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Diagnosis of Plague: an Analysis of the Yersin-Kitasato Controversy

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INTRODUCTION

From time to time certain bacteriological controversies have interested medical historians. Often such disputes concern the priority of discovery, but sometimes the content of the discovery is at question. The case in point is the independent observation of the plague bacillus by Alexandre Yersin and Shibasaburo Kitasato in 1894. The essence of the argument is not who first found the microorganism but whether Kitasato did in fact observe and isolate the agent of plague. Secondary to this is the technical question of how Kitasato could have erred. The scientific origins of this dispute are probably more important than the nature of the controversy itself. There is no question that Yersin correctly described and cultured the bacterium. This fact is reflected in the new name of the bacillus: *Yersinia pestis* (15, 59). Formerly known as *Pasteurella pestis*, the bacterium has been demonstrated, along with *P. pseudotuberculosis*, to be sufficiently unique to form a new genus (82).

Joseph McFarland's classic *Textbook Upon the Pathogenic Bacteria* (60), published 2 years after the discovery, asserted that the "bacillus of bubonic plague seems to have met an independent discovery at the hands of Yersin and Kitasato in the summer of 1894, during the activity of the plague then raging at Hong-Kong. There seems to be not the slightest doubt that the micro-organisms described by the two observers are identical." However, recent English language textbooks and histories of microbiology have been totally inconsistent in crediting Yersin or Kitasato as the discoverer. One mentions only Kitasato (33), many cite only Yersin (26, 28, 34), a few declare both investigators as independent co-discoverers (16, 27, 92), two are noncommital (48, 58), but most ignore the problem by eliminating historical introductions. Furthermore, in the first edition of one microbiology textbook (27) both Yersin and Kitasato were credited with the discovery, but in the second edition (28), which appeared just a few years later, only Yersin was cited.

In our analysis of the dispute we have also noted a confusion in the literature regarding certain aspects of diagnostic microbiology, including morphology, growth, and pathology of the plague bacillus. It seems that many of the bacteriological problems that faced Yersin and Kitasato in the diagnosis of plague are still with us. Using the controversy as a vehicle of review, we will trace the origins of the variance and demonstrate some difficulties that clinical microbiologists might encounter with this dis-

ease. Plague is far from being eliminated, as commonly believed, and even in the United States, where plague is epizootic in the western region (49), human disease and death still occur (18–21). The presumptive identification of *Y. pestis* is deceptively simple. As much of its classification is still based upon morphological features, the early bacteriological history, to which these aspects were central, bears reconsideration.

THE INVESTIGATORS

Shibasaburo Kitasato

Shibasaburo Kitasato, born in 1852 in a mountainous village of the southern island of Kyusho, was one of the first Japanese microbiologists (65). His medical education began in Kumamoto, where the German professor Mansfeld influenced his medical interests and taught him German, and ended at the Tokyo Medical School in 1883.

He joined the Central Hygienic Bureau of the Department of Interior and in 1885 was chosen to travel to Germany for training under Robert Koch. Kitasato was the first of many foreign students in Koch's laboratory. His first studies were on dysentery and cholera, outgrowths of his experience in Japan. After instructing him in the latest techniques of bacteriology, Koch assigned him to investigate tetanus, not expecting much success. The anaerobic techniques developed by Kitasato and the heat resistance of the spores allowed him to isolate *Clostridium tetani* in pure culture for the first time. While studying the pathogenesis of the organism, he discovered and characterized its exotoxin, and in 1890 with Emil von Behring was first to demonstrate the neutralization of toxins in animal blood. When Kitasato's term for foreign study was about to expire, Koch appealed to the Japanese minister in Berlin for an extension. The Emperor of Japan granted a special fellowship through the Minister of the Imperial Household, and Kitasato was able to assist Koch in the investigation of tuberculin as a cure for tuberculosis.

FIG. 1. *Shibasaburo Kitasato (1852–1931). Photograph was taken in Koch's laboratory circa 1890.*

In 1892 Kitasato returned to Japan and the German government awarded him the title of Professor, the first such honor given to a foreign researcher. He was offered positions by English and American universities, but he felt that his duty was to Japan. On arriving in Japan, Kitasato had some second thoughts. He had hoped that the government would establish a laboratory for him. Disappointed, he stated his intention to go to the United States, but one of Japan's great intellectuals, Yukichi Fukuzawa, persuaded Kitasato to remain by funding a small private laboratory.

The government took over the laboratory in 1899, placing it under the control of the Minister of Interior. Next the government established a serum institute and in 1905 both laboratories evolved into the Imperial Institute for Infectious Disease. In 1914 the institute was transferred to the Minister of Education and placed under the authority of the President of the competing Tokyo Imperial University. Kitasato resigned in protest, and the entire staff departed with him to establish the Kitasato Institute of Infectious Diseases.

Later Kitasato was invited by the Keio-Gijiku University to organize their medical faculty. He entered politics in 1917 when the Emperor nominated him to the House of Peers. For his long efforts and noteworthy achievements in developing Japanese medical science, Kitasato was given the title of Baron in 1924. Kitasato, the direct and forceful chief of Japanese microbiology, died in 1931.

Alexandre Yersin

The second figure in this inquiry is Alexandre Émile John Yersin, who was born in Aubonne, Switzerland in 1863 (9). After earn-

FIG. 2. *Alexandre Yersin (1863-1943), as photographed around 1900.*

ing his Bachelor of Arts degree in 1882, he traveled to Lausanne, Marburg, and Paris for medical training. At the Hôtel-Dieu Hospital in Paris, Yersin's interest turned to pathology and infectious disease. Realizing that he lacked the temperament for personal patient care, he fled to the quiet isolation of the pathology laboratory, which had its own disadvantages. A cut incurred during the autopsy of a rabies victim led him to Louis Pasteur for prophylactic treatment.

Yersin was so impressed with the research at the École Normal Supérieur that he entered Emil Roux's laboratory to complete his thesis on experimental tuberculosis. In June 1888, after receiving his medical degree, Yersin went to Berlin to enroll in Koch's course in bacteriology (then taught by Richard Petri and Carl Fränkel) in order to compare the program with that of the French group. Upon his return, Yersin was able to assist Roux in developing the Pasteur Institute's first course in bacteriology. Soon the two scientists took up the investigation of *Bacillus (Corynebacterium) diphtheriae,* recently discovered by Friedrich Loeffler and Edwin Klebs. In 1888 Roux and Yersin announced their own discovery, diphtheria toxin, but after publishing three papers on the subject Yersin suddenly left the institute.

Intrigued with the idea of travel, adventure, and exploration of the newly united French colony of Indo-China, Yersin took a position as ship physician of the Messageries Maritimes that sailed between Manila and Saigon. To secure official sanction and supplies for explorations, he accepted Albert Calmette's offer to join the Colonial Health Corps. Calmette had established a Pasteur Institute in Saigon in 1891. Yersin led three expeditions into the interior which was not without risk, for besides tropical diseases, tigers, cobras, and unruly elephants, the people of what is now Vietnam have always resisted colonialists.

In 1895 Yersin opened a second Pasteur Institute in Nhatrang, which merged with the Saigon facility in 1904 with Yersin as director. Later the laboratories at Saigon, Nhatrang, Hue, Dalat, Pnom-Penh, Vientiane, and Hanoi were consolidated under a common administration. Yersin was also founder and director of the Medical School of Hanoi. He continued the study of tropical diseases and the production of vaccines, but shifted his emphasis to the economic interests of Indo-China, supporting expansion and improvement of agriculture. This complex man, who never used his first name, who preferred to remain alone with his hobbies of astronomy, radio, and photography, and who avoided whenever possible the many meetings of medical and scientific societies, died in 1943 in his home in Nhatrang.

THE DISCOVERY OF THE PLAGUE BACILLUS

The Arrival of Kitasato and Yersin at Hong Kong

Both investigators had come to Hong Kong in June 1894 to study the epidemic of bubonic plague which, spreading through southern China, had claimed over 40,000 lives (41). Kitasato was accompanied by the pathologist Tanemichi Aoyama, several medical students, and assistants. Arriving June 12, members of the Japanese Commission were afforded gracious hospitality by the acting superintendent of the Government Civil Hospital, James A. Lowson, and were given a room at the Kennedy Town Hospital (52).

Yersin, on the other hand, with only a servant and some laboratory equipment, including

a microscope and autoclave, found himself without facilities, quarters, or government support. Yersin had used the epidemic as a pretext for exploring the Yunnan province of China (9). He had been granted funds by the Minister of Public Instruction through the efforts of Pasteur and Roux, but the Governor-General of Indo-China turned down the project. Calmette used his influence to obtain a favorable decision, and Yersin departed Hanoi on June 12, landing in Hong Kong 3 days later.

Yersin was upset more by his lack of access to patients than by his poor reception. He used his experience in impromptu field studies during his explorations to build a straw hut for his laboratory and to obtain the necessary specimens. He bribed the English sailors whose task it was to carry the dead to the cemeteries (57). Later, after an appeal to the Governor, Yersin was permitted patient contact and pathological material.

Kitasato and Yersin met only once, but with German as their common language, communication was poor. Cooperation was certainly minimal. Kitasato, however, did present Yersin a stained preparation of a supposed pure culture of plague bacillus. Upon viewing the slide 30 years afterward, Lagrange, a former assistant of Yersin, stated that he was unable to decide whether the bacteria were truly plague bacilli or pneumococci (57). Yersin noted in his diary that Kitasato seemed to ignore the bubo in his pathological examination (9, 57), a mistake which was immediately corrected.

Yersin's Report

Yersin made his discovery on June 20. As our focus is on the bacteriology of plague, it would be helpful to recall his classic description of the bacillus (97):

> The pulp of the bubo, in every case, was filled with a thick puree of short, thick bacilli with rounded ends, easily colored with aniline dyes but not by the method of Gram. The ends of the bacilli are more stained than the center. Often the bacilli appear to be covered by a capsule. One can recover a great amount from the buboes and lymph nodes of the diseased. The blood also contains them but not in such great numbers and only in very grave and deadly cases.
>
> The pulp of the bubo, when inoculated on agar, gives rise to white transparent colonies whose edges seem iridescent when examined under reflected light. The culture does even better on glycerin agar. The bacilli also grow in coagulated serum.
>
> In broth, the bacilli show a very characteristic aspect similar to erysipelas: clear liquid with flocked particles along the length and bottom of the tube. . . .
>
> These cultures examined under the microscope show true chains of short bacilli, some appearing like a ball. On agar, if you examine these cultures with great care and high magnification, one can see bacilli among normal forms that sometimes are thin and sometimes fat chains of rods joined laterally. These swollen and abnormal forms become more and more numerous in old cultures and stain poorly.

Yersin noted that mice, rats, and guinea pigs, but not pigeons, succumbed within 2 to 5 days to infection when inoculated with diseased tissues or cultures. The spleen was enlarged with eruptions which, to him, resembled miliary tubercles, and the microorganism could be isolated from blood, lymph nodes, spleen, liver, and other organs. Of interest was his remark that the organism, when found in blood, was more elongated than in lymph nodes. Photographs of the plague bacilli in pulp and in broth were included in his report.

Kitasato's Reports

In comparison, there are two notes on Kitasato's studies. The first appeared as an editorial account in *The Lancet* of 11 August 1894 (30). Kitasato's host, Lowson, had sent to the journal's editors some slide preparations and illustrations made by Kitasato and him. Presumably the editors, but possibly Lowson, stated that "the organism—which is a bacterium resembling the bacilli found in the hemorrhagic septicemias, except that the ends are somewhat more rounded—when stained lightly appears almost like an encapsulated diplococcus, but when more deeply stained it has the appearance of an ovoid bacillus, with a somewhat lighter center, especially when not accurately focussed. When, however, it is focussed more accurately it is still possible to make out the diplococcus form."

One of Kitasato's drawings seems to depict a pure culture in broth, although Lowson apologized for not having been able to tell about the appearance of the microorganisms when cultivated in vitro. The figure is ambiguous at best. Drawn from medium magnification (about ×530), bacteria are small with occasional chains. Some thick, elongated structures are discerned, but typical round involution forms are not illustrated. In comparison to Yersin's description, the meager evidence tips toward the discredit of Kitasato, but the hastily drawn figures should not be taken too seriously.

Two weeks later the same journal published Kitasato's preliminary but only bacteriological study, which was sent from Hong Kong on July 7 (52). Because Kitasato knew little English (80), the article probably was translated from

the German or Japanese. This point is important because Japanese is an imprecise language, tending toward descriptions in general terms. Furthermore, it is not known how fluent Kitasato was in German. Although language may have significantly contributed to the controversy, we lack additional information and must set this matter aside.

On June 14, Kitasato, examining some 11-h-old postmortem material, found bacilli in the bubo, but refrained from judging the significance due to the age of the tissue and the opportunity for contamination. From the blood preparation of a patient with severe disease he made his first careful observations (52):

> Under the microscope I found bacilli with capsules, the poles of which were stained much deeper with aniline dyes than the middle part; this gave them a great likeness to the bacilli of chicken cholera (bacillus cholerae gallinarum). On the next day all the serum cultivations which were prepared in the incubator from the different organs of the body and of blood from the finger tips showed a growth of microorganisms, which, under the microscope, were not to be distinguished from those which we found in the blood and in the interior of the bubo at the first post-mortem examination. The bacilli differed only by being a little longer and staining more easily in the middle than those from the blood.

In another part of the paper Kitasato reiterates that "the bacilli are rods with rounded ends, which are readily stained by the ordinary aniline dyes, the poles being stained darker than the middle part, especially in blood preparations, and presenting a capsule sometimes well marked, sometimes indistinct. The bacilli found in the spleen are best stained by a solution of methyl blue."

Then discrepancies with Yersin's observations emerge: "I am at present unable to say whether or no [sic] 'Gram's double staining method' can be employed. . .The bacilli show very little movement and those grown in the incubator, in beef-tea, make the medium somewhat cloudy."

Kitasato's description of the morphology of colonies is more extensive than that of Yersin's paper:

> The different colonies are of a whitish-grey colour and by a reflected light have a bluish appearance; under the microscope they appear everywhere as if piled up with 'glass-wool', later as if having dense, large centres. If a cover-glass preparation is made from a cultivation on agar-agar, and having been stained, is observed under the microscope long threads of bacilli are seen, which might, by careless inspection, be mistaken for a coccus chain, but are recognized with certainty as 'threads of bacilli' under closer observations.

Kitasato found Hong Kong's temperature too high to properly test gelatin media. An agar-gelatin stab formed a fine dustlike growth along the puncture and very little on the surface. The question of optimal temperature was discussed by Kitasato, but his statements were contradictory. He first declared that "the growth of the bacilli is strongest on blood serum at the normal temperature of the human being (34 C.)," but later stated that "as mentioned before the bacilli grow best at a temperature of from 36 C. to 39 C." He apologized for not yet being able to determine the minimal growth temperature. On potato there was no growth after 10 days when incubated from 28 to 30°C, but at 37°C after a few days a small amount of growth was detected. No spores were found in any preparation.

In studying plague victims Kitasato examined their blood daily and observed the same bacillus as found in buboes and internal organs obtained from 15 autopsies. The detection of the bacillus in blood was not consistent and often required the preparation of many slides. Because the appearance of the bacillus differed slightly than those from buboes and internal organs (the latter stained more easily in the middle), Kitasato was careful to study both isolates grown under identical conditions. He determined that serum cultures produced the same form of bacillus. "In any where cultivations are prepared from parts of any internal organs or from the blood taken from the fingertips, with careful observation of all due precautions, pure cultivations of one and the same bacillus are always obtained."

Kitasato's engrossment with blood stemmed from a desire to use the septicemic stage as an easier means of obtaining specimens for laboratory diagnosis rather than piercing the very painful bubo. His report suggested that plague was generally a septicemic disease. Kitasato's question of whether it was possible to make a diagnosis of bubonic plague from examination of blood smears was answered in the affirmative for many cases. He warned, however, that "a good deal of bacteriological practice is required, or such diagnosis is impossible," and further stated that for safety the blood should be cultured as well.

The injection into laboratory animals of blood or tissue from plague victims or of cultures gave essentially the same results as Yersin's trials. In the area of inoculation Kitasato observed a black and red edematous zone that was infiltrated with a gelatinous exudation. The spleen was enlarged and the lymph nodes were sometimes swollen. Animals also died when fed in-

fected tissue or pure cultures. Kitasato, like Yersin, noted the deaths of numerous mice and rats in Hong Kong and also examined the carcasses. The same bacilli were found in a mouse.

He tested the ability of his isolated plague bacillus to withstand the effects of desiccation, heat, phenol, and calcium hydroxide, and concluded with a variation of Koch's postulates:

> (1) This bacillus occurs in the blood, in buboes, and in the internal organs of the plague-stricken only; (2) This bacillus is not to be found in any other infectious disease; (3) With this bacillus it is possible to produce in animals the identical symptoms which the disease presents in human beings. From this evidence we must come to the conclusion that this bacillus is the cause of the disease known as the bubonic plague; therefore, the bubonic plague is an infectious disease produced by a specific bacillus.

Kitasato sent notes and cultures to Robert Koch in Berlin. Yersin mailed his preparations to the Pasteur Institute in Paris, and Émil Duclaux delivered the paper at the Academy of Sciences on July 30, 1894 (98).

A Comparison of Papers

On the whole, Kitasato's description of the plague bacillus is quite similar to that of Yersin's, but on closer inspection one can find four major differences and several minor discrepancies. The more important include the type of growth in broth, the Gram stain reaction, the presence of involution forms, and motility. Others include subtle variation in colony description, involvement of lymph nodes in experimental infections, and appearance of diseased organs. Yersin would have noted motility if he had seen such activity; on the other hand, Kitasato might have easily ignored or simply not have observed involution forms. Kitasato did not commit the bacillus to either category of Gram staining, and it is rare that two investigators describe colonies exactly alike.

Thus, given these two documents alone, the scientific community readily accepted both investigators as independent co-discoverers. The similarities far outweighed the minor variation in observations. Kitasato's paper, being longer and more detailed, presented a greater opportunity for finding fault. Furthermore, because both were already recognized as accomplished scientists, there was little reason to doubt their results. Two independent workers seemed to have described the same microorganism; the plague bacillus must have been found.

Of interest were the respective directions taken once the organism was isolated. Kitasato, a product of the German school, sought the destruction of the bacillus through chemical and physical methods; Yersin, who was a follower of Pasteur, addressed the questions of virulence and immunity.

OPPOSITION

Not everyone accepted the identity of Yersin's and Kitasato's isolated bacteria. The breach occurred in 1895 with the publication in a Japanese journal of an article by Kitasato's associate, Aoyama, in Hong Kong (3). Writing in German, Aoyama mentioned that Kitasato's organism was morphologically different than Yersin's, that it was isolated from the blood, and that it was partially gram positive (gram variable?). Considering the frequency of secondary infections in plague, the pathologist concluded that Kitasato's isolate was merely a streptococcus.

This somewhat obscure report probably did not circulate extensively beyond Japan (although W. Kolle in Berlin did obtain a copy [56]), but Masanori Ogata, who was to suggest the flea as a vector in plague, wrote an article that appeared in a major German journal (67). He related that during the epidemic of plague in Formosa in 1896 the military physician Murakami sent a culture of bacilli isolated from a bubo to the military medical school in Tokyo. There K. Okada studied the organism and concluded that it resembled Yersin's bacillus, not Kitasato's. Ogata remarked that Kitasato himself had declared in the *Journal of the Medical Society of Tokyo* that his plague bacillus was totally different than the bacterium of Yersin.

Ogata then listed the differences which Okada had observed (67). With the exception of adding that the Kitasato microorganism was gram positive, the table clearly described motility, capsule, agar colonies, cellular morphology, agar stab, and experimental pathology in the basic terms as reported by Kitasato and Yersin. Ogata, in addition, referred to Kitasato's lecture in which he had recorded that in lymph nodes of experimental animals both Yersin's bacillus and his blood bacterium could be found and that in most cases lymph node enlargement occurs. Reporting on his own studies on plague, Ogata asserted the difficulty in obtaining positive slides or cultures from blood, and how often other microorganisms could be obtained. He mentioned that in two acutely ill patients he found in the blood bacteria that resembled pneumococci.

Howard-Jones (43) reviewed other examples of second- or third-hand accounts of Kitasato's bacterium by Japanese workers. Finally, in 1900 Tatsusaburo Yabe published a report (96)

describing his personal inspection of Kitasato's bacterium. Noting the distinct capsule, the lancet-shaped diplococci, and the frequency of secondary pneumococcal infections in plague victims, the naval surgeon concluded that the organism was indeed a pneumococcus. He related that Kitasato in November 1899 at Kobe admitted that Yersin's bacillus was the agent of plague, but affirmed that his bacterium coexisted in septicemia and might be important in the pathology of plague.

Two bits of supportive evidence to Kitasato's concession can be found. The first is from Kitasato himself. "An Abstract of the Report upon the Epidemic of Pest in Japan from November, 1899 to January, 1900" by Kitasato et al. was translated in an American medical journal (55). The note stated that "besides the pest-bacilli, septicemic bacilli like those of chicken-cholera, staining deeply by Gram's method, staphylococci, and streptococci were seen." This peculiar sentence can be interpreted two ways. Lagrange (57) differentiated pest bacilli and septicemic bacilli, but Howard-Jones (43) saw no distinction. The latter, after reviewing the abridged German version (54), furthermore concluded that "nowhere is there the slightest hint that there was ever any difference of opinion as to the identity of the plague bacillus."

The second account was by Norio Ogata, the son of the above-mentioned critic. He stated (68) that in Kobe in 1899 in discussing the etiology of plague with Ogata and Nakahama, Kitasato admitted that Yersin's bacillus was the plague organism and his was but a plague-associated bacterium. Despite these private admissions, Kitasato apparently never issued a public statement declaring his error. We will return to this aspect later.

In 1901 Kitasato provided additional bacteriological characteristics of his bacterium. The description is highly significant as it differs considerably in some respects from his first report. The article (53), which was contributed to Stedman's medical encyclopedia, is itself contradictory. Included was obvious paraphrasing from his first report.

He and coauthor Nakagawa assured the bacterium's rodlike character, especially distinct in lymph nodes. They added that "in the lungs, heart, brain, and spinal cord it is not rare to find them presenting an appearance like that of streptococci. Again, in artificial cultures the streptococcus-like appearance is the rule. On attentive observation, however, these cultures will be found to consist of bacillary chains and not cocci groups. On the other hand, cultivations on solidified serum present the real rod-like shape." Bipolar staining was deemed responsible for the polymorphism. The organism was gram positive. They described spheroid, ovoid, dumbbell, and large rodlike involution forms particularly in fluid media. Motility was best detected at 37°C, and, although the activity was slight, we are urged not to confuse it with molecular movement. The bacterium's capsule, seen distinctly in tissue preparations, was lost on artificial media. Colonies on agar and coagulated serum were now described as moist, grey-yellow, transparent, circular, of irregular margins, and minute size. The colonies at first appeared granular, then developed a denser and darker center, but disappeared by day 4 of incubation. Kitasato noted the strong resemblance to *S. pneumoniae* in the nature of the colonies and in the growth of a gelatin stab. Regarding the latter, we find the statement that no growth was seen on the surface. Growth in broth presented a turbid appearance, followed by formation of fine flocculi and sedimentation. Optimal temperature was 36 to 39°C. The bacterium was a facultative anaerobe whose growth was more vigorous in anaerobiosis. Milk was coagulated by 48 h. Further notes include that swelling of lymph nodes was often observed in experimentally infected animals, and, although infection could be achieved through the alimentary tract, inhalation experiments were without success. A toxin, produced in broth, whose activity was reduced by 90% upon heating for 20 min at 60°C, was described.

CONFIRMATION

Clearly, Kitasato's first accounts and those that followed are in variance. To add to the confusion, during the same period researchers in Germany who had received Kitasato's cultures were obtaining somewhat different results. Hugh Zettnow (99) studied and photographed cultures and preparations of plague organisms received from Elie Metchnikoff at the Pasteur Institute (probably Yersin's isolates) and from Kitasato by way of Koch's Institute for Infectious Diseases. He noted at ×320 and with weak light that Kitasato's bacteria could be taken as small chains of cocci, but at higher magnification and with better light bipolar bacilli were observed. A photograph of a 21-day gelatin stab culture of Kitasato's organisms showed continuous growth along the stab, except at the bottom where beadlike colonies could be seen. There was growth at the surface. Zettnow provided an important date. He recorded that Kitasato's preparations were sent to Koch in January 1895, 6 months after the

first isolations. The slides displayed heart blood and bubo exudate taken from plague victims in Hong Kong. Zettnow did not see any differences between Yersin's bacillus and those in Kitasato's exudate slide. However, he did state that the groups of bacteria seen in the blood smear resembled streptococci.

Wilhelm Kolle at the Koch Institute reviewed the bacteriology of plague (56), noting Aoyama's dissent and concern with secondary streptococcal infections. Zettnow provided Kolle with photographs of Kitasato's preparations. In a comparison with strains obtained from various world wide sources, Kolle found no differences in morphology. Again photographs of Kitasato's preparations were published, and these different presentations also seem to be *Y. pestis*. It is important to note that the slides were of exudate and not of blood.

THE PROBLEM

Why does this apparent paradox exist? There are several possibilities: (i) Kitasato did not observe or culture the plague bacillus, but rather a pneumococcus or pneumococcal-like bacterium; (ii) Kitasato observed *Y. pestis* in slide preparations, but his initial cultures were (a) of pneumococci or (b) mixed; (iii) Kitasato's first paper was correct, but either (a) his subcultures were subsequently contaminated or (b) a different organism was described in a later isolation. The fourth alternative, that Kitasato always described *Y. pestis*, is clearly eliminated by his own admission and the many descriptions of another microorganism by colleagues.

The first choice was advocated by some of Kitasato's associates (3, 67, 96), Meyer (64), and Howard-Jones (43). Lagrange (57) indicated that Kitasato might have seen the plague bacillus, but his slide preparations and cultures were of *S. pneumoniae*. Lechevalier and Solotorovsky (58), Wu Lien-Teh (95), and Girard (35) accepted the second hypothesis, giving Kitasato token credit in the discovery. The fourth idea, in spite of Kitasato's own remarks, was supported by Hirst (41), Severn (80), and Bulloch (70) among others. Except for a short statement of belief by Wilson (91) and an acceptance of its possibility by Howard-Jones (44), the third concept has not hitherto been considered in detail. It is the compromise view suggested as an alternative by the authors.

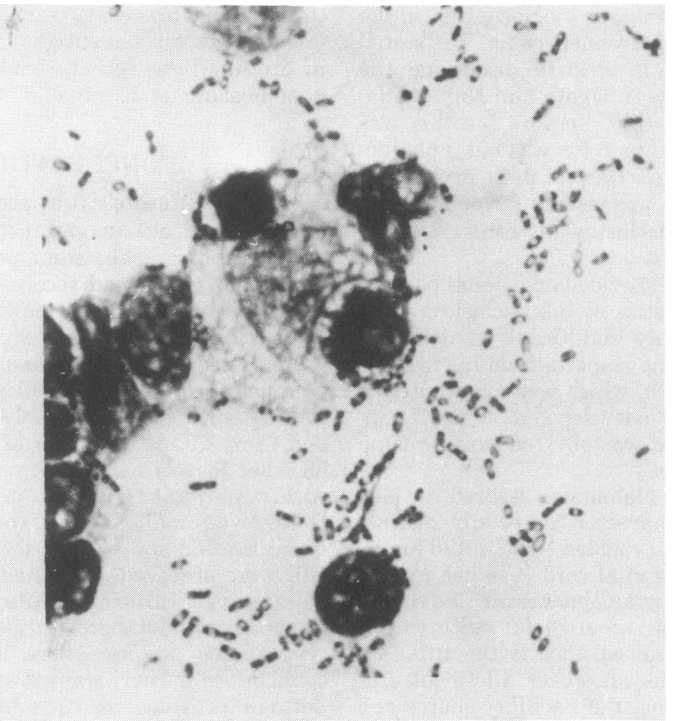

FIG. 3. *Y. pestis from impression smear of spleen of experimentally infected mouse. Wayson's stain. ×1,000.*

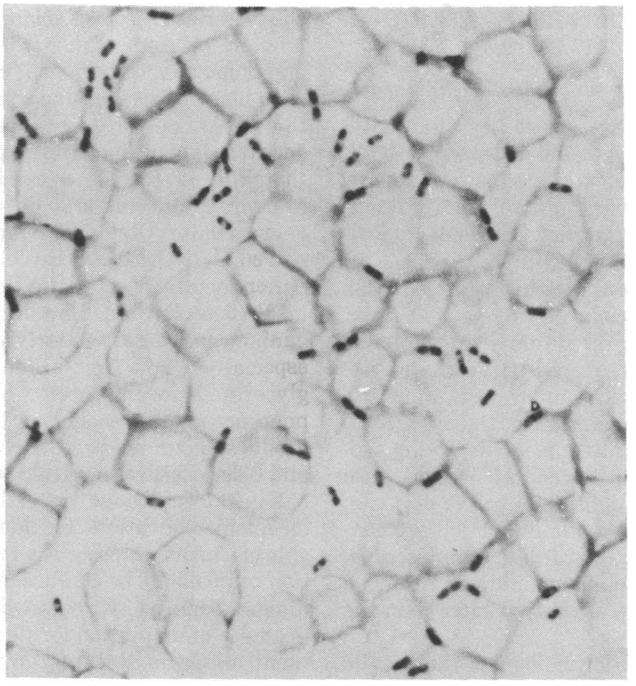

FIG. 4. *Y. pestis from blood of experimentally infected mouse. Wayson's stain. ×1000.*

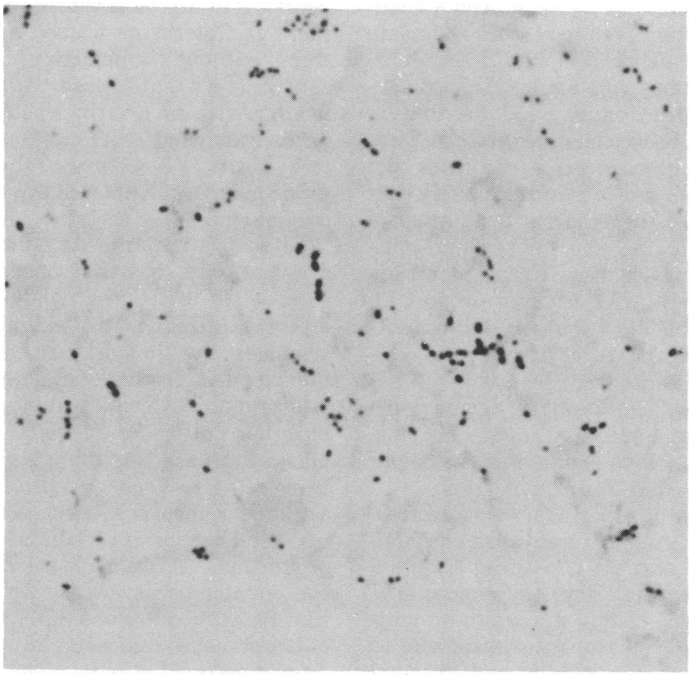

FIG. 5. *S. pneumoniae from 24-hr colony grown on blood agar Gram stain. Kitasato may have confused this organism with the plague bacillus. ×1000.*

AN ANALYSIS OF THE BACTERIOLOGICAL DATA

The Question of *Streptococcus pneumoniae*

Considering first the possibility that Kitasato had both observed and cultured only *S. pneumoniae*, let us re-examine his first report. We are told that the organism resembled *Bacillus cholerae-gallinarum*, now known as *Pasteurella multocida*. The following is what Carl Flügge in 1886 wrote about this organism (32): "They have been described by Pasteur as cocci, but when highly magnified there is no doubt as to their rod character. The fully-grown individuals . . . usually show, when stained, an aggregation of the colouring matter at the ends, as in the rabbit septicemia, the dark poles being separated by an unstained central portion. The bacilli are usually in a state of active division, and thus many forms are found which are constricted in the middle, not unlike a diplococcus, and also numerous young individuals in which the length is only very slightly greater than the breadth."

The similarity in morphology of the plague organism to a diplococcus was pointed out in both the editorial note (30) and Kitasato's report (52) in *The Lancet*. In the former instance the microorganism's nature was ambiguous; Kitasato assured us that it was indeed a bacillus. Obviously the likelihood that Kitasato could have been mistaken is significant; however, two major items rule against this conclusion. Foremost is the quantity and distribution of *Y. pestis* in the body of plague victims. Kitasato might have been in error regarding the bacterium in blood, but it is inconceivable that he did not observe the massive population of the plague bacillus in the buboes and ultimately the internal organs. Kitasato's description is clear enough, and Aoyama's report (3) eliminates the possibility that all the patients were misdiagnosed. However, it was soon realized that some patients were seen who did not suffer from plague (52). Secondly, Zettnow (99) and Kolle (56) agree that at least the slide preparations from tissue exudates show bacilli indistinguishable from Yersin's bacillus.

On the other hand, superficial evidence indicating that Kitasato's cultures were contaminated with, if not entirely consisting of, pneumococci is not wanting. Here the characteristics to examine are colony and cellular morphology, growth on potato, in gelatin, and in broth, temperature relationships, motility, and pathology. Considering the first report, we note at most a very atypical pneumococcal colony. The colonies of *S. pneumoniae* normally are characteristic. Young colonies are 1 mm in diameter, mucoid, transparent, smooth, round, and dome shaped but soon, especially on media with blood or serum, undergo autolysis causing the central region to collapse. The result is a checker-like or nailhead appearance (5). Although type III colonies can become large, they are quite mucoid, and no colony type has a dense center. Rough variants have been observed, but these are mycelial-like in nature. Other factors counter the implication that Kitasato described pneumococcal colonies. The organism grows quite poorly on ordinary media especially when first isolated. He noted that glycerine agar enhanced growth, and although pneumococci do respond in this way (61), they produce lactic acid which causes rapid death and lysis, features not cited in the first paper.

Kitasato's account, nevertheless, leaves room for misidentification. The lancet-shape and capsule of the pneumococcus with careless observation could easily be confused with the enveloped plague bacillus. Kitasato did not record the presence of involution forms or clarify his statement on Gram staining. We should note here that as *S. pneumoniae* grows, it becomes gram negative, and with the coming of autolysis many involution forms are produced (5, 15, 61).

Like the plague bacillus, pneumococci grow poorly, if at all, on potato, and in a gelatin stab there is filiform or beaded growth along the tract without liquefaction. Some doubt is caused by Kitasato's observation that little surface growth on gelatin was seen, which conforms somewhat more to *S. pneumoniae* than to *Y. pestis*. Yet we must remember that Zettnow's photograph showed typical growth at the surface and along the stab.

Kitasato's remarks on optimal temperature are confusing, but they characterize pneumococci at 37°C better than plague bacilli at about 29°C (15). Slight motility characterizes neither organism, but the host range of laboratory animals tends to fit that of plague bacilli. Fowl are resistant to both, and rabbits are not consistently susceptible for *Y. pestis*. However, guinea pigs are usually resistant to pneumococci (90). Interestingly, when susceptible animals are inoculated subcutaneously, edematous exudations and septicemia are found with both microorganisms (61). Death is somewhat faster with the pneumococcus, taking 24 to 72 h. The fact that Kitasato described infrequent involvement of lymph nodes indicates, but does not confirm, a pneumococcal etiology. The route of inoculation, which was not described, can influence pathogenesis, including the swelling of lymph nodes. In general, pathological analysis can dif-

ferentiate the two experimental infections, and Kitasato's description is more suggestive of *Y. pestis*.

The best evidence for streptococci is the slight turbidity of broth. Pneumococci produce broth cultures of low turbidity whereas plague bacilli typically yield flocculent strands. As we shall discuss later, this does not rule out *Y. pestis*.

The evidence, particularly morphology and pathology, is substantial that Kitasato's first paper did not describe solely streptococci, in spite of the accusations by his Japanese contemporaries. Nonetheless, the possibility of mixed cultures is strong. Deferring for the moment further analysis of the contamination hypothesis, we will now attempt to show, through a discussion of the bacteriology of plague, that, except for a few very minor errors, Kitasato's first report may have been essentially accurate.

Bacteriology of Plague

Critics might cite turbidity of Kitasato's broth cultures as proof that he did not isolate the plague bacillus. We should bear in mind that Kitasato's exact words were "somewhat cloudy" which, although nebulous, implies a low turbidity. *Y. pestis*, as Yersin himself reported (97), characteristically produces flocculent strands in liquid media, but the literature indicates that this property is not consistent (13, 29, 63, 64, 86). For instance, in Topley and Wilson's first edition of their text (86) we found the statement that the plague bacillus causes little or no turbidity, and in reference to stalagtite formation this sentence appeared: "This property is not peculiar to the plague bacillus nor is it possessed by all strains of that species." Also, K. F. Meyer (64) mentioned that smooth colony types produce uniform turbidity, whereas rough colonies give granular sediments with a completely clear supernatant. Stalagtite and stalagmite formation were said to be enhanced if sterile oil is placed on the surface, but that smooth colonies recently isolated in vivo do not readily produce these features.

Pollitzer (72) took a cautious stance, warning that "one should not be too dogmatic in excluding the possibility that *Pasteurella pestis* may under peculiar circumstances produce uniform turbidity in broth," but admitted that such growth was rare and indicative of contamination. Petrie (70) never observed turbid growth by *Y. pestis*, but, nevertheless, wrote that variable turbidity might be present and was related to growth conditions and bacterial strain.

With a search of the literature for the origins of the turbid broth characteristic, it soon became evident that the bulk of the fundamental bacteriology was developed between 1900 and 1930 and little new information as to growth and morphology had been added since. The large number of early studies was due to the last great plague epidemic at the turn of the century, which eventually fell upon India. Sparked by the Yersin and Kitasato reports, several plague commissions were organized (41, 70). The Austrians sent Albrecht, Müller, Ghon, and Poech; the German commission included Gaffky, Pfeiffer, Sticker, and Dieudonné; Fraser, Wright, and Ruffer represented the British; the Russian group consisted of Wyssokowitz and Zabolotny. There were also commissions from Egypt, Italy, and Ceylon. Later an advisory committee was appointed by the Secretary of State for India, the Royal Society, and the Lister Institute.

As was mentioned by Meyer (64), broth characteristics of *Y. pestis* were correlated with colony type. Albrecht and Ghon (1) were first to observe two colonial forms: one being small, round, sharply defined, raised, grey-white but bluish in transmitted light; the other being larger with a central nucleus and delicate periphery with wavy edges. Pirie (71) observed that broth inoculated with pure and stable, smooth S type cultures was uniformly but thinly turbid. Others who found S types to yield homogenous growth in broth include Rachinsky (74), Bessanova and Lenskaya (90), and Wats and Pudaval (88). All plague strains tested by Bhatnagar (11) produced many types of colonies, including small, sticky, dew-drop, large, flat, smooth, opaque, and sunflower or fried-egg colonies. In 1943 Jawetz and Meyer (46) again confirmed colonial variation, but suggested that differences in agar thickness and moisture were probably more important than inherent strain characteristics. Both stable S and variable R types were obtained from human buboes by Eisler et al. (31). Peripherally, Wade (87) reported that the type of growth in broth was influenced by various sugars and that glycerin media would not produce stalagtites.

Kitasato might have examined a relatively stable S type, and, therefore, his description of broth would be quite reasonable. His, of course, was a fresh isolate whose activities may have differed a little from microorganisms which had been subcultured numerous times. Certainly, the distinct bipolarity of staining is quickly lost in vitro (64, 95). Stalagtites, which might have developed in Kitasato's cultures with longer incubation, possibly may have been prevented, for shaking or even slight vibration will disrupt

their formation (70, 86). The composition of broth (beef-tea) also may have been influential in production of turbidity. Because there is sufficient room for variation in description and because variation in growth is recognized in the laterature, we believe that Kitasato's claim cannot be denied outright.

Y. pestis is not motile (15, 63). Kitasato's remark that the bacillus was slightly motile is perplexing, for one usually does not record the degree of this characteristic. Brownian movement is the easy explanation, and one should not be distracted by Kitasato's later denials (53). Pollitzer (72) presents the situation succinctly: "Plague strains may show such marked Brownian movement that great experience may be necessary to decide whether a given bacillus is immotile or not." Kitasato, being the first even to see the microorganism, naturally can be excused for his hasty observation.

There are several minor points that would not be worth discussing, if it were not for Ogata and Okada's list of notable differences (67). Kitasato described his organism as producing grey-white colonies that appeared blue by transmitted light. Yersin's was white with iridescent edges. These subjective characteristics commonly are viewed differently with each observer and, thus, are of low significance. Nevertheless, we should mention that other plague workers have interpreted the coloration of *Y. pestis* as did Kitasato (1, 11, 13, 62).

The presence of surface growth in agar or gelatin stab cultures also has been suggested as an important differential feature. Ogata incorrectly listed Kitasato's culture as having no surface growth when, in fact, a small amount was present. More important, but not listed, was Kitasato's description of dustlike points along the stab, which is not quite typical of the plague bacillus. *Y. pestis* normally gives a thin filiform growth along the entire stab with confluent growth toward the top. Older cultures often display small feathery projections, especially near the top of the tube (13, 47, 64, 92). Most workers record a thin growth on the surface, but Petrie (70) asserts that such growth is not always found. Whereas gelatin alone was used by others, Kitasato was forced by the heat of Hong Kong to incorporate agar. The proportion of gelatin and agar, the temperature of incubation, and the size of inoculum could have influenced the appearance of Kitasato's cultures. Perhaps even more important are the variation and lack of uniform consistence in agar and gelatin available at this time.

Furthermore, Ogata's table suggested that Kitasato always observed capsules whereas they were sometimes absent on Yersin's organism. Kitasato reported capsules whose edges were sometimes well delineated but were also sometimes indistinct. We have not been able to ascertain why both investigators' observations of capsules were considered as showing significant differences between the two isolates. Actually, Kitasato's was the more accurate, for *Y. pestis* does not normally produce a true capsule but rather an envelope (2, 77, 95). The existence and nature of the diffused envelope was a subject of controversy. Rowland (77) examined this matter as part of the team investigating plague in India, and found that he could enhance the envelope by growing the organism in broth containing 10% serum, especially at 36°C instead of at 20°C. Capsules were detected occasionally in vivo.

The Gram stain is the most perplexing aspect of Kitasato's report. His statement that he was as yet unable to determine whether or not the procedure is applicable to the plague bacillus could mean that he had not performed the test, an unlikely situation, or that he had difficulty with reagents or in interpretation of results. *Y. pestis* is gram negative, so if some cells retained the stain, then contamination is strongly indicated. Hirst (41) suggested that Kitasato may not have had the necessary aniline dye or may have had problems with the "tricky" method. Kitasato must have had the necessary reagents, since the diagnostic importance of the Gram stain was well recognized by 1894 and Kitasato himself had used it to characterize *Clostridium tetani* (51). However, at this time the technique was not as simple or reliable as today, although even in contemporary times variable performance and misinterpretation of the test have troubled taxonomists (8, 75, 76).

Gram's original procedure of 1883 (7) probably was still in use in 1894. Ehrlich's aniline gentian violet was impure, unstable, and gave inconsistent results (7). High-quality commercial reagents became available only after 1895 (25). Acidity of pus, reagents, or the organisms themselves could cause poor results. An additional difficulty then and now is the decolorization step. Gram employed absolute alcohol. Water-diluted solutions (which occur while washing off of the decolorizer) could increase the rate of decolorization in many instances, but 50% alcohol was found ineffective in decolorizing the plague bacillus (47, 81). Hence, timing is especially important in preventing under- or over-decolorization, and temperature may have some effect as well. Bismark brown was sometimes used by Gram, but other counterstains were found as useful. However,

high concentrations could cause gram-positive microbes to appear negative. Gram and other workers at times did not use any counterstain at all, but this, too, had a disadvantage: gram-negative cells may appear gram positive. Finally, the plague bacillus does not take the usual counterstains well and other stains are used for routine observations (6, 63, 95). Methylene blue was the most frequently used in the 19th and early 20th century. (Incidentally, heat fixation of *Y. pestis* is not as satisfactory as alcohol fixation for demonstration of bipolar staining [63, 72].) Goldenberg et al. (37) contended that Gram's method was decidedly inferior for observing bipolarity.

A clue to the question of Gram staining was provided by Kitasato's assistant Ishigami who, in 1900 (45), noted that unless solutions were freshly prepared, the ends of the plague bacillus (Yersin's) tended to retain weakly the gentian violet dye. In brief, neither the reagents nor the procedure of Gram staining in 1894 was standardized, and results were often inconsistent. There are many reasons why Kitasato would not have been able to ascertain the reaction of the plague bacillus.

We had some difficulty finding a satisfactory explanation of Kitasato's rather damaging data on relationships of temperature to growth. Unless there was a printing error, he was contradictory, for 34°C and later 36 to 39°C were given as optimal temperatures. Hong Kong was too hot for gelatin media, yet he was able to incubate potato at 28°C. No growth was detected on this rather poor medium at 28°C, but at 37°C a thin growth was observed. *Y. pestis* is said to have a minimal growth temperature of −2°C (84), 0°C (48, 63), or 4°C (70) and a maximal temperature of 40°C (15), 43°C (48, 63, 70), or 45°C (84), a very wide range for a pathogen. However, optimal growth is definitely below that presented by Kitasato. Some figures found in the literature include 25 to 30°C (13), 27 to 28°C (84), 28°C (63), 28 to 29°C (48), 30°C (70), and 30 to 35°C (86). Sokhey (83) did report that an inoculum of 500 colony-forming units on nutrient agar would not grow at 27°C, but at 37.5°C would yield some colonies. However, an inoculum of 5,000 colony-forming units grew best at 27°C. When the plague bacilli were plated on blood agar, no differences were found with concentration, and optimal growth was at 27°C. Kitasato did not state how he judged optimal growth. We can only assume, for the sake of argument, that Kitasato's work was limited and not quantitative and that his subjective impressions were wrong.

Certain aspects of plague pathology have been controversial. One aspect pertinent to our discussion is septicemia. Kitasato's associates criticized his emphasis on diagnosis from blood smears and cultures rather than concentrating on the bubo. Except for the period near the victim's death, when the bacillus spread to the spleen and other organs, plague was supposedly limited to lymph nodes. The British Plague Research Commission sampled the blood of 28 patients, taking a total of 74 specimens. Of these, 30 were culturally positive but only 6 showed the bacilli in smears (81). Teissier et al. (85) had rather different results, finding septicemia in nearly every case and often in the early stages of the disease. Kirschner (50), from 237 patients, obtained 212 positive blood cultures. Ohoto (69) was similarly successful with 72.2% positive results, and Schoebl (78) and Gonzaga (38) agreed that bacteremia occurs early. With additional investigations it was soon established that showering of bacilli from the bubo into the blood is frequent, early, generally progressive, and does not necessarily indicate a fatal outcome. Today blood cultures are recommended for early diagnosis (33, 63).

Kitasato's report has been poorly understood. Although he claimed to have observed the plague bacillus in blood smears from 25 to 28 patients, it often required several slides and patient, careful examination to detect even small numbers of the microorganisms. As such, Kitasato readily realized the importance of blood cultures. Kitasato's observations, far from being nonsense or indicative of contamination, seem very reasonable. He apparently was the first to recognize the early bacteremic state and the diagnostic use of blood cultures at a time when absolutely nothing was known about the infectious process. It should be noted that, according to Ishigami (45), most of the patients seen in Hong Kong were in the final stage of the disease.

There were a few minor anomalies in Kitasato's experimental animal infections. Methodology was not specified, but subcutaneous inoculations were the common approach at the time. Dosage was not given either, since quantitation was not yet appreciated. If a large inoculum or a particularly virulent bacillus was used, then death might have been caused by toxemia, with the result that nodules might have been indistinct or even absent on the otherwise swollen spleen (70, 95). Because inbred strains had not yet been developed, Kitasato's results could have varied markedly with each laboratory animal. This might also explain the absence of swollen lymph nodes in every case. It is interesting to note that plague-infested

rodents in the wild normally do not develop enlarged nodes or spleens (J. D. Marshall, Jr., personal communication).

A discussion of *Y. pestis* would not be complete without mentioning its pleomorphism. Cellular morphology is very sensitive to environment, and varieties of coccal bacillary, and even moldlike forms, have been observed (47, 77). Different sugars can influence shape and staining characteristics (87). Within the same broth culture one might find coccal forms at the top and uniformally staining long bacilli toward the bottom (89). Low incubation temperatures can produce elongated forms (12), and Yersin (97) described long bacilli in blood preparations. Photographs (14, 97) of such smears confirmed Kitasato's note that the central area of the plague bacillus took up less stain than those cells located in buboes. Of course, involution forms display a wide assortment of shapes. Hankin and Leumann (40) were able to enhance their formation with media containing 2.5 to 3.5% salt.

We should furthermore point out that Kitasato was working in the midst of an epidemic. The atmosphere for the bacteriological investigation of plague, whose etiology and means of spread were yet unknown, was not conducive to cautious, well-detailed, and controlled work. The first few days probably were intense with first impressions governing the scope of action. Kitasato's report was dated July 7, only 3 weeks after his arrival in Hong Kong. Under the circumstances, it is not unreasonable for even an expert bacteriologist to make some errors. Despite his lack of facilities, Yersin was probably at an advantage working alone, apart from the frenzy of the hospitals and the distracting formalities and social interactions associated with governmental commissions. With these considerations, we feel that Kitasato's report stands up fairly well to criticism. We have little doubt that he did isolate, study, and reasonably characterize the plague bacillus. Kitasato should not be denied this credit.

CONTAMINATION

The matter of contamination remains: when and how did it occur, and why was it ignored? Secondary infections due to streptococci, including pneumococci, were a common occurrence in plague victims (3, 35, 67, 78, 81, 96). It is almost certain that *S. pneumoniae* was Kitasato's contaminant. This was the opinion of his Japanese colleagues (3, 67, 96), Lagrange (57), Wu Lien-Teh (95), and Girard (9). Even Zettnow (99) suggested the streptococcal nature of organisms found in Kitasato's blood slides. We have previously sketched some similarities between the plague bacillus and the pneumococcus.

Many plague investigators have commented on the organism's superficial resemblance to the pneumococcus. The ease in which contamination can occur is manifest. An important point of the inquiry is the determination of the approximate time when Kitasato's cultures became contaminated and ultimately replaced. The major report was completed July 7, 1894 (52); Koch received the cultures January 1895 (99); Aoyama's paper (3) was dated June 1895; and Ogata (67) reviewed the work conducted in 1896.

Although, as we have demonstrated, Kitasato's report may not have reflected contamination, the chances are good that the event occurred in June 1894 with a blood culture. Subsequent growth studies of broth and optimal temperature would conform to pneumococci. By the end of the Hong Kong investigation many cultures would be mixed, including those selected for the return trip. Eventually the contaminant would overwhelm the plague bacillus.

Even if the supposition that the report was essentially accurate is indeed correct, contamination still would have taken place in July. Yet the cultures of the following January yielded only plague bacilli. Girard (35) was intrigued by this paradox and sought the answer in microbial interactions as influenced by temperature. Primarily concerned with pneumonic plague being masked by pneumococcal pneumonia, he found that each organism developed independently and in different lobes of the lung. When cultured together in serum broth at 34°C, the pneumococcus developed faster, retarding *Y. pestis*. Incubating at 20°C allowed greater growth of the plague bacillus, but for up to 48 h the pneumococcus still was dominant. Nutrient broth without glucose or serum did not significantly alter the situation. At 20 to 26°C plague bacilli were always isolated, but at 37°C they were virtually eliminated on subculture. Girard observed similar effects on solid media, but after 5 days the pneumococcal colonies lysed permitting *Y. pestis* to develop. Of great relevance was the result obtained when mixed cultures were injected intraperitoneally into guinea pigs. Only the plague bacillus was found in two of four animals. From this work Girard surmised (9) that on transit to Germany the pneumococcus lysed leaving gram-negative debris. The still inhibited plague bacilli would recover on transfer as a pure culture.

Tantamount with this discussion is the mys-

tery why Kitasato did not recognize the contaminant, and if he did, then why he chose not to admit the error. It is understandable how at first he could miss the presence of pneumococci. Surely, once Yersin's results were published and Kitasato had a chance to examine his cultures leisurely, contamination would have been detected. The pneumococcus was no stranger to the German-trained bacteriologist, for during Kitasato's 7 years at Koch's laboratory the etiology of pneumonia was debated fully (4).

KITASATO'S REEVALUATION

Why then did Kitasato not admit that his cultures had become contaminated? To explain this we must leave bacteriology and speculate on other matters involved with the conduct of science.

Kitasato was a celebrated figure in Japanese medical circles. His discoveries and honor of being selected to work under Koch's tutelage established him as dean of Japanese bacteriologists. He was the perfect candidate for the plague commission. But such recognition still did not fulfill Kitasato's desire for his own research institute. Returning in 1892 to his homeland, he was so disappointed that no laboratory was provided for him that he almost left Japan. Supposing that he recognized the contamination of his culture, he would have to come to a critical decision. He might have lost confidence in his first report, not knowing which characteristic was due to the pneumococcus, yet to deny the data would mean a loss of credit for discovery. An admission of subsequent contamination or mistaken identity would be a grave loss of personal prestige and honor, shattering his dream of a research institute. The competition by envious Japanese co-workers could have been seen as a continuing threat to his position. On the positive side was the possibility that Yersin might have been wrong. Perhaps Kitasato felt that until new investigations were carried out he could stand on his present results. In the interim he could expand his small laboratory and build an even stronger reputation that would withstand the eventual public knowledge of his error. Kitasato diminished his bacteriological research on plague and undertook work in the broader field of public health and hygiene.

In 1899 plague invaded Japan, and with accumulating evidence countering his later descriptions of the plague organism, Kitasato was obliged to admit his error. He did so in private as part of an investigative team studying the epidemic in Kobe. However, he did not agree that his cultures were of pneumococci or any other recognized bacterium. Instead, he mentioned that his isolate was associated with plague and perhaps important in its pathogenesis. This is not quite the same as declaring his organism to be a contaminant, as some have translated or interpreted. The nuance is important since two independent Japanese critics (68, 96) had quoted Kitasato in the same terms. Thus, Kitasato's concession is not complete. Yabe related (96) that Kitasato claimed to have seen Yersin's bacillus in the bubo but chose the septicemic organism as the agent of plague. This statement may have served as a cover for contamination because Kitasato had earlier asserted that the blood-borne organism was the same as that found in the bubo. He also might have actually believed that the pneumococcus was integrally related to the disease process, but it is more likely that he was desperately trying to preserve some creditability.

Howard-Jones (4, 43) concludes that the latter hypothesis was the more probable. Kitasato's assistant in Hong Kong, Tohiu Ishigami, published in 1900 a textbook on plague that was revised by Kitasato. In the book (45) one finds a statement that Kitasato confirmed that Yersin's bacillus was the cause of plague, but believed that on invading the blood it took on a second appearance, becoming gram positive.

In 1899 the government had incorporated Kitasato's private laboratory into the Ministry of Interior. He obtained his dream of directing a major institute in 1905. Secure in his position, Kitasato eliminated the controversy by ignoring it, or, when necessary, by diffusing it. The controversy was never discussed at any international medical congress nor at the 1911 International Plague Conference at Mukden, China, where Kitasato presided over several sessions on bacteriology and pathology and was vice-chairman of the meeting (94).

In 1926 Lagrange attempted to discredit Kitasato's claim to discovery. This paper was very effective, influencing Meyer (64) and Scott (79) among others. Within the article is the following declaration: "However in 1925, as chairman of the Congress of the Far Eastern Medical Association, before 400 members, amongst whom were 250 foreign delegates, Kitasato is to be honoured for having publicly stated that Yersin alone was the discoverer of the plague bacillus." This appears to be the first and only public admission of error by Kitasato. It is not known where Lagrange obtained such information, but it apparently is not true (43, 44).

Our scenario, we believe, is plausible. Others, which may be equally reasonable, may be considered, but the truth of any probably will

never be ascertained. What does stand, however, are the various descriptions of the nature of Kitasato's isolate(s).

CLINICAL APPLICATIONS

The controversy serves as a warning to clinical microbiologists who may someday be required to identify the ancient scourge. The laboratory diagnosis of plague has its pitfalls even today, particularly at the presumptive level.

Standardized Gram stain procedures and reagents have virtually eliminated any chance for confusion with pneumococci or other gram-positive bacteria, but some gram-negative bacilli can at times resemble the bipolar *Y. pestis* (64). Conversely, plague bacilli can mimic other microorganisms. Direct microscopic observation of bubo aspirate or blood smears, although useful, cannot alone render presumptive identification. *Y. pestis* grows quite slowly on agar, requiring 2 days for macroscopic visibility especially at 37°C, and, although morphology may vary, it is this slow grow that is diagnostic. Hence, a negative Gram stain, bipolar staining, slow growth on agar, and optimal growth at 27 to 28°C are normal prerequisites for presumptive identification of *Y. pestis*.

Confirmation is best achieved by specific lysis with bacteriophage at 20°C (6, 17, 37, 39, 63). At 37°C the bacteriophage can also lyse *Y. pseudotuberculosis;* therefore, two sets of cultures are prepared for incubation at the two temperatures.

Another suitable approach is serology, of which hemagglutination is the most specific and sensitive (22–24, 37). In addition, fluorescent-antibody techniques (66, 93) have been employed successfully, although they are not without fault (36, 73). The Center for Disease Control confirms *Y. pestis* by the following criteria (20): (i) microscopic and colonial morphology, (ii) lysis by specific bacteriophage, (iii) staining with fluorescent-antibody conjugate to *Y. pestis* fraction 1, and (iv) production of characteristic lesions in mice that are positive by fluorescent antibody.

CONCLUSIONS

Kitasato's plague studies are as controversial as the nature of the plague bacillus itself. It is difficult to compare the 19th and early 20th century microbiological literature with Kitasato's report when several different descriptions of the organism's growth and colonial morphology can be found. Contemporary textbook accounts are largely based upon the results of early, diverse plague commissions and upon modern investigations of endemic plague strains. It is a significant possibility that the characteristics of the plague bacillus may be altered in an epidemic affecting man. Although specific means of diagnosis are now available, many presumptive bacteriological procedures are subject to the same problems that underline the historical dispute.

From our analysis we are confident that Kitasato had examined the plague bacillus in Hong Kong during late June and early July 1894. For the most part, his report was an accurate description of the bacterium, and the document alone was sufficient for Western scientific circles to give Kitasato a share in the discovery. Aside from this purely historical consideration, Kitasato's note served well as a foundation for further research and field studies undertaken by many investigative commissions. It is only because of the similarity of the plague bacillus to the pneumococcus under specific but common conditions that Kitasato was lead to subsequent error and doubt. Kitasato's face-saving efforts merely furthered the challenges to his claim of discovery. Nevertheless, the contribution of Kitasato to the diagnosis of plague and its history is significant, and this work will endure.

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