# Microbial Persistence and Latent Infection

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Chemotherapeutic agents used in the treatment of bacterial diseases may induce the development of drug resistant microorganisms which tend to persist in the tissues and to become refractory to any further chemotherapeutic treatment. The result of these processes is a condition referred to as a "latent infection". This type of infection although thought to be prevalent does not produce a fatal disease and could be regarded as an adaptation between parasite and host whereby both live in a state of symbiosis. "Latent infections" which, it should be mentioned, may occur naturally as well as in response to the stimulus of drug therapy have not been sufficiently stressed in the past and our purpose is to discuss certain aspects of this problem.

Recent practice has been to designate as "persisters" those bacteria, viruses, etc., which become capable of survival in the tissues either naturally or as a result of prolonged chemotherapy. Terms such as "carrier state", "chronic stage", "latency", and "masked" have been used to describe the pathological state of a disease and do not necessarily imply knowledge to any degree of the underlying and fundamental processes involved. The persistence of a particular pathogen in tissues without manifestations of disease could pose a real problem in its eradication and control. Normally in diseases of bacterial and viral origin the presence of the causative agent in tissues can be demonstrated by standard technical procedures. In the "latent" type of infection however, it appears that our current isolation techniques are not sensitive enough to detect the small numbers of infective particles that are present. Despite these technical difficulties it is hoped that this paper may in same way draw attention to the phenomenon of *microbial persistence* insofar as it applies to those diseases which are prevalent in the domestic animal population and consequently are of importance to veterinarians. For a more informative discussion of the subject reference should be made to the review of McDermott (1) and the publication by Dubos (2).

Microbial persistence is described by McDermott (1) as the capacity of drug-susceptible microorganisms to survive drug attack when subsisting in an animal body. When a group of animals with a drug-susceptible infection is treated with the appropriate antimicrobial drug, there is a rapid disappearance of all symptoms of disease and the animals are apparently well. In the majority of cases symptoms never develop, while in others the illness reappears after a treatment-free interval. The microorganisms recovered from these latter animals are not drug-resistant in the usual sense and retreatment is commonly effective. Microbial persistence may be observed in another situation in which microorganisms which are drug-susceptible when tested "in vitro" are capable of surviving "in vivo" despite intensive therapy with the appropriate drug. In clinical practice this phenomenon obviously has to do with the post-treatment "carrier state" and with post-treatment relapse. McDermott (1) attributes it to the plasticity of microbes

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adapted to their environmental influences and to their ability to "play dead", in which state they are invulnerable to drugs.

Many explanations have been advanced to show why some microbes are able to persist in tissue despite exposure to an antimicrobial drug. The possibility that drug-produced resistant mutants are responsible in all instances is untenable because when their immediate descendants are tested they are found to be sensitive to the drug. Drug dosage has been suggested as another possible factor. However it has been shown by McCune *et al* (3) that in some cases a five-fold increase in the recommended dosage had no effect on these "persisters". A third frequently stated explanation that has had wide acceptance, is that the drug is confronted by "barriers" in the tissue in the form of "fibrotic-walls". The work of Werner *et al* (4) and Tompsett (5) seemingly refutes this theory. When they implanted agar discs in the peritoneal cavity of rabbits and cats it stimulated the formation of a fibrotic wall. Parenterally administered penicillin and streptomycin were found to be present in significant levels within the agar discs. This suggested that there was diffusion of the two antibiotics across such types of "barriers" and that the persistence of microbes behind "fibrotic walls" was not due to a failure of the antibiotics to reach them. In this regard it should be kept in mind that different drugs have different diffusion gradients through fibrotic walls and necrotic tissues. The theory that products of inflammation exert an antagonistic effect on drugs is one which McDermott (1) suggests will no longer stand up to critical scrutiny. The degree to which "organ difference" may influence the development of persisters is also of some importance. For example, it was demonstrated by McCune *et al.* (3) that a microorganism present in the lung of an animal may be more susceptible to a certain drug than it is when it is located in the spleen of the same host. Tubercle bacilli located in the lung tissue of a mouse are generally quite sensitive to streptomycin whereas those present in the spleen are relatively resistant. On the other hand, staphylococci in the spleen tissue of the same animal may show no resistance to streptomycin. Observations such as these indicate that two microorganisms when present in the spleen react differently to streptomycin and infer that there may be a basic difference in their nature. Again it is known that pyrazinamide is inactive on the human tubercle bacillus in the pH range of 7.0 but is highly active if the environment is made acid. The bovine tubercle bacillus remains resistant to the drug regardless of the pH of the environment. In this instance the acid environment renders one organism susceptible to a drug and has no effect whatsoever on the susceptibility of a closely related species.

The form these microbial persisters adopt during their periods of hibernation merits some discussion. Until quite recently there was no convincing demonstration of a correlation between a particular microbial morphology and a physiological state of drug-indifference. The process of bacterial sporulation and the existence of spores in tissues has been known for many years, but it has now been recognized that nonvisible, drug-indifferent forms of bacteria which were not previously known to have complex life cycles exist in tissues. The numerous studies dealing with the ability of penicillin to produce L-forms or protoplasts of certain microbes have suggested this possibility. When the cell wall of a bacterium is lysed under appropriate conditions, the bacterial cytoplasm and its limiting membrane may continue to exist as the protoplast or spheroplast, Weibull (6), McQuillen (7), and Martin (8). This metamorphosis is regarded by several groups of investigators, Lederberg (9), Hahn and Ciak (10), Park and Strominger (11), Pease (12), McKay and Truscott (13), and McKay and Carter (14), as being identical with what happens when certain types of bacteria change from the normal vegetative form to the L-form[1]. Indeed it is now apparent that many more types of bacteria are induced to produce protoplasts or spheroplasts by the action of penicillin, lysozyme and ethylenediaminetetra-acetic acid than had hitherto been recognized. For example Thacore *et al.* (15) have shown that the mycobacteria can be influenced *in vitro* by chemical means to produce these plastic and persistent L-forms. Their presence in tissues and body fluids is constantly attracting

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[1]**L-FORMS usually appear as regular spherical bodies and/or hardly visible granules.**

**VEGETATIVE FORM refers to the classical morphology of a bacterium such as rod, bacillus, cocco-bacillus or coccus.**

more attention and reports have appeared referring to the intracellular L-forms of *Brucella abortus* (16, 17) *Streptococcus* (18) and *Haemophilus* (19). The L-forms of both tubercle bacilli and staphylococci apparently do not increase in number but merely persist and resist all forms of drug therapy. This is to say that the bacterium becomes capable of assuming a static state within the environment of the host where it neither multiplies nor is destroyed and where it can persist for long periods and can cause bacterial infections which become dormant or truly latent.

McDermott (1) feels that perhaps too much emphasis has been placed on the known and unknown reactions of the host as possible mechanisms in maintaining an infection in the dormant or latent state. He defines "dormant infections" as being these which persist at a low but detectable level, while the term "latent" is reserved for those cases which can only be detected in retrospect by the appearance of a relapse. He stresses that more thought should be directed towards the adaptive plasticity of the bacterium in the dormant or latent state of an infection and that the bacterium present therein may not be in the same morphological form and physiological state that it was when it initially produced the disease. The use of cortisone has been shown by Le Maistre *et al.* (20) to evoke pseudo-tuberculosis in rats. This is an example of the conversion of a latent infection to an acute stage of the disease and is an illustration of adaptive changes in the host, at least insomuch as it is understood. The report of Guze and Kalmanson (18) of studies wherein they were able to isolate the protoplast form to *Streptococcus faecalis* from the kidneys of mice which had undergone prolonged antibiotic therapy for pyelonephritis caused by this microorganism presents an example of adaptive changes on the part of the bacterium itself rather than in the cells of the host. Observations by Wittler *et al* (21) on the reversion of a pleuro-pneumonia-like organism to a Corynebacterium in mammalian tissue is another example of such a change. Hatten and Sulkin (16, 17) studied the induction and survival of L-forms of *Brucella abortus* in tissue culture. L-forms were recovered for several days after the elimination of the vegetative form of these bacteria from cultures containing the antibiotics penicillin, streptomycin and tetracyclines. By direct fluorescent antibody staining of infected cells, they were able to establish that the intracellular L-forms were actually related antigenically to the infecting strain of the parent vegetative bacterium. Nelson and Pickett (22) recovered L-forms of *Brucella abortus* from the blood of humans who were suffering from brucellosis and who had been treated with antibiotics. *Brucella melitensis* L-forms were also recovered by Carrère and Roux (23) from sheep exposed to drug therapy. Presumably what was happening was that these infections were initiated by microorganisms that already were "drug-indifferent" and the drugs, while suppressive, did not completely eliminate the infection. When McCune *et al* (3) studied the effect of the antituberculous drug pyrazinamide on the fate of the tubercle bacilli, it was found that injection of the drug apparently resulted in the complete elimination of the bacilli from the mouse tissues because mycobacterium could not be demonstrated therein by microscopy, by culture, and by inoculation of guinea pigs. The drug, in this instance, seemed to be eradicative. However, after a 90 day treatment-free interval, when the mice were examined bacteriologically 40 per cent were found to harbor tubercle bacilli which were susceptible to the drug. Obviously these organisms had survived at a level too low to detect and the infection was latent. In tuberculosis, despite treatment with all the available antitubercular drugs used to date the problem of microbial persistence is of vital concern.

McDermott (1) states that microbial persistence tends to occur more frequently when infections are treated immediately following exposure; that is to say, in the early stages of a disease. Antimicrobial therapy apparently exerts its maximal influence only after the infection has progressed to a certain point. This may be correlated with the time that is required for certain of the hosts defense mechanisms, e.g. an antigenic stimulus, to be mobilized and become fully operative. If such is the case, it is perhaps fortunate that in human and veterinary medicine drug treatment is not usually initiated in the early stages of infection due to the lack of those clinical signs which are indicators of illness and which may only develop in the acute stages of the disease. Consequently the time lag of from 12 to

48 hours which precedes initial therapy most often enhances the action of the drug. This time interval, "the incubation period", naturally depends upon the capacity of a pathogenic organism not only to survive in the tissue of a host but also to establish an infection which is recognizable by clinical signs. The behaviour of these two competitive biological systems (bacterial cells versus tissue cells of the host) is aptly described by Dubos as the "period of scientific darkness".

As was mentioned at the outset, the foregoing discussion has been an attempt on the part of the authors to bring to the attention of the veterinary profession the importance of microbial persistence, the fundamental processes of which are little understood. This phenomenon is widespread and occurs in the presence or absence of drug therapy. Dubos (2) suggests that because the dynamic relationship between the host and the parasite is the outcome of many different factors, it is conceivable that the methods of sanitation and vaccination which so effectively controlled epidemics in the past may not be applicable to these microbial agents which are ubiquitous and which tend to produce dormant infections.

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## Nutritional Hepatic Necrosis in Beef Cattle, "Sawdust Liver"

Results of this study at Cornell University show that sawdust liver or focal hepatic necrosis can be caused by a Vitamin E, selenium deficiency.

Focal hepatic necrosis was produced in Hereford steers by feeding a diet high in polyunsaturated fatty acids and low in protein, Vitamin E and selenium. In addition to typical liver changes, lesions were also produced in the kidney, heart, skeletal muscles and pylorus. The use of additional protein in the diet, or the injection of selenium, partially prevented the condition.

The cause of the focal hepatic necrosis is attributed to the formation of cytoplasmic hyaline which in turn is composed of clumps of mitochondria. The apparent role of the Vitamin E-selenium complex is the maintenance of mitochondrial integrity as these are most sensitive to anoxia.

*Todd, G. C. and Krook, L. Pathologia Veterinaria 3: 379, 1966.*