Experimental Plague in Guinea Pigs Inoculated with *Pasteurella Pestis* of Ecuadorian Origin[1]

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IN ORDER to determine the possible differences existing between the plague bacilli of Ecuador and of other countries, the authors undertook a careful study of guinea pigs experimentally infected with *Pasteurella pestis* of Ecuadorian origin. The clinical course of human plague in the Inter-Andean region of Ecuador and the frequent occurrence of serious outbreaks of pneumonic plague,[2] as contrasted with the relative mildness of bubonic infections, the bacteriological characteristics of the local isolates, already described by the authors,[3] together with other aspects of the disease, offered a rich field for study.

MATERIAL AND METHODS OF STUDY

All strains of *P. pestis* here studied were isolated in the rural districts of the Andean province of Chimborazo, Ecuador, where the disease is now prevalent. The sources of the isolates are given in Table 1.

Experiments were conducted with guinea pigs. A complete autopsy was performed on all of them immediately after death and observations on the type and intensity of the plague lesions in each organ, as well as other pathologic changes of a different nature encountered, were recorded. Both macro- and microscopic postmortem pathology was compared with that given in classical descriptions of the subject.

Smears from the lesions were stained by Gram's method and with Giemsa, when necessary, in order to observe the morphology of *P. pestis* and the relative frequency of its occurrence. Cultures were

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1. Received for publication June 23, 1943. These studies were carried out at the request of the Director of the Pan American Sanitary Bureau, at Washington, D. C., where one of the authors holds the post of epidemiologist, and with the coöperation of the health authorities of Ecuador. The Ecuadorian Government contributed the sum of $10,000 with which it was possible to install a Plague Laboratory in the city of Ambato, where the junior author was assistant.

2. J. R. Murdock, "Pneumonic Plague in Ecuador During 1939," *Pub.Health Rep.*, 55:2172–2178, 1940.

3. A. Macchiavello and D. Urigüen, "Bacteriología de las *Pasteurellas pestis* aisladas en el Ecuador interandino." (In press.)

made routinely from the heart, blood, spleen, liver, lymphatic glands, bone-marrow, lungs, exudates, and so forth. The techniques followed for inoculations, autopsies, and cultures were the same as those employed by one of the authors in a previous study conducted in Brazil.[4] These techniques corresponded essentially to those recommended by many authors abroad.[5]

EXPERIMENTAL STUDIES

P. pestis strains were passed successively through different series of guinea pigs. Table 2 presents a summary of the pathologic characteristics of the infection in each series of animals. In this table are also recorded the span of life of the animals after inoculation, the presence, absence, and intensity of the macroscopic lesions found at necropsy, the presence and the relative number of bacilli found in smears from the viscera, exudates, and so forth, and the relative abundance of colonies formed on culture media inoculated with the same material—especially on triptose agar or nutrient agar with 0.25 of one per thousand of sodium sulphite.

An analysis of Table 2 immediately reveals that, in addition to the usual lesions of plague described for guinea pigs in the literature, there are two other types that occurred with unusual frequency in our experimental animals, though not exclusively produced by the Ecuadorian strains, namely, (1) the intensive and almost constant infection of the pelvic lymphatic gland near the site of subcutaneous inoculation at the posterior extremity, and (2) the extraordinary frequency of pulmonary lesions of various types.

The lesions noted on the pelvic gland above mentioned were often as intense, or more intense, than those of the inguinal glands. As a rule, they revealed a greater number of microorganisms; frequently, this was the first gland of the sacro-lumbar-dorso-aortic chain infected, which might be wholly infected though not to the same degree as the pelvic gland itself. The infection of the inguinal gland and of the sacro-lumbar-dorso-aortic chain represented the primary points of attack of the infecting bacillus, the different glands being infected in succession through the lymphatic channels. The pathologic character of the infection in these glands is in contrast to that of other lymphatic glands—such as the mesenteric—which are infected through the blood stream.

4. A. Macchiavello, *Contribuciones al estudio de la peste bubónica en el nordeste del Brazil* (Washington, D. C.: Pan American Sanitary Bureau, Pub. 165, 1941).

5. C. L. Williams, "Diagnosis and Detection of Rodent Plague," *Am.J.Pub.Health*, 10:851–864, 1920.
Lien-Teh Wu, J. W. H. Chun, R. Pollitzer and C. Y. Wu, *Plague: A Manual for Medical and Public Workers* (Shanghai: Weishengshu National Quarantine Service, 1936).

As far as the pulmonary lesions are concerned, they varied from a simple embolism to an embolism followed by necrosis with or without congestion, or consisted of a lobular or lobar pneumonia.

Due to their low toxicity and high invasive power, *P. pestis* strains of the Andean area produced a general postmortem picture in which the necrotic lesions were predominant and congestive lesions of the viscera very seldom observed. This postmortem picture might have been the result of the low endotoxic power of the bacillus, which permitted a slow necrotic reaction *in situ* but which had practically no effect on tissues at a distance from the infective focus. Even in septicemic infections, the toxic action on the vascular system was practically negligible. The congestion of the blood vessels, extravasation of blood, and actual hemorrhages were not frequent and were never marked or extensive but, when they did occur, they were found almost exclusively in the inguinal glands and with even less frequency in the pelvic glands. This characteristic, however, does not belong exclusively to the Andean strains, foreign isolates of low virulence having produced a similar reaction.

DESCRIPTIONS OF THE LESIONS IN THE LUNGS AND PELVIC AORTIC GLANDS

Pulmonary pathology may be induced in some guinea pigs inoculated with foreign strains of *P. pestis*, but the lesions produced will be discrete and not very conspicuous. In some instances these lesions consist of isolated necrotic points; in others they are necrotic or caseous nodules; in still others the reaction is congestive and limited to a few small areas or to a lobule. Rarely is lobar hepatization observed.

Pulmonary lesions were not common among more than one thousand guinea pigs inoculated in Brazil with eighty different strains of *P. pestis*. Not more than ten of these strains—some of them atypical or attenuated—produced pulmonary lesions of necrotic character, but this happened in only a few of the animals of the large series inoculated. Congestive lesions were also rare, being generally discrete even when not specific. There was only one strain that would systematically produce necrotic pulmonary lesions worthy of consideration.

On the other hand, the Ecuadorian strains isolated in the Andean zone produced in every instance well defined, typical pulmonary lesions that might be classified as follows:

1. *Congestive lesions*

a) Hepatization, lobar or lobular pneumonia with all the characteristics of plague pneumonia. This was an infrequent reaction, easily distinguished from similar processes of different etiology by the enormous number of *P. pestis* present in the lesions, and by its histopathology.

b) Congestion and lobular hepatization. When this type of reaction was located deep in the parenchyma, the foci were spherical; when superficial, they were conical with the base towards the pleural membrane. In the latter instance the lesions became evident superficially as circular congestive zones, varying in diameter from 2 mm. to 1 cm., or more.

c) Cortical lesions, resembling superficially the lesions just described but differing from them by the occasional development of a central, dark hemorrhagic point and, more frequently, by the presence of small necrotic zones measuring from 1 to 2 mm. in diameter. The grayish yellow color of these necrotic areas were in striking contrast with the intense red congestion of the rest of the lesion.

2. *Simple necrotic lesions*

These lesions were generally pinpoint in size, varying in color from yellow to gray. The necrotic points were seldom irregular and occasionally formed extensive patches measuring as much as one square centimeter.

3. *Proliferative lesions*

When the guinea pigs were inoculated with fresh strains of plague possessing a certain degree of virulence, proliferative lesions were not frequently produced. However, when the animals were inoculated with strains kept in the icebox for one year, they showed a large number of nodular pulmonary lesions. These lesions were spherical or polyhedral; they measured up to 3 or 4 mm. in diameter; they were hard, produced bulging of the pleura, and gave the organ a characteristic appearance. Necrotic and caseous changes were often found in the center of these lesions.

The various lesions described may be grouped into two general pathologic processes. One of them was fundamental and consisted of emboli produced by small masses of *P. pestis* in the capillaries. If the bacterial strain possessed a certain degree of virulence, necrosis resulted. If the virulence was low, a nodule was formed. In the center of this nodule a slow but progressive necrosis took place, surrounded by an increasing proliferation of cells among which polymorphonuclears were sometimes seen. The bacillus found in the nodules was often encapsulated. The neutrophylic polymorphonuclear leukocytes showed phagocytic activity for the specific bacillus, a feature that was never observed in the more virulent strains of plague.

The second pathologic process was a congestion that might surround the necrotic area or might occur as purely congestive foci without other apparent changes. This congestive reaction generally revealed the presence of a nonspecific superimposed infection, most frequently of pneumococcic origin. Lobar pneumonia due to plague in the guinea pig was rarely observed.

With respect to the lesions of the pelvic glands, or rather, the lumbar or lumbo-aortic glands, it should be emphasized that we are not referring to the local inconspicuous glandular reaction that follows the inoculation of guinea pigs, in any part of the body, with plague. When the Brazilian strains were used as the inoculum, the infection of the pelvic gland draining the point of inoculation was seldom noticed. It is true that this gland would sometimes present a slight hypertrophy, but its consistency would be soft and the bacilli were rarely abundant. Barely 2 percent of more than one thousand guinea pigs inoculated with Brazilian strains showed a marked infection of the pelvic-lumbar glands; when this happened, the strains were of low virulence. Perhaps not more than three guinea pigs showed total infection of the lumbo-dorso-aortic chain of glands.

On the other hand, guinea pigs subcutaneously inoculated in one of the posterior extremities with Ecuadorian strains of plague showed an infection of the pelvic glands, especially those draining the point of inoculation, which was almost constant and easily demonstrable. This infection formed primary buboes of the second order which were often larger than the inguinal glands and reached at least one centimeter in diameter. These buboes often showed hemorrhage or caseation and were surrounded by an area of hyperemia, also hemorrhagic at times. The hyperthrophy of the pelvic glands, draining the point of inoculation, was accompanied by a less

pronounced infection of the lumbo-aortic glands, which would reach the size of green peas, and by an even smaller reaction of the other paravertebral glands (the dorso-aortic, for example) which showed only a slight, or no, infection at all.

As a rule, these glands underwent caseous changes; congestive lesions were less frequent. Generally, the chain of glands from the pelvis to the diaphragm was all infected and showed enormous numbers of *P. pestis*.

The two pathogenic characteristics described for the Ecuadorian strains of *P. pestis* were not influenced by any constitutional factor in the guinea pigs used in the experiment, since the inoculation of other guinea pigs with foreign strains of the bacillus did not induce the same type of lesions. As will be seen elsewhere, the pathologic picture described was dependent on the intrinsic characters of the particular strains of bacillus found in the Inter-Andean region. Furthermore, it might be stated that the characteristic infection of the deep glands and of the lungs took place through one and the same physiopathologic mechanism.[6]

BEHAVIOUR OF THE ANDEAN STRAINS ACCORDING TO THEIR ORIGIN

The data in Table 2 reveal that the strains of *P. pestis* behave in accordance with the source of isolation.

1. *P. pestis of rat origin.* The four strains obtained from rats revealed a low initial virulence. Strain R-3 increased its virulence from the seventh to the sixteenth, and last, passage. Strain R-1 also showed signs of increased pathogenicity in the last two passages.

While rat strains retained their low virulence, the lesions produced in the experimental animals were indistinguishable from those produced by strains from other sources. However, as soon as the virulence of the former increased, it was noted that, in addition to the usual lesions of plague, the infection of the deep glands and of the lungs became apparent.

2. *P. pestis of flea origin.* The two strains obtained from fleas revealed a low degree of virulence that was further reduced after a series of passages through guinea pigs. The possible explanation for this phenomenon has been given elsewhere.[7] The infection of the pelvic glands and lungs with flea strains was extremely slight yet,

6. A. Macchiavello and D. Urigüen, "Peste pneumónica experimental." (In preparation.)
7. A. Macchiavello, *Comportamiento experimental de cepas de peste aisladas en el nordeste brasileño* (Washington, D. C.: Pan American Sanitary Bureau, Pub. 165, 1941).

in the third and fourth passages of the strains obtained from *X. cheopis*, both types of lesions were observed.

3. *Human strains.* Eighty-five passages were made through 90 guinea pigs with thirteen human strains; of these, 8 were virulent (H-15 to H-19, H-25, H-35, H-39), while 4 (H-6, H-10, H-14, H-34) did not reach the degree of virulence common to ordinary strains until a series of passages had been effected. Strain H-11 retained its low virulence throughout the period of the experiment. An interesting observation was the fact that at the time of isolation Andean human strains in Ecuador rarely possessed the high degree of virulence noticed in strains considered virulent in India, for example.

Table 3 presents the total number of guinea pigs that were inoculated in series with *P. pestis*. These animals were separated into two groups: (1) those in which postmortem findings revealed an attenuated infection, and (2) those showing the usual signs of virulent plague. The table illustrates the frequency with which plague lesions of the pelvic glands and lungs occurred in both groups.

The findings in this experiment showed (1) that immediately after isolation the Andean strains of Ecuador possessed a low degree of virulence, this being particularly true of rat and flea strains; (2) serial inoculation generally produced an increase in virulence that never reached the degree considered normal in foreign strains; (3) the strains that were virulent on isolation, as well as those in which virulence was artificially increased, were capable of producing lesions of the pelvic glands and of the lungs with unusual frequency when compared with previous observations registered in the literature.

For the purpose of the present study, it should be stated that a strain of *P. pestis* was considered avirulent when it failed to produce lesions in guinea pigs; slightly virulent, when the animals survived over six days and the postmortem findings did not reveal the classical lesions of plague; virulent, when the animals revealed the typical postmortem picture of plague, and hypervirulent, when death occurred from hyperacute plague infection. Although this gross classification is considered satisfactory for our purposes here, it must be admitted that the present conception of virulence with respect to *P. pestis* needs revision. This point will be taken up at greater length in another paper.[8]

8. A. Macchiavello and D. Urigüen, *op. cit.* (6).

STABILITY OF THE PATHOLOGIC CHARACTERISTICS INDUCED BY *P. Pestis* OF ECUADOR

During this study the authors thought it worthwhile to determine whether the pathogenic characters of the Andean strains of *P. pestis* were related to accidental conditions or depended on the intrinsic constitution and biology of the bacterium. The following observations would seem to answer this question.

The original cultures—those obtained directly from the glands of plague patients, from the viscera of the first guinea pig inoculated with this material, or from the viscera of plague rats—were carefully sealed and kept in the icebox at a temperature ranging from 0° to 5° C. for the period indicated in Table 4. This table contains additional information on the culture medium employed and the type of lesions induced in the guinea pigs, especially of the pelvic glands and of the lungs.

Summing up these data, it will be seen that ten of the original cultures kept in the icebox for approximately one year lost their virulence, although it was easily recuperated in subcultures (strains H-14, H-15, H-18, H-19, H-34, H-35, H-40, H-41, H-44). One strain (H-43), which retained its virulence, did not produce lesions of the pelvic glands nor of the lungs; two strains (H-16, H-25) produced lesions of both the glands and the lungs. In other words, irrespective of their virulence on isolation, many cultures—generally the more pathogenic—lost their pathogenicity. Other cultures retained their virulence in a reduced degree, but the lesions which they produced on the lungs and pelvic glands were of the same type already described and as frequent. Indeed, 7 of 8 strains (87.5 percent) produced infection of the glands, while 5 of 8 strains (62.5 percent) produced lesions of the lungs. The pulmonary lesions were much more pronounced than those produced with the original strains and were nodular in type, a feature which we considered an indication of a reduction in virulence. The formation of the nodule would be a defensive process of the tissues around the necrotic focus, which was the fundamental lesion produced by *P. pestis* in this organ.

At the end of approximately two years, another group of the original cultures of the same strains, kept in the icebox under the same conditions, was inoculated to another lot of guinea pigs with the results registered in Table 5. The data contained in this table show that the virulence of the bacteria had been further reduced when compared with the results of the previous experiment. This

reduction in virulence was accompanied by a reduction in the frequency and intensity of the pulmonary lesions, although the pelvic glands still showed well defined lesions. The reduction in virulence could be noted also in the general appearance of the postmortem picture. The spleen, for example, did not show the characteristic pinpoint necrotic lesions. The lesions found in this organ were often nodular; in most instances, there were no typical plague lesions whatsoever, although the bacillus was still abundant in smears from the tissue. An additional sign of the attenuation of the bacillus was shown by the great varietyof involution forms noted.

In the next experiment, cultures from the organs of the last lot of guinea pigs were taken and inoculated into other guinea pigs in series, in the hope that the bacteria would regain their pathogenic habits by artificially increasing their virulence. The results of this experiment are tabulated in Table 6 and disclosed the following facts:

1. In the first series of guinea pigs, inoculated directly with the cultures, only three of the ten bacterial strains used produced lesions of the pelvic glands; none of them produced pulmonary lesions.

2. The invasive power of the bacteria was increased with variable rapidity as they were passed through the animals. The increasing invasive power was first noted in the pelvic glands, while pulmonary lesions did not begin to appear until the third or fourth passages.

3. A proportion of 28 percent of the guinea pigs inoculated in this experiment was negative both for glandular and pulmonary lesions. Most of the animals included in this percentage belonged to the first passage of the series.

4. Another 28 percent of the animals showed infection of the pelvic glands only and 8 percent pulmonary lesions. Of the four animales included in the last percentage, the infection (plague, pneumonia, and embolism) could have been effected through the blood stream and not through the lymphatics. In 36 percent of the animals the pelvic lesions were accompanied by pulmonary infections, the latter being very pronounced in more than half of the cases.

5. Lesions of the glands and lungs were more frequently observed in the last passage.

6. The general findings may be summarized as follows:

	Total	*Percentage Inoculated*	*Percentage with Lesions*
a. Guinea pigs inoculated	50		
Guinea pigs without lesions of glands or lungs	14	28	
Guinea pigs with lesions of pelvic glands or of lungs, or both	36	72	
b. Guinea pigs with lesions of the pelvic glands	32	64	88.9
Guinea pigs with pulmonary lesions	22	44	61.1
c. Guinea pigs with lesions of the pelvic glands only	14	28	38.9
Guinea pigs with pulmonary lesions only	4	8	11.1
Guinea pigs with both types of lesions	18	36	50.0

From the results of this experiment we may conclude:

1. That the majority of the strains, which had lost their virulence, regained it almost completely by serial passages through guinea pigs.

2. That the bacteria not only diminished their initial low toxicity with the loss of virulence but also their invasive power, as evidenced by a reduction of the lesions of the pelvic glands and lungs.

3. That the ability to produce such lesions was regained with the recuperation of virulence.

4. That, with serial passages through guinea pigs, the virulence of the bacteria could not be raised to any degree higher than that originally possessed.

Since it was impossible to increase the virulence of the strains to a point where they would kill the guinea pigs before lesions of the lungs had time to develop, it was impossible to prove the theory that these lesions developed when the virulence of the infecting bacillus ranged within certain limits—not sufficiently low to eliminate the invasive power nor too high to produce an early death of the animal.

Previous observations had led us to confirm the experience of other investigators[9] who found that serial passages through susceptible animals would not increase the virulence of certain plague

9. A. Yersin, A. Calmette, and Borrel, "La peste bubonique," *Ann.Inst.Pasteur*, 9:589–592, 1895.
W. Kolle and E. Martini, "Ueber Pest," *Deutsche med. Wchnschr.*, 28:1, 1902.

bacilli above certain limits. We believe that an increase in virulence beyond the degree noted at the time of isolation would mean that the strain in question had already undergone a spontaneous attenuation in its host, a phenomenon which is, of course, reversible.

If the observations above mentioned are true, it would follow that the antigenic constitution of the Andean plague is not compatible with a degree of virulence higher than that found by us. In other words, the intrinsic nature of the bacillus would permit a certain maximum degree of invasive power but never a high degree of toxicity. This is in line with the epidemiological facts, with the pathologic habits of the bacillus, and with its persistent pneumotropism.

It is evident that *P. pestis* of Andean origin cannot increase its invasive power beyond a certain established limit; that it cannot function as a highly virulent organism due to its low toxicity which gives to the pathologic processes, excited by it, a characteristic mildness.

It is evident that the toxic power of the Andean strains of plague varies within very narrow limits, while the invasive power has a much wider range of variation. A high invasive power and a low toxicity have apparently come to be permanent qualities of *P. pestis* of Andean origin, these qualities being responsible for the special type of lesions found in our experiments.

Therefore, no matter how many times the strains are passed through susceptible animals—rat, guinea pig, or man—the Andean plague always shows a characteristic tendency to attack the lungs. This tendency would have probably disappeared if the bacillus had been able to increase spontaneously and simultaneously the two qualities of its virulence already discussed. It may thus be concluded that high invasive power and low toxicity are stable characters of the Andean strains of *P. pestis*. This may be seen in Table 7 in which the lesions of the pelvic glands and of the lungs are compared in the three series of guinea pigs studied herein.

Series 1 and 3 are similar in the percentage of lesions produced. Series 2 reveals lower percentages, but this series is not comparable with the others because (1) they barely represent the first passage of the cultures which were kept in the icebox for one year and (2), they include nine strains that were found to be nonpathogenic for guinea pigs.

If the strains of this second series had been subjected to animal inoculations in series, it is possible that the percentage of lesions

produced would have been higher. This may be assumed from the high frequency with which characteristic lesions appeared in the guinea pigs of this group that showed infection (Table 7: the numbers in parenthesis correspond to the second series).

It may be finally concluded, therefore, that the production of the two types of lesions described constitutes a permanent, effective, and intrinsic quality of the strains of Andean plague.

DISCUSSION

According to the experiments here presented, it is evident that the Andean strains of *P. pestis* from Ecuador, when inoculated into guinea pigs, show a peculiar tendency to produce pulmonary and pelvic-gland lesions that are characteristically more pronounced than when similar infections are produced with foreign strains. This tendency of the Ecuadorian strains appears to be a quality inherent to their virulence. However, the virulence of the organisms must remain within certain limits in order that they may produce the characteristic pathologic changes described.

The differences in pathogenic behaviour between our local strains and those of foreign origin cannot be explained on the basis of differences in the degree of virulence only. We believe that the differential characters of the Andean strains lie chiefly in their antigenic constitution on which the biology and properties of the microorganisms depend. Differences in the antigenic qualities of our local strains would answer for the differences in the type of reaction induced by them.

Let us consider now our belief that the Andean strains of plague are not as virulent as foreign strains. Epidemiologic observations have revealed this to be true. In the mountainous area of Ecuador human plague is milder, and plague patients frequently present a localized infection of the cervical glands that we believe is a sign of low virulence,[10] these glands showing minimal congestive and inflammatory signs. Notwithstanding the great increase in size of the glandular structures, the tendency to suppuration is not constant, and the pus, when present, chiefly reveals neutrophilic polynuclears wherein large number of phagocytized bacilli are found. It has been observed that *P. pestis*, isolated from these glands, shows a low virulence for guinea pigs, a fact that is also true for the strains isolated from infected rodents.

10. A. Macchiavello, *La ingua de frío, o fiebre de carozo, es una forma de peste ambulatoria* (Washington, D. C.: Pan American Sanitary Bureau, Pub. 165, 1941).

We have stated elsewhere that in the rural areas of the Andean region the Indians raise colonies of domestic guinea pigs as a source of food. It has been often noted that the first sign of plague in that region is the unexplained mortality of these guinea pigs for several weeks preceding the human outbreak. When an epidemic of plague is preceded by a guinea pig epizootic, the disease is usually much more serious and lethal than when such epizootics do not occur.[11]

A possible explanation for these phenomena is given below:

1. The natural reservoirs of rural plague are the fleas in which the plague bacillus undergoes a progressive attenuation.[12]

2. At the beginning of the rainy season, wild rats invade the human dwellings of these rural areas. Upon coming in contact with infected fleas, they contract plague and give rise to small domestic epizootics that are most frequently underground and unnoticeable to man.[13]

3. Due to the small number of rats, the epizootic dies out before the plague virus has had a chance to increase its virulence.

4. Due to climatologic conditions and to confinement of the fleas to their nests,[14] the cases of human plague occur as mere accidents and are generally isolated. The mildness of these cases is evidence of the low virulence of the virus.

When an outbreak of plague, which has already exterminated the rat population, has a chance to continue its propagation among guinea pigs, which are so highly susceptible, the possibility for an increase in virulence is greatly enhanced. When this possibility is effected, the subsequent human infection is more serious and the mortality higher.

As noted by Saenz Vera,[15] experience has shown that the comparatively mild plague of the Andean regions leads more frequently to outbreaks of pneumonic plague. This is particularly evident when the low incidence of pneumonic plague in the coast is compared with that of the mountainous area. In Guayaquil, for example, pneu-

11. A. Macchiavello, "Epidemiología de la peste en el Ecuador con especial referencia a la zona interandina," *Bol.Soc.Méd.Quir.del Guayas.* (In press.)

12. P. V. George and W. J. Webster, "Plague Inquiry in Cumbum Valley, South India," *Indian J. M. Research*, 22:77–104, 1934.
A. Macchiavello, H. Paracampos and C. Arcoverde, *La pulga, reservorio rural de la peste del N.E.del Brasil* (Washington, D. C.: Pan American Sanitary Bureau, Pub. 165, 1941).

13. A. Macchiavello, "Peste en Ecuador," *Am.J.Pub.Health.* (In press.)

14. A. Macchiavello, *op. cit.* (11).

15. C. Saenz Vera, "Breves observaciones sobre neumonía pestosa en el Ecuador," *Bol. ofic.san.panam.*, 20:11–17, 1941.

monic plague is only rarely observed. In this city the rat population is plentiful and widely distributed and the transmission of the infection from rat to rat may last for considerable periods of time, thus leading to an increase in the virulence of the infecting bacillus.

Although the low rat population would explain the mildness of the human infection in the mountainous areas, it cannot explain the high incidence of pneumonic plague in this same region. That the great frequency of pneumonic plague is not merely the result of attenuation of the infecting bacillus becomes evident from the fact that in other countries like Brazil, where rural plague is frequently of a mild type, secondary pneumonic complications do not appear.

A careful study of the morphology of the colonies of *P. pestis*, isolated in the Andean region, has revealed that almost without exception they belong to the "S" type, notwithstanding the scant reports about this form of the bacillus published in the literature and the general belief that *P. pestis* is only known in its "R" form.[16]

Our observations, which will be published elsewhere,[17] have established that the "S" forms found by us are related to the antigenic structure of the Andean strains of plague. We believe that the pathologic characteristics of the experimental infection in guinea pigs, as here described, depend on this particular antigenic structure. For a better understanding of our theory, it might be convenient to discuss briefly the present conception of virulence and the interpretation of our experimental infections in the light of that conception.

Many authorities define virulence as the capacity of an organism to invade the tissues of a given host. Topley and Wilson[18] believe that this invasive power alone does not constitute virulence. It must be borne in mind that certain microorganisms that are not essentially invasive—the diphtheria bacillus, for example—may still be extremely harmful through their toxins. Virulence should be defined as the quality that renders a microbe pathogenic for a given host, irrespective of the mechanism through which it acts. In some microbes the invasive power is predominant, while in others the toxic power is more pronounced.

The invasive power of bacteria may depend on mechanical conditions rather than on any active property of the microbe. In plague, for example, the virus may be transported passively through the lymphatic circulation, possibly as any other inert particle, and it may invade the general circulation through the permeability of the vessel walls and the high pressure of the intraglandular fluid.

16. H. Schütze, "Studies on *P. pestis* antigens as prophylactic agents," *Brit.J.Exper.Path.*, 20:235–244, 1939.

17. A. Macchiavello, "Constitución antigénica de la *P. pestis*." (In press.)

18. W. W. C. Topley and G. S. Wilson, *The Principles of Bacteriology and Immunity, 2d ed.*, (Baltimore: Williams and Wilkins, 1937).

The vascular lesions that disturb permeability are of toxic nature. Since *P. pestis* does not possess an exotoxin, any local action produced by the bacillus requires its presence and disintegration *in situ*, which results in the liberation of the endotoxin. In certain organs there is a reaction to the presence of the microorganism and its endotoxin; in others, the presence of the microorganism does not induce any lesion but toxic changes may take place.

From what has been stated, it would seem *prima facie* that the virulence of *P. pestis* depends essentially on its invasive power. That this is correct is shown by the strains of *pestis minor*, or of the so-called Brazilian *ingua de frío*,[19] which are incapable of invading the organs of the host but which produce circumscribed glandular lesions without disturbing the general condition of the host. However, certain strains of plague which have a low or high invasive power may possess, at the same time, endotoxins that may be very powerful or, on the contrary, inactive or almost inactive. In other words, different strains of the bacillus may show different degrees of predominance of one or the other of the two qualities that constitute virulence. Thus, we may theoretically conceive strains that are both toxigenic (endotoxins) and invasive; strains that are toxigenic but not invasive; strains that are invasive but not toxigenic, and strains totally avirulent that are deprived of both qualities.

It should be stated that no strain of plague possessing one of these qualities is entirely devoid of the other, and that there are innumerable intergrading proportions of the two qualities in different strains of plague. The Andean strains of plague in Ecuador are of the invasive type with a mild toxicogenic power.

Of the two qualities just described, the toxic power is responsible for the destructive character of the microbe. A strain, which is highly invasive but nontoxic, does not cause death. In such a case, the strain is considered avirulent although the bacilli may be isolated from the organs and the blood. This illustrates the inconvenience of describing virulence in terms of invasive power. The avirulent bacilli circulate in the blood as inert particles rather than as living

19. Ambulatory plague.

organisms, and they are finally destroyed by phagocytic action because they are unable to develop a negative tropism, or the ability to destroy the phagocytes.

As will be seen elsewhere, we believe that the toxic and invasive qualities are related to the antigenic structure of the bacteria. Let us state here, however, that the Andean strains of plague in Ecuador are of the "S" type, and that we positively correlate this character with their particular invasive qualities and low toxicity.

Guinea pigs infected with strains possessing a low toxicity survive for a long time. Strains that are highly invasive will rapidly infect the deeper glands and viscera. The liberation of only a small amount of endotoxin favors the development of small necrotic changes and the formation of proliferative lesions with the accumulation of defensive cellular elements, including polymorphonuclear neutrophils having evidence of phagocytic activity. The possibility that *P. pestis* may reach the lungs depends on the law of chance which is influenced by several factors such as the rapidity of invasion, defensive power of the glands, survival of the animal, and so forth.

Summing up these facts, we believe that the special type of pathogenicity, which characterizes the Andean strains of plague in Ecuador, represents a biologic character dependent on the intrinsic constitution of the microorganisms. This character lends to the bacilli a high degree of invasiveness and a low toxic power that lead to the development of extensive lesions in the viscera, including the lungs. Since the lungs are usually attacked late during the course of infection, these organs are frequently free from lesions in ordinary cases of plague in which the animals are sacrificed within a short period.

Series of blood cultures carried out on the infected guinea pigs resulted in the isolation of *P. pestis* within a few hours after subcutaneous inoculation. Determinations of the toxic power (endotoxin) of the infecting strains revealed that they were from 50 to 200 times less virulent than foreign strains.

An analysis of the last experiment, which led to the reversion of the bacterial strains to their original virulence, or to a higher degree of virulence, would seem to indicate that the lesions of the pelvic glands and lungs are produced only when the degree of virulence lies within certain limits. This may be true for ordinary strains of plague bacilli, the virulence of which varies directly with their invasive and toxic qualities. An increase in virulence would imply a higher toxicity, leading to the death of the animal in a short period,

and that there would be little chance for the involvement of the lungs which, as already seen, are invaded late in the course of the infection. On the other hand, a reduction in the virulence of the bacillus would mean a corresponding reduction in its power to invade and kill so that even under a long survival there would be no chance for lesions to develop in the lungs or other tissues.

This is just the contrary to what occurs when animals are inoculated with our Andean strains of plague. The Ecuadorian isolates have shown a dissociation not only of the two qualities that constitute the virulence but also of the quantitative variations of these qualities. As a result of this dissociation, our plague bacilli are capable of inducing a special type of pathologic response that is dependent on the invasive power of the strain and, above all, on the degree to which this power is present at the moment of infection.

The above observations would seem to confirm that the properties of Andean strains of plague in Ecuador are different from those of foreign strains, although no explanation is offered as to the original cause of the differences. A discussion of the latter point will be presented in a future paper.[20]

SUMMARY

Guinea pigs, experimentally infected with Andean strains of plague from Ecuador, reveal two types of interdependent lesions that are not usually observed when the infection is produced with foreign strains: (1) great involvement of the pelvic-aortic glands and (2) pulmonary lesions, especially of the necrotic type.

The production of these lesions is possibly due to the high invasive power and relatively low toxicity characteristics of the Andean strains of plague.

It is suggested that the proportion in which these two qualities are manifested in the Andean strains is a phenomenon dependent on their peculiar antigenic structure.

Translated—A.L.C.

20. A. Macchiavello and D. Urigüen, *op. cit.* (6).

Strain	Date of Isolation	Source	Method of Isolation	Route of Inoculation
R 1	1–7–41	Viscera of 4 dead *R.r.* found in nest—Guasuntos	Guinea Pig	Subcut.
R 2	1–7–41	Viscera of 5 *R.r.*	Guinea Pig	Subcut.
R 3	1–6–41	Viscera of 2 *R. alexandrinus*	Guinea Pig	Intracut.
F 5	1–12–41	115 dead *X. cheopis* found in nests—Guasuntos	Guinea Pig	Subcut.
H 6	1–11–41	Pus from lymph gland of cervix, C. M., Guasuntos	Culture	
H 10	1–13–41	G. cadaver, R. Ch., Schuid	Guinea Pig	Intracut.
H 11	1–13–41	Pus g., Fr. Ch. Schuid	Guinea Pig	Subcut.
H 14	1–25–41	Pus g., Tr. A., Schuid	Guinea Pig	Subcut.
H 15	1–29–41	Pus g., M. N., Schuid	Guinea Pig	Intracut.
H 16	1–17–41	Pus g., cadaver M. M., Compuone	Guinea Pig	Subcut.
H 17	1–41	Pus g., V. Ch., Schuid	Guinea Pig	Subcut.
H 18	2–41	Pus g., E. G., Schuid	Guinea Pig	Subcut.
H 19	2–17–41	Pus g., P. M., Guasuntos	Guinea Pig	Subcut.
H 25	4–2–41	Pus g., F. B., Farm Sosoles	Guinea Pig	Intracut.
H 26	4–4–41	Viscera *Phyllotis fruticulus*, Sosoles	Guinea Pig	Subcut.
F 31	4–6–41	12 *C. londinensis*, found in empty rat nest in home of infected person, Sosoles	Guinea Pig	Subcut.
H 34	3–31–41	Pus g., C. N., San Antonio—Charaza	Guinea Pig	Intracut.
H 35	4–8–41	?? Patient, San Antonio—Cebadas	Culture	
H 39	4–27–41	Pus g., N. C., Samborondón	Culture	
H 40	6–9–41	Pus g., N. A., Farm "El Encalado"	Culture	
H 41	6–9–41	Pus g., E. X. V. A., Farm "El Encalado"	Culture	
H 42	6–9–41	Pus g., C. Y., Farm "El Encalado"	Culture	
H 43	10–41	Pus g., ?, Town of Cocol (culture from Riobamba)	?	

Abbreviations:

R.r. = *Rattus rattus*
g. = Lymph gland
Subcut. = Subcutaneous
Intracut. = Intracutaneous
? = Information unknown
F = Flea strain
H = Human strain
R = Rat strain

Resumé:

Strains: murine: R 1 – R 2 – R 3 – R 26 :: 4
flea: F 5 – F 31 :: 2
human: H 6 – H 10 – H 11 – H 14
H 15 – H 16 – H 17 – H 18
H 19 – H 25 – H 34 – H 35
H 39 – H 40 (Orig. 69) –
H 41 (Orig. 70) – H 42 (Orig. 71) –
H 43 (Orig. 104) :: 17

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TABLE 2

Resumé of Postmortem Findings in Guinea Pigs Inoculated with Strains of Plague Isolated from Rats, Fleas, and Humans

Strain	No. of Serial Cultures	Life Duration of Guinea Pigs	Autopsy Findings: Intensity and Number of Guinea Pigs with: Subcutaneous Neg.	Pos. S	Pos. I	Inguinal Lymph Gland Neg.	Pos. S	Pos. I	Pelvic Lymph Gland Neg.	Pos. S	Pos. I	Spleen Neg.	Pos. S	Pos. I	Liver Neg.	Pos. S	Pos. I	Lung Neg.	Pos. S	Pos. I	Cultures from: Blood Neg.	Pos. S	Pos. I	Viscera Neg.	Pos. S	Pos. I
R 1	6 (6)	5.2 (1)	2	2	2	1	3	2	3	1	2	0	3	3	1	2	3	4	2	0	3	1	2	2	1	3
R 2	11 (13)	7.2 (8)	0	2	2	1	9	3	10	2	1	1	10	2	3	8	2	12	1	0	6	1	0	1	5	1[a]
R 3	16 (19)	3.0 (6)	5	6	8	2	4	13	7	3	9	0	8	11	3	7	9	11	2	6	6	8	4		8	10[b]
F 5	6 (6)	5.0 (2)	3	2	1	2	3	1	4	2	0	1	3	2	1	4	1	4	2	0	3	3	0	1	3	2
H 6	15 (16)	4.4 (0)	0	4	12	2	1	13	4	4	8	0	1	15	0	2	14	4	6	5	0	5	5	0	0	10[c]
H 10	6 (6)	5.4 (3)	1	2	3	0	3	3	2	1	3	0	1	5	0	2	4	2	3	1	4	0	2	0	2	4
H 11	6 (6)	4.5 (4)	3	3	0	2	4	0	5	1	0	1	4	1	0	5	1	5	1	0	4	0	0	0	3	1[d]
H 14	3 (3)	5.0 (1)	0	0	3	0	0	3	1	1	1	0	0	3	0	0	3	1	0	2	0	2	1	0	1	2
H 15	3 (3)	4.6 (0)	0	1	2	0	0	3	0	0	3	0	0	3	0	0	3	1	0	2	0	2	1	0	0	3
H 16	11 (12)	4.7 (1)	1	2	9	0	1	11	1	2	9	0	1	11	0	3	9	7	4	1	1	2	2	0	2	3[e]
H 17	11 (13)	4.3 (2)	2	3	8	1	3	9	3	2	8	0	2	11	0	4	9	4	5	4	5	4	3	0	2	10[f]
H 18	5 (6)	4.0 (2)	2	1	3	0	3	3	3	2	1	1	2	3	0	4	2	3	3	0	5	0	1	1	3	2
H 19	5 (5)	4.4 (0)	0	2	3	0	1	4	1	1	3	0	1	4	0	1	4	1	2	2	1	0	4	0	1	4
H 25	2 (2)	4.0 (0)	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	1	1	0	0	2	0	0	2
R 26	3 (3)	? (3)	3	0	0	0	1	2	3	0	0	0	2	1	0	2	1	3	0	0	3	0	0	0	2	1
F 31	4 (4)	? (4)	0	3	1	0	2	2	2	1	1	0	0	4	0	2	2	4	0	0	4	0	0	0	1	3
H 34	7 (7)	3.0 (3)	2	0	5	0	0	7	3	0	4	0	1	6	0	1	6	3	3	1	3	1	3	0	3	4
H 35	9 (9)	4.2 (1)	0	2	7	0	0	9	1	1	7	0	3	6	0	3	6	5	1	3	2	6	1	0	1	8
H 39	2 (2)	6.5 (0)	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	1	0	1	0	2	0	0	0	2
19	131 (141)	5.8 (41)	33	35	73	11	38	92	53	24	64	4	42	95	8	50	83	75	36	30	50	37	31	5	38	75
Percent Negative:			23.4			7.7			37.5			2.8			5.7			53.1			42.3			4.2		
Percent Positive:			76.6			92.3			62.5			97.2			94.3			46.9			57.7			95.8		

Note: In the column marked "No. of Serial Cultures," the number in parenthesis indicates the total number of animals used in the series. In the column marked "Life Duration of Guinea Pigs," average duration is based on animals that died spontaneously; it does not include those sacrificed; that number is indicated in parenthesis. Two guinea pigs of R 2 strain were not included, either, because their autopsies were incomplete. Autopsy findings considered were: congestion and edema of the subcutaneous tissues; hyperthrophy, congestion and caseification of lymph glands; hypertroply, congestion, necrotic foci and degeneration of viscera; focal hepatization or pneumonia.

[a] Of 13 guinea pigs, only 7 were cultured.
[b] Of 19 guinea pigs, only 18 were cultured.
[c] Of 15 guinea pigs, only 10 were cultured.
[d] Of 6 guinea pigs, only 4 were cultured.
[e] Of 12, only 5 were cultured.
[f] Of 13, only 12 were cultured.

Relation between the Virulence of Andean Plague Strains and the Degree of Infection Found in Pelvic-Lumbar-Aortic Lymph Glands and Lungs

Strain	Frequency of Lesions Found in Lymph Glands of Pelvis and Lung of Guinea Pigs:							
	Whose Autopsies Showed Little, or No, Virulence				Whose Autopsies Showed Signs of Normal Virulence			
	No. of Serial Cultures	Total No. of Animals	No. of Guinea Pigs Found with Lesions of Lymph Glands of Pelvis	No. of Guinea Pigs with Pulmonary Lesions	No. of Serial Cultures	Total No. of Animals	No. of Guinea Pigs Found with Lesions of Lymph Glands of Pelvis	No. of Guinea Pigs with Pulmonary Lesions
I. Murine								
R 1	4	4	1	0	2	2	2	2
R 2	11	13	3	1	0	0	0	0
R 3	7	8	2	1	9	11	10	7
R 26	3	3	0	0	0	0	0	0
	25	28	6 (21.4%)	2 (6.66%)	11	13	12 (92.3%)	9 (69.2%)
II. Flea								
F 5	6	6	2	2	0	0	0	0
F 31	4	4	2	0	0	0	0	0
	10	10	4 (40.0%)	2 (20.0%)	0	0	0	0
III. Human								
H 6	4	4	1	2	11	12	11	10
H 10	2	2	0	0	4	4	4	4
H 11	6	6	1	1	0	0	0	0
H 14	1	1	0	0	2	2	2	2
H 15	0	0	0	0	3	3	3	2
H 16	0	0	0	0	11	12	11	5
H 17	0	0	0	0	11	13	10	9
H 18	0	0	0	0	5	6	3	3
H 19	0	0	0	0	5	5	4	4
H 25	0	0	0	0	2	2	2	2
H 34	3	3	0	0	4	4	4	4
H 35	0	0	0	0	9	9	8	4
H 39	0	0	0	0	2	2	2	1
	16	16	2 (12.5%)	3 (18.75%)	69	74	64 (86.4%)	50 (67.5%)
IV. Resumé:								
4 murine	25	28	6 (21.4%)	2 (6.66%)	11	13	12 (92.3%)	9 (69.2%)
2 flea	10	10	4 (40.4%)	2 (20.0%)	0	0	0 (0.0%)	0 (0.0%)
13 human	16	16	2 (12.5%)	3 (18.75%)	69	74	64 (86.4%)	50 (67.5%)
19 strains	51	54	12 (22.2%)	7 (12.5%)	80	87	76 87.2%	59 (67.8%)

TABLE 4

Postmortem Findings Encountered in Lymph Glands of Pelvis and Lungs of Guinea Pigs Subcutaneously Inoculated with Plague Strains Recently Isolated and Kept in the Icebox at 0° to 5° C.

Strain	Source of Culture	Culture Media	Date When Placed in Icebox	No. of Days in Icebox	Life Duration of Guinea Pigs	Postmortem Findings		
						General	Pelvic Lymph Glands	Lungs
R 2	Guinea Pig Spleen—5th Series, Intracutaneously	Blood Agar	5–8–41	450	8 days	Typical	Intense	Moderately Intense
R 3	Guinea Pig Lymph Gland—10th Series, Intracutaneously	Sulphite-Agar	6–10–41	417	7 days	Typical	Moderately Intense	Moderately Intense
H 6	Guinea Pig Liver—9th Series, Intracutaneously	Blood Agar	5–4–41	423	Sacrificed	Negative	Negative	Negative
H 14	Pus from human lymph gland	Blood Agar	5–41	430 (ap.)	Sacrificed	Negative	Negative	Negative
H 15	Guinea Pig Liver—2nd Series, Intracutaneously	Blood Agar	5–41	430 (ap.)	Sacrificed	Negative	Negative	Negative
H 16	Pus from human lymph gland	Blood Agar	5–22–41	436 (ap.)	5 days	Attenuated	Moderately Intense	Negative
H 17	Guinea Pig Lymph Gland—11th Series, Intracutaneously	Blood Agar	3–29–41	490	8 days	Typical	Intense	Intense
H 18	Guinea Pig Inoculated with Human Strain	Sulphite-Agar	5–41	430 (ap.)	Sacrificed	Negative	Negative	Negative
H 19	Guinea Pig Liver—4th Series, Intracutaneously	Blood Agar	5–41	430 (ap.)	Sacrificed	Negative	Negative	Negative
H 25	Guinea Pig Blood—1st Series, Intracutaneously	Blood Agar	4–7–41	481	6 days	Attenuated	Moderately Intense	Negative

Description of Lesions of Lymph Glands of Pelvis and Lungs

Strain R 2. Two lymph glands of left pelvis, measuring 2 x 0.6 cm., more or less; friable, congested; hypertrophy of lumbar-aortic lymph gland chain reaching to below diaphragm. Lungs: zones of congestion and hepatization but little nodular necrosis.

Strain R 3. Hypertrophy of lymph glands of pelvis with intense congestion. Lungs: necrosis surrounded by irregular zones of gray hepatization; pleural exudate.

Strain H 16. Hypertrophy of lymph gland of left pelvis with hyperemia. Normal lungs.

Strain H 17. Hypertrophy of lymph gland of pelvis with intense caseification; lumbo-dorsal-aortic lymph chain. Lungs with numberless necrotic nodules about 0.1 to 0.2 cm., with central hemorrhagic areas.

Strain H 25. Very hypertrophied and very congested lymph gland of pelvis. Normal lungs.

TABLE 4—*Continued*

Strain	*Source of Culture*	*Culture Media*	*Date When Placed in Icebox*	*No. of Days in Icebox*	*Life Duration of Guinea Pigs*	*Postmortem Findings*		
						General	*Pelvic Lymph Glands*	*Lungs*
H 34	Guinea Pig Blood—2nd Series, Intracutaneously	Sulphite-Agar	5–7–41	430 (ap.)	Sacrificed	Negative	Negative	Negative
H 35	Guinea Pig Liver—6th Series, Intracutaneously	Blood Agar	5–21–41	437	Sacrificed	Negative	Negative	Negative
H 39	Guinea Pig Blood—2nd Series, Intracutaneously	Sulphite-Agar	5–15–41	443	8 days	Typical	Intense	Intense
H 40	Guinea Pig Lymph Gland—1st Series, Intracutaneously	Sulphite-Agar	6–16–41	411	Sacrificed	Negative	Negative	Negative
H 41	Guinea Pig Lymph Gland—1st Series, Intracutaneously	Sulphite-Agar	6–13–41	414	Sacrificed	Negative	Negative	Negative
H 42	Guinea Pig Spleen—2nd Series, Intracutaneously	Blood Agar	6–13–41	414	9 days	Typical	Intense	Intense
H 43	Mixed culture	Sulphite and Blood Agar	11–41	260 (ap.)	4 days	Attenuated	Negative	Negative
H 44	Mixed culture	Sulphite and Blood Agar	11–41	260 (ap.)	Sacrificed	Negative	Negative	Negative

Strain H 39. Hypertrophy of pelvic-aortic lymph glands; no congestion. Lungs having abundant and prominent necrotic nodules with gray hepatization.

Strain H 42. Hypertrophy of pelvic-dorsal-aortic lymph glands. Lungs having numberless prominent necrotic granules, grayish-yellow; abundant pleural exudate.

Strain H 43. No deep lesions of lymph glands or lungs. Other symptoms of plague very attenuated.

TABLE 5

Postmortem Findings Encountered in Lymph Glands of Pelvis and Lungs of Guinea Pigs Inoculated Subcutaneously with Plague Strains Kept in the Icebox at 0° to 5° C. Culture Medium : Sulphite-Agar

Strain	*Source of Culture*	*Date When Placed in Icebox*	*No. of Days in Icebox*	*Life Duration of Guinea Pigs*	*Postmortem Findings*		
					General	*Pelvic Lymph Glands*	*Lungs*
R 3	Pelvic Lymph Gland, Guinea Pig—2d Series	5–21–41	646	6 days	Attenuated	Intense	Negative
R 3–1	Pelvic Lymph Gland, Guinea Pig—9th Series	5–21–41	646	5 days	Attenuated	Intense	Negative
H 14	Guinea Pig Liver	5–20–41	645	Sacrificed on 10th day	Negative	Negative	Negative
H 14–5	Guinea Pig Spleen	5–20–41	645	Sacrificed on 10th day	Negative	Negative	Negative
H 15	Guinea Pig Inguinal Lymph Gland	2–27–41	731	Sacrificed on 10th day	Negative	Negative	Negative
H 16	Guinea Pig Spleen	3–30–41	700	Sacrificed on 10th day	Very attenuated	Negative	Negative
H 19	Guinea Pig Heart Blood	4–7–41	692	Sacrificed on 10th day	Very attenuated	Negative	Negative
H 19	Guinea Pig Liver	4–7–41	692	Sacrificed on 10th day	Negative	Moderate intensity	Negative
H 25	Guinea Pig Spleen	4–6–41	691	6 days	Very attenuated	Negative	Negative
H 44	Guinea Pig Spleen	11–10–41	473	Sacrificed on 10th day	Negative	Negative	Negative

TABLE 6

Degree of Infection of Lymph Glands of Pelvis and Lungs of Guinea Pigs Inoculated with Plague Strains

Strains	*No. of Series*	*No. of Guinea Pigs without Lesions of Lymph Glands of Pelvis or Lungs*	*No. of Guinea Pigs with Lesions of Lymph Glands of Pelvis or Lungs*	*No. of Guinea Pigs with Lesions of Lymph Glands of Pelvis Only*	*No. of Guinea Pigs with Pulmonary Lesions Only*	*Total of Guinea Pigs with Lesions of Lymph Glands of Pelvis*	*Total of Guinea Pigs with Pulmonary Lesions*
36	12	5	1	5	1	6	2
14–5	9	4	2	2	1	4	3
15	11	1	6	3	1	9	7
16	3	1	2	0	0	2	2
19	10	1	5	4	0	9	5
25	5	2	2	0	1	2	3
	50	14 (28%)	18 (36%)	14 (28%)	4 (8%)	32 (64%)	22 (44%)

TABLE 7

Frequency of Lesions Found in Lymph Glands of Pelvis and Lungs of Three Series of Guinea Pigs Inoculated with Strains of Plague (1) Immediately after Isolation, (2) after a Year, More or Less, in the Icebox at 0°–5° C., and (3) after Two Years in the Icebox

Series	*No. of Guinea Pigs Inoculated*	*No. of Guinea Pigs with Lesions of Lymph Glands of Pelvis*	*Positive Percentage of Group*	*No. of Guinea Pigs with Pulmonary Lesions*	*Positive Percentage of Group*
(1)	141	88	62.5	66	46.9
(2)[a]	17 (8)	7 (7)	41.1 (87.5)	5 (5)	29.3 (62.5)
(3)	50	32	64.0	22	44.0

[a] In the second series, the number of guinea pigs with indications of disease has been placed between parenthesis; the frequency and percentage of each type of lesion has been calculated on the basis of this number, without taking into consideration those guinea pigs that gave negative results (see text).