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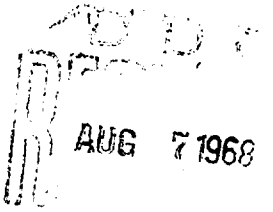

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

EXPERIMENTAL CONSERVATION OF PLAGUE IN THE SOIL

A report by H. H. Mollaret, of the Institut Pasteur, Paris, published in the French-language bi-monthly Bulletin de la Societe de Pathologie exotique, vol. 56, no 6, for November-December 1963 - pp 1168-1182.

The study of the epidemiology of infections by the Malassez-Vignal bacillus, in which we had been engaged since 1957, provided our first suspicion of the soil as the reservoir for this germ. This was our working hypothesis, a logical outgrowth of certain observations we had made in nature, specifically and particularly the fact that here we had a disease involving primarily the digestive tract, both because of the usual manner of contamination, and of the crucial role played by internal lesions and the fecal elimination of the germs. Of course, we were well aware of the primary importance of latent infection and animal carriers in the spread of the disease. We had also checked the findings of Lorey (1911), Dessy (1925), Flamm and Kovac (1958) on the importance of urinary elimination of the bacillus by infected animals who had contracted the disease by mouth. This explained many of the outbreaks in herds and zoos, on the basis of contamination of forage supplies by infected rodent urine. However, even taken all together, these observations served only to explain certain isolated cases, and failed to resolve the fundamental problem of the way the germ survives in nature.

The persistence of endemic plague in certain places demanded a more satisfying explanation than that of constant reinfection from animal to animal, or that of intermittent imports of infected animals. Myxomatosis, which almost completely wiped out the rabbit population (the primary animal host of the Malassez-Vignal bacillus) in many areas, did not thereby banish the bacillus, which still attacked some completely new rabbit populations, from the very moment of their constitution. Therefore, we reasoned, there must be some way, in addition to the occasional, intermittant transmission of the germ by direct animal-to-animal contact, some mechanism for survival in nature. It must be adequate to assure a degree of continuity of infection, quite independent of the hazards of animal contacts. The steady return to the soil of the Malassez-Vignal bacillus in the feces and urine of infected individuals raised the question: what becomes of these bacteria? Can it be that they are held dormant in the soil?

In order to check the survival potential of the M-V bacillus in the earth, we proceded as follows: our initial experiment called for pre-sterilized earth. This was a natural short-cut to assure that the control media would not be invaded by saprophyte germs. Two glass pots, 20cm in diameter, were filled with 4 liters of garden soil, which we sterilized in several small batches. We then watered them with distilled water. On the 3rd of March, 1960, we sprinkled the surfaces with a suspension of washed microbial bodies (strain 5-I) taken from gel cultures in Roux containers - one container per pot.

The pots were then covered with a sheet of glass to delay drying, and one of them was kept at room temperature, the other out-of-doors, exposed to all the variations of temperature and weather, with the exception of direct sunlight. We watered the earth from time to time, whenever it looked too dry.

We checked for bacteria every month, first with gel cultures, then, when moulds appeared on the surface of the soil, with guinea pigs. We gave them a few millilitres of soil in a physiological suspension in water, by mouth. We had long been accustomed to this technique in our search for the bacillus in feces.

In October 1960, the eighth month of our experiment, we were certain that the bacillus was still alive. The technique we used to ascertain this also provided ample evidence that it had lost none of its virulence.

We performed the experiment a second time, with two identical glass pots, one kept indoors, the other out-of-doors, except that this time we used ordinary garden soil, unsterilized. We performed the same monthly check for bacteria. Both experiments were stopped in September of 1961, when the pots of earth had become very dry. But they had proved that the M-V bacillus can survive for 18 months in sterilized earth, and for 11 months in unsterilized earth.

The inter-epizootic survival of plague in resistant foci posed a problem similar in many respects to that we confronted with the Malassez-Vignal bacillus: How to approach the year-to-year persistence of the virus in nature? This was the way our teacher, G. Girard (21) wrote of it in 1957. Even when we shall have managed to clear up the problem of the nature of this natural reservoir: even when we have singled out the etiological agent, as this or that species of rodent or insect in the plague foci that undergo this pendulum-swing from quiescence to activity, we shall still be in the dark as to the factors which actually govern epidemic cycles. Until now, we have been chiefly concerned with the hiding-places the virus may find in animals, but not at all with the virus itself. And that may well be where the great mystery lodges, after all.

Another of our teachers, Baltazard, ran up against this same mystery in Teheran. He was kind enough to keep us informed of the progress

of his inquiries. This potential for survival of the plague bacillus in nature, during the hiatus between animal epidemics, fitted right in with our earlier interest in the survival patterns of the Malassez-Vignal bacillus. This curious analogy, coupled with the close relationships of the two diseases, encouraged us to take the positive results we were already getting from our experiments with the Malassez-Vignal bacillus, and apply them to a new series of identical experiments with the plague bacillus. This was when we suggested to Dr. Baltazard our hypothesis of soil-dormant survival of plague.

This hypothesis was by no means new. By the time we began our experiments (as recounted below), the "telluric plague" theory had already collected quite a history.

In 1894 YERSIN (75) published his report on Bubonic Plague in Hong-Kong, announcing his discovery of the causative agent of the malady in the pus extracted from buboes. But he went on: "I succeeded in isolating the plague bacillus from earth collected as much as 4 or 5 centimeters below the ground surface in an infected house where attempts at disinfection had already been made. It was similar in every respect to the bacillus isolated from buboes, except that it was not virulent." What bacillus was this, really, so "similar in every respect" to plague, but not virulent? (Three years later, Yersin clarified the point a bit, when he referred to it as "less virulent," rather than "non-virulent.") Was it really plague bacillus?

According to a report by LOWSON (1896) (42) delivered to the Epidemiological Society, KITASATO and TAKAKI flatly refused to admit any such possibility, on the grounds that their own efforts to find plague bacillus in the soil had failed. You can find an echo of this noisy battle in the British Medical Journal (1896-1897), as LOWSON (42, 43), CANTLIE (8), A. RUTTER (63) and others had their way. HANKIN (1897) (28) and the Indian Plague Commission also helped cast doubt on the nature of the germ. HAFFKINE (1898) (26) went so far as to question whether Yersin himself was certain about it:

"When Dr. Yersin, who described also plague microbes found in soil and floors, was in Bombay, I particularly enquired in what way he diagnosed those microbes; and I am not certain whether he was absolutely sure to have dealt with fully authenticated plague microbes." Nobody but BATZANOFF (6) in 1899 would admit that this was "probably" an attenuated form of the plague bacillus.

And yet, in the same year with Yersin, KITASATO (35) (36) announced in turn that he had isolated plague bacillus from dust-inoculated guinea-pigs. He writes: "With the dust of dwellinghouses from which the plague-stricken had been removed I made several experiments upon animals. Some of the animals died from tetanus. In one case only a guinea pig died with plague symptoms, and in this animal the same bacilli were found in the internal organs as in those of plague patients who had succumbed."

We don't know whether Yersin made any more experiments in isolating the bacillus in the soil. His 1894 publication makes mention of only one, and yet LOWSON (42) and HAFFKINE (26) were to refer subsequently not to one, but to several plague strains Yersin extracted from the soil.

/NOTE: There is the same vagueness concerning the quantity of bacteria found in the soil. Only PROUST (1897) wrote: "Yersin found it (the plague bacillus) in abundance in the soil."/

Whatever the case may be, YERSIN (77) applied his findings to a generalization three years later when he wrote:

"During plague epidemics, and even after the sickness has disappeared, it is possible to find in the soil of an infected area a microbe similar in every respect to that of the plague, but less virulent than the germ taken from buboes. This microbe can survive in the earth, and it is conceivable that rats might contract infection from it if circumstances are favorable. This is the way epidemics are started."

Yersin went on to quote PASTEUR, CHAMBERLAND, and ROUX (52):

"With remarkable prescience, Mr. Pasteur, in his celebrated Memorandum on the attenuation of the virus and its return to virulence, wrote of the spontaneous outbreak of plague in Benzhazi in 1856 and 1858: 'Let us assume, being guided by the new knowledge that is ours today, that the plague, a virulent malady peculiar to certain countries, has long-lived germs. In all those countries, its attenuated virus must exist, only awaiting the cay when it can take on its active form again, the day when the ideal conditions of climate, of famine, and of poverty crop up again." Quotations like this have their effect. These produced several converts to the new hypothesis: LANDOUZY, 1897 (38), E. ROUX, 1897 (62), SALIMBENI, 1900 (64), and more recently, our own teacher, G. GIRARD (21).

Actually, even before the plague bacillus was discovered, some authors still enamored of the PETTENKOFER Bodentheorie (soil theory) were tempted to apply it to plague. Among them was R. ARNAUD, 1876 (4), who wrote: "I should not be at all surprised if, in following up the theory suggested by Pettenkofer in relation to cholera, we were to find similar relationships between plague and the soils upon which it appears and develops." Others, meanwhile like ROCHER, 1878 (59), will be quoted by SIMOND (1911) (69), and later by DOPTER and de LAVERGNE (1927) (15). All of them were to be struck by the unvarying pattern of rat epizootics immediately preceding human epidemics, and to point to the soil as the reason why small animals living underground were attacked first.

This belief in the telluric origin of plague was given particular impetus by CREIGHTON (12) in his History of Epidemics in Britain (1891). While one can find fault with him for ignoring even the second edition (1905) (13) of Simond's work, he still deserves full credit for being the first to attempt to establish an epidemiological causal relationship

between the soil and man, with his hypothesis of respiratory contamination, long before the idea of inoculation by cutaneous abrasion was aired.

Yersin's theory had considerable effect. In their reports to the Academie de Medecine, (1897), both E. Roux (62) and Proust (55) drew on it for prophylactic admonitions: "Particular attention must be paid to the soil, which seems to be the principal reservoir for the pathogenic agent." BROUARDEL (7) says this in his report on the conclusions reached by the International Conference of Venice in 1897:

"It has been established, through research by Kitasato, Yersin, and others, that the plague microbe can be found as far as 4 to 5 centimeters below the surface of the earth in plague-infested dwellings. There is no evidence that these microbes multiply outside these houses, at least at any considerable distance. In the soil of these dwellings, however, their virulence seems to diminish fairly fast. Dessiccation seems to have much the same effect. As to the length and degree of dessiccation necessary to remove all virulence, however, as well as on the possibility of reanimation of the virus after this process, we have still no reliable data."

METCHNIKOFF (46), at the Congress of Moscow in 1897, had a few more reservations:

"The natural history of the plague bacillus, despite a body of valuable and well-proven facts concerning it, is still far from complete. Specifically, we have no idea of the manner in which the plague bacillus manages to survive in nature over long periods. Ever since Kitasato published his work on the great sensitivity of the plague bacillus to dessiccation, direct sunlight, and antiseptics, it has been generally assumed that this microbe cannot survive outside a living organism for more than a very short time, relatively speaking. Even in so doing, it loses most of its virulence. These facts, of themselves, are not adequate to explain certain epidemiological observations which show that plague is communicated by some agent that has survived in a dry state over long periods of time... On the basis of these data, we must assume the existence of some survival form, some highly resistant form of the plague bacillus which we have hitherto not encountered."

According to the Plague Commission of Alexandria (1899) (11), "The chain of infection is unbroken. It is simply that we cannot see some of the links...For days, even weeks at a time, the disease appears, superficially, to be stamped out. Disinfection has destroyed all the germs. The victims have been placed in isolation. If there are any microbes hiding in a corner somewhere, they'll die soon enough, we are told, of dessiccation... And yet, history has shown us that Yersin's bacillus is considerably tougher than that... From time to time, out of the subsoil, plague germs climb back to the surface in the body of a sick animal. It crawls away to die in some hidden corner of a house, and there it is: people

stricken with plague again. It's like the earthworms, in PASTEUR's cursed fields, who slowly and patiently bring up to the surface, long months and years afterwards, the spores that lodged in the tissues of cattle who had died of anthrax."

According to Salimbeni (1900) (64): "The bacillus is attenuated in the soil, to the point where it is probably incapable of causing sickness in a human being. But at a given moment, influenced by a variety of factors, the microbe can become pathogenic for animals as sensitive as mice and rats. One rat falls ill, and, through the pattern we are familiar with, spreads his disease to others. In the passage from rat to rat, the germ grows more active with each transfer. Therefore it is the soil that keeps the microbe alive, the rats who give it the virulence it needs to infect men, the virulence it had lost during its dark sojourn in the soil."

Some authors find that the most interesting point in Yersin's hypothesis is his explanation of the persistence of certain age-old plague foci. Netter is one such (1900) (49):

"It may be that, under particular conditions, the plague bacillus lodged in the soil can maintain its vitality infinitely longer, in some sort of involuted form or state that is quite resistant to regeneration. The persistence of plague in endemic foci cannot be explained on any other basis."

PELLISSIER (1902) (53) feels the same way:

"Could we not assume that the bacillus can survive for a very long time in the earth, without any patnogenic activity, and then, under the influence of specific conditions, regain its virulence, and spark an epidemic that had been extinguished years ago? And mightn't this be the secret of these endemic foci we have all reported?"

Now listen again to J. PENNA (1902) (54):

"The plague germ also survives and reproduces in the soil, and its telluric origin was long known to the natives of Yunam, Gurhwall, and Kymaon..."

Here is ABBATUCCI (1911) (2):

"I can think of no better simile for the bacillus than that of the seed, which, according to the soil in which it is sown and the conditions of the climate, will germinate at a given time... The comparison is further justified by the fact that the bacillus lives in the soil. The safest assumption is that the bacteriologists have not yet managed to find its spore form, which can survive for long months in a dormant state, to be reactivated when the seasonal conditions necessary to its development appear."

To conclude, we quote BASTHARD-BOGAIN (1911) (5):

"Although it is common knowledge now that the plague bacillus dies swiftly once it is expelled from the host organism, ... GAXIA and GOZIO were led to assume the existence of a still-unknown survival form. Whatever it is, it is tough enough to keep the germ alive for months, even for years. Therefore it can be assumed that the plague bacillus never actually leaves a country, once it has settled there. It merely metamorphoses into its long-survival form, to wait until the immunity acquired from the first epidemic has thinned out, and its protective force has been weakened to the point where another epidemic can spring up."

While the hypotheses were piling one on top of the other, two main branches of research emerged. One was on the trail of the natural conditions that would bring out the bacillus in the soil, and the other aimed at laboratory tests in vitro of the bug's survival potential.

In Hong-Kong, TAKAKI (70) and LOWSON (42) failed, as we said earlier, to find the germ either in culture or by inoculation of rats and rabbits. OGATA (50), during the Formosa epidemic, carried on his unsuccessful attempts to produce plague in guinea-pigs by inoculation with sweepings from plague-infested dwellings. In Bombay, HANKIN (1897) (28) decided it was impossible to isolate the germ by direct culture of the soil. On CALMETTE's advice, he tried sub-cutaneous inoculation of rats and mice, following this up by scattering their minced cadavers about. His very meticulous research proved fruitless, he reported, except for one probable exception. This did not prevent him from writing, a year later, in the Annales de l'Institut Pasteur (1898) (29):

"Rats can spread the infection from one neighborhood to another, but it does not appear that they, alone, can keep the microbe strain virulent. There must be some other propagation agent in the chain. It may be that, in order to stay virulent, the microbe must leave the rat for another host or environment: earth, stagnant water, or perhaps the body of an insect... Somehow or another, the microbe leaves the rat and makes itself a "nest" in the new neighborhood. We don't know how this happens. In the laboratory, the plague bacillus looks and acts like a very fragile germ indeed. But in its natural state, it would seem to be extraordinarily tough."

HAFFKINE (1898) (26), Ch. CAYLEY (1898) (9), GIBSON (1898) (20) and MILNE (1898-1899) (47) also failed in their efforts to find the plague bacillus in the soil. Only one of the research men on the Indian Plague Commission, RANSOME (57) got a plague culture from a sample of dust sweepings. According to HAFFKINE (1903) (27), even this result might have been attributed to accidental contamination.

LEUMANN (1898) (40) managed to get three cultures from 25 dust samples gathered from sweepings in plague-infested dwellings. In similar

houses and in the Bombay hospital, VALASSOPOULO (73) reports that LAWRIC achieved the same results with inoculation of rats. SCHOTTELIUS is reported to have isolated the bacillus in earth at 20 centimeters distance from the unburied cadavers of rats. (We were unable to find this text of Schottelius, which is quoted by both NETTER (1900) (49) and ABBAS KHAN (1908) (1).) TOYAMA (72), also found the bacillus in the soil during the Tokyo epidemic of 1900. (We were unable to consult Toyama's Japanese text. It is reviewed in the Centralblatt fur Bakteriologie, 1910, 47, pp 266-268. Toyama's success at isolating the bacillus is also referred to by DIEUDONNE and OTTO (14).

And still, most research ran into a blank wall: the few positive findings, simply because they had not been made during the inter-epidemic lull, but rather in the full rage of epidemic, and almost always in hospital wards or in plague-stricken homes where the bodies of the dead had only recently been removed, were thus robbed of any statistical significance.

The next question was: would laboratory experiment at culture-growing prove any more rewarding? A lot of men made experiments along these lines, beginning in 1897 and running clear to 1912: ABEL (1897) (3); de GIAXA and GOSIO, 1897 (19); the German Commission, 1899 (10); GERMANO (1897) (18); NEISSER (1898) (48); GLADIN (1898) (22); BATZAROFF, 1899 (6); E. LEVY, 1899 (41); GOSS, 1905 (24); GOTTSCHLICK, 1912 (25), and many others. All of them were trying to prove one thing: that it is possible for the plague bacillus to survive in dust. All agree that the survival period is very short, although GIAXA and GOSIO found 30-day survival periods, and GLADIN reports one strain that lasted 67 days.

Still others tried to approximate natural conditions by burying the cadavers of animals inoculated with plague. There is no need here to report on the numerous inquiries conducted on the cadavers themselves. Ever since KLEIN, in 1899 (37), SATA, in 1901 (65) and ZLATOGOROFF in 1904 (79), we are all familiar with the rapid disappearance of the bacillus, once the host animal dies. However, only the inquiries whose authors had the idea of looking outside the cadavers themselves, in the surrounding earth, such as YOKOTE (1898-78), whose work with the earth surrounding plague-infested mice buried in little wooden coffins, proved fruitless. The same applies to SCHOTTELIUS (1901 - 66) and SATA, who sought the plague bacillus both in the buried animal cadavers and in the surrounding earth. Comparing results obtained with sterilized and unsterilized earth, SCHOTTELIUS concludes:

"While, in the struggle against soil bacteria, most of the plague bacilli die, all those remaining become far more infectious."

Other experimenters followed the same procedure we adopted, adding microbe cultures or killed bacteria to sterilized and unsterilized earth samples, subsequently conducting regular checks on the survival rate. GABRITCHEWSKY (1897 - 17), working with sterilized earth, found a survival

In Madagascar, ROBIC (1942 - 58) buried linen contaminated with the sputum of pulmonary plague victims in laterite soil at temperatures of 16°-18°C, and found no living bacteria at the end of four weeks. However, control samples of the same linen kept under refrigeration were still virulent after six months. Yersin's theory was consigned to the limbo of other hobgoblins of "telluric miasma," to be cited only in cursory footnotes to exhaustive treatises.

And yet, here we are back at this same theory ourselves. We believe it is the only possible explanation for the problem of virus survival in nature. Our revered teacher, G. GIRARD, used to tell us that he considered it the one true and still unsolved problem connected with plague.

We began checking on May 2, 1960. Except for contamination with a plague strain, we produced the same conditions we had set up for our experiments with the bacillus of Malassez and Vignal. We used two jars of earth, of which only one had been sterilized. Both were covered with a glass plate and left on a laboratory table.

Beginning with non-sterilized earth, we looked for the germ each month by means of subcutaneous inoculation of guinea pigs with suspensions of about 1 ml of earth. The guinea pigs died of plague showing the usual symptoms. (Of course, the same strain of plague was recovered in the organs and carefully checked.) We continued these experiments until the month of December, at which point we could no longer find the bacillus. Hence, the soil survival period we found was seven months. Several guinea pigs died of gas gangrene in less than 48 hours, before the plague symptoms had had time to become evident. These were a foretaste of the troubles our friend, Y, KARIMI (34) was to run into three years later, when we went to help him with the same technique in his quest for the germ in the soil of Kurdistan.

Starting with the jar of earth, which had previously been sterilized, we looked for the bacillus both by inoculation and by scatter-culture on gelatin, using a hose which we had inserted into the earth to a depth of several centimeters. Some of the tubes remained sterile, but as we varied and increased the number of our sampling points, finding the bacillus became a matter of routine for a period of more than 16 months, until September, 1961 at which time the earth had dried out to such a degree that we stopped our experiment.

And there was our proof: Yersin's bacillus stayed alive and virulent in the earth, and, for the first time, its survival span had a precise significance.

Naturally, this first rudimentary experiment required further orderly repetition on a larger scale, with attention given the role of the several factors: The kind of soil, the organic matter content, sterilization, pH, humidity, temperature, normal soil flora, etc.

Obviously, it was still necessary, once we had provided experimental proof of the survival potential of plague in the soil, to single out the channels that would bring it out, and finally, going back to the state of nature, to isolate the bacillus from the untreated denizens of the soil. But experimental proof of the possibility of plague survival in the soil was accomplished. That was the essential prelude.

SUMMARY

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