

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

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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).

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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 bacilliary 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 straptomycin 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 drug, 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.

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