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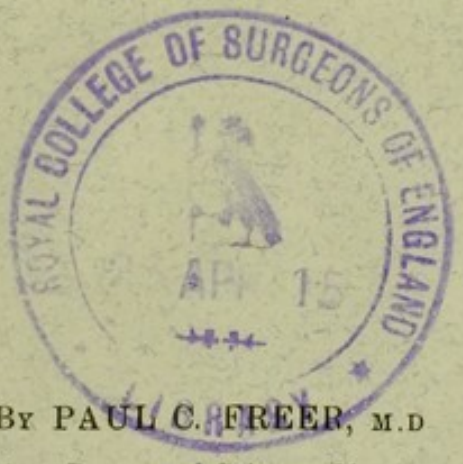

By PAUL C. FREER, M.D

Director, Bureau of Science, Manila, P. I

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THE INOCULATION ACCIDENT IN MANILA, P. I., IN 1906.

BY W. M. HAFFKINE,

AN article by Dr. Paul C. Freer, Director of the Bureau of Science, Manila, P. I., was published under the title "Accidental Inoculation with the Virus of Plague," in the *Journal of the American Medical Association* of 13th April 1907. The article is to the following effect:—

"ACCIDENTAL INOCULATION WITH THE VIRUS OF PLAGUE.

BY PAUL C. FREER, M.D.,

Director, Bureau of Science, Manila, P. I.

"LAST fall, as the unfortunate result of the accidental contamination of the cholera vaccine with a culture of plague bacilli, a number of natives were inoculated with plague and several died. The occurrence makes of interest the following account of the work with the cholera vaccine.

"For the last three years Dr. Richard P. Strong of this bureau has been working on a cholera vaccine which consisted of an extract of the killed, digested and filtered organisms, later modified to a mixture of such an extract with one obtained by shaking on a shaking machine the living organisms in distilled water and then filtering. Obviously this product is always carefully tested and is absolutely sterile, and the health department has used it on about seven thousand persons in the Philippines. One-half of the prisoners in the Government prison at Bilibid were vaccinated with this vaccine and one-half unvaccinated. The same was done in a number of villages in the surrounding territory. Needless to state, no bad results followed from these vaccinations. There is a moderate general reaction following the inoculation, but the local reaction is very slight. The reaction from vaccination with this cholera vaccine is not as serious as that which frequently follows the small-pox vaccination, yet a high blood immunity results.

"Subsequent experiments in the town of Angat, where one thousand and seventy-eight persons were vaccinated, showed that after vaccination there were one hundred and twenty-two cases of cholera, one hundred and twenty-one of which were among the unvaccinated and only one among the ones vaccinated. In the prison we had no further epidemic of cholera after the vaccination, but no conclusions can be drawn because the cholera was stopped by sanitary measures. It may be stated, however, that since the vaccination we have had twenty cases of cholera, eighteen of which were among the unvaccinated and two among the ones vaccinated.

"Dr. Strong continued his work on immunity against cholera by vaccination, as he was convinced that this means was one which would aid in the prevention of large epidemics in the future, since it is known that apparently healthy people frequently carried about in their intestines cholera spirilla, which are passed in the stools, and hence quarantine and sanitary measures cannot always be considered effective safeguards against a cholera epidemic. Certainly, the end sought for is a great one and the results satisfactory.

"Last year, in a Medical Congress at Berlin, the method of vaccination by means of the entire organism and not by its extracts was advocated. Haffkine has urged the use of the living organism and believes that by its use a higher immunity can be obtained. Dr. Strong, of necessity to complete his argument, needed to make some vaccinations by this method, as he was convinced that it was not superior to the one generally employed by this laboratory, but, obviously, with such arguments in literature others would come forward attacking his results. In employing the living organism, it is necessary to use a 24-hour old culture owing to the fact that after this time changes take place in the culture owing to the formation of ferments, death of a large number of bacteria, etc., and, consequently, while the method allows of every test as regards the purity of the cultures up to 24 hours before their use, after this time (when many cultures are employed) no satisfactory test of its purity can be made and no test on animals can be carried on, since the cultures must be inoculated 24 hours after their preparation. Inoculations had been carried on in Bilibid several days without accident. On the day of the misfortune

twenty-four men were inoculated, but this was not compulsory. The history of this vaccination is about as follows:—

"The culture was carefully carried out and identified, and was known to be a pure culture of the cholera organism up to 24 hours before the time when the vaccination was made. A large number of tubes of cholera media were inoculated from these pure cultures and placed in the incubator. On the following morning the cultures were each suspended in one cubic centimeter of saline solution, the whole mixed, and inoculations, as was necessary, made immediately after the preparation of the suspension. It is supposed that some one placed a 48-hour virulent plague culture among the cholera cultures, the blue pencil marks which designated the culture having been erased from the glass by handling. This view is supported by the fact that on the afternoon of the day of inoculation a 48-hour virulent culture was missed from the incubator. It was known certainly that one of the five plague cultures prepared two days previously, as was shown by Dr. Strong's note-book, had been removed from the incubator, but it was not until two days later, when he wished to examine the opsonic index of five guinea-pigs, for each one of which a 48-hour richly-grown culture had been prepared, that the plague culture was missed.

"It was proved by a technical committee of three physicians appointed by the Governor-General to investigate the matter that a 48-hour richly-grown plague culture spread over the entire surface of the entire slant resembled some of the cholera cultures so strongly that they were unable to identify or to pick out such a plague culture by its gross appearance when it

was placed among a number of the cholera cultures of the strain employed. It was also shown that a hanging-drop preparation and the stained microscopic one made from the mixed suspension of all the cholera cultures contaminated with the plague one (as used for the human inoculations) did not reveal the suspicion that the fluid was contaminated with plague bacilli, since no bipolar staining organisms were visible and, evidently, the plague bacilli were in too small numbers or those present did not show any sufficiently distinctive morphology to separate them from the pleomorphic cholera organisms in the saline solution. Nevertheless, this same suspension, in which plague bacilli were not detected by microscopic examination, when injected subcutaneously into guinea-pigs and a monkey, caused, after several days, death from plague infection. It has not been ascertained who placed the plague culture among the cholera ones after all possible tests of the cholera culture had been made. An investigation of the entire matter was pursued by the Government prosecuting attorney at our request, and later the Governor-General, in order to satisfy public opinion, appointed a committee of eight, six Filipinos, one American and one Englishman, three of which committee constituted a technical committee, for investigation.

" While the serum division of this laboratory is also in charge of Dr. Strong, and while this division prepares our regular cholera vaccine, it must be clearly understood that the regular preparation of our cholera prophylactic had absolutely no connection with this matter and that the vaccine used by Dr. Strong on the day of the accident is entirely distinct from the preparation with which he is identified. The

method used on the last twenty-four prisoners is the one which is best known by the names of "Ferran" and "Haffkine." It is perhaps unnecessary to add that the laboratory has prepared several million units of vaccine virus and several thousand doses of plague prophylactic which have been used (in addition to the cholera prophylactic) with good results and without accident."

I.

The study of vaccination against cholera, according to the method worked out in 1890-93, in the Paris Pasteur Institute, began in India in April 1893, that is, some 13 years previous to the Manila accident. The procedure which was adopted for cultivating and inoculating the vaccines was described, with great detail, in a pamphlet published in Lahore, Punjab, in 1894, by Mr. E. H. Hankin, M.A., Chemical Examiner and Bacteriologist to the Government, Surgeon Lt.-Colonel Ch. H. Owen, I.M.S., Medical Adviser to the State of Patiala, and myself, under the title of "Technique of Haffkine's anti-cholera inoculation." The pamphlet was reproduced in the *Indian Medical Gazette*, Calcutta, in June, 1894. A more detailed version of the same "Technique" was published in the journal just mentioned two years later, in June 1896, by Lt.-Colonel (then Surgeon-Captain) Hare, I.M.S., the present Sanitary Commissioner of Eastern Bengal and Assam. In the issue of the same journal for November, 1896, in an article entitled "Technique of Haffkine's method of preparing fixed cholera vaccine," the same officer described the method of transforming cholera virus into the vaccine strain.

The extent to which the above technique rendered the operation safe, and the facility with which it was learned by non-specialists and by subordinates, may be gauged from the following facts.

The inoculations were at first carried on by myself, and between 1893 and 1896 were introduced in over 100 towns and villages in the Indian Plains and the Himalayas. I prepared the vaccines in trains, while travelling from place to place, in the ordinary passenger carriages, and at Railway stations, while waiting for the arrival of trains; also in tents, in "dâk-bungalows" and rest-houses; in rooms placed at my disposal for a day or two in dwelling-houses and transformed for that time into "laboratories;" and sometimes (in Calcutta and Agra) in established laboratories, in which various work was carried on at the time by other workers. Between April 1893 and July 1895, 42,197 people, who received in all close on 70,000 injections, were inoculated under these conditions, and in 1896, a further 30,000 were so operated on. A large proportion of the inoculated lived under medical and administrative supervision, so that any unusual effect of the inoculation could not have escaped notice. Thus, the operated on of 1893-95 comprised officers, non-commissioned officers and men belonging to 64 British and Indian Regiments; contract labourers of 45 tea plantations in the Brahmaputra and Surma Valleys of Assam; inmates of nine civil jails; children of boarding and other schools, etc.

In 1894 the Municipal Corporation of Calcutta voted a grant to its Health Office for applying these inoculations experimentally. A Hindu Medical Inspector, Mr. Jonomanjoy Chowdry,

was put on to this duty. He was assisted, in the preparation of the vaccines, by two other Hindu officers, Messrs. Jogendranath Dutt and Sasi Bhusan Ghose, of the Calcutta Health Office. None of these doctors had been acquainted with bacteriological work before. Apart from the preparation of the cholera vaccine, which, after a period of instruction, they were left to carry on independently, they became soon engaged in a variety of other kindred work, notably in connection with infectious diseases affecting the ponies, buffaloes and bullocks in the "gow-khanas" of the Municipal scavenging department; with outbreaks of rinderpest and other epizöotics reported from Calcutta and Howrah; and in connection with the then Health Officer's studies of pustules and vaccine lymph, in the Municipal vaccine depôt. Cholera inoculation in the bustees and suburbs of Calcutta, with cultures prepared in the Health Office, was carried on daily for two years, and the results were closely followed by various members of the Municipal Corporation. The number of persons inoculated was 7,690. About two-thirds of them underwent inoculation twice, at an interval of five days, so that the number of injections of vaccine performed was about 13,000.

In 1896 anti-cholera inoculation was started at Purulia, on the Bengal-Nagpur Railway. At first Surgeon-Captain (now Major) J. C. Vaughan, I.M.S., Superintendent of the Campbell Medical School, Calcutta, then Deputy Sanitary Commissioner for the Chota Nagpur Circle, was in charge of the work. Two Hindu assistant-surgeons were appointed to operate under his orders. Surgeon-Captain Vaughan was, after a few months, ordered away to the Tirah campaign; and from that time on, one of

the assistant-surgeons, Gopal Chunder Mukerjee, was left in independent charge, the other co-operating with him. In this depôt the operations were performed every day for eight and-a-half years, till the end of 1904; and the number of persons inoculated was as follows :—

In	1896	...	... 4,413
„	1897	...	... 10,950
„	1898	...	... 4,296
„	1899	...	... 2,388
„	1900	...	... 13,291
„	1901	...	... 3,453
„	1902	...	... 3,144
„	1903	...	... 2,202 and
„	1904	...	... 1,623
In	all	...	... 45,760

The material for these inoculations was manufactured, examined and used in an improvised laboratory, by workers who had had before no bacteriological training. The inoculated were almost exclusively coolies contracted for transport to Assam, and who were, at the time of inoculation, and during their subsequent journey and service, under the supervision of Government and labour supply officials. The slightest mishap would have at once been known to the emigration authorities and reported upon.

In none of the above operations has an untoward result at any time come to knowledge; and quite certainly no accident of any gravity has ever occurred. Anti-cholera vaccination has thus been demonstrated to be as free from danger as any method devised by man.

II.

Just as in vaccination against small-pox and inoculation against hydrophobia, as well as in Professor Kolle's inoculation which the Manila laboratory has advocated and applied against

plague, the vaccine used in anti-cholera inoculation is a live virus, and is not sterilized before injection. Nevertheless the details mentioned in the preceding paragraphs and referring to a long testing in India, indicate that the methods followed in the preparation and use of that vaccine render it, even in relatively inexperienced hands, safe from contamination, and eliminate sources of mishap.

Again, neither in the anti-cholera vaccine, in the emulsion of spinal cords containing live hydrophobia virus, as used for anti-rabic inoculation, nor in the live virus of small-pox vaccine lymph, nor in any of the sera and drugs used in hypodermic injections in man and animals can contamination with harmful germs be detected with certainty by the microscope; but obviously this does not mean that either of the methods mentioned, now so extensively practised, is insecure. In the anti-cholera inoculation, the examination by the microscope is an adjunct so important that, when applied in the way in which it has always been applied in India, an accident such as occurred in Manila is impossible; and an extraneous culture like that of plague would be detected immediately. Nevertheless, entire elimination of mishap is secured, obviously, not by microscopic examination alone, but by an *ensemble* of operations of which some precede and others follow that examination, and by the general dispositions of the work.

III.

In the accident at Manila it is essential to note that the cholera vaccine did not get contaminated by plague *spontaneously*. Such an eventuality may be treated as outside all prac-

tical possibilities. Every bacteriologist of experience will take on himself to say that the spontaneous invasion of a bacterial culture by germs of cholera, plague, glanders, anthrax, diphtheria, tubercle and certain other specific microbes is not to be thought of any more than the spontaneous contamination of such a culture with arsenic or strychnine. There are a few pathogenic species,—like those causing abscesses, for instance,—which are, upon occasions, found to contaminate cultures and other materials. In Manila a plague cultivation, presumably free from admixtures of any kind, was put by the operator into a watery suspension of cholera cultures, the latter probably being at the time quite pure and uncontaminated also.

The accident became possible by the operator deviating, amongst other points, from the following two rules prescribed in the anti-cholera inoculation, *viz.*, the contents of the culture tubes should not have been mixed ; and each tube, immediately before being used, should have been, apart from other examination, submitted to an examination by the microscope. Under these circumstances a plague culture would have never passed for a cholera culture.

It is not stated that the material injected into the men in Manila had been examined by the microscope ; but the Technical Committee of enquiry have found that when they mixed, in an experiment *ad hoc*, the contents of one cultivation tube of plague with that of a large number of cultivation tubes of cholera (as had been actually done at the time of the human inoculation), and examined a drop of the mixture, the plague bacilli were overlooked under the microscope. Such a result is, of course, only too probable.

IV.

The details of the Manila accident reported by Dr. Freer tend to show that its occurrence did not stand in connection with the degree of perfection or deficiency which belong to cholera vaccination or to any bacteriological method as such.

In all pharmacies and shops where collections of drugs are kept, simple dispositions are adopted, on the responsibility of those in charge, for making it impossible for dangerous materials to get mixed with harmless ones. Obviously these measures had, at the time of the accident, not been in force at Manila; and it must be presumed that some particular circumstances which existed at the time did not allow of the necessary dispositions being taken.

It is, further, a practice with those in possession of materials of various kinds, particularly harmful ones, to differentiate these by inscriptions or marks of identification. The first action of an apothecary, when handling his phials, is to look at the label, independent of any other mode of examination,—chemical, physiological, or other,—which may be at his disposal for identifying the materials. The labelling of cultivation tubes is one of the articles of instruction mentioned in bacteriological text-books and lectures. In the pamphlet on the "Technique of the Anti-cholera Inoculation" referred to above the procedure is enjoined on p. 8, paragraph 12, where it is stated: "Mark the inoculation tubes unmistakeably, in order that the kind of vaccine they contain and the date of their inoculation shall be known." The operator who omits to provide his preparations with clear inscriptions, or omits to take notice

of them when using the preparations, renders obviously nugatory the whole of the safeguards, however perfect, which have been devised for preparing his materials in a pure condition.

In Manila, where the tubes of vaccine for inoculation in man had to be incubated in the same box as tubes of virulent plague, and other persons than the vaccinator had access to the same incubator and to the same batches of tubes, it is stated that the inscription on the tube was not ascertained previous to using it. The accident was, therefore, in every way of the same kind as would be incurred by a pharmacist mixing up a poisonous substance with the drug which he is preparing; and it was preventible also in the same way as such accidents are prevented in pharmacies.

V.

The idea that the Indian method afforded no possibility of avoiding the accident appears to have been based on the following considerations mentioned by Dr. Freer:—"While the method allows of every test as regards the purity of the cultures up to 24 hours before their use, after this time (when many cultures are employed) no satisfactory test of their purity can be made and no test on animals can be carried on, since the cultures must be inoculated 24 hours after their preparation." The accident was caused by the mixing up of a plague culture with the cholera vaccine at the very moment of using the latter. Obviously, no test applicable 24 hours or any longer interval before that moment, nor any test applicable a shorter period, even one hour, before, could have prevented the result of a confusion thus made. To avoid such a confusion,—once the general

dispositions in force at the time permitted of its occurrence,—a test or tests were required applicable at the moment of using the tubes. The most direct of such tests were those mentioned already, *viz.*, (1), the reading of the inscriptions made for that purpose on the receptacles; and (2), the examination under the microscope of the contents, in the manner prescribed for the anti-cholera inoculation. There were also certain other aids, but the special object of the present article does not seem to require entering into them.

VI.

The Manila Officers have gracefully recognized as conclusive the results of the Indian cholera vaccination studies, and have themselves contributed not a little to the subsequent investigations on the matter. A few years ago, before introducing that vaccination in the Philippines, the very able director of the laboratory there made, in the Institute for Infectious Diseases in Berlin, a study of the vaccine used in these operations. The vaccine,—as described in the publications referred to higher up,—is a strain of cholera germs transformed into a virus of exalted, fixed potency, by cultivating it, in accordance with certain rules, in the peritoneal cavity of the guinea-pig. The bacteriologists in Berlin compared this vaccine, from the point of view of its immunization properties, with the natural strains of cholera germs maintained by cultivation in laboratories, and convinced themselves of the significance of the transformation imparted to the vaccine. Consequently, in the Phillippines, a strain has been adopted,—for preventive inoculation in man,—which is prepared and maintained in the way in which this is done in India; but

instead of operating with that substance itself, they advocate the plan of leaving it to soak in water, at the temperature of the incubator, and using the resulting soluble extraction ;—a plan to which, as Dr. Freer mentions, it has been objected at the last International Medical Congress in Berlin that the extraction might not have the protective effect which the vaccine itself had. The Manila officers are under the impression that one of the advantages of the watery extraction is that it is free from the possibility of misadventure which they have had with the vaccine. The same department of the Manila institute that prepares the extraction of the cholera vaccine prepares also soluble products of other microbes, such as the toxine of diphtheria ; that of tetanus, of which the admixture of a few c.c.s would, of course, suffice to kill a horse ; probably, solutions of snake venoms for the preparation of anti-venene ; and so forth. If these microbial toxines and solutions, or, for the matter of that, any alkaloids or other drugs were to be so kept as to permit of their being inadvertently mixed with one another ; if, before using them under such circumstances, the inscriptions on the receptacles were not ascertained ; physical differences of the contents overlooked ; and the contents mixed together and used ; that is, if a concurrence of circumstances took place identical on all points with that which they have had the misfortune of having at the time of the late accident,—the extraction of the cholera vaccine would obviously be exposed to the same possibility of misadventure as has occurred in the use of the vaccine. Of course, it is not suggested that the above is the condition prevailing normally in the Manila laboratory. The latter has, in a few years,

and most deservedly, taken a place amongst the first class institutions of its kind in the world. It is only unavoidable now to make it clear that their accident has not been conditioned by the peculiarities of the anti-cholera vaccination method, as, they believe it has.

VII.

Referring to the facts mentioned in section 3 of Dr. Freer's article (" Subsequent experiments in the town of Angat.....In the prison we had.....and two among the ones vaccinated "), – they do not unfortunately convey indications as to whether the extraction of the cholera vaccine confers on man the immunity against asiatic cholera as has been obtained with the vaccine. Such an indication would have been most welcome to me, but obviously Dr. Freer had no data yet for making any definite affirmation on the matter. In the case of the town of Angat, the number of non-vaccinated inhabitants, among whom 121 cases of cholera occurred, and the degree of exposure to infection, in the case of the vaccinated and of the non-vaccinated, have not been made known, and consequently a deduction from the figures given is impossible. In the Government prison in Bilibid, where one-half of the prisoners had been vaccinated and one-half not, after which event 18 cases of cholera occurred among the non-vaccinated and 2 among the vaccinated, the result would have been quite important, and, under requisite conditions, even conclusive ; but, presumably, between the time of vaccination and the date of the cholera occurrence, changes of which details are not related in the article had taken place in the composition of the prison population ; for Dr. Freer states that no conclu-

sion could be drawn from the facts related about that prison.

It is to be hoped that the above explanations will not be viewed as implying any want of consideration for the Manila scientists, whose efforts, ever since the establishment of their laboratories, have enriched science with numerous contributions of a truly remarkable character.
