehabilitation centres. The Conference will be open to doctors and non-medical workers in the Chest and Heart field.

Indian Academy of Medical Sciences

The Indian Academy of Medical Sciences will conduct its next post-graduate examination on an all India basis to admit candidates to the Membership of the Academy. The examinations will be held in two parts from 3rd July, 1967. Further particulars can be obtained from the Executive Director, Indian Academy of Medical Sciences, C.H.F.B. Buildings, Temple Lane, Kotla Road, New Delhi.

The Indian Journal of Tuberculosis

ABSTRACTS

Vol. XIV

March 1967

Abst. No. 2

Reversion to Drug Sensitivity in Tubercle Bacilli

Sheila M. Stewart.

Tubercle 1966, 47, 190.

Reversion to bacterial sensitivity following known drug resistance has been demonstrated in 2 out of 46 patients (4%) with streptomycin resistant cultures and in 14 out of 62 (23%) with INH resistant cultures. No case of reversion was detected in 41 patients with PAS resistant cultures. All these patients had been without treatment for at least 3 months before reversion was noticed, the mean period for 3 drugs being 21.7, 16.1 and 22.2 months respectively.

Reversion to INH sensitivity occurred most frequently in patients treated for 6 months or less before emergence of resistance; in those who had been without treatment for more than 12 months and in those whose bacilli had only a low degree of resistance (1.0 µg/ml.).

Three out of 4 patients re-treated with adequate combination of drugs became sputum negative and have remained so for 5 to 7 years. These three included 2 patients whose cultures had reverted both to streptomycin and INH sensitivity. The mechanism of reversion to sensitivity may be due to a back mutation to sensitivity in the absence of drugs or alternatively the few sensitive bacilli in course of time out-growing the resistant organisms which are known to be slow growing.

S.S.P.

Late Emergence of Isoniazid-susceptible Organisms in Guinea Pigs Challenged with a Homogeneous Isoniazid-resistant Strain of *Mycobacterium Tuberculosis*

H. Noufflard-Guy-Loe and Solange Berteaux

Amer. Rev. of Resp. Dis.; 1966, 94, 540.

Twenty guinea pigs were challenged with a highly and homogeneously isoniazid resistant strain of decreased pathogenicity, that gave benign regressive lesions, but persistent tuberculin sensitivity. One year later, relapse occurred in all 16 surviving guinea pigs, the

lymph nodes enlarged again in the neighbourhood of the site of infection, and protracted systemic tuberculosis developed in several guinea pigs.

Half the animals had been given cortisone for 3 weeks at the start of the experiment i.e. almost a year before the tuberculous lesions developed. Curiously enough, both the local lesions and the systemic tuberculosis were definitely more extensive in animals that had received cortisone.

The numerous cultures yielded by different lesions were never identical in the pattern of drug-resistance to the culture used for infection; none was catalase-negative, and none was highly isoniazid-resistant. All included a vast majority of organisms giving an entirely normal response to isoniazid. However, in many of these cultures, highly resistant organisms were present in a definitely higher proportion than is the case in wild strains.

The proposed explanation for this situation is reverse mutation to isoniazid-susceptibility in a highly isoniazid-resistant microbial population.

S.P.P.

Ambulatory Treatment of Pulmonary Tuberculosis

Samuel Clive Cohen, Leonard I. Steinfeld, John T. Foley & Edward Blacker

Diseases of the Chest. 1966, 50, 21.

Nine years experience in the ambulatory treatment of tuberculosis at the Boston Health Department Clinics is reviewed. Out of total of 3,859 patients attending the various clinics viz. chemotherapy clinic, post chemotherapy (follow-up of arrested cases) clinic, surgical clinic etc., 854 patients were randomly selected for assessment of follow-up. Only 69% of these cases had been hospitalized prior to domiciliary treatment and of these nearly 30% (almost half being alcoholics) left the hospital against medical advice or were discharged on disciplinary grounds.

Of all the patients reviewed, 12% gave up the treatment (domiciliary) prematurely after taking treatment regularly from 1 to 12

Ind. J. Tub., Vol. XIV, No. 2

! • ' & ' \

months; 81 % were regular, 11 % irregular and 8% were very erratic in their attendance.

69% were negative for AFB to begin with, 12% were converted from positive to negative, 2% continued to be consistently positive and 6% were intermittently positive. Incidence of drug resistance was 1.7%. Radiologically 92% remained stable or showed improvement and 4.9% had evidence of relapse, radiological or bacteriological or both.

No active clinic case of tuberculosis was found amongst 850 children of these patients who were given isoniazid prophylaxis.

S.P.P

Natural History of Tuberculosis in the Human Body

J. Arther Myers; J.E. Bearman and Alma Botkins

Diseases of the Chest, 1966, 50, 120.

The 2,908 medical students joining Minnesota University from 1930 to 1953 were followed up. Whereabouts of 12 were not known and 220 died of non-tuberculous diseases. The remaining 2,676 have been followed for 78,635 person-years.

Among 904 of these, who reacted to tuberculin on entrance and were followed for 25,538 person-years, 31 (3.43%) developed clinical pulmonary or extra-pulmonary tuberculous lesions. One died from tuberculosis.

Among the 447 who became infected while in the medical school with a follow up of 13,667 person-years, 29 persons (6.2%) developed clinical tuberculosis. Two died from tuberculosis.

There were 652 who were infected after graduation with a follow up of 16,977 person-years. Amongst them there were 30 cases (4.8%) of clinical tuberculosis with no death. This leaves 768 persons who remained non-reactors to tuberculin up to the end of the study with a follow up of 17,469 person-years. The present mean age of this group ranges from 59.1 years for the 1931 class to 38.1 years for the 1953 class. The last clinical case among this material developed in 1956. None developed meningitis or miliary tuberculosis.

S.P.P.

Relation between the loss of Acid-Fastness due to ultraviolet irradiation and the pathogenicity of tubercle bacilli

Toyoho Murohashi & Konosuke Yoshida

Amer. Rev. of Resp. Dis.; 1966, 94, 86.

Ultraviolet irradiation for a certain period

Ind. J. Tub., Vol. XIV, No. 2

of time caused tubercle bacilli to lose complete acid-fastness. Time required for this loss was longer in the virulent strains than in the avirulent or attenuated ones. This finding suggests a difference of cell wall structure between these two that might determine the existence or survival of the bacterial cell at its entrance into host cell. This finding may enable us to relate the pathogenicity of tubercle bacilli to the constitutional cell wall structure. A very simple staining method was devised to differentiate these two forms of strains from each other.

S.P.P.

Pulmonary Lesions of Chickenpox—Annotations

The Lancet; 1966, 11, 431.

A convincing case has been made out for the opinion that Chickenpox is a likely cause for pulmonary calcification in addition to pulmonary tuberculosis and histo-plasmosis which were hitherto considered as the only two conditions which led to calcifications in the lung. Adults, where pulmonary lesions may be present in 16 to 30% of the cases of Chickenpox, are more usually affected than children. The lesions start as extensive bilateral nodular opacities varying in size from several millimeters to more than a centimeter. The apices may be spared. Radiological resolution is much slower than clinical, taking up to 8 weeks. In many of them, the nodules may go on to calcification which usually takes more than 2 years from the attack of the Chickenpox but in some cases may be delayed up to 10 years.

S.P.P.

Multiple Skeletal Muscle Tuberculosis Abscesses by Hematogenous Spread

Irvin Herman

Amer. Rev. of Resp. Dis.; 1966, 94, 233.

The case of a 50 year old negro female who worked in the isolation ward of a hospital and developed multiple skeletal and subcutaneous abscesses due to tuberculosis is reported. She had bilateral basal lesions in both lungs with positive sputum. Tubercle bacilli were also isolated from the pus aspirated from the abscesses. Thirteen cases have so far been reported earlier in literature where skeletal abscess is considered to be due to haematogenous dissemination from a pulmonary focus. The rarity of skeletal muscle tuberculosis is postulated to be due to high lactic acid content, low oxygen tension and paucity of reti-

ABSTRACTS

culoendothelial tissue in the striated muscles. The difficulty of diagnosis especially in the absence of a definitive pulmonary lesion is stressed.

S.P.P.

The Hyperlucent Lung, a Problem in differential Diagnosis

Morris M. Culiner

Dis. of the Chest. 1966, 49, 578.

Radiological finding of hyper-lucency is often considered as being only due to emphysema, compensatory or obstructive. It may however also be due to unilateral absence or hypoplasia of the pulmonary artery.

Idiopathic unilateral hyperlucent lung, usually designated 'Swyer-James Syndrome' is usually seen in adults and frequently misdiagnosed in clinical practice. The diagnosis in these cases is based on the following features in addition to hyperlucency:—

1. Impaired pulmonary ventilation.
2. Decrease in calibre of pulmonary artery and its branches.
3. Marked paucity of bronchial sub divisions with or without associated bronchiectasis seen in the bronchogram.
4. Decreased volume of right hemithorax of lung.
5. Susceptability to frequent recurrent lower respiratory tract infections with resultant post-inflammatory fibrosis.

S.P.P.

Thoracoscopy and Pleural Biopsy in the Diagnosis of Pleurisy

S. Bergovist & H. Nordenstam

Scand. J. Resp. Dis.; 1966, 47, 64.

The material consisting of 130 patients with

pleurisy was investigated, inter alia, by thoracoscopy, pleural biopsy and exudative cytology. Thoracoscopy was carried out in the usual way under local anaesthesia and prietal pleura about 2x2 mm from a suitable site was excised after anaesthetising.

Tuberculosis was found to be the cause of pleurisy in 59 cases, cancer 46, other miscellaneous conditions 13, leaving 12 patients where no definite cause for pleurisy could be obtained.

Out of the 59 tuberculous pleurisies, bacteriology of the fluid and pleural biopsy were both positive in 34 cases and in the remaining 15, pleural biopsy alone gave positive results.

The authors conclude that thoracoscopy combined with pleural biopsy can be performed without any risk of complications and is much more reliable diagnostically than needle biopsy and is not inferior to open thoracotomy.

S.P.P.

A Study of the Isoenzymes of Lactic Dehydrogenase in Pleural Effusions

E. Raabo, Knud N. Rasmussen & T. Chr. Terkildsen

Scand. J. Resp. Dis.; 1966, 47, 150.

Neither by examination of the total content of lactic acid dehydrogenase (LDH) activity in serum and pleural effusion nor by determination of the 5-LDH activity has it been possible to demonstrate and define difference between exudates resulting from infectious (tuberculous) and malignant conditions. Pleural effusions resulting from chronic stasis have always shown a low LDH content, both in exudate and in serum.

S.P.P.

