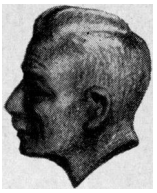
The R. E. Dyer Lecture



Inapparent Infection

Relation of Latent and Dormant Infections To Microbial Persistence

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BOTH microbes and man have an extraordinary degree of adaptive plasticity with reference to their respective environments. For centuries, man has been constantly changing his external environment and using his adaptive capacity to survive therein. In recent decades, however, man has greatly increased his capacity to alter his internal environment. And, it is man's now frequently altered internal environment to which the microbes that inhabit man must adapt if they are to survive.

When the Cornell University Medical College opened for the new term in the fall of 1958, I happened to draw the assignment of discussing the first case at the clinical-pathological conference. The case record started off as follows:

A 43-year-old white Italian stock clerk was admitted to the New York Hospital-Cornell Medical Center for the first time June 4, 1957, complaining of swelling and tenderness of the right shoulder of 12 weeks' duration.

The patient was in good health until 3 months prior to admission when he noted the onset of swelling of the right arm from shoulder to wrist. This was shortly followed by fever and night sweats. Eight weeks prior to admission the right arm became painful. Shortly thereafter the skin over the right elbow became red and warm and the swelling increased. Despite initial improvement with corticotropin, steroids, and physical therapy, the discomfort in the right arm increased and admission was advised. A firm small right supraclavicular mass had been present for about 18 months. He had had an episode of "dry" pleurisy at age 21. One brother was known to have multiple cutaneous lipomata.

Physical examination revealed a well-developed, well-nourished white male who appeared neither acutely nor chronically ill. . . .

I shall omit the detailed presentation of the remainder of the findings in this case. I simply wish to call to your attention that the man was 43 years old, had considered himself to be per-

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Dr. McDermott, who is Livingston Farrand Professor of public health and preventive medicine at Cornell University Medical College, delivered the Dyer Lecture on December 16, 1958, at the National Institutes of Health, Public Health Service. A leading investigator in the field of infectious diseases, he has authored a large number of articles for scientific journals and contributed to medical classics on chemotherapy, pneumonia, and syphilis. He has also held posts of distinction on advisory and study panels of the Public Health Service.

fectly well until 3 months previously, and on physical examination had appeared neither acutely nor chronically ill.

Revival of Microbial Slumberers

During the next 7 months this man had many trials and tribulations including such things as widespread tuberculosis of lymph nodes and lymphosarcoma. His multiple serious ailments were all brought under excellent control by the careful and wisely chosen application of some of the wonders of modern medical science. Indeed, he attained a symptom-free state and was able to leave the hospital for a short period. Nevertheless, this gallant patient and his most dedicated physicians were finally conquered by his developing an infection with *Monilia albicans*, a common microbe that characteristically lives harmlessly in man.

Presumably, when the patient was born he was not carrying *Monilia* but neither is there any reason to believe that he "caught" the monilial infection in the weeks preceding his death. In all probability, throughout most of his adult life the microbes of *Monilia* were living quietly somewhere in his tissues. This was presumably the case whether or not by our relatively crude diagnostic methods it would have been possible to detect them there amid the welter of other microbes for which he played the host. The point that concerns us is the fact that from this welter of other micro-organisms it was this particular one, *Monilia*, that found the environmental conditions for arising and conquering. In short: Why *Monilia?*

Let us turn now from the bedside to the laboratory and regard other examples of infection. Some 7 or 8 years ago, as was correctly fashionable at the time, we were engaged in our laboratory in studying the influence of cortisone and corticotropin on infections, notably experimental infections with tubercle bacilli.

In one part of these studies, LeMaistre and Tompsett chose a model consisting of avian tubercle bacilli and the rat, in order to have an infection that was generally mild in character (*1*). In figure 1 may be seen the type of caseating necrotic lesion observed in the lungs of the rats infected with avian tubercle bacilli and maintained on cortisone. The avian tubercle bacilli could be recovered by culture of these tissues.

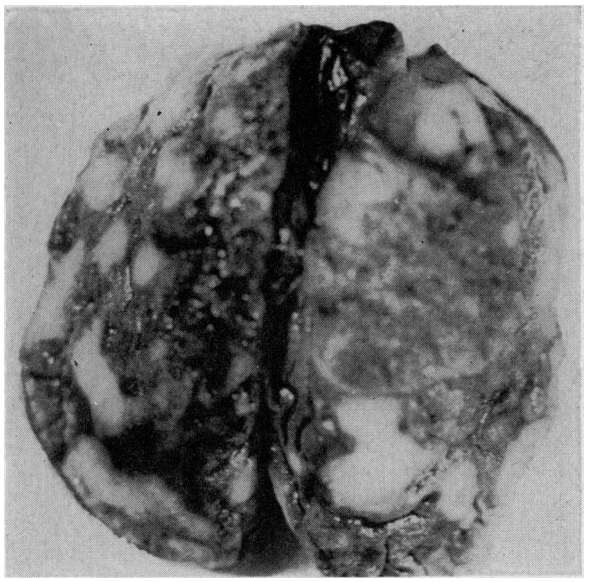

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Figure 1. Lesions of the lung of a rat which received cortisone but was not inoculated with tubercle bacilli.

The initial inference was the natural one that a characteristically mild tuberculous infection had been enhanced to the point of fulminating caseonecrotic disease by the influence of the cortisone. On more careful study, however, it was revealed that the avian tubercle bacilli had nothing to do with these caseonecrotic lesions. Instead, it was found that the entire process was a disease known as pseudotuberculosis produced by quite a different microbe, *Corynebacterium pseudotuberculosis muris.*

In the absence of cortisone, the members of the rat colony uniformly showed a high degree of natural resistance to the corynebacteria when attempts were made to infect them by various routes. For this and other reasons it seemed wholly improbable that the corynebacterial infection in the animals given cortisone represented a cross-infection occurring after the start of the cortisone. Despite careful and detailed investigation, however, it was not possible to demonstrate that the rats were already harboring the corynebacteria at the time they received the cortisone.

In other words, by the use of all available

diagnostic methods it was not possible to detect the presence of corynebacteria in the tissues of the rats. Yet the evidence was quite convincing that the corynebacteria in fact were there; it was just not possible to demonstrate that they were there. Once the rats received cortisone, this infection—that could not be introduced experimentally from the outside—exploded "from the inside," so to speak, and formed a tissue-destroying and eventually fatal disease.

The question arises as to whether it was the presence of the latent corynebacterial infection or the absence of the appropriate tissue environment that created the host resistance against our attempts at experimental infection. In any case, once the cortisone influence was established, it was the corynebacteria, and not any of the other microbial species residing in the rats, that were resurrected to the production of destructive disease. Once again we may well inquire: Why *Corynebacterium?*

In this particular case we do have a little more of a lead from studies, some by LeMaistre and others and some by György and by Zucker and their associates in the precortisone era (*2*, *3*). These studies have shown that certain specific dietary deficiencies can provide the appropriate environment for the resurrection of *Corynebacterium* in the tissues. In short, we have a glimpse here of the sort of specificity that we sense must be present in these microbial adaptations to their environment.

Retreat Into Latency

The two examples cited thus far have been concerned with one end of a phenomenon: the apparent resurrection of a microbial slumberer that is then able to surpass its fellow competitors so successfully that it finally overcomes its host. Let us turn now to what might be viewed as the other end of the phenomenon, namely, how a microbe that is surrounded by a tissue environment wholly appropriate for its flowering as disease nevertheless assumes the latent state. How does the microbe that is living openly in the host "go underground?" Or, more precisely, can either the microbe or the ideal tissue environment be artificially modified so that microbial latency is the outcome?

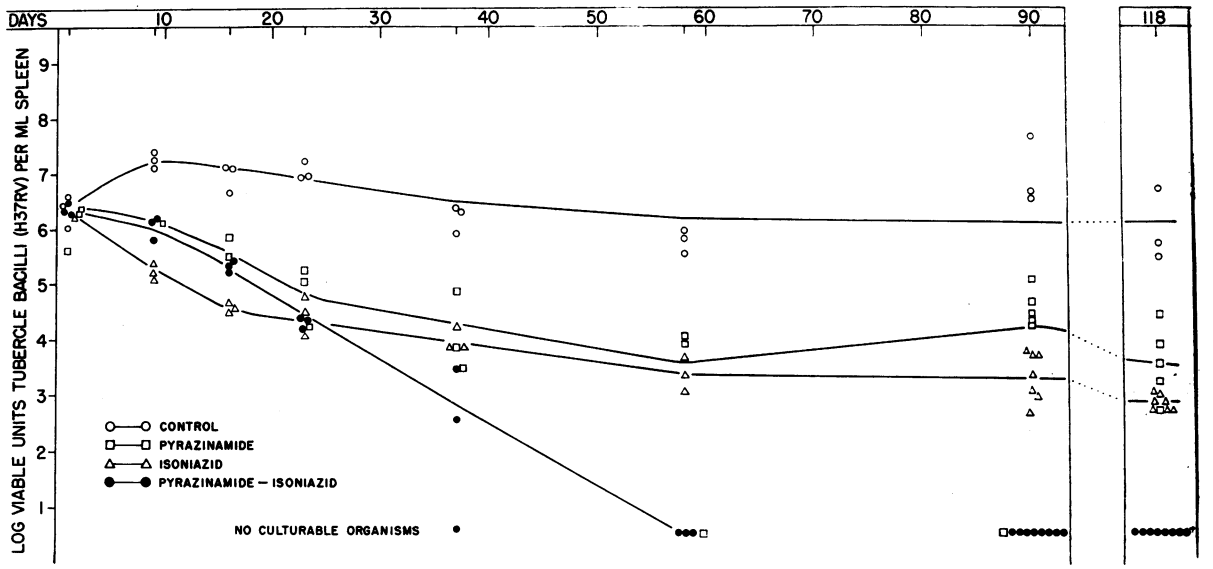
An approach here that immediately comes to mind is to modify the tissue environment by infiltrating it with one or more antimicrobial drugs. For the past 15 years or so our group at Cornell has been attempting to do just this. And, I can say that we have found only one situation in which it has been possible regularly to make an infection vanish from the tissues.

In all other situations with a variety of microbial species and a large number of antimicrobial drugs, the most that it has been possible to do is to keep an infection suppressed at very low but detectable levels in the tissues. The one exception has to do with tubercle bacilli of human origin and a derivative of the nicotinamide series known as pyrazinamide.

When tubercle bacilli subsisting in the tissues of the mouse are simultaneously exposed to pyrazinamide and another antituberculous drug for an appropriate period, all of the tubercle bacilli vanish from the tissues of the animals. By "vanish" is meant that the presence of tubercle bacilli in the animal tissues can no longer be demonstrated by the most elaborate techniques of microscopy, culture, or animal inoculation. The administration of pyrazinamide alone will likewise cause the tubercle bacilli to vanish if they have just previously been exposed to isoniazid for an appropriate period. When the pyrazinamide is administered entirely alone, that is, without either prior or concomitant administration of another drug, the vanishing phenomenon does not occur, and the effects of pyrazinamide seem to be of the same type as those of other antituberculous drugs, notably isoniazid. In the last-named circumstances, the populations of tubercle bacilli in the animal tissues steadily fall during the early weeks of chemotherapy and then stabilize at a low census at which they persist throughout many months of continued chemotherapy. These two phenomena, the vanishing of "drug-influenced" tubercle bacilli when exposed to pyrazinamide and the persistence of tubercle bacilli when exposed to isoniazid or to pyrazinamide alone, may be seen in figure 2, which depicts an experiment that has been repeated many times (*4*).

In actuality, the vanishing of the bacilli does not represent their complete elimination from the tissues of all the animals. When a 90-day period of pyrazinamide-isoniazid administra-

Figure 2. Influence of pyrazinamide and isoniazid used singly and together on populations of tubercle bacilli in mouse spleens during 118 days of therapy.[1]



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[1] Infecting inoculum : 2.0 x 10^6 culturable units of tubercle bacilli.

tion was followed by a 90-day treatment-free interval, the bacilli reappeared in approximately one-third of the animals. In these animals, therefore, a truly latent infection had been induced with spontaneous resurrection after a drug-free interval of 60 to 90 days. Whether the other animals that showed no microbial revival in the 90-day period of observation also had latent infection cannot be stated, but we believe it to be the case.

There are obvious parallels between this artificially induced latent infection with tubercle bacilli and the latent monilial and corynebacterial infections of the man and the rats. In all three cases there was a stage at which there is every reason to believe that the microbes were present in the tissues, yet not the slightest trace of their presence there could be detected. In all three cases the resurrection from the latent state did not occur until the tissue environment, the external environment of the microbe, had undergone some modification. With the corynebacterial infection of the rats, the change in the nature of the environment provided by the tissue represented some consequence of cortisone.

With the fatal monilial infection of the man, the nature of the environmental change in the tissues is not clear, but the various microbial species in natural competition with *Monilia* had been suppressed and cortisone had also been given. With the infection made latent artificially, the usual environment provided for the tubercle bacilli by the tissues of the mouse was obviously perfectly suitable for the full expression of the microbes. Consequently, for microbial resurrection no tissue modification was necessary other than to free the environment of the pyrazinamide.

The two examples of "naturally" occurring inapparent infection that have been chosen were simply those most readily at hand and represent the expression of the phenomenon by a fungus and by a bacterium. A familiar example of inapparent infection with a virus is provided by the commonplace happening of the breaking out of cold sores around the lips caused by resurrection of the virus of herpes simplex. In an infected person, this event will occur predictably when the environment provided for the virus by the tissues has been modified by such factors as fever, excessive sun-

light, digestive upsets, or menstruation. As techniques for tissue culture have been developed it has become clear that the cells employed are not infrequently the site of an inapparent infection. Another case in point is the activation of an inapparent viral infection of bacteria, a bacteriophage, by appropriate change in the environment, with resultant destruction of the bacteria. Thus it is becoming increasingly recognized that inapparent infection is a broad biological phenomenon involving all sorts of microbes and every sort of host including man, lower animals, plants, and even the microbes themselves.

In the present discussion no attempt will be made to cover this field throughout its entire breadth. Dubos has a recent publication on latent infection (*5*), and one of his associates, Dr. Harold Simon, has virtually completed a comprehensive review of the subject which is scheduled to appear as a monograph in the latter half of 1959. Consequently, in the following discussion, attention is limited to the phenomenon of inapparent infection as it applies to bacteria and fungi because here, with antimicrobial drugs, it is possible to manipulate inapparent infections and to draw certain relatively elementary inferences concerning their nature.

The Dormant State

Thus far, the discussion of inapparent infections has centered around latent infections. It seems to me that a latent infection can be regarded as an extreme form of microbial adaptation. For, inapparent infection can exist and eventually give rise to serious disease without the microbes ever having assumed a truly latent form. In a sense, this is merely semantics, but I have found it helpful to follow the practice of subdividing infections into those which are latent and those which are dormant.

The term "latent infection" is reserved for situations in which the presence of the microbes cannot be demonstrated by any method now available and the fact that infection is present can only be demonstrated in retrospect by the emergence of overt disease (usually as a relapse).

A dormant infection is one in which the presence of the microbes can be easily demonstrated but they are not producing disease. In a dormant infection, the micro-organisms may be living openly as the so-called commensals in the respiratory or enteric flora or less obviously in the healed lesions of previous disease as in those of the tuberculin reactor and probably also the typhoid carrier.

As we view things today, the emergence from inapparent infection to openly progressive disease occurs far more frequently with dormant infections than it does with latent infections. This could change, however, and especially what could change are our techniques for detecting the presence of latent infections.

* Seven paragraphs on p. 493-4, should follow here.

The Antimicrobial Drug

In attempting to analyze the phenomenon of microbial persistence it has seemed to me that there are five possibilities that merit consideration:

The first two are the possibilities that drug resistance of the genotypic form or inadequate drug dosage might be responsible. These can be dismissed from further consideration by virtue of the fact that experiments designed to test these possibilities have shown that they do not apply. Likewise a third possibility that microbial persistence is a result of a failure of the delivery of the drug to the parasite because of impenetrable barriers by abscess walls, fibrin membranes, or areas of necrosis can also be dismissed on the basis of appropriate experiments conducted both in our own laboratory and elsewhere.

One aspect of this barrier question does deserve special mention, however, and that is the effectiveness of intracellular residence as a sanctuary from drugs present in the intracellular fluid.

We are accustomed to hear the statement that such and such an antimicrobial drug "does not penetrate the monocyte" or some other type of cell. It is not generally realized that the type of experiment cited is not usually designed to measure whether the drug is or is not transferred across the cell boundary. Instead what is actually shown in most such experiments is that a particular microbial species, when situated within a cell, is less susceptible to a particular drug introduced into the extra-

* See Ed. Correction, V. 74, #6, p. 1142.

cellular environment than is the case when both the microbe and the drug are allowed to come together outside the cell.

In short what one observes is that microbes within cells are less drug susceptible than microbes outside cells; what one *infers* is that the drug was not transferred into the cell.

In reality, in the few cases in which the actual transfer of a drug has been measured, such as in Eagle's careful studies with isotopically labeled penicillin at the National Institutes of Health (*7*), it is found that quite substantial quantities of drug are transferred. Indeed, the quantities of penicillin transferred are more than sufficient to exert full effectiveness, so when the intracellular microbes are not fully susceptible, and sometimes they are not, some other explanation must be found.

From Eagle's studies on penicillin transfer and from some observations on penicillin-staphylococcus-leucocyte systems by Tompsett (*8*), it does not appear that the persistence of a minority of the intracellular staphylococci is due to a failure in drug delivery. Instead, the persistence within the phagocyte seems to be merely a replica in miniature of microbial persistence in the body as a whole.

The fourth possibility is that the environment of the inflammatory lesion exerts an antagonistic influence on the activity of the antimicrobial drug.

Although in the past I have done as much as anyone to promulgate this concept, I no longer believe it will stand up to critical scrutiny (*9*). It is easy to show that environmental changes result in changes in drug effectiveness. This is particularly striking in the fact that the same microbial species situated in the different organs of the same animal show widely different susceptibility to the same drug. For example, tubercle bacilli in the lung of the mouse are far more susceptible to isoniazid than tubercle bacilli in the spleen. Moreover, tubercle bacilli in the spleen are far less susceptible to streptomycin than the staphylococci in the spleen of the same animal species.

When tested in conventional circumstances in vitro, tubercle bacilli are quite unaffected by pyrazinamide. When the environment of the bacilli is altered, however, either by making it more acidic or by situating the bacilli within monocytes, tubercle bacilli of human (but not bovine) origin become highly susceptible to pyrazinamide. A recent report by Williamson (*10*) shows that a change from an anaerobic to an aerobic environment doubles the effectiveness of dihydrostreptomycin on *Escherichia coli* over a wide range of pH. By contrast, with *Aerobacter aerogenes*, the enhanced drug activity in the aerobic environment occurs only at pH 7 or above. Since in both sets of circumstances the drug is the same, the effect of the environmental change on drug influence must be something directly related to the parasite and not a direct environmental antagonism of the drug.

Thus a number of examples exist wherein a change in environment has been accompanied by a change in drug effectiveness. In each case, however, the result could equally well have represented an influence of the environment on the parasite. Some of the examples of environmental influence, moreover, represent influences that could only have been exerted on the parasite. In view of these considerations together with the fact that a large excess of drug should usually be present in the lesion, it appears that environmental antagonisms of drug activity may well occur, but that seldom, if ever, should it attain a magnitude that would provide a satisfactory explanation for the phenomenon of microbial persistence.

The State of Drug Indifference

The fifth possibility is the one that in my opinion fits the evidence. In brief, the concept is that microbial persistence is the result of the ability of microbial populations to assume a state in which they are neither permanently incapacitated by a drug nor do they multiply freely in its presence as do the genetically drug-resistant microbes.

I have designated this state, which can be demonstrated in vitro and in vivo, as "drug indifference."

The concept of drug indifference does not imply marked suppression of all metabolic functions of a microbe but merely those related to a particular drug. The metabolic functions do not necessarily continue throughout the life

of the microbe but might have to do exclusively with activities of its early youth, for example, cell-wall synthesis. In such a case, the newborn microbe might resemble a protoplast with its capacity to carry on metabolic functions including limited cell division but without the capacity to multiply freely (*6*, *22*).

It is believed that the assumption of this state of drug indifference is induced or favored by the influence of the environment on the microbe. Included in these environmental influences are other antimicrobial drugs and intermicrobial relationships as well as the influences of the cellular and humoral defense reactions of the host and the reactions of inflammation. An environmental change of a particular sort may make the same parasite display widely different behavior to different drugs or may make different parasites display less than their maximal susceptibility to the same drug. For these reasons it is believed that it is the adaptive plasticity of the microbe that is the important factor in the influence of environment on drug effectiveness and not a chemical or physical antagonism exerted directly by the environment on the drug.

Expressed differently, pus does not neutralize drug activity, but in adapting to a necrotic environment a microbe may become less susceptible to a drug. Although instances of drug enhancement from environmental adaptation of the microbes do occur, thus far they have not been observed to lead to total eradication of a microbial population. Consequently, the net overall effect of the various environmental influences on drug effectiveness is in the direction of providing situations that favor microbial persistence.

Presumably this state, or these states, of drug indifference closely resemble, perhaps are identical to, the microbial states associated with latent or with dormant infections. Thus in concept, if not in actuality, microbial persistence may be regarded as merely the induction of the latent or the dormant microbial state by drugs.

The Parasite's Morphological Changes

Consideration of the phenomenon of microbes in the latent or in the dormant state naturally gives rise to speculation on the possible nature of such states. This is particularly the case with the latent state because the question immediately arises as to the form that could be assumed by the tubercle bacilli or the corynebacteria so that they might be able to exist in the tissues without our being able to find them.

In the first place, it must be recognized that our methods for microbial detection are so relatively crude that the microbes need not change their form in order to "vanish" completely. All that would be necessary would be for the microbes to lose the ability to grow on our artificial culture media. A population of tubercle bacilli, for example, must be quite large before it is readily detectable by direct microscopy so that if it had lost its ability to adapt to artificial media, it would be undetectable.

In recent years the concept that microbes might possibly exist in more than one form has become respectable, almost fashionable. Moreover, with full realization that our inability to detect microbes during latency may simply reflect the crudity of our diagnostic methods, there remain, nevertheless, certain features of latency that suggest a major change in the form of the microbe.

The principal feature has to do with the time sequences involved in the pyrazinamide-induced latent tuberculous infection. For the uniform induction of latency, a total period of 12 weeks of chemotherapy was required. The pyrazinamide and companion drug could be administered together for the entire 12-week period or 4 weeks of treatment with isoniazid followed by 8 weeks of pyrazinamide could also suffice. But variations of these time-dose relationships, keeping the 12-week period constant, would not suffice. For example, merely reversing the order in which the two drugs were given in the sequential experiments or reducing the initial isoniazid therapy to 2 weeks instead of 4 weeks would result in a failure to induce latency. Obviously, a number of inferences are possible from these observations, but the relative precision of the time-dose requirements suggests the operation of some process that requires considerable time.

The resurrection of the tubercle bacilli from the latent state likewise required a considerable period. It must be remembered moreover, that the tissue environment in which the resurrec-

tion occurs is presumably quite favorable for the proliferation of the tubercle bacilli. Yet in the animals in which the bacilli reappear after a 90-day treatment-free interval, it has not been possible to demonstrate their presence at the 30-day or the 60-day observation point. Even when sufficient cortisone is given to evoke other microbes during the first 30 days after therapy, no resurgence of the tubercle bacilli has been demonstrated.

This appreciable delay in the reappearance of the tubercle bacilli in an apparently favorable environment and our inability as yet to hasten the process by artificial "stresses" suggest that a significant modification of the parasite was necessary before resurrection could occur. In other words, the time relations suggest that the microbial adaptation represented by the latent state of tubercle bacilli is not something that is rapidly responsive to transient fluctuations in the status of the host defenses. By contrast, resurrection of tubercle bacilli from the dormant state can occur with rapidity. The speed with which resurrection from the latent state (as contrasted with awakening from the dormant state) occurs might vary considerably among the microbial species and might likewise depend, to some extent, on the rapidity with which the appropriate environmental alterations could be accomplished.

What could be the nature of a morphological change that might accompany the physiological state of latency? Certain studies of this general subject of the existence of other forms of well-known microbes have employed antimicrobial drugs, notably penicillin, to induce the novel microbial forms. This was the case with the minute colonial forms of staphylococci, the so-called dwarf forms, and more recently with the studies of L-forms and bacterial protoplasts.

When the outer, rigid wall of a bacterial cell is removed under appropriate conditions (or its synthesis prevented), the bacterial cytoplasm and its cytoplastic membrane may continue to exist. This surviving unit is known as the protoplast. The protoplast can carry on many, perhaps all, of the metabolic functions of the original cell except cell-wall synthesis.

It is the process of cell-wall synthesis that appears to represent the site of action of penicillin and the protoplasts of penicillin-susceptible microbes are unaffected by penicillin. The protoplasts also appear to possess the capacity to multiply but to what extent such newborn protoplasts can survive is not yet established. These minute "peeled grapes," so-to-speak, are quite fragile and are especially susceptible to oxygen and to changes in the osmolarity of the environment.

There is some reason to believe that all protoplasts from the same microbial species are not alike. Consequently, Weibull, who is one of the outstanding investigators in this field, believes it is valuable to distinguish between true protoplasts in which no trace of the outer cell wall remains, and "protoplasts" or protoplast-like structures to which some fragment of a cell wall may remain (*11*). Weibull regards true protoplast formation as being something that has been clearly demonstrated for only a few microbial species and the protoplast-like phenomenon as being something more common.

In any case, the literature today contains a number of reports of observed "protoplast" formation among such micro-organisms as staphylococci, tubercle bacilli, enterococci, and many others. Moreover, a number of workers have come to regard the process of protoplast formation as being identical with what happens in the first stage of the formation of the L-form from the normal vegetative form of certain bacteria.

Despite their fragility, protoplasts can survive in vitro with appropriate manipulations of the environment. Moreover, if L-forms are protoplasts or protoplast-like structures, they would represent one form that survives in vitro where indeed the osmotic homeostasis might be expected to be more protective.

Wittler and associates in Washington have shown that the L-form of corynebacteria can be converted to the familiar vegetative form in HeLa cells by appropriate changes in the environment of the host cells (*12*). Wittler has also shown the same phenomenon for *Haemophilus pertussis* in mouse tissues (*13*).

Thus there is accumulating quite a respectable body of evidence to the effect that many microbial species are capable of responding to

environmental change by assuming quite a different form and that this may occur within the animal body. It is of interest that the corynebacteria and mycobacteria involved in the two latent infections I have been using as illustrations are included in the list of organisms that can assume a protoplast-like form. Moreover, it is especially relevant to the present discussion to note that for some microbial species protoplast formation can be regularly induced by appropriate exposure to penicillin. The protoplasts so induced are not destroyed by penicillin, and when the penicillin is removed from the environment the protoplasts revert to the vegetative (and penicillin-susceptible) form of the microbe.

Obviously, it would be intriguing to attempt to study the pyrazinamide-tubercle bacillus relationship from the standpoint of protoplast formation especially in view of the long period necessary for microbial resurrection in the tissues.

As mentioned previously, however, we must keep constantly in mind the fact that our detection techniques for the customary forms of bacteria are so relatively crude that nonculturable but morphologically typical microbes could survive undetected in the tissues.

It might well be questioned whether there is any value in attempting to divide inapparent infections into those which are dormant and those which are latent on so artificial a basis as the ability or inability to detect the infective agent at a particular point in time. As long as this contrivance does not make us prisoners of our thinking, I believe it serves a useful purpose at this time when our knowledge is so elementary. For, the distinction keeps open the prospect that in a latent infection the microbe may be structurally and functionally in a state quite different from the microbe of overt disease or the carrier state. Along with this goes the corollary possibility that in the phenomenon of resurrection from latency to overt disease, the activation of complicated mechanisms within the parasite may be as essential as the appropriate changes in the environment provided by the host. By contrast, with a dormant infection all that may be necessary is a lapse in host defenses for the infection to become disease.

Microbial Persistence

Let us return now to consideration of experimentally induced latent and dormant infections in laboratory animals. As you will recall it was emphasized that the vanishing of the drug-influenced tubercle bacilli when they were subsequently or concurrently exposed to pyrazinamide in the tissues represented the only instance in our experience in which a microbe became truly latent as a consequence of chemotherapy. This experience includes studies with staphylococci, streptococci, *Klebsiella*, and *Brucella* as well as tubercle bacilli, and it includes all of the available antimicrobial drugs. In the case of tubercle bacilli alone, some 13 drugs with demonstrable antituberculous activity have been studied when administered singly and in various multiple drug regimens. With two compounds, a thioamide of nicotinic acid and streptovaricin, results approaching those of the pyrazinamide phenomenon were noted, but true latency could never be produced with uniformity throughout all the animals studied.

Obviously, tubercle bacilli possess the equipment to assume the latent state, but the process apparently has to be invoked in some highly specific way. Some notion of the degree of specificity that must be involved may be seen from the fact that this capacity of human tubercle bacilli to respond to pyrazinamide by assuming latency is not shared by the very closely related tubercle bacilli of bovine origin. At least one other microbe, *Treponema pallidum*, probably possesses the capacity to assume the latent state both in rabbits and man as a result of drug exposure. This point cannot be established with certainty, however, because of our lack of culture techniques whereby the tissues could be subjected to reasonably searching scrutiny to show the nondetectability of the treponemas during a latent state.

In contrast with the rarity of drug-induced latent infections, the production of dormant infections occurs regularly with all microbes studied when exposed in the tissues to an appropriate antimicrobial drug. Indeed in the experiments with latent tuberculous infections, dormant infections were produced in the groups of animals that received isoniazid or pyrazinamide as single drug therapy (fig. 2). In each case, the populations of tubercle bacilli fell

The following seven paragraphs on p. 493-4 should proceed The Antimicrobial Drug, on p. 489.

sharply after the start of drug therapy but then stabilized at a low census at which they remained throughout the many weeks of continued therapy.

As mentioned previously, this survival of the microbes in the tissues at a constant low census despite appropriate drug therapy can be shown for a number of microbial species and all available antimicrobial drugs. Indeed this phenomenon, which I like to designate microbial persistence, represents a biological property of very broad but perhaps not unlimited generality. In the treatment of infections in humans, the existence of the phenomenon of microbial persistence need not necessarily lead to therapeutic failure but most failures that occur stem from it. Moreover, in addition to providing the basis for post-treatment relapse, microbial persistence is obviously responsible for the post-treatment carrier state. In short, it is this phenomenon which is responsible for our inability to eradicate an infection uniformly from a group of patients or from a community by the use of drugs.

Our group at Cornell has been specially preoccupied with this phenomenon of microbial persistence ever since early 1946. An extensive review of these studies has been published recently (*6*). Accordingly, I shall attempt to present the subject only in sufficient outline to provide a proper basis for a subsequent consideration of latent and dormant infections. In so doing it will be necessary to employ bold assertions at certain points without bringing forward the experimental evidence that has seemed to me to be convincing. This evidence is offered in the published presentation.

In this brief consideration of microbial persistence, we start from the demonstration both in patients and in laboratory animals that microbes that are drug susceptible in the orthodox sense are nevertheless able to survive in the tissues despite the prolonged administration of the appropriate antimicrobial therapy. The horizontal trend lines of the census of microbes during therapy seen in figure 2 are merely one graphic representation of this point. Indeed, about the only situations in which antimicrobial therapy can be totally eradicative in humans are with the relatively fragile *Neisseria* and possibly also with dysentery bacilli.

The close resemblance of the microbial states of drug indifference to those of the naturally occurring latent and dormant infections may be seen in the phenomenon of drug-resistant "persisters." It is quite clear that the general phenomenon of microbial persistence is not a result of the presence or emergence of genotypically drug-resistant microbes. Indeed with the experimental model employed in figure 2, when a microbial population is resistant to a particular drug the census in the tissues does not fall on administration of the drug. Moreover, when the population is drug susceptible at the beginning but is transformed in the course of therapy to one that is drug resistant, the drug-induced downward trend of the census is reversed and the microbes proliferate freely in spite of the continued administration of the drug. But sometimes this microbial resurgence does not occur. Instead the population that has been markedly reduced by drug therapy (and is now predominantly drug resistant) simply remains at the low census throughout long periods of continued drug therapy. In short, the drug-resistant microbes behave like drug-susceptible persisters.

Unfortunately, it has not been possible to conduct the obvious experiments on the fate of these drug-resistant persisters in the period after the antimicrobial therapy has been discontinued. It is possible, however, that the situation here may resemble the paradoxical situation discussed below in connection with chemoprophylaxis in which a microbial population that is drug indifferent is nevertheless under the continued influence of the drug. What can be said at this point about the resistant persisters, however, is that microbial populations (including the drug-resistant cells) obviously can become dormant or latent within an animal body by processes not necessarily dependent on antimicrobial therapy.

The possible morphological changes in the microbe to go along with the altered metabolic states have been considered in terms of latent rather than dormant infections. The latent state appears to represent a more extreme form of microbial adaptation than the dormant state, and hence it is to the latent state that attention is understandably directed. Obviously, it cannot be stated at this time whether the mi-

crobes in a dormant infection have the capacity to assume different morphological states. In contrast with a latent infection, however, there is in a dormant infection no delay or difficulty in cultivating the microbes in their orthodox form.

Before leaving this question of possible morphological changes in the parasite in the tissues, I should like to make a brief comment on the matter of the role of the host in maintaining an infection in the dormant or the latent state. Presumably, the host plays an important role here both through the known host mechanisms of defense, by similar mechanisms as yet unrevealed, and by not providing certain types of environment such as those produced in the tissues during starvation or in the cortisone-treated animal. What I would like to point out, however, is that in quite properly focusing our attention on the host in the host-parasite reaction we have tended to regard the parasite as something that is passive and relatively constant in nature whether it is actively producing disease or living quietly in the tissues. In so doing we have tended to regard the difference between the latent and the active stages of an infection as depending almost entirely on the momentary status of the host defenses and have been neglecting the wide range of individual expression possessed by the parasite. It seems quite likely that evocation of an infection from the latent state might require some rather substantial adaptive changes by the parasite and not merely a failure of some defense mechanisms of the host. The difference between a dormant infection such as an arrested tuberculous lesion in the lung and a latent infection such as syphilis might hinge on this very point: that for resurrection to disease, the latter would require changes in the microbe itself.

Point of Drug Susceptibility

While considering the role of the host in maintaining an infection in the dormant or latent state it is also appropriate to consider to what extent antimicrobial therapy can exert an influence in this respect. The question might be phrased by inquiring whether microbes that are not actively producing disease are likely to be drug susceptible. A fair amount of information is available on this point. The information is derived both from laboratory studies and from five clinical situations in which it has been possible to observe the effects of drug therapy administered in the early hours after the moment of infection. In all five clinical examples (syphilis, malaria, scrub typhus, tuberculosis, Q fever), the antimicrobial therapy was not eradicative, but simply held the situation frozen, so-to-speak, for as long as its administration was continued.

The observations on these various diseases suggest that there exists a stage to which the host-parasite reaction must mature before the infection is fully drug susceptible as measured by post-treatment relapse (*9*). In the Tigertt-Benenson studies of Q fever it was shown that this particular stage of maturity is not necessarily so old as the stage of evolution to the full clinical illness (*14*). When the treatment was started before this stage, however, it was clearly ineffective and served only to produce a slight prolongation of the incubation period of the clinical illness.

From these five clinical examples (*6–9*) and especially the two that were conducted experimentally by Smadel and Woodward and their associates (*15*) and by Tigertt and Benenson, it is clear that the phenomenon of drug indifference or microbial persistence can be present from the very beginning of an infection. Even at the earliest stages of an infection when the untreated microbial population is presumably at its lowest census, the introduction of antimicrobial therapy is by no means totally eradicative. In these circumstances, moreover, it is important to note that microbial persistence can occur without evoking the host-immune response characteristic of that particular infection. As a practical matter it makes no real difference whether certain microbes in an infecting population are drug indifferent at the time of implantation or whether the whole process of conversion to drug indifference occurs after infection has been accomplished and drug therapy started. In either case drug indifference can be present in the early moments of infection, and this fact is obviously of crucial importance with respect to chemoprophylaxis.

Many factors are presumably involved in the successful transmission of one or more microbes from one host to another, and the actual physi-

cal transfer of an infecting population to a new host is not necessarily followed by their successful maintenance there. The presence of a prophylactically administered drug in the fluids of the new host might well make the difference between the success or the failure of the implanted microbes to survive. What must be recognized, however, is that even if an antimicrobial drug is present in the tissue fluids at the time of the initial microbial seeding there is no reason to doubt that some of the microbes may survive as persisters. Consequently, the possibility seems highly likely that chemoprophylaxis—to the extent that it is employed to try to prevent actual infection—will only be uniformly successful with the very few highly fragile microbes, such as gonococci, meningococci, or dysentery bacilli, that appear to have little capacity to survive as persisters.

Although the premature drug therapy was ultimately unsuccessful in the five examples cited, it was clear that the infections were suppressed as long as the antimicrobial therapy was continued. This implies that the microbial populations were both drug indifferent and drug influenced at the same time. Presumably, what happens in this apparently paradoxical situation is that a significant proportion of the microbial population is in fact drug indifferent and remains so. But as the tissue environment contains antimicrobial drug, whenever individual microbes revert from drug indifference to a state of drug susceptibility or new cells are born into this state they are promptly inhibited by the drug. As a result the population as a whole is kept constantly suppressed, a condition I like to describe as being in a state of physiological imprisonment.

In the clinical instances cited, the physiologically imprisoned microbes were able to resurge to the point of producing clinical illness once the antimicrobial drug was removed from the tissue environment. It is conceivable that if the physiological imprisonment were maintained for a sufficiently long period, the microbial populations might die off completely in some cases and in others assume a latent or dormant state that would continue even in the absence of drug. Presumably something akin to the last-named possibility occurs in the puzzling case of drug-resistant persisters.

It would hardly be proper, in a discussion of inapparent infection, to fail to note the possibility that latent or dormant infections, either natural or drug associated, are capable of damaging the tissues by their continued presence there. This is the sort of speculative exercise that can rapidly get out of hand. Nevertheless, it seems appropriate to mention a few diseases, associated with microbial infection, in which demonstration of the indicted microbe in the tissues in its recognizable form has either never been accomplished or has been accomplished only rarely. The two principal diseases in this category are syphilis and rheumatic fever. Sarcoidosis might possibly represent a third example, but the indirect association of these lesions with tubercle bacilli is considerably less definite than the indirect association of rheumatic carditis with streptococci or tabes dorsalis with *T. pallidum.*

The fact that tabes dorsalis is caused by *T. pallidum* is generally accepted, yet the presence of the spirochete in the lesions has never been convincingly demonstrated. To some extent this situation is not comparable with that of other infections because no method exists whereby *T. pallidum* can be cultured. Nevertheless, *T. pallidum* can be demonstrated by microscopy of stained tissue in certain other lesions of syphilis, notably parenchymal disease of the brain and in the lungs and liver of syphilitic newborn. In the pre-penicillin era, the lesions of tabes dorsalis were generally considered to represent the sterile end result of a previous involvement with *T. pallidum.* It came as a considerable surprise, therefore, to note clinical improvement in the so-called "lightning pains" of tabes dorsalis when afflicted patients were treated with penicillin. To be sure, evaluation of improvement in "lightning pains" is notoriously difficult, but long-experienced clinicians were convinced that penicillin influenced them. Thus an antimicrobial drug appears to exert its influence in lesions that hitherto were not considered to contain microbes. As noted above, however, the possibility remains that the lesions of tabes dorsalis represent an active syphilitic process, and it is merely our inability to cultivate *T. pallidum* that makes the situation seem at all unusual.

The lesions of rheumatic carditis are not gen-

erally believed to represent a direct tissue reaction to the presence of streptococci but some type of unusual host reaction to previous streptococcal infection elsewhere in the body. Moreover, on microscopic examination of the lesions of an acute rheumatic carditis, streptococci are not to be seen. Nevertheless, reports recur of the successful cultivation of streptococci from the acute lesions of acute rheumatic carditis in patients who have died during the acute stage of the disease (*16–19*). These reports have been made by knowledgeable people who have presented their findings with great diffidence. In today's climate of opinion, however, the general tendency with respect to the observations has simply been to look the other way. In experiments in rabbits, however, Denny and Thomas have shown that group A streptococci could persist in the tissues for many weeks and could then be evoked to the stage of bacteremia by the administration of cortisone (*20*). Whether, prior to evocation, they were dealing with a dormant or truly latent infection cannot be stated because the experiments were not designed for the specific study at this point.

Thus, with all due respect to the many more widespread beliefs concerning the relationships of *T. pallidum* to tabes dorsalis and streptococci to rheumatic fever, the possibility that these microbes in dormant or latent form are actually present in the respective lesions cannot be excluded.

Our Contemporary Challenge

What is the magnitude of the problem we face today with respect to this resurrection of infections from the inapparent state to the state of progressive disease? It is quite clear that the problem is a formidable one and by its very nature is bound to become even more so in the future. Indeed, it is not always appreciated that in the economically developed countries today it is these endogenous infections that constitute virtually the entire load of illness caused by all forms of microbial disease except those caused by viruses. In short, on an individual basis today, but on one that is bound to steadily widen, man is beginning to succumb to his own microbes.

All that is necessary to see this is to enter an adult ward of a general hospital any morning and inquire what problems are present that day with respect to infection. This is an experience that I happen to have every 6 weeks as part of our teaching program, and it is most illuminating. Certain points immediately become evident. First, a surprisingly important portion of the disease load on a general hospital ward on any one day is still caused by microbial infection. Second, no one microbial species or genus is particularly involved but rather the disease load is caused by a relatively wide assortment of microbes. Third, despite the heterogenicity, these disease-producing microbes have one outstanding characteristic in common: they all form what is customarily regarded as the normal microbial flora of man.

Thus the microbes causing the greater portion of our morbidity in hospitalized patients today are the familiar microbial species that through the years we have grown accustomed to regard without fear. On any one day the disease assortment will include infections produced by *E. coli*, *A. aerogenes*, *Proteus*, *Pseudomonas*, nonhemolytic streptococci, and *Monilia*. Two other microbes, staphylococci and tubercle bacilli, likewise deserve inclusion on the ground that although they are capable of producing disease they commonly inhabit our bodies without doing so. Approximately one-half of any group of adults are carriers of potentially pathogenic staphylococci. With tubercle bacilli the situation depends on one's age. It is estimated that 40 percent of the population of the United States over the age of 30 are harboring living tubercle bacilli so that in effect both tubercle bacilli and staphylococci can be considered to be part of man's normal microbial flora.

All of these microbes have the capacity to survive for long periods in man without causing either benefit or harm insofar as can be detected by today's methods. But when man's internal environment is suitably altered, as is so often the case today, all of these microbes have the capacity to arise and produce serious illness.

The ways whereby man's internal environment is subject to alteration today are familiar to us all and represent the unsought portion of the consequences of our great therapeutic

advances. In ever-enlarging measure these advances are making it possible for us to permit the survival of the socially desirable but biologically unfit. This sociobiological force is not limited in its impact to infancy and childhood but is operative to a considerable extent at all ages of life. As a consequence there are many more of us who consider ourselves to be in "good health" but who have some defect with respect to our ability to make our own adaptation to the microbes around us. The defect may be in our structure or in our experience.

Even those of us who were structurally sound to begin with and who have not been overly protected against life's microbial experiences may rapidly lose our veteran status if we have to give up certain of our tissues to the surgeons or of necessity must be saturated with certain hormones. When man's internal environment was modified by politically induced states of extreme deprivation, such as in a Nazi concentration camp, the disease produced was largely microbial and was caused by the more prominent of the microbes that inhabit man (*21*). When crudely comparable states of deprivation are produced today by long-continued corticosteroid administration, the results are the same. And, if we accompany the tissue deprivation by a drug-induced suppression of the more prominent microbes, we get disease by the less prominent microbes such as *Monilia.*

We have been relatively slow in recognizing the importance of this problem of endogenous infection today. One part of it we seem to have almost suddenly discovered, namely, the hospital infections due to staphylococci. But we are slow in recognizing that important as staphylococci are—and I have no desire to minimize their importance—they represent, nevertheless, only a part of what is a considerably larger contemporary problem. Indeed, in terms of serious or fatal microbial disease in our adult hospital services, the intestinal bacilli and the fungi are usually even greater offenders than staphylococci. We are also too prompt to attribute successful microbial survival in this drug-ridden world to the phenomenon of genotypic drug resistance. It is not so much that drug-resistant staphylococci or the drug-resistant members of other microbial genera are not important today as it is that they represent only one of the many ways whereby microbes can successfully adapt to the changes around them. The serious illness and death due to microbial disease today are not chiefly a result of once-susceptible microbes that have now become drug resistant. Instead, to an increasing extent, these diseases are being caused by microbes that never were susceptible to our drugs but hitherto have managed to persist in an inconspicuous fashion in our tissues.

As we have seen, not only does man lack the power to create life but his ability to destroy it, at least at the microbial level, is sharply limited. To the extent that this means that microbial adaptability will continue to blunt our attempts at therapy and prophylaxis, the situation might be regarded as being somewhat gloomy. But to the extent that what holds for the microbes holds for us as well, any restriction on man's ability to exterminate life has its good side too.

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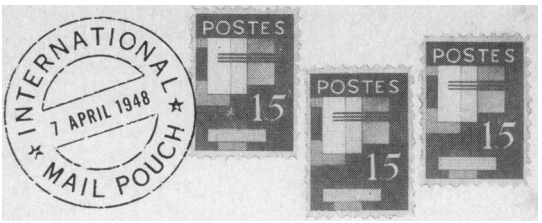
Antibiotic Use for Preserving Fish

The use of an antibiotic to aid in keeping fresh-caught fish in sound condition was authorized for the first time by the Food and Drug Administration in an order, effective April 21, 1959, which sets a safe limit on the amount that may remain on such food without harm to the consumer.

The order allows commercial fishermen to use the antibiotic, chlortetracycline, on fresh-caught whole, headed, and gutted fish, shucked scallops, and unpeeled shrimp. Its use is not permitted on processed seafood products, including fish cuts, steaks, and fillets, peeled shrimp, and shucked oysters. Chlortetracycline has been used on poultry since 1955 and extensive data on its safety are available.

The maximum amount that may remain on the seafood has been fixed at 5 parts per million. This quantity will not always be removed in cooking, but the agency has determined that it may be consumed without harm even by persons sensitive to antibiotics. Treated seafood products must be labeled to show that they contain the antibiotic and that it has a preservative effect.



Computers and Bicycles

Malaria surveillance field teams in Thailand expect to protect 5 million people each month in house-to-house visits. For this full-scale program, a report form for rapid analysis by electronic computers has been developed.

Village surveillance records are summarized for each canton, an administrative grouping of about 10 villages. The canton surveillance summary reports, about 1,000 a month, require analysis for location, population, control operations, workload, fever cases, treatment, and malaria by age groups.

A report from the northern region explained that the first cycle of the program will probably take 4 months to complete, because time is needed to improve the procedure and many employees are new to the work. Also the shortage of bicycles has meant that men must travel on foot over trails between scattered villages that cannot be reached by jeep. However, Thai officers and men treat the program as a personal challenge and are working enthusiastically to see it through.

—J. MILES BUTLER, Ph.D., *malaria eradication adviser, Chiengmai, U.S. Operations Mission, Thailand.*

The Pitha Turned Red

By the time we reached Tarabou, a village near Dacca, East Pakistan, to investigate a report of sudden fatal illnesses, 6 children had died and 8 other people had been ill. A 20-year-old woman had the same symptoms as the younger victims, cyanosis, restlessness, abdominal pain, and signs of circulatory collapse. We learned that the children, whose ages ranged from 1 to 6 years, had died within half an hour after they became ill. The others had recovered in 3 to 5 hours and had no apparent residual effects. A chemical poison seemed to be the most likely cause of the illnesses.

The detailed food histories showed that rice and water were the only items consumed by all the people who were affected. The rice had been boiled and nothing had been added to it. We suspected the water which came from the river, and checked the alum used to clarify it. Then we discovered that the last family we interviewed drew water from a nearby pond and did not use alum.

Meanwhile a sanitarian, questioning the families independently, found that some of those who became ill had eaten a rice cake called pitha. We questioned all the families again; everyone who became ill had eaten pitha.

Analysis of the rice cake showed the salt ingredient contained sodium nitrite. We learned later that the woman who made the cake had asked a bus driver to get salt for her. He had a package of salt in the bus and handed it to her. She remembered, as she prepared pitha that day, that the rice cake turned red.

—MOHAMMED FAHIMUDDIN, M.D., *director of public health, East Pakistan, and* GLENN S. USHER, M.D., *special assistant for medical activities, Communicable Disease Center, Public Health Service, and co-leader, epidemic aid team to East Pakistan, May–July 1958.*

Rural Occupational Medicine

Occupational medicine and rural hygiene are combined in the research work carried on by the State Institute of Rural Occupational Medicine and Rural Hygiene in Lublin, Poland. Prof. Dr. Józef Parnas is director of the institute and Prof. Dr. F. Wysocka is its scientific secretary.

The institute's origins are connected to Prof. Dr. Witold Chodźko, Poland's first minister of health and a member of the sanitary organizations of the League of Nations, who helped to set up the nation's first health stations in 1905.

Since the institute was established in 1951, it has conducted laboratory and field research on the effects of chemicals on agricultural workers, the working conditions of drivers of farm machinery, rural housing, and the personal hygiene and prevalence of parasites in the rural population. It has dealt with outbreaks of tularemia, Q fever, leptospirosis, toxoplasmosis, and brucellosis; and has trained rural surgeons and workers for sanitary and epidemiological stations.