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Bacterial Persistence in Leprosy[1]

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Bacterial persistence is a phenomenon commonly defined as the capability of microorganisms to survive in the host despite adequate[3] antimicrobial treatment. Bacterial persistence has been observed during infection with a number of organisms and during treatment that kills the normally susceptible organisms of the same bacterial population from which persisters originate. The phenomenon of bacterial persistence thus appears to be of considerable generality and very likely pre-existed the advent of antimicrobial chemotherapy. However, its occurrence and importance have been recognized only since bactericidal drugs became available. Thus, for more than 30 years, it has been known that persistence is one of the common reasons for failure of the treatment of tuberculosis, yet our knowledge of how bacterial persistence develops and how to cope with it is still fragmentary.

OBSERVATIONS AND STUDIES OF BACTERIAL PERSISTENCE

In 1942, Hobby and her coworkers reported ([15, 16]) that hemolytic streptococci were readily killed *in vitro* by penicillin but that about 1% of the drug-susceptible organisms were able to survive appropriate concentrations of the drug. It was noted that penicillin was most effective against actively multiplying microbial cells.

One of the earliest investigations of the phenomenon of bacterial persistence was reported by Bigger ([2]), who coined the term "persisters." After exposure of staphylococcal cultures to suboptimal temperatures, he found that the time of penicillin exposure had to be prolonged from 3 to as long as 11 days, depending upon the duration of the period of cooling. Even then, two of ten bottles were not sterilized by penicillin in the high concentration of 2 units per ml broth. He excluded drug-resistance as the explanation of bacterial survival; after transfer to a drug-free medium, the survivors were killed by 0.125 unit per ml within 48 hr. Bigger concluded that persisters were not drug-resistant cells, but ". . . normal cocci, which happen to be when exposed to penicillin in a phase in which they are insusceptible to its action," and that the persister state could be induced in normal cocci by changing the environment, e.g., by cooling.

In one experiment, cultures of staphylococci to which penicillin had been added were exposed to three different temperatures. Sterility was attained only in the culture tubes maintained at 37°C at which temperature conditions for multiplication were optimal. No sterilization, but only inhibition of multiplication, occurred in the culture tubes held at room temperature, and at 4°C even a high concentration of penicillin was almost without effect. These results strongly indicated that, under conditions in which the multiplication of susceptible microbial cells was suppressed, the bactericidal effect of penicillin was greatly reduced.

In another experiment, bacteriostatic conditions were produced by adding distilled water or boric acid in low concentration to the medium. Penicillin, in a concentration of 1 unit per ml, and acting for 18 to 24 hr at 37°C, reduced only slightly the original number of staphylococci; lower concentrations of penicillin were not appre-

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[3] The term *adequate* chemotherapy or antimicrobial treatment will be used throughout this paper. The essential characteristics of an adequate chemotherapeutic regimen are:

a) the regimen contains at least two drugs to which the organisms are susceptible, particularly when the patient is harboring large numbers of bacilli;
b) high efficiency and acceptability of the regimen have been established by controlled clinical trial; and
c) the drugs are administered regularly, in the dosage, rhythm and duration established by controlled clinical trial.

ciably bactericidal. These experiments strengthened to a considerable degree the hypothesis ". . . persisters are cocci which . . . survive contact with penicillin because they are in a dormant, non-dividing phase."

It was further observed that, after incubation with penicillin for 72 hr and inactivation of the penicillin by penicillinase, not all of the persisters began to multiply at once. About one third began only after 48 hr, and about 7% after 3 to 9 days. The author concluded from these experiments that:

a) among the millions of cocci present, there were a few which were insusceptible to penicillin;
b) a small, inconstant proportion of the bacteria is already in the persister state at the time of incubation. In other bacteria, the condition is induced later in the new environment;
c) there is no evidence that persistence is produced by penicillin although the state may be induced in its presence;
d) persisters are believed to be insusceptible to penicillin because they are in a non-dividing, dormant state and because penicillin kills bacteria only when they are dividing or are about to divide;
e) persisters are individually (phenotypically) insusceptible to penicillin, but their progeny are no more insusceptible than are the normal bacterial cells;
f) when penicillin is inactivated and the culture continued, the majority of the persisters soon develop into normal cells, and divide quite quickly, but a few remain in the persister state for several days; and finally,
g) "it is believed that despite the presence of penicillin, the biological urge to multiply may ultimately force persisters to undergo a process preparatory to division, with the result that they become susceptible to the action of penicillin and are killed by it [2]."

From clinical experience with the initial response of osteomyelitis to treatment by penicillin, followed by relapse after cessation of treatment, Bigger inferred that persisters are more common *in vivo* and remain dormant longer *in vivo* than *in vitro*; they are ultimately eliminated by dying (during dormancy) or by the drug when active metabolism resumes and also partly by the action of polymorphonuclear leukocytes. The author speculated about the intermittent application of penicillin, hoping that during the drug-free intervals persisters would begin to divide and be killed when penicillin was recommenced.

In 1957, McDermott [22] presented a comprehensive review of the experimental and clinical studies on bacterial persistence conducted by his and other research groups. He analyzed the factors that might determine the survival of organisms in the various tissues and examined the possibilities of a direct adverse influence of certain organs, tissues, and inflammation on drug action. Reference was made to special studies [20, 21] undertaken to investigate the then-popular theory that persistence was the result of "underdosage," i.e., that for various reasons the drug reached the microbes in insufficient concentration. Particularly, tissue barriers, such as fibrotic walls around abscesses or tuberculous cavities, avascular necrotic areas, or biological membranes, were made responsible for protecting the organisms against drug action. However, in studies in animals and *in vitro,* the drugs, in the presence of which microbial persistence had been observed, were demonstrated to enter all organs, tissues, and lesions in sufficiently high concentrations. Thus, McDermott concluded that persistence was very unlikely to be the result of a direct environment-drug antagonism. Even if such an antagonism occurred, it could seldom, if ever, attain a magnitude sufficient to explain the phenomenon of bacterial persistence.

McDermott subsequently turned his attention from the relationship between drug and environment to that between environment and organism. Based on observations that changes of the environment could produce morphological changes in certain microbes such as *H. pertussis* [35] and *M. fortuitum* [7], a hypothesis was developed that assumed that environmental changes could alter the susceptibility of the organism to drugs. Such an environmental change could result in a loss of the effectiveness of a drug. This hypothesis was supported by the results of a series of experiments in animals

and *in vitro*. It was demonstrated, for example, that changes of temperature induced reversible drug indifference of staphylococci ([2]) and that under anaerobic conditions, streptomycin lost its effectiveness on staphylocci and tubercle bacilli but not on *E. coli* ([23,24]). It was further shown that in an acid environment *M. tuberculosis* becomes susceptible to pyrazinamide whereas *M. bovis* does not. That a change in the environment produces susceptibility to a drug in one mycobacterium but not in another may indicate an influence of the acid environment on *M. tuberculosis*. Pyrazinamide has been shown to have little effect *in vitro* when tested at a neutral pH, suggesting that the susceptibility of *M. tuberculosis* to pyrazinamide at acid pH is related to the capacity of the organism to undergo some specific alteration in response to an environmental change. Another reason why pyrazinamide is active against *M. tuberculosis* but not against *M. bovis* (and *M. leprae*) is probably that the latter organism(s) lack the amidase necessary to degrade pyrazinamide to pyrazinoic acid, which is thought to be the active moiety (G. A. Ellard; personal communication).

In another investigation, Berntsen, in McDermott's laboratory, examined the relationship between the total number of organisms in the bacterial population and the frequency of persisters. Experiments *in vitro* and in animals proved that penicillin may reduce an actively growing population of Group A β-hemolytic streptococci of about 10^6 organisms to low levels and often to zero within a few hours. However, so rapid a decrease of the number of organisms did not occur in populations of 10^8 to 10^9 organisms, the peak values attained by staphylococci, β-hemolytic streptococci, and tubercle bacilli in culture and in some organs of the mouse. Thus, populations of penicillin-susceptible staphylococci of 10^8 to 10^9 organisms were not markedly reduced *in vitro* by penicillin and thus were "drug-indifferent." Also, in the experimental animal, populations of 10^8 to 10^9 streptococci, penicillin-susceptible staphylococci, and tubercle bacilli were reduced to a lesser degree by penicillin or isoniazid than were populations of 10^6 in the same animal.

In a further series of experiments ([22]), streptococci were grown on a large scale for a short period, and from 10^8 to 10^9 organisms were resuspended in fresh medium. It was demonstrated that a population of this size less than 4 hr old was as susceptible as a small population to the action of penicillin. Similar observations were made when 4 hr old populations of the same size were inoculated into mice. McDermott concluded that the occurrence of bacterial persistence was not merely a function of the size of the bacterial population. Larger populations have been growing for a longer time than have small populations and, therefore, are older and have been in contact longer with the environment than smaller, younger populations. During the more prolonged contact, the environment had a greater opportunity to react with the older population and give rise to drug indifference. Thus, microbial persistence is likely to be induced by environmental changes and is more likely a function of the age than of the size of the bacterial population.

The relationship between inflammation, necrosis, or other unfavorable environmental conditions and drug indifference was explained by additional experiments and observations. McDermott and his coworkers ([22]), Canetti ([5]), and Wood ([31,36]) showed that environmental factors that inhibit multiplication of bacterial populations, such as aging, crowding, inflammation, pus, and necrosis, may induce changes in the organisms by which they adapt to the unfavorable conditions. These adapted cells have been found to be dormant, in which state they are invulnerable to drug action.

Finally, McDermott ([22]) considered the possibility that persistence was the result of an adaptation of the organisms to the environment that occurred only during chemotherapy. The authors reviewed clinical studies and observations of the chemotherapy of syphilis, malaria, scrub typhus, tuberculosis, and Q-fever. It had been shown that, when effective chemotherapy was begun shortly after infection, the result was often only postponement of the onset of clinical disease. However, the same treatment was highly effective when it was begun after illness was manifested clinically, or shortly before the manifestations of the

illness were expected to appear. Thus, it was inferred that microbial persistence may occur before antimicrobial therapy has been administered.

BACTERIAL PERSISTENCE IN TUBERCULOSIS

Relatively speaking, considerable information about bacterial persistence has been derived from experimental tuberculosis and from controlled clinical studies of the chemotherapy of tuberculosis. Particularly since the development of short-course regimens, by which the conventional period of treatment of 12 to 18 months may be reduced to one half or less, the frequency of relapse resulting from persistence has become a major issue.

Bacterial persistence in experimental tuberculosis. Persisting *M. tuberculosis* were found *in vitro* in the early days of treatment with streptomycin and isoniazid ([30]). Workers at Cornell University reported in 1956 on the survival of drug-susceptible *M. tuberculosis* despite prolonged antimicrobial therapy ([20]). In one study, the effect of various drugs (isoniazid, streptomycin, pyrazinamide, and para-aminobenzoic acid [PAS]) on murine tuberculosis was explored. The drugs were given singly or in combination every day for about four months. The behavior of the bacterial populations in the various tissues was observed during and at the end of drug administration. There was uniform persistence of *M. tuberculosis* in the spleen except after treatment with regimens containing isoniazid and pyrazinamide. Even in mice that had received a triple-drug regimen, such as isoniazid, streptomycin and PAS, a few persisting organisms could be detected by means of a very sensitive technique. The cultures of the bacilli that had survived in the tissues despite adequate chemotherapy were highly susceptible to the drugs *in vitro*. However, when isoniazid and pyrazinamide were administered together, it was not possible to detect a single *M. tuberculosis* in the lungs or spleen of treated animals by microscopy, culture, or animal inoculation. Tubercle bacilli could not be demonstrated even when a special culture technique, described by Hobby and her coworkers ([17]), was used. This technique, which required incubation for as long as nine months, can detect viable *M. tuberculosis* not detected by standard culture methods.

When animals treated with the combination of isoniazid and pyrazinamide were observed for three months without further treatment, about one third were found to relapse ([21]). With but one exception, the organisms that could be cultivated were susceptible to both isoniazid and pyrazinamide. The authors assumed that the number of relapses could be minimized by appropriate prolongation of treatment, but that the elimination of persisting bacilli during prolonged treatment might result not only from drug action but also from natural death of the persisters after a prolonged period of dormancy.

Extensive studies of experimental chemotherapy were carried out at the Pasteur Institute in Paris. In a series of experiments, the relapse rate was measured of mice that had been treated with rifampin and/or pyrazinamide-containing regimens ([10]) which included also isoniazid or streptomycin. The regimens were administered daily or intermittently to mice for various periods of time, and the relapse rate was measured, as had been done in the study of McCune and his coworkers ([21]).

It was found that the tubercule bacilli susceptible to H, S, PAS, T, R, Z[4] and other drugs *in vitro* were able to survive long-continued exposure to these drugs in the mouse. After an initial decrease, once the number had reached a certain minimum, the bacterial census remained constant despite continuation of the therapy for three to six months. That was not the result of emergence of drug resistance because the residual populations were proven to be susceptible to the drugs administered. The effect of the various regimens—except a few (see below)—differed only quantitatively, depending on the drugs, their combination, the dosages, and the rhythm of administration. Microbial populations were usually decreased to very low levels, and the persisting bacilli could be demonstrated only in the spleen.

[4] H = isoniazid, S = streptomycin, PAS = para-aminobenzoic acid, T = thiacetazone, R = rifampin, Z = pyrazinamide.

This uniform persistence, easily demonstrable by microscopy or culture at the end of treatment, was, however, strikingly altered when R or Z was given in association with other drugs, provided one of them was H. These three key drugs when given together or paired, i.e., H + R or H + Z could reduce the population of tubercule bacilli below the limit of detectability at the point in time where treatment was stopped. Nevertheless, even when H + R, which appeared the most effective "sterilizing" combination, was given for less than nine months, 20% of the treated animals relapsed with susceptible organisms.

Finally, recent experiments in mice ([13, 14]) have shown that, in spite of 1 year of isoniazid and rifampin, it was possible to achieve a relapse rate of 60% by the administration of cortisone in high dosage for two months after the end of antimicrobial therapy. It was concluded that even highly effective chemotherapy in the mouse achieves latency rather than sterilization of the tuberculous lesions.

Bacterial persistence in tuberculosis of humans and relapse after chemotherapy. With the introduction of short-course chemotherapy, the problem of relapse resulting from microbial persistence became of crucial importance. With isoniazid and streptomycin as well as with regimens containing also rifampin or pyrazinamide in combination, sputum negativity by culture can be achieved by the end of 6 months in almost all cases. Therefore, the chief criterion of efficacy of short-course regimens is the rate of relapse after completion of treatment.

A series of controlled clinical trials pioneered by the East Africa/British Medical Research Council (BMRC), the Singapore Tuberculosis Services, and the Hong Kong Tuberculosis Treatment Service in cooperation with the BMRC ([8]), and additional controlled studies in Europe and Latin America, all employing regimens of four to nine months' duration, have shown that it is possible to achieve results comparable to those achieved with standard regimens of 12 to 14 months' duration. Thus, even in previously untreated patients with severe pulmonary tuberculosis, discharging large numbers of *M. tuberculosis*, bacteriological quiescence[5] can be attained in well over 90% of the patients. A brief account of these trials together with a review of their relevance to the problem of persisters follows.

The principal findings of short-course chemotherapy were evident in the first study ([8]). Compared were five regimens with about 150 to 180 patients in each treatment group (see the Table). The four experimental regimens—SHR, SHZ, SHT and SH[6]—were given for six months; a standard regimen—SHT for two months, followed by HT for 16 months—served as "control." All regimens were administered daily. At the completion of six months of treatment, all four regimens showed a very high rate of bacteriologic conversion. Among the 690 patients initially sputum positive by microscopy and culture, only two still had culturable bacilli in the sputum. However, in contrast to the uniformly favorable results at the termination of treatment, the rates of relapse, i.e., the reappearance of culturable bacilli in the sputum after at least three months of bacteriological negativity after treatment showed striking differences. The six month regimen, SHR, was followed by a 3% relapse rate, SHZ 8%, SHT 22%, and SH 29% whereas the 18 month control regimen, SHT/HT, was followed by a 2% relapse rate. Patients on the regimens SHR and SHT/HT were followed up for 60 months, those on the other regimens for 36 months. Other important findings were:

a) regimens containing rifampin or pyrazinamide reduced the bacterial population much faster that did the other regimens;
b) the great majority of relapses occurred within 6 months after cessation of therapy. Some relapses occurred in the following 6 months but rarely later;

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[5] Bacteriological quiescence is the absence of culturable tubercle bacilli for a prolonged period of time confirmed by repeated sputum cultures particularly during the first 3–6 months after conversion to negativity ([21]).

[6] S = streptomycin, 0.75 to 1 g; H = isoniazid, 300 mg; T = thiacetazone, 150 mg; R = rifampin, 450 to 600 mg; Z = pyrazinamide, 1.5 to 2 g daily.

TABLE. *Bacteriological relapses after short course chemotherapy in tuberculosis patients with organisms drug-susceptible before treatment.*[a]

Treatment regimen[b]	Total patients assessed	Total bacteriological relapses		Relapse with sensitive organisms	Relapse after cessation of treatment in months				
		No.	%		1–6	7–12	13–18	19–24	25–60
6 SHR	145	5	(3)	5	3	1	0	0	1
6 SHZ	153	13	(8)	10	10	3	0	0	–
6 SHT	104	23	(22)	20	20	2	1	0	–
6 SH	112	33	(29)	32	28	3	2	0	–
18 SHT/HT (control regimen)	125	3	(2)	2	0	0	2	0	1

[a] After East African/BMRC study; Am. Rev. Resp. Dis. **116** (1977) 3–8.

[b] S = streptomycin, 0.75–1.0 g, H = isoniazid, 300 mg, T = thiacetazone, 150 mg, R = rifampin, 450–600 mg, Z = pyrazinamide, 1.5–2.0 g, 6 = 6 months daily (supervised administration), 18 = 18 months daily (supervised only 2 months).

c) 90% of all relapses were associated with organisms susceptible to isoniazid and streptomycin. All five patients who relapsed after treatment with SHR had fully susceptible organisms. Only a small proportion of the relapses was associated with drug resistance.

It was concluded that relapse was caused either by residual, drug susceptible organisms that had not been eliminated because of treatment deficiencies, or more likely, by drug-susceptible organisms that survived treatment because they had been in the persister state and had revived after treatment had been stopped. Irregularity of drug intake could be largely excluded because the patients were treated under careful supervision.

The findings of the first large-scale trial have been confirmed by a series of studies carried out among different populations in various parts of the world. Short-course regimens containing isoniazid or isoniazid and streptomycin and supplemented by rifampin or pyrazinamide, given daily, intermittently, or only for an initial period, showed a success rate exceeding 90%. Relapse, which occurred in 8% of the patients or less, was almost always caused by drug susceptible persisting *M. tuberculosis*. By extending the duration of treatment to nine months, some regimens may yield a relapse rate of zero during a follow-up period of three or more years ([3, 4]). However, the low relapse rates reported have all been achieved under stringent research conditions under which drugs were administered regularly, mostly under direct supervision, and adverse reactions were readily managed.

An important task was to determine the minimal time required for short-course therapy because the acceptability of a regimen decreases progressively with its duration. Also, the high costs of rifampin and pyrazinamide and the risk of adverse effects made it necessary to investigate the minimal dose and duration required for the administration of these two drugs. The duration of chemotherapy is generally determined by the proportion of patients achieving bacteriological negativity by the time of completion of therapy and by the subsequent rate of relapse. Thus, it was necessary to examine the reasons for relapse. Because these relapses occur usually with drug-susceptible organisms, it was logical to explore the problem of microbial persistence in greater depth.

The antituberculosis drugs used most often in regimens of short duration, isoniazid, streptomycin, rifampin, and pyrazinamide, are bactericidal. However, experimental studies *in vitro* or *in vivo* in mouse and guinea pig as well as controlled clinical trials indicated that rifampin and pyrazinamide are capable of a special antimicrobial activity. One working hypothesis, developed by the group of bacteriologists affiliated with the series of clinical trials ([9]),

draws on the experimental studies carried out at Cornell University and the Pasteur Institute as well as on their own investigations ([6, 25]). According to this hypothesis, there are two different drug actions in the chemotherapy of tuberculosis: first, a bactericidal activity against *M. tuberculosis* in the phase of multiplication and second, a bactericidal action against the organisms that are multiplying very slowly after a period of dormancy. One may ask why all known bactericidal drugs readily kill metabolically-active *M. tuberculosis* but are not equally effective against nearly dormant organisms.

Rifampin is no more bactericidal against *M. tuberculosis* H37Rv than is isoniazid, nor is there a synergistic action when the organisms are exposed to both drugs simultaneously. Moreover, rifampin does not kill slowly-multiplying bacilli much more rapidly than does isoniazid, nor does rifampin penetrate membranes better than does isoniazid. The difference between the two drugs may be explained by the exceptional speed with which rifampin attacks once-dormant organisms that may have become metabolically active for a few hours only. Whereas rifampicin may begin to kill these organisms almost immediately, isoniazid may require at least one day of continuous exposure, i.e., too long to act on organisms that start growing for short periods.

A "simulation model" produced strong evidence in favor of this hypothesis *in vitro* ([25]). Cultures of *M. tuberculosis* were grown under four different conditions. In the first set of cultures, incubated continuously at 37°C, there was rapid growth in the drug-free medium, and killing occurred with equal speed in the cultures containing isoniazid and those containing rifampin. In the second set, incubated continuously at 8°C, no growth occurred in the drug-free control cultures, and neither drug was bactericidal. In the third set, incubated at 8°C with daily 6 hr periods of incubation at 37°C, the drug free control cultures grew slowly; rifampin killed more quickly than did isoniazid, which required about 15 days more to achieve the same level of kill. In the fourth set of cultures incubated at 8°C with daily 1 hr periods of incubation at 37°C, the drug-free cultures did not grow, and there was no appreciable kill in the cultures containing isoniazid. However, in the cultures containing rifampin, the viable count fell steadily during the 31 days of the experiment. Thus, the shorter the period of metabolic activity, the greater the bactericidal effect of rifampin in comparison to that of isoniazid.

The authors inferred that in a bacterial population four different groups of organisms may be distinguished, which differ metabolically and react differently to antituberculosis drugs:

a) Group I, consisting of fully metabolizing, actively-growing organisms. These are located chiefly inside tuberculosis lung cavities that are in communication with open bronchi. In cavity walls, the pO_2 is normal and the pH neutral. Under these conditions, the rapidly multiplying organisms are killed rapidly by isoniazid, rifampin, and streptomycin, and the more slowly multiplying organisms are killed proportionately more slowly by these drugs;

b) Group II, consisting of organisms dormant most of the time but occasionally multiplying for very brief periods. A period of about 1 hr is sufficiently long for rifampin to kill such organisms but not long enough for isoniazid or streptomycin;

c) Group III, consisting of organisms that are multiplying very slowly because of the low pH that exists inside macrophages. These intracellular organisms appear to be coated with antibody and within phagolysosomes ([1]). An environment with an acid pH is known to favor the bactericidal action of pyrazinamide. Although isoniazid is also able to penetrate cell membranes, its action is rather slow because of the slow growth of intracellular organisms. Streptomycin is ineffective at a low pH;

d) Group IV, consisting of organisms that are fully dormant and therefore unaffected by any drug. These organisms rarely regain metabolic activity and die usually of inanition, or are killed by host defense mechanisms.[7]

[7] Such defense mechanisms are believed to be lacking in lepromatous leprosy.

Relapse after cessation of chemotherapy results most often from organisms of Groups II and III.

Another hypothesis, advanced by Grosset [11] and compatible with the first, emphasizes the role of the environment. According to this hypothesis, the effect of antituberculosis drugs is, apart from the proportion of drug-resistant mutants in the bacterial population, largely determined by:

a) the location of *M. tuberculosis*, either intra- or extracellular; and
b) the capability of drugs to pervade the intra- and extracellular environments of the organisms in bactericidal concentrations.

This hypothesis further assumes that the rapid disappearance of *M. tuberculosis* from the sputum after the onset of chemotherapy represents the effect on extracellular organisms. Intracellular bacilli do not appear in the patient's sputum and are able to survive the administration of drugs that are unable to enter the cell. When persisting intracellular organisms in macrophages are released after termination of treatment, relapse may occur. When they are released during treatment, they may be killed by the drugs that kill extracellular bacilli. The longer bacilli persist in macrophages, the longer must be the duration of treatment. Sputum conversion during chemotherapy or at its completion is not proof that all intracellular bacilli have been eradicated.

The author supports this hypothesis by comparing the results of controlled clinical trials of various long-term and short-course regimens. He concludes that those regimens that contain drugs that kill only extracellular bacilli must be administered for a long time, at least 12 months; otherwise, the relapse rate will be unacceptably high. Regimens containing isoniazid and rifampin or pyrazinamide are suited for short-course chemotherapy because they effectively attack intracellular organisms.

In a more recent paper [12], the same author calls attention to the important role in the antibacterial effect of chemotherapy played by the immune status. In the immunocompetent mouse, *M. tuberculosis* are eradicated more quickly and more reliably than in the immunodeficient "nude" mouse in which relapses invariably occur as soon as treatment is stopped [33]. A similar result was observed in immunocompetent mice that received corticosteroids after apparently successful chemotherapy [14]. He concludes that, at least in mice, cell-mediated immunity determines to a great extent the outcome of antimicrobial treatment of tuberculosis.

SUMMARY OF BACTERIAL PERSISTENCE POSSIBLY RELEVANT TO THE TREATMENT OF LEPROSY

Persisters are individual organisms that have the capacity to survive in the host despite adequate chemotherapy. They form only a small proportion of the total bacterial population in the body. Their frequency is estimated to range from about $1:10^4$ to $1:10^6$; in populations smaller than this number, the occurrence of persisters is uncertain. They may be more common *in vivo* than *in vitro*. Persisters may already be present in bacterial populations that have never come into contact with antimicrobial drugs, and they may also develop after the onset of chemotherapy.

Persisters cannot be directly identified in a bacterial population; their presence may only be inferred. Persistence is probably induced by factors unfavorable to multiplication or those that inhibit bacterial metabolism such as aging and crowded bacterial populations, old necrotic lesions, sparsely vascularized sites with a low pO_2, and intracellular location within macrophages where the pH is acid. Persisters are cells that are capable of adapting to such adverse conditions in their environment by reducing their metabolism to a minimum. They may remain in this state for some time; a proportion of them dies, but some cells may regain normal metabolism and multiply as do normal organisms. Because of reduced metabolism, persisters are not affected by drugs that act primarily on metabolically active organisms.

Treatment failures resulting from persistence or drug-resistance may be easily confused. The chief differences between these two phenomena are:

Resistance	Persistence
Drug resistance results from a selective process. By destroying the drug-susceptible organisms of the population, effective drugs "select" the pre-existing drug-resistant mutants, which overgrow the susceptible organisms of the strain and eventually replace them.	Microbial persistence is the result of an adaptive process. Under adverse conditions some cells reduce their metabolic requirements to a minimum, assuming a state of dormancy that coincides with insusceptibility to drug action.
Resistant organisms are genetically changed microbial cells. Their descendants will be resistant to the same drug as the parent cells. Thus, drug-resistance is inherited.	Persisters are metabolically changed microbial cells. Those that "revive" behave like normal organisms. They and their descendants are as susceptible to a drug as are the normal cells of the strain. Microbial persistence is not inherited.
Treatment failure resulting from drug-resistance occurs most often during inadequate antimicrobial treatment. Continuation of treatment with the same drugs usually does not produce a favorable response.	Treatment failure resulting from microbial persistence usually occurs after adequate chemotherapy has been stopped. Reinstitution of the same treatment will almost always produce a favorable response ([18]).

RELAPSE CAUSED BY BACTERIAL PERSISTENCE AND TREATMENT FAILURES FOR OTHER REASONS IN LEPROSY[8]

The phenomenon of microbial persistence in leprosy has been rather recently observed in a small number of patients under prolonged chemotherapy by Waters, *et al.* ([34]), Russell, *et al.* ([29]), Rees, *et al.* ([28]) and Pattyn ([26]).

Reasons for treatment failures have been reviewed by Levy ([19]). The occurrence of bacterial persisters as a reason for treatment failure was given great importance. The fact that viable, drug-susceptible *M. leprae* could be found after years of treatment with dapsone, clofazimine, rifampin, and thiambutosine has been taken as evidence that none of these drugs is capable of eradicating the viable organisms from the body of patients with multibacillary leprosy. The fact that some drug-susceptible organisms capable of adapting to adverse conditions may survive adequate antimicrobial chemotherapy and cause relapse has been established during the last four decades in a large number of infections and should have led to the assumption that persisters also occur in leprosy. Even without the evidence recently presented, the presence of persisters in the large bacterial populations that exist in patients with lepromatous leprosy could have been predicted.

There is little reason to believe that, in the near future, a new drug or combination of drugs will be found that is capable of eradicating persisting *M. leprae*. It may be useful, nevertheless, to reconsider whether the sporadic demonstration of viable, drug-susceptible organisms after antimicrobial treatment of variable duration and efficacy warrants a policy of life-long chemotherapy. It has been repeatedly asserted that patients with lepromatous leprosy are unable to cope with even a small number of viable *M. leprae*. Because these patients lack specific cell-mediated immunity, it has been taken for granted that once chemotherapy is stopped, even a few persisters will uniformly begin to multiply and inevitably cause relapse.

At the Workshop on Leprosy Chemotherapy ([27]), the question was raised as to whether and how many patients would relapse once antimicrobial chemotherapy of high efficacy and sufficient duration was stopped and whether such relapses were related to persistence of *M. leprae*. No firm evidence was available. Preliminary data were presented from a study of apparently cured patients who had been followed up

[8] Common reasons for treatment failures are illustrated in Figs. 1–4.

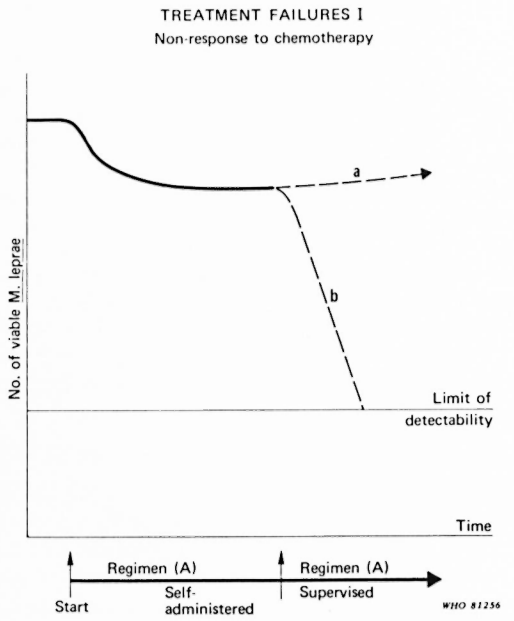

FIG. 1. No response, no appreciable reduction of AFB. If after continuation of treatment with the same regimen but under strict supervision:

(a) no appreciable response
Conclusion: Treatment failure due to probable initial drug resistance.

(b) definite response, evident reduction of AFB
Conclusion: Treatment failure due to non-compliance; the patient had not taken the drugs.

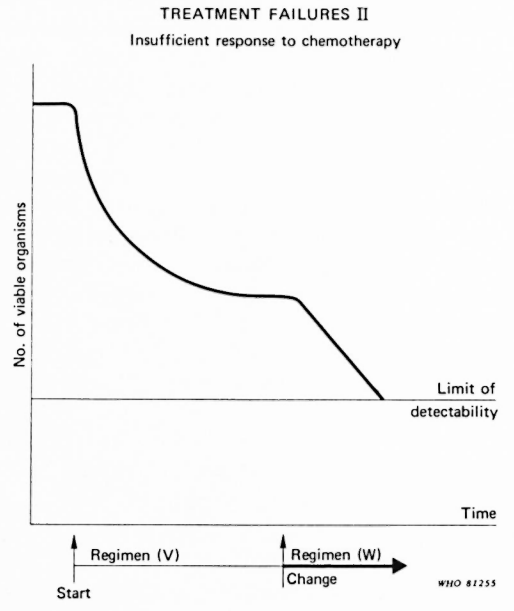

FIG. 2. Slow response, reduction of AFB perhaps due to only bacteriostatic drug effect or under-dosage. Prompt response to changed regimen.
Conclusion: Treatment failure due to inadequate drug regimen, corrected by change to an adequate regimen.

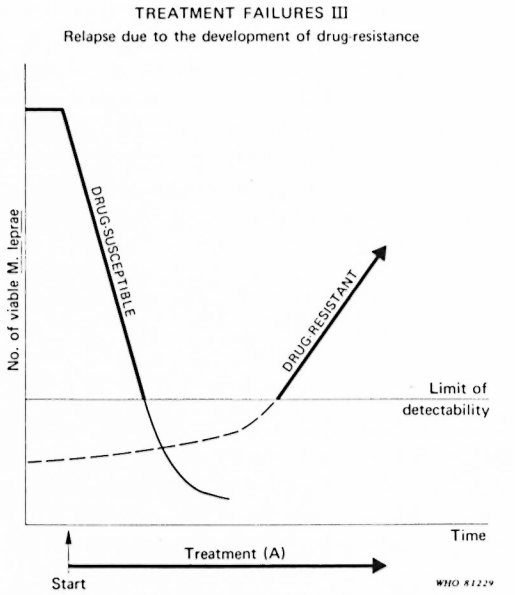

FIG. 3. Initial response, temporary negativity for a relatively short period followed by reappearance of AFB in increasing numbers and viable (drug-resistant) organisms. Deterioration or non-response in spite of *continuing treatment* with the same regimen even when administered under supervision.
Conclusion: Relapse with drug-resistant organism

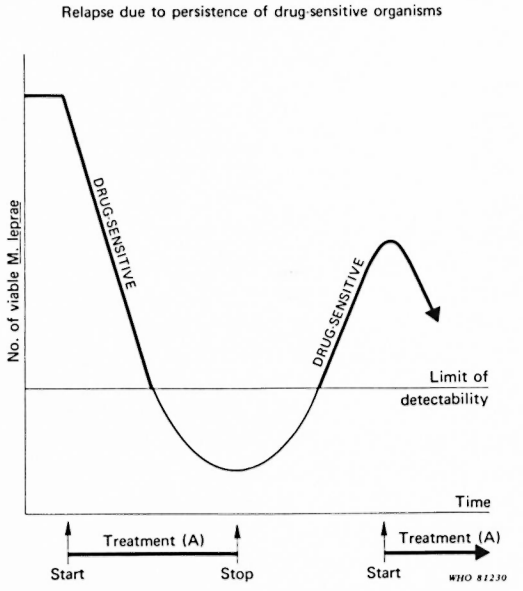

FIG. 4. Favorable response to treatment, disappearance of viable organisms for a prolonged period. Reappearance of AFB and viable organisms in increasing numbers after termination of treatment. Prompt response to reintroduction of the same treatment.
Conclusion: Relapse with drug-susceptible organisms responding to the original treatment.

for five years after cessation of regular treatment of at least 10 years' duration. Of a total of 200 lepromatous patients, in a large proportion of whom persisters might have been expected to be present, only about 10 to 20 were thought to have relapsed. A similar relapse rate has been observed in patients with multibacillary tuberculosis after chemotherapy with standard regimens of 12 months' duration ([32]). Thus, it is not yet precisely known how many adequately treated lepromatous patients would relapse after cessation of chemotherapy and what proportion of the relapses would be associated with the multiplication of drug-susceptible organisms. It cannot be denied that relapse caused by bacterial persistence is a minor problem compared to the number of treatment failures that occur because of shortcomings of treatment delivery. Therefore, there exists a definite need to reexamine the justification for life long chemotherapy, not only because treatment of lepromatous patients for a lifetime is impractical in most communities but also because the need remains to be verified.

SUMMARY

Bacterial persistence is the capability of microorganisms to survive in the host despite adequate antimicrobial treatment. This is a general phenomenon and has been observed during infection with a number of organisms. The subject is reviewed in relation to the treatment of leprosy. The presence of persisters in the large bacterial populations that exist in patients with lepromatous leprosy is not surprising. It is unlikely that in the near future a new drug or combination of drugs will be found that is capable of eradicating persisting *M. leprae*. It may be useful, nevertheless, to reconsider whether the sporadic demonstration of viable, drug-susceptible organisms after antimicrobial treatment of variable duration and efficacy warrants a policy of life long chemotherapy in lepromatous leprosy.

RESUMEN

Persistencia bacteriana, es la capacidad de los microorganismos para sobrevivir en el huésped a pesar de un tratamiento antimicrobiano adecuado. Este es un fenómeno general observado durante la infección por diversos microorganismos. Se revisa este tema en relación al tratamiento de la lepra. La existencia de microorganismos persistentes dentro de la enorme cantidad de bacilos que se encuentra en los pacientes con lepra lepromatosa no es un hecho que sorprenda. Es poco probable que en un futuro cercano se encuentre una nueva droga o una combinación de drogas que sean capaces de erradicar a los *M. leprae* persistentes. Sería útil, sin embargo, reconsiderar si la demostración esporádica de organismos viables, susceptibles a la droga, después de un tratamiento antimicobacteriano de duración y eficacia variables, justifica o garantiza una política de quimioterapia de por vida en los pacientes con lepra lepromatosa.

RÉSUMÉ

On entend par persistance bactérienne, la capacité de microorganismes à survivre dans la cellule de l'hôte en dépit d'un traitement antimicrobien adéquat. Ceci est un phénomène général qui a été observé au cours d'infections par un certain nombre d'organismes. Le sujet est passé en revue en relation avec le traitement de la lèpre. La présence de bacilles persistants dans les larges populations bactériennes qui existent chez des malades atteints de lèpre lépromateuse, n'est aucunement surprenante. Il est improbable que dans un avenir rapproché on puisse découvrir un nouveau médicament, ou une nouvelle combinaison de médicaments, qui soit capable d'éradiquer *M. leprae* persistant. Il peut toutefois être utile de se demander, à nouveau si la démonstration sporadique d'organismes viables et susceptibles au médicament, après un traitement antimicrobien efficace de durée variable, justifie une politique de chimiothérapie prolongée pour la vie entière, chez des malades souffrant de lèpre lépromateuse.

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