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W. M. HAFFKINE.

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PREFACE.

The present Note has been written in connection with certain official correspondence regarding vaccination against cholera and the preparation and study, in India, of a devitalized form of anti-cholera vaccine. The purpose of the paper has rendered it desirable to make certain references to the support which the author's personal views have received so far from the results of other workers.

DIVISION OF THE CONTENTS.

Part I deals with the preparation of an anti-cholera vaccine ;
Part II with immunization of man against cholera ;
and Part III with the anti-cholera vaccine after its devitalisation.

PART I.

ANTI-CHOLERA VACCINE.

PART I.

PREPARATION OF AN ANTI-CHOLERA VACCINE.

THE VIRUS OF CHOLERA.

The preparation of a vaccine comprises a number of separate problems, the first of which is that of identifying and selecting an appropriate strain of the specific virus. In the case of cholera this problem is rendered complicated by the fact that the various specimens of virus found in patients and in inanimate nature present considerable differences, and that, further, the properties of individual specimens undergo comparatively rapid alteration in the course of their cultivation in the laboratory.

The Cholera Virus as found in Nature.

The following details may illustrate the matter as regards the condition of the virus in nature. The form of the germ varies from a curved rod, or "comma", to a small oval speck, or to a long and straight, or slightly undulated bacillus. The germ is endowed with spontaneous movement; but the mode of that movement and the apparatus which produces it are not always the same, while some specimens show no mobility at all. Again, the cholera microbe, like a number of other germs, secretes a ferment with which, when planted out on solidified gelatine, it digests and liquefies the jelly. The dissolution is seen to take place to some distance around the mass of microbes and to form a clearly delineated fluid area. As is well known, the figure of this area is typical for various species of germs and for the cholera bacillus in particular, and is made use of as a diagnostic feature, for distinguishing species of microbes; but in the case of the cholera germ there may occasionally be no liquefaction at all, or, on the contrary, the whole mass of gelatine contained in the culture tube may become rapidly and completely dissolved. The secretion of

other ferments by these germs is similarly uncertain. As a rule, the bacilli grow in milk without causing in it visible alteration; yet some strains bring about a rapid coagulation of that medium. On vegetable substances certain representatives grow luxuriantly, and others do not grow at all. A few specimens found in patients were phosphorescent in the dark, but the vast majority do not manifest this property. The serum of immunized animals and man, when put in a cultivation vessel in contact with a watery emulsion of cholera germs, agglutinates these into lumps and causes them to fall to the bottom of the fluid. Many of these germs are sensitive to the minutest additions of such serum; but others remain practically unaffected. A fairly characteristic feature of a cultivation of microbes is its ability or otherwise to dissolve the red corpuscles of the blood. In the case of cholera some apparently true strains have been observed to produce this effect; but the vast majority do not do so.

Practically all other properties of the cholera germ show similar variations; but the most essential are obviously those which concern its relations with animals and man. Cholera bacilli have generally no virulence for the lower animals, or are harmful to them only when inoculated in certain peculiar ways. Even then the morbid symptoms caused by the inoculation have no resemblance to human cholera. Strains have, however, been met with which caused death even to the resistant species of animals, in whatever way inoculated, while the character of the disease produced in them was singularly analogous to the disease in man. The virulence of the germs also differs greatly, for a lethal dose may vary as much as from 1 to 500. Again, in man, the main seat of the development of these bacilli is the mucous membrane of the small intestine; they do not invade the circulating blood, the subcutaneous tissue or the muscles, and when experimentally introduced into those tissues in animals, cholera germs, as a rule, rapidly die out; yet specimens have been found which, in these circumstances, speedily penetrated into the whole system of the inoculated animal.

Variability of some degree is proper to all germs, as it is to higher animals and plants; but the cholera microbe is one of those

in which, owing to their organization and mode of life, that variability is particularly marked; so much so that not unfrequently, after an examination with all available tests, it is impossible to say whether the germ dealt with is, or is not, a representative of the cholera species. An idea of that variability may, I believe, be gathered from the fact that a close observer, Brigade-Surgeon-Lieutenant-Colonel D. D. Cunningham, I.M.S., who was bacteriologist to the Government of India when the cholera germ was first discovered, and who continued his studies of the subject for a number of years afterwards, formed the conclusion that the comma bacilli met with in cholera patients belonged to several distinct species of microbes; and practically up to the date of his last publication in India that investigator hesitated to recognize in a group of germs so unequal in their nature the cause of one and the same well-determined disease, such as cholera. (D. D. Cunningham in *Scientific Memoirs by Medical Officers of the Army of India*, Part I, 1884, pp. 1-20; Part II, 1886, pp. 1-14; Part III, 1887, pp. 4-7 and 11-16; Part IV, 1889, pp. 1-20; Part V, 1890, pp. 1-39; Part VI, 1891, pp. 1-49; Part VIII, 1894, pp. 1-57; Part X, 1897, pp. 1-28.) As far as I am aware, no such difficulty appeared to the same observer in regard to anthrax, tetanus, diphtheria, plague, etc.

The above *résumé* will, I think, be sufficient to show that, in dealing with the problem of preparing a vaccine for cholera, it is essential first very carefully to consider the various points in favour of and against the particular variety of the germ which should be selected as authentic and appropriate.

The Cholera Virus in the Laboratory.

The fact next confronting the operator is that, in the course of, sometimes, a short interval, the specimen selected undergoes "spontaneous" alterations of considerable importance, some of a temporary, others of a seemingly permanent character; so that a plan based on the properties of the virus as seen originally does not hold good for very long. A cholera germ, for instance, which, when first obtained, has been dissolving blood corpuscles, coagulating milk, digesting gelatine and albuminous matter and deoxidizing colouring substances, such as lithmus safranine and methylene blue,

may, after a time of laboratory cultivation, show none of such effects. (V. and A. Bourovie, "Particularités biologiques du vibrion cholérique de l'épidémie de 1908-1910," *Archives des Sciences biologiques*, St. Pétersbourg, 1912, XVII, No. 1.) In the most important properties—those concerning the pathogenic effects of the virus—a decrease in strength, in a short time, in the proportion of, say, 75 to 1, and ultimately the total disappearance of virulence is of no unusual occurrence. This means that, if, at first, a given dose of that virus has been prescribed for producing a certain desired effect, later on any quantity smaller than 75 times the original one may fail to give the expected result. Instability of virulence has come to be regarded as a characteristic feature of the cholera microbe, and where this peculiarity is not sufficiently pronounced, the fact has been treated as an objection to admitting the cholera nature of the germ. (Händel und Woithe, "Vergleichende Untersuchungen frisch isolirter Cholerastämme mit älteren Cholera und El Tor Kulturen," *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, Vol. XXXIV, f. 1., March 1910.) The marked mutability thus observed in *one and the same* specimen renders, of course, less paradoxical the differences which distinguish various specimens of these bacilli.

VIRULENCE AND IMMUNIZING POWER.

Their Inter-relation.

Turning now to the main subject of Part I of this Note, namely, the preparation of a vaccine against cholera, it is of importance to examine into the question as to whether variation of observable characteristics, such as mentioned above, carries with it variation in the immunizing faculties of a virus.

In the experiments which I carried out on this subject in 1890-1892, I observed repeatedly that a cholera germ of a low degree of virulence conferred on animals less immunity than a germ of high virulence. The special features of the preparation of the cholera vaccine the effects of which I subsequently studied in India, were based on this fact. The inter-relation just stated may, I think, be taken as a basis for a general working rule; but this need not exclude the possibility of so called "exceptions". For it is

conceivable that, in isolated instances, some, yet undefined, peculiarities of a germ may intervene and disturb the habitual position of the case.

Observations by other Workers.

The fact of the relationship in question between the virulence and immunizing faculty of a cholera germ has now been confirmed by many experimentalists. Some three years after the publication of the above-mentioned process of cholera vaccination (*vide* W. M. Haffkine, "Sur le choléra asiatique chez le cobaye," *Comptes-rendus des séances hebdomadaires de la Société de Biologie de Paris*, 9th July 1892), on the conclusion of my first inoculation studies in India, I had the privilege of discussing the matter with the late Robert Koch and his then co-workers, Drs. R. Pfeiffer and W. Kolle, of the Institute for Infectious Diseases in Berlin. I had in view both anti-cholera and anti-typhoid vaccination* in which I was then interested. Soon after that conference Drs. Pfeiffer and Kolle tested the subject in connection with the typhoid bacillus, and, in the subsequent year (1896), with the bacillus of cholera. In 1897, having visited Bombay with the German Imperial Plague Commission and studied the plague there, Dr. Pfeiffer, in collaboration with Dr. Friedberger, investigated the same point in application to the plague bacillus. It must be remarked that the latter germ, as found in patients or preserved in a laboratory, differs from the germ of cholera by a much greater stability of properties. On various occasions bacilli of plague, kept under observation for several years, even when subjected to multifarious processes, of which some were of a nature to weaken, and others to increase their virulence, showed no appreciable modification of strength. (*Vide* W. Kolle, H. Hetsch and R. Otto, "Weitere Untersuchungen über Pest, in Besonderen über Pest-Immunität, *Zeitschrift für Hygiene*, 1904, Vol. XLVIII, f. 3; Richard Strong, "Studies in plague immunity," *Philippine Journal of Science*, II, No. 3, June 1907; Sydney Rowland, "Investigations into plague vaccines," *Journal of Hygiene*, X, November 1910, No. 3; and others.) This

* *Vide* reference on p. 76.

circumstance simplifies greatly the work of preparing a prophylactic from the plague germ. Nevertheless, specimens of that germ of *different* virulence are met with in nature and can also be produced artificially, and so the question as to the relationship between virulence and immunizing power can be tested on this virus also. In all instances—cholera, typhoid and plague—the above investigators found that the result of immunization stood in direct connection with the virulence of the germ used ; that as the virulence rose or fell, the protection afforded to the inoculated was greater or less. (*Zeitschrift für Hygiene*, Vols. XX and XXI, 1896 ; *Berliner Klinische Wochenschrift*, Vol. XXXIX, 1902.)

In 1903, the point under consideration came again under discussion. The problem of combating cholera had, at the time, acquired importance in the new American possessions in the Philippines, and the matter was submitted to a fresh study in the Institute in Berlin, this time under the direction of the well-known German pathologist, Professor Wassermann. The experiments were conducted by the Director of the Government Biological Laboratory of Manila, Dr. Richard Strong, who published, in the subsequent year, detailed accounts of that enquiry. (R. Strong, "Protective inoculation against Asiatic Cholera," *Bulletin No. 16, Biological Laboratory, Bureau of Government Laboratories*, Manila, September 1904 ; "Some questions relating to virulence of micro-organisms, with particular reference to their immunizing power," *Bulletin No. 21*, October 1904.) Dr. Strong applied in this study such procedures of measurement and calculation as the subject admitted. In his words, Pfeiffer and Friedberger's experiments on the relationship of virulence and immunizing power "seemed of such great importance that it was decided to repeat them, and, in addition, to perform them in as accurate a comparative way, with relation to the virulence of the stem, as practicable. This seemed desirable because in Pfeiffer's and Friedberger's work, as far as can be ascertained from their article, no attempt was made previously to determine the exact relationship of virulence of the different stems to one another."

I believe the investigation was undertaken (and very usefully so) in a spirit of scepticism as regarded the relationship in question; but Dr. Strong's findings were ultimately summarized as appears below. He experimented with two different specimens of cholera germs, "virulent" and "avirulent," the strength of which stood in the proportion of 15 to 1. The variation in virulence was, therefore, very far from reaching the limits mentioned on pp. 10 and 12 above; yet it sufficed to give clear indications of its effects on the immunizing power. Dr. Strong states :—

"It became evident that the rabbits inoculated with the virulent culture always furnished better serum than those inoculated with the avirulent one; but that the value, in both agglutinative and bactericidal properties, of the serum from the animals treated with the former was in no case (?) more than two and one-half times that of the serum furnished by the animals treated with the latter stem."

"By the intravenous injection of the living organisms in quantities of one-half *Oese*, the ratio representing the bactericidal value of the sera of the animals inoculated with the virulent and the avirulent organisms was never greater than $4\frac{1}{2}$: 1; that is, the virulent organisms never furnished a serum more than four and one-half times as potent as the avirulent one. Therefore, it cannot be said that the immunity obtained was directly proportional to the virulence of the organisms, since the latter was 15 to 1 before inoculation. However, with the digested extracts of the organism of different strains and the killed organisms of the different degrees of virulence this may, within certain limits, be said to be the case."

"As the results were somewhat at variance with the ideas of Haffkine and quite different from what R. Pfeiffer and Friedberger found upon the intravenous injection into rabbits of dead cholera spirilla of different degrees of virulence, it was decided to repeat them. Accordingly, a second series of animals was inoculated just as the first, and on the day of inoculation, as in the previous series, the virulence of the injected organisms was verified as 15 to 1. The result was practically the same, for at the end of eight days the examination of the sera showed that the virulent stem had in only one case given a serum of more than about two and one-fourth times the bactericidal value of that produced by the avirulent one.

In this one case the avirulent serum was between one-fourth and one-fifth as strong."

While thus confirming, as Pfeiffer, Kolle and Friedberger had done before, the fact that by a stem of higher virulence higher immunization effects were produced, Dr. Strong opposed the view (which, I must remark, I had not advanced) that there was a simple numerical proportion between the two values. The divergence, even thus restricted, is, however, lessened further, inasmuch as in Dr. Strong's plan of experiment there appear to be certain features which, I believe, tended to mask the true proportions—namely, to reduce part of the numerical values recorded by him ; and as, further, on devitalizing the virus, or using its extractions, he observed, as stated in the above quotation, results actually approximating to the proportions he expected.

In conformity with these findings, in 1907, the same experimentalist emphasized the importance of using, for the preparation of cholera vaccine, stems of germs of the highest virulence, namely, in connection with the inoculations which he carried out for the suppression of the cholera outbreak in Manila in 1905 (*Philippine Journal of Science*, II, No. 5, 1907, p. 413). Similarly, Dr. Pfeiffer, in a communication to the International Congress of Hygiene and Demography in Berlin, in September 1907, took the opportunity of reiterating his conclusion as to the importance of using fully virulent stems for the preparation of *typhoid* vaccine. On yet another occasion, when testing the matter in application to *plague* and examining the immunity of animals treated with live cultures of different strength, Dr. R. Strong ascertained, as R. Pfeiffer had done in 1897 in regard to devitalized cultures, that the virulence of the stem was of decisive importance in this instance also, the immunity conferred on the inoculated animals rising concurrently with the rise of the virulence. (*Philippine Journal of Science*, II, No. 3, June 1907.) Accordingly, in some of the laboratories situated in non-infected centres and having no facilities for regularly renewing their stock of plague bacilli, special measures are taken for maintaining the virulence of the germ used in manufacturing the plague prophylactic. (*Vide*

Mauro Jatta and Romano Maggiora, on the operations of the Pianosa Plague Prophylactic Laboratory, in the Proceedings of the Direzione generale della Sanita Publica, Rome, 1904.)

The necessity of using fully virulent strains of germs for the preparation of the cholera vaccine has been acknowledged also by the Japanese bacteriologists. (*Vide* Mabry and Gemmil, in the *Journal of the American Medical Association*, 20th December 1902.)

Similarly, in 1910, Dr. Aaser, in Christiania, laid stress on the same point in preparing vaccine for the cholera inoculations which he carried out in that town. (*Berliner Klinische Wochenschrift*, 22nd August 1910, No. 34.)

I am aware of only one dissentient view formed on the subject as a conclusion from an original study, namely, that of Meincke, Joffe and Flemming. Their memoir is, however, not accessible to me at present.

Before leaving this subject I should mention that the facts observed in India in 1894-96 tended to show that a vaccine of higher virulence produced an immunity not only of a higher degree, but also of longer duration than a vaccine of lesser virulence, as will be seen lower down (*vide* pp. 43, 67 and 70-71).

Microbial Virulence and some of its Elements.

In the present exposition I use the words *virulence* and *pathogenic power* as meaning the capacity of a stem or species of germs to live in an animal and produce in it morbid effects. To this end a germ requires a variety of properties, some of which enable it to resist the adverse effects of the animal's tissues and humours, others to derive food from the same elements, and others again, to produce in the animal morbid symptoms. A stem of pathogenic germs which has lost, or has been artificially deprived of, one or more of these faculties, loses its virulence for the animal, though the rest of its characteristics may remain intact.

The actual nature of the properties here referred to varies with the species of germ and with the nature of the animal for which the germ is virulent, but a few peculiarities common to considerable groups of pathogenic microbes have been observed and some of them minutely studied. Such are, for instance,

(1) the faculty of resisting various specific substances in the animal body—"agglutinines," "bactericidines," "alexines," "bacteriolysines"—which tend to destroy the germ; or of absorbing and thus neutralizing these substances; or of producing others—"aggressines"—which seem to ward off and injure the protective elements of the body;

(2) the faculty of calling forth in the system of the animal the formation of the defensive substances mentioned in the foregoing paragraph, or of others of similar purpose, such as "stimulines," "bacteriotropines," etc.;

(3) the faculty of comparatively rapid growth and multiplication;

(4) the faculty of producing and setting free poisonous substances or "toxines," which cause morbid symptoms in the animal; and so on.

I believe that at present it would not be a safe procedure to consider a germ *pathogenic* or *virulent* if it possesses one or some of the above properties, singled out above the others; or to declare an animal *immune*, if it resists a germ "virulent" in that restricted sense. A true vaccine is perhaps best defined as one which prepares the individual to face the virus in its most common manifestations in outbreaks. I shall have occasion to touch again upon this subject later.

GENERAL AND SPECIAL FEATURES OF THE PROBLEM UNDER CONSIDERATION.

Peculiarities dependent on the Nature of the Germ.

The above observations go to show that the immunizing power of a germ varies with its virulence; and in order to produce a vaccine of given definite efficacy, a strain of virus is required of as definite a degree of pathogenic strength. The germ of cholera being of the varying and unstable nature mentioned above, the problem of anti-cholera inoculation, as a measure of practical application, becomes dependent on the possibility of steadying that virus on an adequate level of potency, namely, on a level sufficient for conferring on man immunity from epidemic cholera.

These requirements were overlooked in an experiment of cholera vaccination which was tried in Spain in 1885 by the Barcelona physician Dr. J. Ferrán. In this attempt use was made, for inoculating man, of different specimens of cholera germs obtained from patients and employed alive in the condition in which they were obtained, or which they assumed spontaneously afterwards. The plan corresponded to that, still prevalent in some parts of the East, of inoculating man with virus from a patient attacked with a mild form of small-pox, and is known as variolization, in contradistinction from vaccination. The procedure takes no account of the peculiarities of the virus found in the patient, that is to say, of the question as to whether the virus is actually mild and yet possesses the requisite degree of potency to be immunizing, or whether the mildness of attack is due to the patient's individual resistance which masks the true character of the germ. These circumstances must have had some part in influencing the outcome of the Barcelona experiment; for, according to the enquiries made at the time by numerous government commissions deputed to observe the operations, the trials gave uncertain, in some instances clearly negative, and on the two special occasions when a Spanish government commission, associated with Dr. Ferrán, watched the events, directly disadvantageous results. The procedure was therefore not adopted in other countries and was discontinued in Spain. *Vide* reports of the 1st and 2nd French Government Commissions (*Annales d'Hygiène publique et de Médecine légale*, Vol. XIV, August 1885; *Comptes-rendus de l'Académie des Sciences*, August 1885; *Bulletin de l'Académie de Médecine*, 1885, Nos. 28, 29 and 33); the reports of the 1st and 2nd Spanish Government Commissions ("La inoculación preventiva contra el cólera morbo asiático," por J. Ferrán, Valencia, 1886, pp. 194 and 225—229. Dr. E. Duhourcau's translation, "L'inoculation préventive contre le choléra morbus asiatique, par J. Ferrán," Paris, 1893, is, on some points, incomplete); the report of the Belgian Commission (*Bulletin de l'Académie de Médecine*, Paris, 1885, No. 33, and *Deutsche Medicinische Wochenschrift*, 16th July 1885); the report of the Italian Government delegate (*La Riforma Medica*, 25th July 1885, and *Giornale internaz. d. Sci. Med.*, Napoli, 1885, fasc. 9 and 10);

the report of the Portuguese Government Commission (Dr. Abreu's reference in J. Ferrán's book, pp. 264-268) ; the report of the Russian delegate (*Vratsch*, St. Petersburg, 17th April 1886, p. 287); the report of the Royal Academy of Medicine and Surgery of Barcelona (J. Ferrán, pp. 178-180); the report of the Royal Academy of Medicine of Madrid (*ibidem*, pp. 198-201) ; the report of the delegate from the State of Massachusetts (*L'Union Médicale*, Paris, 23rd July 1885) ; the information recorded by the Local Government Board, England (Minute by Sir George Buchanan, Medical Officer of the Board) and the details of the enquiry made by Dr. Shakespeare, United States' Commissioner, who visited Spain after the cessation of the epidemic and was generally in favour of Dr. Ferrán's views. (Edward O. Shakespeare, "Report on cholera in Europe and India," Washington Government Printing Office, 1890. *Vide* Dr. Shakespeare's personal results, on page 687 *et seq.*, and the 18 replies received by him from the localities visited by the cholera, on the subject of the effects of the operations.)

In 1892 Pasteur offered, on my behalf, to the Russian Government, through H. H. the Prince Alexander of Oldenburg, to try my method of cholera vaccination in the then cholera-stricken provinces of Russia ; but the offer was not accepted on the ground of the above results in Spain in 1885. The view adopted on the matter by most of the authoritative bacteriological schools is referred to on pp. 35-37.

Peculiarities of the present Problem as concerns Animals.

The problem set forth in the preceding lines, of creating and preserving in a virus stable and uniform qualities, necessitates as an essential condition that the germ be maintained in appropriate uniform circumstances of nutrition and multiplication. When the particular properties which it is desired to fix in a microbe are those which concern its relation to the animal body, the plan for achieving the object is to make the germ live in that definite relation to the animal selected. Thus, in the classical instance of Jenner's vaccine, the desired uniform qualities of cow-pox lymph are maintained by continued cultivation in the skin of the calf. In this instance the plan

offers no complications, as the virus has its natural abode in the skin of cattle.

In the case of cholera the method which suggests itself for maintaining the germ on a steady level of virulence is, similarly, that of cultivating it in the tissues of an animal ; but man is the only animal known to suffer from the disease ; lower animals do not contract it spontaneously, and when the virus is injected into them artificially, it is, as a rule, rapidly destroyed, as mentioned higher up.

THE VIRUS OF CHOLERA IN ANIMALS AND ANIMAL HUMOURS.

Experimental Cultivation in the Intestine.

The first attempt at solving this phase of the problem was made in 1888 by Dr. Gamaleia, a pupil of Pasteur's, who tried the plan of introducing the cholera germ into the intestinal canal of the guinea-pig and transferring it subsequently from the intestine of the first animal into that of another, and so on, in a continuous series of cultivations—the obvious reason for the plan being that, in its development in man, the cholera germ affects the intestinal tract. The digestive organs are, however, an inconvenient medium for the artificial cultivation of a germ ; for, among other reasons, these organs are already inhabited by vast masses of microbes, which, according to their species, interfere in one way or another with the development of the new comer. In the case of guinea-pigs and other lower animals the circumstances are unfavourable to the growth of the bacillus of cholera ; so, after a certain number of transfers from animal to animal, the series generally breaks down, and the germ disappears from the intestinal contents, as was seen repeatedly in trials made in Paris. A modification of the procedure was then attempted in which the cholera bacillus, after its cultivation in the intestine of one animal, and preliminarily to being transferred into that of another, was freed from extraneous germs, so that it might get a fresh start in each new host. This plan involved operations tending partially to counteract the desired effect ; for the technique of separating the cholera germ from others necessitates its maintenance, for some generations, in *artificial media*, that is, outside

the tissues of the animal to which it is desired to acclimatize it. From this or other causes, Pfeiffer and Nocht, who performed many experiments on the intestinal infection of animals with the germ of cholera, found it impossible to raise the strength of that virus to such a degree, for instance, as would make it virulent to birds. (*Zeitschrift für Hygiene*, VII, 1889, Heft 2.)

Cultivation in Animals outside the Intestine.

To meet the above difficulties, Gamaleia proposed to cultivate the cholera virus in the thoracic cavity of animals, namely of pigeons, the thorax being free from extraneous germs. This plan proved, indeed, successful in the case of a microbe much akin to that of cholera and designated by Gamaleia, who discovered it, *Vibrio Metchnikovi;* but when the same procedure was applied to the strains of cholera germs then available, it was found that such animals as birds remained immune against infection.

The plan which was tried next was that of Professor Hüppe of Prague, who suggested the growing of the cholera bacillus in the peritoneal cavity of animals, between the intestines and the outer walls of the abdomen, a region ordinarily free from germs. This plan had failed some time previously in the hands of Dr. L. Vincenzi (*Deutsche Medicinische Wochenschrift*, No. 26, 1887); but the idea was nevertheless taken up by R. Pfeiffer, who found that a fatal form of cholera peritonitis could, indeed, be induced in an animal in that way, but that the germ itself perished in the process. In cases in which the virus was still recoverable on the death of the first animal and was transferred into the peritoneal cavity of another, it perished in the latter, or in the third animal, and so continuous cultivation was again found to be impossible. (R. Pfeiffer, *Zeitschrift für Hygiene*, XI, 1891, No. 3). The accuracy of these observations was verified at the time and on various occasions subsequently by many observers, including Dr. E. Roux, of the Paris Pasteur Institute, and myself. (*Vide* Pfeiffer and Wassermann, *Zeitschrift für Hygiene*, XIV, Heft I, of 3rd March 1893; D. Pane and C. Lotti, "Nuovi studi sull' infezione peritonale," *Annali d'Igiene Sperimentale*, 1908, XVII, f. 3.)

Under these circumstances I tried to modify the nature of the germ of cholera preliminarily to introducing it into the animal body; namely, to mithridatize or immunize it first against the animal. By a series of cultivations in test-tubes, I gradually accustomed the bacillus to live in meat-broth mixed with increasing quantities of *fresh blood serum*, which ordinarily is inimical to it; until, after a time, the germ became capable of growing luxuriantly in blood serum pure. When it reached this condition, I injected it into the circulating blood of an animal. (*Vide* E. H. Hankin, "On Haffkine's method of protective inoculation against Cholera," *British Medical Journal*, 10th September 1892; W. M. Haffkine, "Vaccination against Asiatic Cholera," *Fortnightly Review*, 1st March 1893.) Some years previously I had made similar experiments with the bacillus of typhoid fever, in regard to which analogous difficulties existed. In this instance I had acclimatized the germ, instead of to blood serum, to the *humor aqueus* of the anterior chamber of the eye. (*Vide* W. M. Haffkine, "Adaption au milieu chez les Infusoires et les Bactéries," *Annales de l'Institut Pasteur*, III, 1890.) When the bacilus became so acclimatized, I injected it into the anterior chamber of the animal.

In both cases—cholera and typhoid—the mithridatized germ, on being introduced into the animal body, instead of rapidly perishing, as is ordinarily the case with cholera and typhoid bacilli, struggled on successfully and caused the animal an attack of illness which often ended fatally. The duration of that illness, however, varied greatly, *viz.*, from a few days to several months; and occasionally the animal ultimately resisted. This irregularity of results rendered it impossible to utilize the plan for practical purposes.

Identical or analogous facts were observed by investigators who attacked the subject subsequently, and the significance which was attributed to them may possibly have been, at least in one direction, exaggerated. In 1902, E. W. Ainley Walker and, soon after him, P. Th. Muller, ascertained that the cultivation of *typhoid bacilli* in broth mixed with serum of an immunized animal increased their

resistance to the agglutinating and dissolving effects of the serum and enhanced their virulence for animals. The same facts were observed by Edna Steinhardt in 1905. In 1908, K. Tsuda, by cultivating typhoid bacilli in sera of normal, instead of immunized, animals obtained similar results. In regard to the *cholera* microbes, F. Hamburger, in 1903, found that cultivation in anti-cholera serum rendered these also capable of resisting the agglutinating effect of such serum and increased their virulence for animals. Symmers observed in these circumstances a similar rise in virulence, and H. T. Marshall, in 1908, found that such cultivation imparted to cholera bacilli the power of dissolving red blood corpuscles. Even germs not generally endowed with, or possessing only small pathogenic power, have been seen to acquire virulence by cultivation in animal sera. *Vide*, for instance, Dudley W. Day's results with *Bacillus prodigious*, *Bacillus proteus vulgaris* and *Bacillus fluorescens non liquefaciens*; *Journal of Infectious diseases*, 25th November 1905.

In 1903, F. Wechsberg observed an analogous phenomenon with the bacilli of *diphtheria:* through cultivation in broth mixed with anti-diphtheritic serum, they became capable of producing more toxine than before; but in the case of these germs the uncertainty in results observed by me in 1890 and 1892 became apparent. Indeed, in another series of experiments, which were made under the same conditions as Wechsberg's first series, but in which the preliminary acclimatization had possibly been pushed a few stages further, it was seen that the procedure not only did not increase, but diminished the toxine production of those germs. Such were, *e.g.*, Edna Steinhardt's results in 1905. A similar diminution, instead of increase, of pathogenic properties was proved by the latter author in the case of the *dysentery* bacillus cultivated in immune serum. (*Proceedings of the New York Pathological Society*, IV, January 1905.)

The uncertainty of the mithridatization plan is perhaps due to the fact that a germ introduced into an animal is faced with *many* peculiarities in the tissues and fluids of the body, as alluded to previously. These peculiarities are only partially and imperfectly represented in a sample of *humor aqueus* or serum, placed in a test tube. By mithridatizing the germ against the action of such fluids

we train it only to resist some of the effects which it has to overcome in the contest with the animal organism, while the rest of the germ's properties are allowed to deteriorate. The further such a procedure is carried, the more do we disturb the balance of faculties which goes to make the pathogenic character of the germ. The best that can be expected from such a preliminary training is to enable the virus, when injected into the animal, to maintain itself during some of the initial stages of the struggle. The ultimate plan for making a germ pathogenic and keeping all the component elements of this property balanced on the requisite level would still seem to be that of cultivating it in the animal body, after the original procedure of Jenner.

THE METHOD ULTIMATELY ADOPTED.

Cultivation in animal Series.

The problem was eventually solved by reverting to the Vincenzi-Hüppe intra-peritoneal injection and working out, from that starting point, a plan which permitted the cultivation in animals of the germ of cholera, in a state of purity, indefinitely, generation upon generation ; the raising of it to a well-determined degree of virulence, sufficient for the protection of man ; and its maintenance at that level for an unlimited period of time, with the same certainty of result as obtains in the preparation of small-pox vaccine lymph and of Pasteur's antirabic virus.

Cholera bacilli do not live in the body of man *uninterruptedly*. We express this fact when we say that " cholera is not a contagious disease " in the sense of measles or small-pox, *i.e.*, it is not transmitted direct from man to man. The germ obviously needs to spend part of its existence outside the human body; and, indeed, it grows readily in various natural and artificial media, most commonly in drinking water. A peculiarity of its culture in such media is its great avidity for the air, in apparent contradiction of the fact that, when it infects a human being and grows in the intestinal tract, it lives in a medium devoid of oxygen. The alternation of aerobic and anaerobic conditions appears thus to be a marked peculiarity of this germ ; and this peculiarity may account for the fact, observed in

the course of my experiments of 1890-1892, *viz., that the chief obstacle to the cultivation of the cholera virus in an uninterrupted series of animals is the continuous deprivation of air.*

Another and unexpected condition was found to be the necessity of *varying the size of the animals selected for grafting the virus upon.*

The formula given by me in 1892, in the above-mentioned transactions of the Paris Biological Society, for obtaining a cholera virus of stable properties, contains the following three clauses :—

1. The series of cultivations must be begun by giving the first animal a *superlethal dose of virus*, so as to obtain a rapid effect and to find, upon the death of that animal, in the fluid exudating into the peritoneal cavity, a remnant of resistent germs surviving the destruction of others.

2. On the death of the first and of each succeeding animal, the serous fluid found in the peritoneum (or, else, a culture of cholera germs made from that fluid) must be *aërated* for a few hours, before being injected into the peritoneum of another animal ; and, lastly,

3. For this latter injection, an animal of *greater body weight* than the previous one must be taken, if the amount of serous fluid found in the peritoneum is small ; and *vice versâ.*

I ascertained at the time that this formula was applicable not to the cholera germ alone, but also to others, in particular to the bacillus of typhoid fever.

The Properties of the cultivated Cholera Virus.

The peculiarities imparted to the virus by the above process of animal cultivation are as follows :—

As the germ is being passed from animal to animal, its virulence gradually rises, so that smaller and smaller doses of it become sufficient to cause an animal a fatal illness. With the particular strain of virus with which I then experimented, the dose lethal to

an adult guinea-pig gradually sank to one-twentieth, one-thirtieth and eventually to one-fiftieth of what it had been originally.

The animals successively inoculated succumb after a shorter and shorter duration of illness. In my Parisian breed of guinea-pigs, the time for animals of 350 gr. weight was reduced from 20 hours to 8, the latter period then remaining constant.

The virus becomes fatal to rabbits and pigeons in doses which have been harmless to them.

Inoculation into the depth of the muscles becomes fatal to guinea-pigs, which has not been the case before.

Inoculation under the skin, in the animals just mentioned, causes mortification of cutaneous tissues and the subsequent sloughing off of the region concerned. (*Vide* above-quoted Transactions of the Biological Society of Paris, 9th July 1892. Also W. M. Haffkine, "A lecture on anti-choleraic inoculation," *British Medical Journal*, 11th February 1893, and "Vaccination against Asiatic Cholera," *Indian Medical Gazette*, April 1893.)

Apart from the above effects, some of the properties which have so far been observed in groups of pathogenic germs, as alluded to on pp. 17-18, become accentuated with the rise of the germ's virulence. (Cf., *e.g.*, R. Strong's study already mentioned, on "*Some questions relating to virulence of micro-organisms, with particular reference to their immunizing power*," Manila, October 1904.)

Observations by other Workers.

The method above described for raising the virulence of the cholera germ and keeping it on that level has been many times verified and has become familiar to workers in laboratories. It was first confirmed, soon after its publication, by Professor Max Grüber of Vienna (*Wiener Klinische Wochenschrift*, 1892). In the same year I had an occasion to introduce it in the Army Medical School in Netley, and the operation was afterwards often repeated there. (Wright and Bruce, "On Haffkine's method of Vaccination against Asiatic Cholera," *British Medical Journal*, 4th February 1893.) Pfeiffer and Kolle used the plan throughout their

work of 1896-1898 (*vide, e.g., Deutsche Medicinische Wochenschrift,* 1st January 1897). In describing the operations performed in Berlin by Professor Wassermann and himself, in 1903, Dr. Strong states (*l.c.*, September 1904, p. 18) :—

"Some time was spent in accurately standardizing these ('virulent' and 'avirulent') cultures, and the minimal lethal dose for guinea-pigs of 250 grams weight was carefully determined. *After numerous passages of 'virulent' through animals a lethal dose of 0·1 of a standard (2mg.)* Oese *of a twenty-hour agar culture was reached.* With 'avirulent,' on the other hand, one and one-half standard *Oesen* of a twenty-hour agaragar culture, when injected intraperitoneally, were required to produce death within the same time in such an animal. Throughout the course of the work this *relationship between the organisms has been carefully preserved* and continuously tested by animal inoculation. *As the virulence of the cholera spirilla grown on laboratory media changes in a few days, it is necessary to make daily animal inoculation in the case of the virulent strain*" (vide p. 39) "*and always to use the same generation of the stem.* With the avirulent culture considerable care was also necessary to keep its virulence fixed." *L.c.*, October 1904, p. 15 : "*The 'avirulent' was next passed successively through the abdominal cavities of twelve guinea-pigs and then examined in regard to its virulence. This was found to have considerably increased, since now three-fourth* Oese *of the organism produced death in a guinea-pig of 250 grams' weight within twenty-four hours.*" (The italics have been inserted in the present quotation.)

H. T. Marshall ("Studies on Cholera," *Philippine Journal of Science*, III, f. 2, 1908) obtained a similar confirmation of the method.

The procedure was tested also in application to the *typhoid bacillus* and gave analogous results ; *vide* "Étude expérimentale sur l'exaltation, l'immunization et la thérapeutique de l'infection typhique," par MM. Chantemesse et Widal, *Annales de l'Institut Pasteur*, November 1892, p. 755 ; Rodet et Lagrifoul : "Quelques faits relatifs à la virulence du bacille d'Eberth. Exsudats de passages et bacilles de passages," and "Influence de certaines conditions du milieu sur le pouvoir infectant des cultures du bacille

d'Eberth, notamment des bacilles de passages," *Comptes-rendus, Société de Biologie de Paris*, 2nd and 16th December 1905.

THE CHOLERA VACCINES.

Vaccine "II."

It was *a virus in live condition, brought by the above described procedure of successive cultivations in guinea-pigs to a uniform and stable degree of virulence*, such as is obtained in small-pox vaccine lymph by cultivation in the calf, that I designated, in 1892, following the example of Jenner and Pasteur, the *cholera vaccine;* and, for reasons mentioned below, I referred to it as cholera vaccine "II." The subcutaneous inoculation of an appropriate dose of this vaccine protected the guinea-pig, the pigeon, the rabbit and the dog against all such forms of infection with cholera virus as, without previous immunization, were fatal to them. The results were thus found to apply to *several species of animals—mammals and birds*—which appeared susceptible to the same process of immunization. Later on Dr. R. Strong confirmed the similarity in this respect between the rabbit and the guinea-pig, while R. Pfeiffer and Friedberger showed that this similarity extended also to goats ("Weitere Beiträge zur Theorie der bakteriolytischen Immunität," *Centralblatt für Bakteriologie*, I, Vol. XXXIV, No. I, 1903). Considering that the differences of organization between various mammals, including man, are less than those between mammals and birds, the possibility of the conclusions extending also to man was rendered by these facts more plausible than it would otherwise have been.

Cholera Vaccine "I."

The hypodermic injection into the guinea-pig of the cholera vaccine proper mortifies, as already mentioned, the skin at the seat of the injection and leaves an open wound, which becomes, however, regularly covered with granulations and heals up without suppurating.

To prevent the formation of this wound I prepared, by a process which it is now unnecessary to describe in detail, an *attenuated*

derivation of the same vaccine, which I designated cholera vaccine "I," and which, when inoculated in a guinea-pig, not alone caused no mortification, but immunized the skin against the deleterious effect of the cholera vaccine proper. The inoculation of this preliminary vaccine alone conferred on the animal also a certain degree of immunity against a lethal infection. These two vaccines I applied to the preventive treatment of man and used in my studies in India in 1893-95 and part of 1896.

Inoculation Reaction of anti-cholera Vaccine.

In 1895 I ascertained, however—first on myself and afterwards on others—that vaccine "II," when inoculated, in the doses prescribed, *without preliminary immunization with vaccine "I,"* had no mortifying effect on the human skin. This result is possibly due to the subcutaneous tissues of man being more inimical to the germs of that vaccine than are the corresponding tissues of the guinea-pig, so that the activity of those germs is arrested before any harmful effects are produced. In accordance with this observation, in all operations on man performed in the summer of 1896 and subsequently, up to 1904 inclusive, and in the operations in Bengal in 1908, vaccine "II" alone was employed. Under these conditions the inoculation with that vaccine causes a rise of temperature and a local reaction, developing, on the average, in ten hours, and then gradually subsiding. The length of time required for getting over most of these effects has been ascertained from long observation, and an idea of the subject may be gathered from the correspondence which took place, in 1904, between the emigration authorities of Bengal, who had had several years of experience in the matter. (Vide *Detailed Report of the General Committee of the Indian Tea Association, Calcutta, for 1904*, p. 250.) The information is contained in letters No. 452 of 7th March 1904, from the Superintendent of Emigration, Calcutta, and No. 43, of 9th *idem*, from the Assistant Superintendent, Asansol, and bears on the question as to how soon after inoculation emigrants availing themselves of the treatment at the hands of the officers of the Purulia Inoculation Station

were able to proceed on their railway and steamer journey *en route* for Assam. The information is to the effect that :

(1) emigrants arrived at Asansol from the Central Provinces daily at 9 A.M. ;

(2) were inoculated between 3 P.M. and 5 P.M. of the same day, after they had had their midday meal and some rest; and

(3) left Asansol by the 4 P.M. train of the next day.

This arrangement had been in operation uninterruptedly for 8 years and was proved to work satisfactorily.

PART II.

IMMUNIZATION OF MAN AGAINST CHOLERA.

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The Nature of the Problem.

The foregoing explanations refer to the preparation of a stable cholera virus of given strength and to the study of its immunizing effect on the lower animals, which, under ordinary conditions, enjoy natural immunity from the disease. Special research of a different nature is obviously required in order to find out whether the same or another virus may be efficient in immunizing man against the disease which affects him specifically, and, indeed, whether immunization of man against cholera is realisable at all.

At the time of my undertaking the study of the matter scientific and medical authorities were divided on the latter question; or, perhaps I should say, the balance of opinion was in favour of a negative reply. I have mentioned above (p. 20) the effect which the results of the operations in Spain, in 1885, had on the views of the Russian authorities. In India observers were impressed with the fact that cholera had been known to recur in persons who had previously suffered from the disease,* and who seemingly had not benefited by what should be a highly efficient course of immunization. Others considered that the *comma bacilli* which served for the preparation of the vaccine were not the primary cause of cholera, but only a harmful concomitant of it, and that consequently the vaccine could not affect man's susceptibility to a cholera attack, though it might affect the rate of recovery from such attack. (*Vide* Brigade-Surgeon-Lieutenant-Colonel D. D. Cunningham, I.M.S., "The results of continued study of various forms of comma bacilli occurring in Calcutta," *Scientific Memoirs by Medical Officers of the Army of India*, Part VIII, 1894, pp. 54-57.)

* Regarding this and similar observations made in small-pox and measles, as well as their interpretation, *vide* debate on "Anti-choleraic Inoculations in India," at the First Indian Medical Congress: *Transactions* of the Congress, Thacker, Spink and Co., Calcutta, 1895; *Indian Medical Gazette*, February 1895.

(*Cf.* p. 76 below.) Among authorities in Europe, Pfeiffer and Wassermann, Directors of the Scientific Department of Koch's Institute in Berlin (*Zeitschrift für Hygiene*, XIV, 1893, p. 60), Sobernheim (*ibidem*, XIV, 1893, p. 499; *Ind. Med. Gaz.*, September 1893, p. 313), Metchnikoff, Director of the Laboratory of Research in the Pasteur Institute, Paris (*Annales de l'Institut Pasteur*, VIII, 1894, pp. 572, 574, 577, 578), Zabolotny, Bacteriologist, Imperial Institute of Experimental Medicine, St. Petersburg (*Centralblatt für Bakteriologie*, 1894, p. 150), and other notable authorities held immunization against intestinal cholera impossible, or at least very unlikely, both on grounds of theoretical consideration and of the result of laboratory experiments on animals and man. Metchnikoff aptly summarized the views then prevailing in the following passage (*Annales de l'Institut Pasteur*, Vol. VIII, August 1894, pp. 574-575) :—

"As we have seen, intestinal cholera of rabbits is an intoxication by the poisons prepared in the digestive canal. Now, it has been shown in several investigations that vaccination does not protect against poisoning of the organism. One can, therefore, easily conceive *à priori* that an animal, very well vaccinated against the cholera vibrio introduced into the tissues, may not resist intoxication by a poison manufactured in the intestinal contents. The experiments described in the preceding chapter have given a result which accords with the view of authors who conclude that vaccination, by live or sterilized cultures, against intestinal cholera produced by Koch's method is ineffectual. In the memoir in which they treat this subject, Pfeiffer and Wassermann come to the same conclusion as regards the prevention of this experimental disease by means of a very active serum, obtained from a person who recovered from cholera. While very weak doses of that fluid sufficed for preventing choleraic peritonitis in guinea-pigs, considerable quantities (up to 5 c.c.) were powerless to vaccinate these animals against infection by Koch's method."

Professor Metchnikoff had also been led to the above view in another way, namely, by the result of an experiment on two men whom I had inoculated against cholera in Paris in 1892, from six to

seven months previously, and whom, on my departure for India, he had occasion to submit to an artificial infection, by the mouth, with measured doses of cholera virus. The result of the experiment appeared to be as follows :—

"Mr. Haffkine's subcutaneous vaccinations, done six and a half months before the experiment, did not prevent, therefore, either the diarrhœaic effect of the cholera vibrios, or the causation of a condition of general malaise. . . . . If the two persons inoculated with Haffkine's vaccines had less diarrhœa, they experienced a pronounced general malaise, which was absent in M. B." (a non-inoculated individual). "Of the two persons who underwent Mr. Haffkine's vaccinations, the one who had been vaccinated twice was more affected by the vibrios than M. S., who was vaccinated only once.

"As in the old experiments of Dr. Ferrán's, it is in no way possible to consider as proved that hypodermic inoculations of vibrios prevent the action of those microbes when they are introduced in the digestive canal." (*Annales de l'Institut Pasteur*, Vol. VII, July 1893, pp. 581 and 582). (*Vide* p. 92 below.)

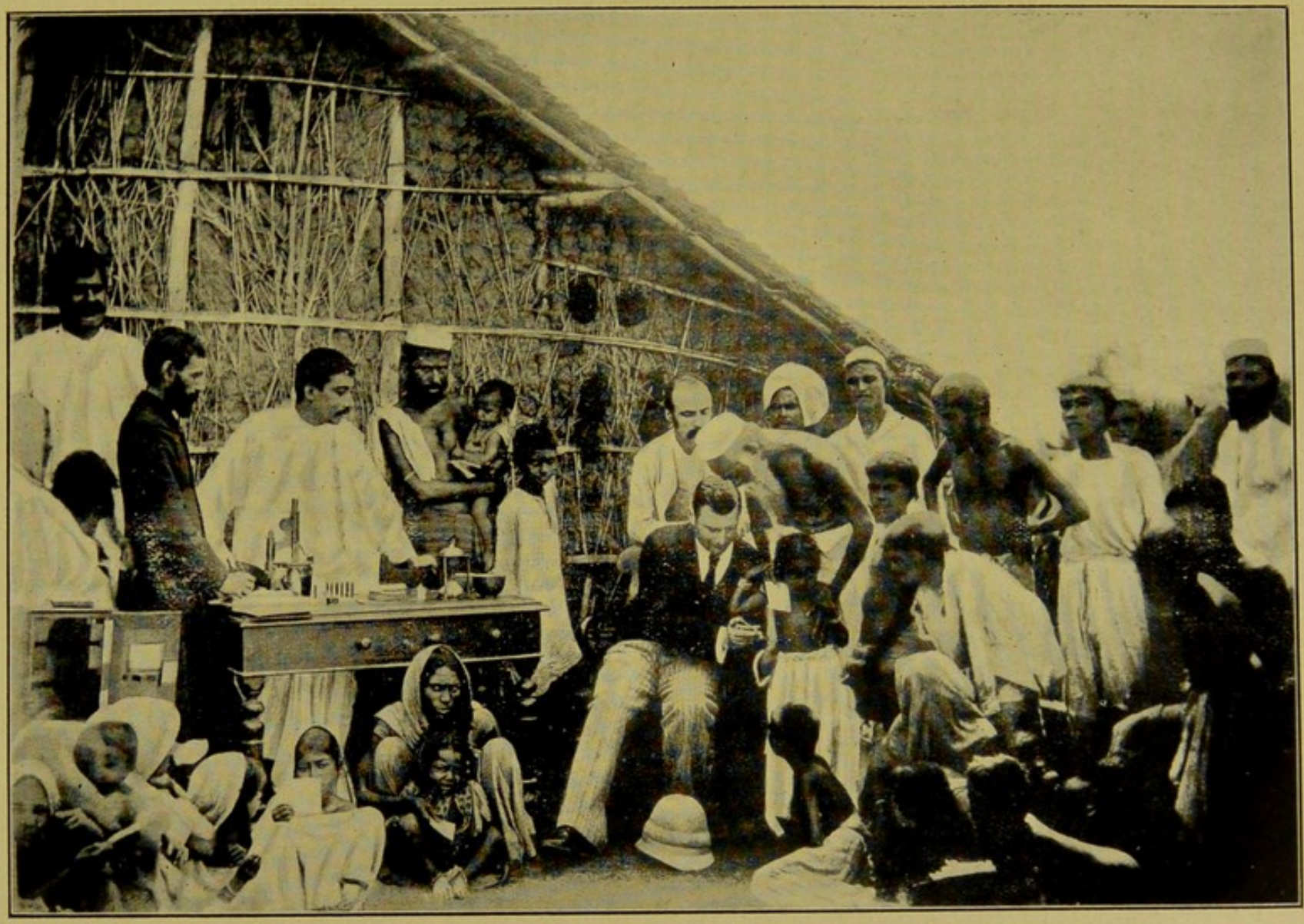
The Plan of Research in the Indian endemic Areas.

It was thus of essential importance to investigate first the above question of principle, that is to say, to find out whether cholera immunization of man was possible at all ; and for this reason, throughout my studies in India of 1893-96, I employed the most promising form of vaccine which I could evolve, irrespective of the amount of labour and technical difficulties which this implied ; and such a preparation I considered *vaccine "II" in a live condition.* I had already worked out then and used on animals and man, as is stated in various publications of the time (*vide* p. 81), the *devitalized* form of that vaccine, namely, the form which, lately, has not unfrequently been referred to as Kolle's vaccine ; but I held the live variety to be by far the more reliable, and this from two points of view : on account of its immunizing power and of the duration of immunity likely to result from it. The latter point was an essential one, as it was not known how long an interval of time would elapse between the date of inoculation and the occurrence

of an epidemic in which the immunity of the inoculated was to be put to the test, a consideration the significance of which appeared clearly in the events which afterwards took place in India (*vide* pp. 67-68). The superiority of immunization by *live* vaccine became apparent early in my laboratory experiments, and is referred to in the publications above-mentioned. The fact of this superiority has been, during the last ten years, confirmed by experimentalists working on various diseases, namely, in plague, by W. Kolle and R. Otto ("Untersuchungen über die Pestimmunität," *Zeitschrift für Hygiene*, 29th December 1903) and R. Strong ("Studies in Plague Immunity," *Philippine Journal of Science*, Vol. II, No. 3, June 1907); in typhoid, by H. Vincent (" Les bases expérimentales de la vaccination antityphique," *Comptes-rendus de l'Académie des Sciences*, Vol. CL, 7th February 1910) and Metchnikoff and Besredka (" De la vaccination par les virus sensibilisés," Dr. Besredka's "Revue" in the *Bulletin de l'Institut Pasteur*, 30th June 1912, pp. 536-537); and so on.

In the course of the years 1892 to 1895, I submitted therefore to study the effects of the above-described two live vaccines and inoculated, first, myself and a number of my personal friends, so that the reaction produced by the injection could be carefully observed, and its harmlessness established. Then I extended the operation to 42,197 persons inhabiting ninety-eight different localities in India, *viz.*, in Bengal, Behar, the then North-Western Provinces and Oudh, the Punjab, the Brahmaputra Valley, and Lower Assam. In 1895-96 I inoculated a further 30,000 people in Bengal, Behar, Assam, the Central Provinces and the Bombay Presidency. It was necessary to spread the operations in this manner, as it was not known exactly where cholera outbreaks might occur, while in some of the localities most threatened with such outbreaks, namely in Bengal, where I carried out subsequently the most instructive of my operations, I was unable, for a considerable time, to obtain assent to my work. My efforts were directed to inoculating people under such conditions as would afterwards render possible an accurate study of results. In this manner I inoculated part of the officers, non-commissioned officers and men in 64 British and native

Introduction of Anti-cholera Inoculation in Calcutta in March 1894.



Dr. G. N. Mookerjee,
Medical Inspector, Calcutta.

Dr. Jogendra Nath Dutt,
Analyst to the Health Officer.

Dr. W. J. R. Simpson,
Health Officer of Calcutta.

Dr. R. Sen,
Assistant Health Officer.

Mr. W. M. Haffkine.

(To face p. 38.)

time, I was able to procure and bring with me only six of the required animals. The most essential part of my method, which forms its distinguishing feature, could only be carried out, during the whole time of my nomadic operations in the country, in a most unsatisfactory manner." (*Transactions of the First Indian Medical Congress*, Thacker, Spink & Co., Calcutta, 1895; *Indian Medical Gazette*, January and February 1895.)

Nevertheless, the results obtained, though varying in direct relation to the variety of vaccine employed, the duration of the particular epidemics and the lapse of time between the date of inoculation and the date of exposure to cholera infection, *all* pointed to the fact that effective protection was conferred by the operation, as will be detailed presently.

Safeguards adopted for securing Accuracy of Results.

The arrangements for a systematic study of the subject culminated in a special organization made in Calcutta, where elaborate machinery for the purpose, under the then Health Officer, Dr. W. J. R. Simpson, at present Professor, King's College, London, was kept in operation for 33 months. The facts brought to light in that city coincided accurately with those observed in *all other parts of India*, and may be said to form the basis of our knowledge on the immunization of man against natural cholera. In view of this circumstance it is, perhaps, desirable to mention the particulars of the arrangements by means of which the results were gathered, and the safeguards adopted for securing accuracy. The statement may be the more opportune as the question of anti-cholera inoculation has been coming up lately for animated discussion, and the precise details of the Indian studies, which were consigned mostly to special reports and publications, are perhaps not sufficiently known.

The information as regards the arrangements in Calcutta is given in the report submitted by the Health Officer, on the 1st July 1896, to the Chairman of the Municipal Corporation concerning the first 24 months of the observations in that city. (Reprinted, without tables, in the *Indian Medical Gazette* for August of the same year.) The details of the service

which was employed in the work, and which formed part of the Municipal Health Office, are described in the report thus :—

"The following records of the inoculations are kept in the Health Office :—

"(1) a daily register filled up at the time of inoculation, containing name, father's name, sex, age, caste, occupation, residence and place of inoculation ; also any relative who may have been inoculated ;

"(2) an alphabetical register containing the names of the inoculated with the above details, so that ready reference can be made as to whether a person attacked with cholera has been inoculated ;

"(3) a ward-register showing the residences of the inoculated people, so that when any particular locality is affected with cholera, the inoculated in that locality may be easily found."

"Cases of cholera are notified to the Municipal Office by the following agencies : the registrars of births and deaths of each of the wards ; the sub-registrars at the burning ghats and burial grounds ; the authorities of the local hospitals, jails and the Fort ; the police ; the medical practitioners who are asked, in conformity with the Municipal law, to notify all cases of cholera on special postcards with which they are provided ; and by officers of the Health Department. All notified cases are immediately subjected to an enquiry by the Medical Inspector in charge of the district, who has personally to visit the house and take the necessary precautionary measures to prevent the spread of the disease. In his enquiry as to the circumstances of the attack, he has to fill up a printed form embodying the necessary information ; and one of the questions, since the introduction of the inoculation, is whether there are any inoculated people in the house, and whether the attacked person was inoculated or not. This information is at once communicated to the Health Office ; and when the case occurs in a house where inoculated people live, the Medical Officer in Charge of the Inoculations immediately visits the house, accompanied by the Medical Inspector of the district, and makes a thorough investigation as to the particulars of inoculated and uninoculated inmates and the incidence of cholera on these respectively. The accuracy of the statement as to who is inoculated is checked by the inoculation registers,

which have already been referred to. A list of these cases is given to the Health Officer who periodically visits the cases and verifies the results.

"On two occasions the results have been subjected to a further scrutiny. In July 1895, when the number of houses in which observations had been made was 36, M. Haffkine re-visited with me ten of the most important, which he had not previously seen, and satisfied himself that the returns were absolutely accurate. He would have seen all, but he was not well at the time. And quite recently, during my absence in England, Surgeon-Captain Robson Scott, I.M.S., Deputy Sanitary Commissioner of the Presidency Circle, made a special investigation on the subject, which lasted several weeks. Dr. Mookerjee and Dr. Chowdry, of the Municipal Medical Service, were specially deputed to assist him in his enquiry, and the local Medical Inspectors were asked to give him every assistance. The enquiry consisted in visiting affected localities and those in which inoculation had been carried out, and in collecting information from the members of the households and neighbours, which, on being brought to the Health Office, was checked by the inoculation registers and by the cholera death registers. The result of this investigation confirmed the accuracy of the previous observations, and Surgeon-Captain Robson Scott has furnished me with the following note :—

"'In compliance with your request of yesterday, I now send you a short account of my visits to the bustees in Calcutta during last May.

"'In the beginning of last May, M. Haffkine asked me to visit those wards in the town, in which anti-choleraic inoculation has been performed on the inhabitants, with the view of testing the accuracy of already recorded observations, and to try and find out if any observations had been missed.

"'During the eleven afternoons or mornings that were devoted to the work, fourteen wards were visited, and enquiries were made in 164 different bustees and houses. I was either accompanied by a Medical Inspector or by the Town Inculator (Dr. Chowdry), and

was taken by him to the various quarters where inoculation had been carried out.

"'First of all I found out from the inhabitants those dwellings in which cholera had broken out; and afterwards the individual residents in those particular dwellings were questioned, and their statements taken down by me.

"'I have been able to convince myself that the method of recording the observations in Calcutta is most satisfactory, and that the recorded observations are correctly stated.

"'I intend to continue these visits to the bustees in Calcutta from time to time, and I shall be glad to furnish you with any new information I may gain.'"

Results of the Studies on Man exposed to Cholera Infection.

From the end of March 1894 to the end of December 1896,—7,908 people were inoculated in Calcutta, mostly in the cholera-stricken suburbs. The disease visited subsequently 85 of the houses in which inoculated persons lived, and one of the ships in the harbour, the *Majestic*, part of the crew of which had been inoculated (*vide* statement LXX on p. 59). The total number of inhabitants in these houses and on the ship was 1,395. The subjoined tables summarize the facts observed. The details embodied in the tables comprise the addresses, the number of inoculated members in each house and on the ship, and the date of their inoculation; the number of non-inoculated members; the name, sex, age and caste of the individual or individuals attacked with cholera, and a statement as to whether the attacked had been previously inoculated or not, and, if inoculated, whether with preparatory vaccine only, *i.e.* vaccine "I," or with vaccines "I" and "II"; the date of attack and the interval which had elapsed between the date of inoculation in that particular house or on the ship and the date of the cholera occurrence; and a statement as to recovery, or, in the event of death, the date of this. Only one instance of failure in a patient inoculated with the two vaccines was observed (*vide* statement LXIII on p. 57), the attack in that case occurring 688 days after the date of inoculation.

OBSERVATIONS ON THE EFFECT OF ANTI-CHOLERA INOCULATION IN CALCUTTA, IN 1894-96.

Address of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
I.—8, Cantopher's Lane, Ward 20.	2 persons inoculated on 28-3-94. Prankrishto, Hindu male, 10 years of age, inoculated with vaccine "I" only, died of cholera on 30-3-94,—2 days after inoculation.	13 persons non-inoculated. No non-inoculated attacked.
II.—Monglo Jemadar's house, Kattal Bagan, W. 19.	11 ps. inoculated on 31-3-94. No inoculated attacked.	7 ps. non-inoculated; after the first 4 days, average number present 5. Four non-inoculated attacked with cholera : 1. Raju Bewah, Mahomedan female, 40 years, died on 2-4-94,—2 days after date of inoculation in the house. 2. Burasitana, M. f., 5, died on 6-4-94,—6 days after inoculation in the house. 3. Amirana, M. f., 40, died on 12-4-94,—12 days after inoculation in the house. 4. Pyrun Bibee, M. f., 25, attacked on 16-4-94,—16 days after inoculation in the house ; recovered.
III.—Ramdhun Dutt's h., Kattal Bagan, W. 19.	6 ps. inoculated on 31-3-94 to 7-4-94. No inoculated attacked.	2 ps. non-inoculated. A non-inoculated, Bilashmani Dassi, Hindu female, 45 years of age, attacked with cholera on 9-4-94,—9 days after introduction of inoculation in the house ; died on the day following the attack.
IV.—Shaik Subratee's h., Kattal Bagan, W. 19.	7 ps. inoculated 11-4-94. No inoculated attacked.	7 ps. non-inoculated. A non-inoculated, Shaik Babu, Mahomedan male, 30, attacked on 18-4-94, —7 days after inoculation in the house ; recovered.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
V.—Karam Ali's h., Kattal Bagan, W. 19.	5 ps. inoculated on 31-3-94. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Tetar Bewab, M. f., 35, attacked on 7-5-94,—37 days after inoculation in the house ; died on date of attack.
VI.—Golam Rohoman's h., Joratolla bustee, W. 21.	5 ps. inoculated on 14-3-94. No inoculated attacked.	6 ps. non-inoculated. A non-inoculated, Sabujan Bibee, M. f., 50, died of cholera on 17-6-94,—95 days after inoculation in the house.
VII.—Issoo Mistree's h., Beg Bagan bustee, W. 21.	4 ps. inoculated on 15-3-94. No inoculated attacked.	2 ps. non-inoculated. A non-inoculated, Makal Bibee, M. f., 30, died of cholera on 22-6-94,—99 days after inoculation in the house.
VIII.—Shaik Hingoo's h., Beg Bagan bustee, W. 21.	6 ps. inoculated on 19-6-94. No inoculated attacked.	3 ps. non-inoculated A non-inoculated, Daulat Bibee, M. f., 19, attacked on 22-6-94,—3 days after inoculation in the house ; recovered.
IX.—Gonessee Bewah's h., 16, Jorabagan Street, W. 5.	4 ps. inoculated on 15-5-94. No inoculated attacked.	1 p. non-inoculated. The non-inoculated, Balkristo Das, Hindu male, 40, died of cholera on 11-7-94,—57 days after inoculation in the house.
X.—Jodunath Chakravarty's h., 155, Upper Chitpore Road bustee, W. 1.	4 ps. inoculated on 11-8-94. No inoculated attacked.	7 ps. non-inoculated. A non-inoculated, Nanda Lal, H. m., 13, attacked with cholera on 13-8-94,—2 days after inoculation in the house ; recovered.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XI.—Mr. H. L. Mukerjee's Cooly Depôt, 2, Chattoo Baboo's Lane, W. 19.	25 ps. inoculated on 10-8-94. No inoculated attacked.	33 persons non-inoculated A non-inoculated, Bhagoo, H. m., 30, attacked with cholera on 13-8-94,—3 days after inoculation in the Depot; died on 15-8-94.
XII.—Narain Mistri's h., 155, Upper Chitpore Road bustee, W. 1.	3 ps. inoculated on 11-8-94. No inoculated attacked.	8 ps. non-inoculated. A non-inoculated, Digambari Dassee, H. f., 55, attacked with cholera on 14-8-94,—3 days after inoculation in the house; died on 15-8-94.
XIII.—Narain Marick's h., 155, Upper Chitpore Road bustee, W 1.	3 ps. inoculated on 11-8-94. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Kali Dassee, H.f., 22, attacked with cholera on 12-10-94,—62 days after inoculation in the house; died on 15-10-94.
XIV.—Bipath Chamar's h., Neechoo Bagan bustee, W. 21.	3 ps. inoculated on 14-3-94. No inoculated attacked.	6 ps. non-inoculated. A non-inoculated, Bipath Chamar, H. m., 40, attacked with cholera on 10-12-94,—271 days after inoculation in the house; died on 11-12-94.
XV.—Ambica Churn Roy's h., Tallygunge Road, W. 22.	3 ps. inoculated on 1-1-95. Ambica Churn Roy, H. m., 40, inoculated with vaccine "I" only, attacked with cholera on 5-1-95,—4 days after inoculation; died on 6-1-95.	2 ps. non-inoculated. No non-inoculated attacked.
XVI.—Satcouri Nikari's h., Kattal Bagan bustee, W. 19.	2 ps. inoculated on 31-3-94. No inoculated attacked.	6 ps. non-inoculated. A non-inoculated, Satcouri Nikari, H. m., 32, attacked with cholera on 25-1-95, 300 days after inoculation in the house; died on 27-1-95.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XVII.—Pitambur Sircar's h., Kopidanga bustee, W. 25.	4 ps. inoculated on 17-1-95. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Pelye, H. f., 65, attacked with cholera on 21-3-95,—63 days after inoculation in the house; died on 22-3-95.
XVIII.—Banikanto Mukerjee's h., Kalikumar Mukerjee's Lane, Shibpur.	6 ps. inoculated in August 1894. No inoculated attacked.	1 p. non-inoculated. The non-inoculated, Babu Banikanto Mukerjee's wife, H. f., 25, died of cholera in March, 1895,—240 days after inoculation in the house.
XIX.—Guru Prosad Khotta's h., 23, Nimtolla Ghat Street bustee, W. 5.	2 ps. inoculated on 15-5-94. No inoculated attacked.	10 ps. non-inoculated. A non-inoculated, Chanchala Bewa, H. f., 25, attacked with cholera on 30-3-95,—319 days after inoculation in the house; died on 31-3-95.
XX.—Surendra Nath Ghose's h., 33, Shampooker Street bustee, W. 1.	3 ps. inoculated on 17th to 25th March 1894. No inoculated attacked.	11 ps. non-inoculated; average number present 10. Three non-inoculated attacked with cholera : 1. Soilendra Kumar Ghose, H. m., 1½, attacked on 29-3-95,—377 days after inoculation in the house, died on 30-3-95. 2. Netto Dassee, H. f., 6, attacked on 1-4-95,—380 days after inoculation in the house, died on 1-4-95. 3. Indromutty Dassee, H. f., 5, attacked on 4-4-95,—383 days after inoculation in the house, died on 9-4-95.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XXI.—Sookhon Khan's h., Kurya Bustee, W. 21. (A case of cholera ended here fatally on 2-4-95 and another on 6-4-95).	1 p., a girl, inoculated on 4-4-95 with vaccine "I" only, attacked with cholera the same day and died on 6-4-95.	2 ps. non-inoculated. No non-inoculated attacked after the date of inoculation in the house.
XXII.—Gopal Das's h., 33-H-13, Shampooker Street, W. 1.	12 ps. inoculated on 17th to 25th March 1894. No inoculated attacked.	13 ps. non-inoculated. A non-inoculated, Kusum Behara, H. m., 40, attacked with cholera on 5-4-95,—384 days after inoculation in the house; died on 7-4-95.
XXIII.—Lakhimoni Dhye's h., 66-1, Boloram Dey's Street, W. 5.	5 ps. inoculated on 13-12-94. No inoculated attacked.	8 ps. non-inoculated. Two non-inoculated died of cholera : 1. Mati Bewah, H. f., 21, attacked on 6-4-95,—114 days after inoculation in the house; died on 9-4-95. 2. Garabini Dassee, H. f., 32, attacked on 10-4-95,—118 days after inoculation in the house; died on 11-4-95.
XXIV.—Durga Churn Bose's h., 20-H-I, Middle Road, Entally, W. 19.	2 ps. inoculated on 1-4-94. No inoculated attacked.	6 ps. non-inoculated. Two non-inoculated attacked with cholera : 1. Radhamoni Dassee, H. f., 30, attacked on 13-4-95,—377 days after inoculation in the house; recovered. 2. Surendra Chunder Bose, H. m., 3, attacked on 28-4-95,—392 days after inoculation in the house; died on 28-4-95.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XXV.—Nistarinee Devi's h., 2, Nokoolesartolla Lane, W. 22.	2 ps. inoculated on 13-2-95. No inoculated attacked.	6 ps. non-inoculated. A non-inoculated, Nogendra Bala, H. f., 3½, attacked with cholera on 25-4-95,—71 days after inoculation in the house ; died on 26-4-95.
XXVI.—Shaik Hossain Box's h., 8-H-12, Middle Road, Entally, W. 19.	1 p. inoculated on 1-4-94. Not attacked.	9. ps. non-inoculated. A non-inoculated, Shaik Fakir, M. m., 25, attacked with cholera on 29-4-95,—393 days after inoculation in the house ; died on 5-5-95.
XXVII.—Munshi Maniruddin's h., 6, Marquis Street, W. 13.	9 ps. inoculated in May 1894. No inoculated attacked.	43 ps. non-inoculated; average number present, 42. Three non-inoculated died of cholera : 1. Mangloo, H. m., 11, on 3-5-95,—356 days after inoculation in the house. 2. Rashmoni, H. f., 4, on 6-5-95,—359 days after inoculation in the house. 3. Chamroo, H. m., 5, on 7-5-95,—360 days after inoculation in the house.
XXVIII.—Abdul Aziz's h., 17-H, Muchiparah Road, W. 19.	3 ps. inoculated on 29-3-94. No inoculated attacked.	8 ps. non-inoculated. Two non-inoculated attacked with cholera : 1. Marihur Bibee, M. f., 80, attacked on 3-5-95,—400 days after inoculation in the house ; died on 7-5-95. 2. Shaik Abbas, M. m., 45, attacked on 6-5-95,—403 days after inoculation in the house ; died on 7-5-95.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XXIX.—Mohit Chamar's h., Neechoo bustee, W. 21.	10 ps. inoculated on 13-12-94. No inoculated attacked.	24 ps. non-inoculated. A non-inoculated, Dhonpatia, H. f., 7, attacked with cholera on 6-5-95,—144 days after inoculation in the house ; died on 7-5-95.
XXX.—Choonilal Koormie's h., 16, Jorabagan Street bustee, W. 5.	3 ps. inoculated on 16-1-95. No inoculated attacked.	18 ps. non-inoculated. A non-inoculated, Panchoo, H. m., 45, attacked with cholera on 28-5-95,—132 days after inoculation in the house ; died on 28-5-95.
XXXI.—Babar Ali's h., Neechoo bustee, W. 21.	5 ps. inoculated on 10th and 12th March 1894. No inoculated attacked.	7 ps. non-inoculated. Two non-inoculated attacked with cholera 449 days after first introduction of inoculation in the house : 1. Abdul Jabbar, M. m., 3½, attacked on 2-6-95 ; died the same day. 2. Sheanath, M. m., 32, attacked on 2-6-95 ; died on 3-6-95.
XXXII.—Karim Box's h., Neechoo bustee, W. 21.	3 ps. inoculated on 14-3-94. No inoculated attacked.	8 ps. non-inoculated ; average number present 7. Three non-inoculated attacked with cholera : 1. Modon, H. m., 8, attacked on 2-6-95,—445 days after the date of inoculation in the house ; died on 4-6-95. 2. Phool, H. m., 2, attacked on 6-6-95,—449 days after inoculation in the house ; died on 6-6-95. 3. Rashu, H. m., 5, attacked on 9-6-95,—452 days after inoculation in the house ; died on 9-6-95.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XXXIII.—Narain Chandra Banerjee's h., 58, Haldarparah Road, W. 22.	4 ps. inoculated on 13-2-95. No inoculated attacked.	11 ps. non-inoculated. A non-inoculated, Nogendra Bala, H. f., 23, attacked with cholera on 13-6-95,—120 days after inoculation in the house ; recovered.
XXXIV.—Abdul Hakeem's h., Kattal Bagan bustee, W. 19.	2 ps. inoculated on 31-3-94. Somsunnessa, Mahomedan female, 8, inoculated with vaccine "I" only, attacked with cholera on 4-7-95,—460 days after inoculation ; died on 5-7-95.	5 ps. non-inoculated. No non-inoculated attacked.
XXXV.—Sreedhur Poyrah's h., 114-H, Jaunbazar Street, W. 13.	15 ps. inoculated on 8th February and 21st March 1895. No inoculated attacked.	26 ps. non-inoculated. A non-inoculated, Radhakissen, H. m., 22, attacked with cholera on 9-7-95,—151 days after first introduction of inoculation in the house ; died on 9-7-95.
XXXVI.—Rati Kanto Das's h., 62, Anund Gopal Palit's Lane, W. 19.	1 p. inoculated on 6-9-94. Not attacked.	6 ps. non-inoculated. A non-inoculated, Subode, H. m., 10, attacked on 13-7-95,—310 days after inoculation in the house ; died on 14-7-95
XXXVII.—Sara Bewah's h., 19, Moonshigunj Road, W. 25.	2 ps. inoculated on 4-1-95. No inoculated attacked.	11 ps. non-inoculated. A non-inoculated, Brindaban Naik, H. m., 32, attacked with cholera on 15-10-95,—284 days after inoculation in the house ; died on 15-10-95.

Name of locality where cholera occurred in a household containing inoculated persons	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XXXVIII.—Puddo Bewah's b., 15, Jorabagan Street bustee, W. 5.	3 ps. inoculated on 11-4-95. No inoculated attacked.	17 ps. non-inoculated. Two non-inoculated attacked with cholera : 1. Balbhadra Pan, H. m., 22, attacked on 17-10-95,—189 days after inoculation in the house ; died on 17-10-95. 2. Nidhiram Mullick, H. m., 23, died on 19-10-95,—191 days after inoculation in the house.
XXXIX.—19, Prosonno Coomar Tagore's Street, W. 5.	3 ps. inoculated on 11-4-95. No inoculated attacked.	7 ps. non-inoculated. A non-inoculated, Chundro Kissore Nundy, H. m., 52, attacked with cholera on 31-10-95,—203 days after inoculation in the house ; died on 2-11-95.
XL.—Gagan Chundro Mazumdar's h., Kalighat Rd., Satyapirtolla, W. 22.	1 p. inoculated on 22-12-94. Not attacked.	6 ps. non-inoculated. A non-inoculated, Bunshi Tanti, H. m., 40, attacked with cholera on 5-11-95,—318 days after inoculation in the house ; died on 5-11-95.
XLI.—Joynarain Boyragee's h., 5-4, Chingreehatta Road, W. 19.	2 ps. inoculated on 13-11-95. Madhub Chandra Paul, H. m., 50, inoculated with vaccine "I" only, attacked with cholera on 13-11-95,—15 hours after inoculation ; died on 14-11-95.	1 p. non-inoculated. Not attacked.
XLII.—Ram Khetra Nath's h., Bara Bagan bustee, Bokul Bagan Road, W. 22.	9 ps. inoculated on 15-11-95. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Bidu, H. f., 60, attacked with cholera on 8-12-95,—23 days after inoculation in the house ; recovered.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XLIII.—Parbati Churn Bhattacharjee's h., Kalighat 2nd Lane, W. 22.	6 ps. inoculated on 11-1-95. No inoculated attacked.	9 ps. non-inoculated. A non-inoculated, Gourmoni, H. f., 70, attacked with cholera on 12-12-95,—335 days after inoculation in the house; died on 13-12-95.
XLIV.—Chintamoni Dutt's house, Nepal Bhattacharjee's 2nd Lane, W. 22.	3 ps. inoculated on 4-12-95. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Raimoni, H. f., 24, attacked with cholera on 17-12-95,—13 days after inoculation in the house; died on 18-12-95.
XLV.—Kamini Dassee's h., 8, Obhoy Halder's Lane, W. 10.	8 ps. inoculated on 4-1-96. No inoculated attacked.	12 ps. non-inoculated. A non-inoculated, Sohdora Dassee, H. f., 29, attacked with cholera on 9-1-96,—5 days after inoculation in the house; died on 13-1-96.
XLVI.—Ram Kristo Ghugu's h., Western Berh, Kidderpore, W. 25.	5 ps. inoculated on 10-1-96. No inoculated attacked.	9 ps. non-inoculated. A non-inoculated, Ramsadoy Pandah, H. m., 45, attacked with cholera on 14-1-96,—4 days after inoculation in the house; recovered.
XLVII.—Kamikhay Nath Banerjee's h., 56, Issur Ganguly's Lane, W. 22.	6 ps. inoculated on 19-1-95. No inoculated attacked.	7 ps. non-inoculated. A non-inoculated, Gurupoddo Devi, H. f., 36, attacked with cholera on 14-2-96,—391 days after inoculation in the house; died on 15-2-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
XLVIII.—Sree Rattan Chamar's h., 12, Baliaghatta Road., W. 19.	3 ps. inoculated on 17-2-96. One of the inoculated (vaccine "I" only) attacked with cholera on 17-2-96,—5 hours after inoculation ; recovered.	6 ps. non-inoculated. No non-inoculated attacked.
XLIX.—Thakur Das Gunin's h., 53, Sakareetola Lane, W. 11.	2 ps. inoculated on 13-10-95. No inoculated attacked.	7 ps. non-inoculated. Two non-inoculated attacked with cholera : 1. Benode Behary Chowdry, H. m., 24, attacked on 20-2-96,—130 days after inoculation in the house ; died on 28-2-96. 2. Jodu Nath Ghosh, H. m., 45, attacked on 1-3-96,—140 days after inoculation in the house ; died on 2-3-96.
L.—Gagan Mazumdar's h., 46, Kalighat, W. 22.	1 p. inoculated on 22-12-94. Not attacked.	3 ps. non-inoculated. A non-inoculated, Kamal Bewah, H. f., 75, attacked with cholera on 28-2-96,—433 days after inoculation in the house ; died on 29-2-96.
LI.—Madhub Paramanick's h., Kopidanga bustee, W. 25.	2 ps. inoculated on 17-1-95. No inoculated attacked.	4 ps. non-inoculated. A non-inoculated, Sadee, H. f., 26, attacked with cholera on 29-2-96,—408 days after inoculation in the house ; died on 29-2-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LII.—Alokemoni Dassee's h., 64, Strand Rd., W. 22.	6 ps. inoculated on 30-11-95. No inoculated attacked.	4 ps. non-inoculated. Two non-inoculated attacked with cholera on 5-3-96,—96 days after inoculation in the house : 1. Chonnon Dass, H. m., 40, died on 5-3-96. 2. Hurry Churn Hazra, H. m., 30, died on 7-3-96.
LIII. — Shiboo Naik's h., 134-H-14, Machooa Bazar Street, W. 6.	10 ps. inoculated on 2-7-95. No inoculated attacked.	30 ps. non-inoculated. A non-inoculated, Madhu, H. m., 22, attacked with cholera on 9-3-96,—251 days after inoculation in the house ; died on 10-3-96.
LIV. — Mr. Carlyle's Cooly Depôt, 139, Baliaghatta Road, W. 9.	63 persons inoculated on 10-3-96. Rani, Hindu female, 32, inoculated with vaccine "I" only, attacked with cholera on 13-3-96,—3 days after inoculation ; died on 14-3-96.	25 ps. non-inoculated ; average number present 24. Three non-inoculated attacked with cholera : 1. Senmongul, H. m., 9, attacked on 11-3-96,—1 day after inoculation in the Depôt ; recovered. 2, Chattai, H. m., 35, attacked on 13-3-96,—3 days after inoculation in the Depôt ; died on 14-3-96. 3, Bhanta, H. m., 7, attacked on 14-3-96,—4 days after inoculation in the Depôt ; died on 15-3-96.
LV.—Shaik Budhu's h., 211, Lower Circular Road, W. 21.	2 ps. inoculated on 14-3-94. No inoculated attacked.	23 ps. non-inoculated. A non-inoculated, Shaik Gomani, M. m., 80, attacked with cholera on 14-3-96,—731 days after inoculation in the house ; died on 16-3-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LVI.—Abdul Rohoman's h., 10, Syed Ismail's Lane, W. 13.	6 ps. inoculated on 9-8-95. Abu Anthony, Christian male, 4, inoculated with vaccine "I" only, attacked with cholera on 15-3-96,—219 days after inoculation; died on 16-3-96.	14 ps. non-inoculated. No non-inoculated attacked.
LVII.—Ethbari's h., 6-9, Noorwoolla Doctor's Lane, W. 21.	4 ps. inoculated on 14-3-94. 2 inoculated attacked with cholera : 1. Omran, M. f., 7, inoculated with vaccine "I" only, attacked on 19-3-96,—736 days after inoculation ; died on 20-3-96. 2. Eshahac, M. m., 5, inoculated with vaccine "I" only, attacked on 22-3-96,—739 days after inoculation ; died on 22-3-96.	5 ps. non-inoculated. No non-inoculated attacked.
LVIII.—Tarini Churn Chatterjee's h., 53, Chasadhobapara Str., W. 6.	3 ps. inoculated on 24-5-94. No inoculated attacked.	14 ps. non-inoculated. A non-inoculated, Bepin Behary Chatterjee, H., m., 28, attacked with cholera on 28-3-96,—674 days after inoculation in the house ; died on 30-3-96.
LIX.—Bama Churn Dutt's h., 161, Upper Chitpore Road bustee, W. 1.	1 p. inoculated on 23-11-94. Not attacked.	8 ps. non-inoculated. Two non-inoculated attacked with cholera : 1. Kailashi Dassee, H. f., 60, attacked on 30-3-96,—493 days after inoculation in the house ; died on 30-3-96. 2. Kadu, H. f., 30, attacked on 5-4-96,—499 days after inoculation in the house ; died on 7-4-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LX.—Ramkristo Ghose's h., 27-H., 8, Ramkanto Mistri's Lane, W. 9.	1 p. inoculated on 14-5-95. Not attacked.	7 ps. non-inoculated. A non-inoculated, Kiron Chundro Ghose, H. m., 12, attacked with cholera on 6-4-96,—328 days after inoculation in the house; died on 6-4-96.
LXI. -Mohessia Bewah's h., 16, Jorabagan Str., W. 5.	4 ps. inoculated on 15-4-94. No inoculated attacked.	10 ps. non-inoculated. Two non-inoculated attacked with cholera. 1. Fagu, H. m., 16, attacked on 8-4-96,—724 days after inoculation in this house; recovered. 2. Larwan, H. f., 80, attacked on 23-4-96,—739 days after inoculation in this house; died on 23-4-96.
LXII.—Etcha Bewah's h., Kalighat Rd., Potooapara, W. 22.	1 p. inoculated on 20-11-94. Mohini Bewah, H. f., 42, inoculated with vaccine "I" only, attacked with cholera on 15-4-96,—512 days after inoculation; died on 16-4-96.	4 ps. non-inoculated. No non-inoculated attacked.
LXIII.—Rakhal Mondal's h., 8, Manicktolla Str., W. 6.	2 ps. inoculated on 29-5-94 and 21-1-95. Sarat Chundra Mondal, H. m., 12, inoculated with vaccine "I" on 29-5-94 and vaccine "II" on 3-6-94, attacked with cholera on 16-4-96,—688 days after inoculation; died on 21-4-96.	6. ps. non-inoculated. No non-inoculated attacked.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LXIV.—Mohunbansee Kur's h., 14, Ultadanga Road bustee, W. I.	4 ps. inoculated on 26-2-95. Monoranjan Kur, *alias* Hurrypudo Kur, H. m., 7½, inoculated with vaccine "1" only, attacked with cholera on 18-4-96,—417 days after inoculation ; died on 23-4-96.	8 ps. non-inoculated (on 18-4-96 the number was 7). A non-inoculated, Mohunbansee Kur, H. m., 49, attacked with cholera on 17-4-96,—416 days after inoculation in the house ; died on 18-4-96.
LXV.—Santo Lall's h., 16, Jorabagan Str., W. 5.	5 ps. inoculated on 15-4-94. No inoculated attacked.	14 ps. non-inoculated. A non-inoculated, Mongal Sonar, H. m., 30, attacked with cholera on 19-4-96,—785 days after inoculation in this house ; recovered.
LXVI.—Bhairub Chundra Dass's h., Moonshiganj Rd., W. 25.	1 p. inoculated on 4-1-95. Not attacked.	9 ps. non-inoculated. A non-inoculated, Urjoon Samule, H. m., 25, attacked with cholera on 20-4-96,—472 days after inoculation in this house ; died on 20-4-96.
LXVII.—Public Works Department's h., 1, Bakery Road, W. 18.	1 p. inoculated on 13-2-96. Not attacked.	69 ps. non-inoculated. A non-inoculated, Jiton, H. m., 20, attacked with cholera on 25-4-96,—72 days after inoculation in this house ; died on 25-4 96.
LXVIII.—Birn Pandah's h., 34, Market Str., W. 13.	10 ps. inoculated on 16-4-96. No inoculated attacked.	15 ps. non-inoculated. A non-inoculated, Kassy Pandah, H. m., 30, attacked with cholera on 25-4-96,—9 days after inoculation in this house ; died on 25-4-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LXIX.—Shiboo Naik's h., 134, Machooa Bazar Str. bustee, W. 6.	7 ps. inoculated on 12-3-96. No inoculated attacked.	8 ps. non-inoculated. A non-inoculated, Shams Jans, H. m., 35, attacked with cholera on 26-4-96,—45 days after inoculation in this house ; died on 28-4-96.
LXX.—On Board the Ship *Majestic*.	14 seamen inoculated on 25-4-96. No inoculated attacked.	8 seamen non-inoculated. A non-inoculated, Ivan Ferwing, 24 years, attacked with cholera on 28-4-96,—3 days after the date of inoculation on board ; died on 28-4-96.
LXXI.—Alladhi Bewah's h., 4, Muddon Dutt's Lane, W. 11.	4 ps. inoculated on 17-11-95. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Madhoosoodan Das, H. m., 80, attacked with cholera on 28-4-96,—163 days after inoculation in this house ; died on 4-5-96.
LXXII.—26, Hidaram Banerjee's Lane, W. 11.	1 p. inoculated on 20-4-95. Not attacked.	16. ps. non-inoculated. A non-inoculated, Rutton Moni Dassee, H. f., 25, attacked with cholera on 29-4-96,—375 days after inoculation in this house ; died on 29-4-96.
LXXIII.—Sree Nath Bearer's h., 88, Champatolla Lane, W. 8.	1 p. inoculated on 13-1-96. Not attacked.	19 ps. non-inoculated. A non-inoculated, Chundro Bearer, H. m., 36, attacked with cholera on 4-5-96,—112 days after inoculation in this house; died on 4-5-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LXXIV.—Tulla's h., 16, Jorabagan Str. bustee, W. 5.	3 ps. inoculated on 15-5-94 and 16-1-95. No inoculated attacked.	13 ps. non-inoculated. Two non-inoculated attacked with cholera : 1. Larwan, H. f., 30, attacked on 6-5-96,—722 days after first introduction of inoculation in this house ; died on 8-5-96. 2. Chattoo, H. m., 50, attacked on 8-5-96,—724 days after first introduction of inoculation in this house ; died on 11-5-96.
LXXV.—Jogun Bewah's h., 161-H, Upper Chitpore Rd. bustee, W. 1.	6 ps. inoculated on 3-4-96. No inoculated attacked.	7 ps. non-inoculated. A non-inoculated, Nathoo, H. m., 16, attacked on 7-5-96,—34 days after inoculation in this house ; died on 8-5-96.
LXXVI.—Munshi Naiamuth Ali's h., Western Berh, Kidderpur, W. 25.	7 ps. inoculated on 10-1-96. No inoculated attacked.	4 ps. non-inoculated. A non-inoculated, Samsher Nessa, M. f., 8, attacked with cholera on 9-5-96,—120 days after inoculation in this house ; died on 9-5-96.
LXXVII.—13, Shib Narain Das's Lane, W. 4.	2 ps. inoculated on 6-8-95. No inoculated attacked.	5 ps. non-inoculated. A non-inoculated, Dhonoda Soondari Dassee, H. f., 29, attacked with cholera on 14-5-96,—283 days after inoculation in this house ; died on 14-5-96.
LXXVIII.—120, Bulloram Dey's Street, W. 6.	2 ps. inoculated on 29-5-94. No inoculated attacked.	34 ps. non-inoculated. A non-inoculated, Bhochon, H. m., 2½, attacked with cholera on 19-5-96,—721 days after inoculation in this house ; died on 19-5-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LXXIX.—Bhoobonesur Bhattacharjee's h., 29-2, Jelliapara Rd., W. 22.	5 ps. inoculated on 30-11-95 with vaccine "I" only. 2 inoculated attacked with cholera : 1. Jotessur Bhattacharjee, H. m., 8, attacked on 8-6-96,—189 days after inoculation; died on 10-6-96. 2. Adissur Bhattacharjee, H. m., 6, attacked on 11-6-96,—192 days after inoculation ; recovered.	3 ps. non-inoculated. No non-inoculated attacked
LXXX.—Mr. Carlyle's Cooly Depôt, 139, Baliaghatta Road, W. 9.	14 ps. inoculated on 26th March and 2nd April 1896. No inoculated attacked.	31 ps. non-inoculated. 3 non-inoculated attacked with cholera on 9-6-96,—75 days after inoculation in the Depôt : 1. Chiton, H. m., 3, died on 10-6-96. 2. Rakhi, H. f., 30, died on 12-6-96. 3. Chita, H. f., 6, died on 16-6-96.
LXXXI.—Kyen Mistri's h., 30, Free School Str., W. 13.	1 p. inoculated on 19-3-96. Not attacked.	2 ps. non-inoculated. A non-inoculated, Chik Chik, H. m., 20, attacked with cholera on 26-6-96,—99 days after inoculation in the house; died on 27-6-96.
LXXXII.—Anondo Bearer's h., 4, Muddun Dutt's Lane, W. 11.	4 ps. inoculated on 17-11-95. No inoculated attacked.	11 ps. non-inoculated. A non-inoculated, Fakir Patro, H. m., 28, attacked with cholera on 27-6-96,—223 days after inoculation in the house ; died on 28-6-96.

Name of locality where cholera occurred in a household containing inoculated persons.	*Number of inoculated persons in the house and particulars of cholera patient, if among the inoculated.*	*Number of non-inoculated persons in the house and particulars of cholera patient, if among the non-inoculated.*
LXXXIII.—Nobin Shah's h., 133, Jaunbazar Str., W. 13.	8 ps. inoculated on 16-1-96. No inoculated attacked.	15 ps. non-inoculated. A non-inoculated, Angoni, H. m., 50, attacked with cholera on 14-7-96,—180 days after inoculation in the house ; died on 14-7-96.
LXXXIV.—Bhagamani Bewah's h., 3-1, GoorooprosadGhose's Lane, W. 13.	9 ps. inoculated on 19-6-95. No inoculated attacked.	32 non-inoculated. A non-inoculated, Lutchman, H. m., 22, attacked with cholera on 28-7-96,—405 days after inoculation in the house ; died on 30-7-96.
LXXXV.—Poora Soondari Dassee's h., 5, Nilmoney Halder's Lane, W. 13.	1 p. inoculated on 5-6-94. Not attacked.	8 ps. non-inoculated. A non-inoculated, Behariram, H. m., 60, attacked with cholera on 13-8-96,—800 days after inoculation in the house ; died on 14-8-96.
LXXXVI.—Tooni Bewah's h., 31, Kurraya Road, 21.	1 p. inoculated on 4-4-95. Not attacked.	6 ps. non-inoculated. A non-inoculated, Jainab, M. f., 24, attacked with cholera on 27-11-96,—603 days after inoculation in the house ; died on 27-11-96.

A special inoculation *Bulletin* was published by the Health Office in the monthly issues of the *Indian Medical Gazette*, which, in addition to ordinary general circulation, is supplied by the Government to the Medical Schools, Hospitals and other medical institutions of the country (*vide* Vol. XXIX, 1894, pp. 275, 316, 355, 395, 435 and 474 ; Vol. XXX, 1895, pp. 47, 84, 126, 166, 206, 246, 284, 321, 366, 398, 455 and 503 ; Vol. XXXI, 1896, pp. 39, 79, 118, 150, 229, 309, 346 and 385 ; and Vol. XXXII, 1897, p. 79). In these *Bulletins* the details of the inoculations performed during the month and all occurrences of cholera in

houses where inoculated individuals lived were published as the events were taking place. The information included all the details summarized in the above tables. In this manner the medical and sanitary authorities and the medical practitioners of Calcutta, of whom many were keen critics of the method, were kept informed of the progress of the work and placed in a position to verify the facts. Further, throughout the period of investigation, detailed reports, showing the occurrences of cholera in households containing inoculated members, were submitted periodically to the Municipal Council of Calcutta, at whose expense the inoculation service was maintained. The Council includes, as is well known, a high grade Government officer, a member of the Civil Service, as Chairman ; officials of the Government of Bengal; lawyers; officers of the Indian Medical Service and medical practitioners; newspaper proprietors and editors; house and land-owners; members of European and Indian mercantile and trading firms, etc.—who represent and reside in each of the localities referred to in the tables. The Municipal Commissioners were thus enabled to control the statements embodied in the reports. The facts and the whole inoculation system were challenged whenever a Commissioner had doubts about them ; questions were put officially to the Chairman of the Municipal Corporation ; the matter was debated publicly in the Corporation meetings, of which reports appeared in the daily press and in the medical papers (*vide*, e.g., *Indian Medical Record*, 1894, p. 261 ; *Indian Medical Gazette*, 1894, pp. 234-238, 435-437 ; 1896, pp. 358, 363, 386-888, etc. ; "*The Englishman*," 5th June 1896 ; and others) ; and a detailed re-investigation was on each such occasion made. In the course of these re-investigations the following four errors were discovered and corrected in the recorded facts :—

(1) In statement VIII, referring to Shaik Hingoo's house, at Beg Bagan bustee, Ward 21 : inoculation had been done 3 days before the occurrence of cholera in that house, not 5 days, as had been stated, probably by a copyist's slip, in the original report.

(2) In statement XLI, referring to Joynarain Boyragee's house, at 5-4, Chingreehatta Road, Ward 19 : "*none* non-inoculated" had been erroneously mentioned for "*one* non-inoculated."

(3) In statement XLII, referring to Ram Khetra Nath's house, at Bara Bagan bustee, Bokul Bagan Road, Ward 22 : the number of inoculated persons was 9, not 11, as stated originally (2 of the 11 inoculated members of the family were not staying in the same, but in a contiguous house) ; and

(4) Similarly, in statement LXXVI, referring to Munshi Naiamuth Ali's house, at Western Berh, Ward 25 : the number of inoculated members living with the others was 7, not 8, as originally stated.

(*Vide* Health Officer's report to the Chairman of the Municipal Corporation, dated 24th August 1896. Reprinted in the *Indian Medical Gazette* for October 1896, pp. 363-369.)

Had it appeared at any time that the study was being impaired by inadequate investigation, by exaggerating the results or by minimising cases of failure, or by any other manifestation of carelessness or of bad faith, the progress of the work would have been at once arrested, as it depended wholly on the confidence which the public and the authorities had in the operators. (*Vide* Surgeon-Lieutenant-Colonel A. Crombie, I.M.S., Dr. Koilas Chunder Bose Rai Bahadoor, Dr. W. J. Simpson and Surgeon-Lieutenant-Colonel Joubert, I.M.S., on "Anti-choleraic Inoculation," in the *Indian Medical Gazette*, September 1895, pp. 354-357.)

In examining the above tables, households in which the inoculated escaped the disease, but were present proportionately in such small numbers as to be little exposed to infection, may be left out of account. Such were the five households Nos. LV, LXVII, LXXII, LXXIII and LXXVIII, in each of which the inoculated numbered less than 1 to 10 non-inoculated, the aggregate population of these houses being 161 non-inoculated, among whom 5 attacks of cholera occurred, and 7 inoculated, who remained free.

In the other 80 houses and on the ship *Majestic*, the question as to the number of days which elapsed between the date of inoculation and the date of the occurrence of cholera in the house and on the ship should first be examined.

The result is as follows :—

Number of days which elapsed between the date of inoculation in a household and the attack of cholera in a non-inoculated or inoculated member of that household. Also number of cases, when more than one attack is referred to.	
In a non-inoculated member.	In an inoculated member.
Days.	Days.
——	0 (3 cases)
1	——
2 (2 cases)	2
3 (5 cases)	3
4 (2 cases)	4
5	——
6	——
7	——
9 (2 cases)	——
12	——
13	——
16	——
23	——
34	——
37	——
45	——
57	——
62	——
63	——
71	——
75 (3 cases)	——
95	——
96 (2 cases)	——
99 (2 cases)	——
114	——
118	——
120 (2 cases)	——
130	——
132	——
140	——
144	——
151	——
163	——
180	——
189	189
191	——
——	192
203	——
——	219
223	——
240	——
251	——
271	——
283	——
284	——
300	——
310	——
318	——
319	——
328	——
335	——
356	——
359	——
360	——
377 (2 cases)	——
380	——
383	——
384	——
391	——
392	——
393	——
400	——
403	——
405	——
408	——
416	——
——	417
433	——
445	——
449 (3 cases)	——
452	——
——	460
472	——
493	——
499	——
——	512
603	——
674	——
——	688
722	——
724 (2 cases)	——
735	——
——	736
739	739
800	——

This table at once reveals the fact that the incidence of cholera among the inoculated varied according to three periods. During the first 4 days after the date of inoculation cases were observed both among the inoculated and the non-inoculated; after the first 4 days there was a period of nearly 14 months (412 days) in which 3 attacks occurred among the inoculated, while among the non-inoculated, in the same houses, cases were taking place at short intervals throughout the whole of that period; and from the 417th day, during the remaining 13 months of observation, cases re-appeared among the inoculated. As time went on, the field of observation gradually contracted owing to the usual migration of the occupants of suburban tenements.

Analysing the tables according to the above three periods, it appears that—

I.—In the 12 houses and on the ship *Majestic* where cases of cholera occurred during the first 4 days after inoculation—a period in which the protective effect of the vaccine gradually asserted itself—there lived a total of

123 non-inoculated individuals, who had 6 cholera deaths (4·88 per cent.) and 4 attacks ending in recovery, and

142 inoculated, who had 5 deaths (3·52 per cent.) and 1 attack with recovery.

II.—In the 54 houses where cholera occurred during the second period, extending over 14 months, *i.e.*, from the 5th to the 416th day after inoculation, there lived

539 non-inoculated, who had 61 deaths (11·32 per cent.) and 5 attacks ending in recovery; and

279 inoculated who had 2 deaths (0·72 per cent.) and 1 attack with recovery.*

III.—Lastly, in the 16 houses where cholera occurred during the third period, *i.e.*, between the 417th and 800th day after inoculation, there were

126 non-inoculated, who had 15 deaths (11·90 per cent.) and 2 attacks with recovery, and

---

* Household No. II figures both in the I and II periods of observation.

41 inoculated who had 6 deaths (14·63 per cent.).*

During the immunization period, which occupied 4 days, the number of deaths among the inoculated was, therefore, 1·39 times smaller than among the non-inoculated. During the period of immunity, lasting 412 days, the number of deaths among the inoculated was 15·79 times smaller than among the non-inoculated ; which is to say that of every 100 deaths from cholera, which were to take place in that period of 412 days, 94 could be averted by the use of the vaccine. Lastly, during the third period, from the 417th to the 800th day after inoculation, when the effects of the immunization had vanished, the number of deaths among the non-inoculated was 1·23 times smaller than among the inoculated.

Of the 6 inoculated belonging to the last group, who were attacked more than 416 days after inoculation, 5 had received only one injection, with the first, or preparatory, vaccine ; and the 6th, inoculated on the 29th of May and 3rd of June 1894, and attacked with cholera on 16th April 1896, 688 days after the first inoculation, had had the two vaccines in very weak doses, as was practised before the date of the observations made in July and August 1894, in the East Lancashire Regiment, at Lucknow. It was the latter observations that indicated for the first time at what rate the effect of the doses with which the inoculations had been commenced in India tended to disappear as time went on. Since the summer of 1894 the doses and the strength of the vaccine were increased, with the object of extending, if possible, the duration of immunity, as will be stated lower down (*vide* pp. 70-71).

The results observed throughout the rest of the country agreed in every instance with and confirmed the Calcutta observations.

In the 1st Battalion, East Lancashire Regiment, mentioned above, inoculation was carried out in May 1893, soon after I had begun work in India, and cholera, in a severe form, broke out in July and continued in August 1894, that is, during the 14th and 15th month after the date of inoculation.

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* Household No. LXIV figures in the II and III periods of observation.

(In the plan followed in 1894-96 for summarizing totals, a household in which 2 or more cases of cholera occurred was, I believe, considered as a new unit on each such occasion.)

Among 640 non-inoculated officers, non-commissioned officers and men there occurred 120 cases (18·75 per cent.) with 79 deaths (12·34 per cent.) ; and among the 133 inoculated, 18 cases (13·53 per cent.) with 13 deaths (9·77 per cent.). The inoculation had been done with vaccines "I" and "II" given in very small doses.

Among the British Troops at Cawnpore, in July and August 1894, 13 months after inoculation, there occurred, among 797 non-inoculated, 19 cases with 13 deaths, and among 75 inoculated, no cases. Inoculation in this Regiment had been done by Mr. E. H. Hankin of Agra.

In the 2nd Battalion, Manchester Regiment, at Dinapore and Camp Beta, in July-August 1894, a few cases occurred 2 to 6 days after inoculation, *viz.* :—

Among 729 non-inoculated, 6 cases with 3 deaths, and among 193 inoculated, no cases.

On the Tea Estates in Lower Assam, *viz.*, Kalacherra, Chargola, Pollarbund and Lungla, there occurred in the summer of 1895, during the first few weeks after inoculation,

among 4,747 non-inoculated coolies, 12 cases (0·25 per cent.) with 9 deaths (0·19 per cent.) , and

among 1,374 inoculated, all with vaccine "I" only, 1 case (0·07), fatal.

On one Estate only, at Adam Tila, 657 non-inoculated remained free from cholera, while among the 318 inoculated, all with the preliminary vaccine only, there occurred 2 cases (0·63 per cent.) with 1 fatal issue (0·31 per cent.).

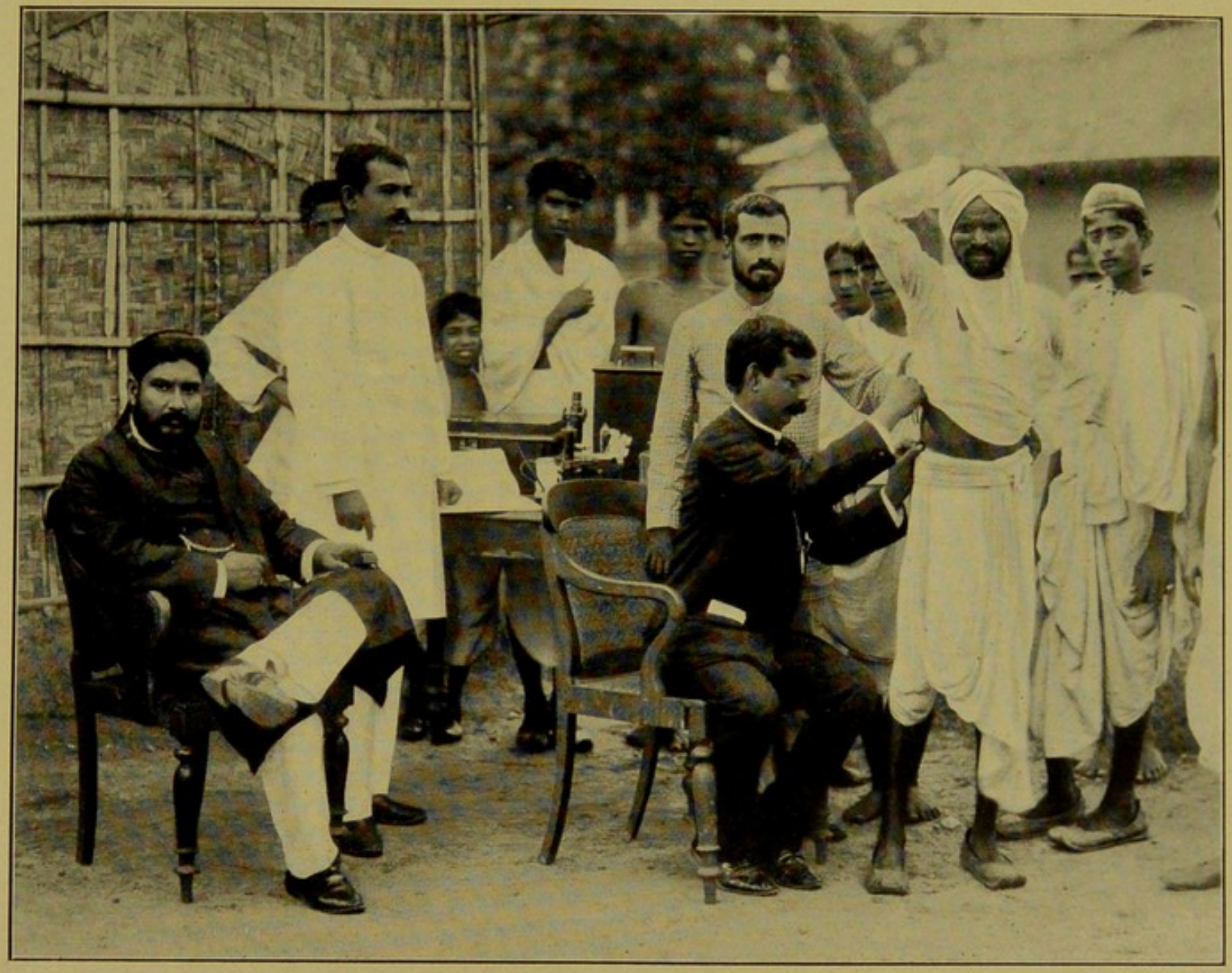
In the Gya Jail inoculation—first with vaccine "I," and five days later, with vaccine "II," both in small doses—was applied in July 1894, while an epidemic of cholera was in progress. In the 15 days during which the epidemic continued, and including the cases which occurred during the first days, *i.e.*, while the protective effect of the vaccines was still asserting itself, there were,

among a daily average strength of 202 non-inoculated prisoners,* 20 cases (9·91 per cent.) with 10 deaths (4·95 per cent.) ; and

* The daily strength was 218, 218, 213, 210, 210, 206, 206, 201, 199, 195, 199, 189, 193, 190 and 194 respectively.

Anti-cholera Inoculation in Calcutta.

Operations by the Officials of the Calcutta Municipal Corporation
under Dr. W. J. R. Simpson, Health Officer.



Dr. Sasi Bhusan Ghose,
Assistant Analyst,
Calcutta Health Office.

Mr. Panna Lal De,
Superintendent of Records,
Calcutta Health Office.

Dr. J. Chatterjee,
Medical Inspector.

Dr. Jonoman Joy Chowdry,
Inoculating Officer,
Calcutta Health Office.

(To face p. 68.)

among an average strength of 196 inoculated, 4 cases (2·04 per cent.), all fatal.

Two inoculations, with vaccines "I" and "II," in increased doses, were given in this instance. Colonel Hare, in reporting upon this outbreak and referring to the similarity of conditions under which the inoculated and the non-inoculated lived until the camp was broken up, makes the following observations :—

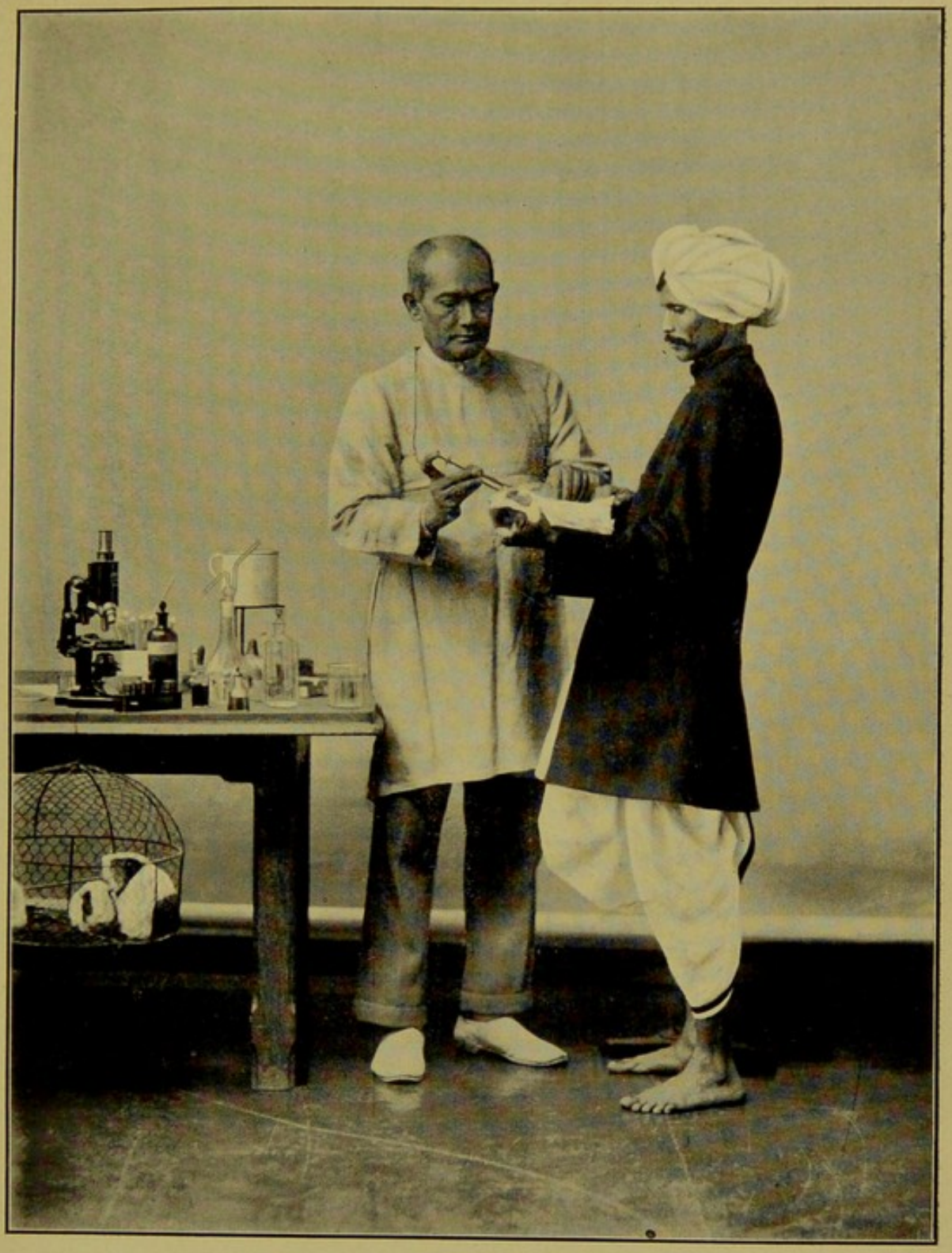
"The results from one group are especially worthy of record. It consisted of the first 50 men whose names were written consecutively on the general register (Nos. 1 to 50), with Nos. 356 and 357 belonging to the same party; they formed a natural group, living together in the same sheds. Of these 52 individuals 36 were inoculated; among the 36 there was 1 attack and 1 death (2·7 per cent.), compared with 11 attacks and 11 deaths (68·75 per cent.) among the 16 uninoculated." (Surgeon-Captain Ch. E. Hare, I.M.S., "Notes on an outbreak of cholera among the Khasia coolies of the Assam-Burma Connection Railway Survey, and on the effect of anti-cholera inoculation," *Indian Medical Gazette*, December 1895, pp. 503-505.) The coolies belonged to the same Khasia or Synting caste and shared, at the time, food, drinking water, etc. The camp was situated away from other habitations and was guarded by a detachment of military police, so that no occurrence of disease or death escaped notice. (The police guards, who formed a group apart and remained free from cholera, are not included in the above figures.)

The plan of inoculating strong doses of vaccine "II" in the hope of extending the period of immunity of the inoculated was tried on a large scale on coolies of the Cachar Tea Estates, in 1895-96. The observations upon the inoculated were continued till the end of 1899, and the following incidence of cholera was recorded and published by the medical officer then in charge of the Estates, Dr. Arthur Powell, at present Police Surgeon and Coroner's Physician of Bombay :—

among 6,549 non-inoculated coolies there occurred 198 cases (3·02 per cent.) with 124 deaths (1·89 per cent.); and

among 5,778 inoculated, 27 cases (0·47 per cent.) with 14

Operations at the Health Office, Calcutta, for keeping anti-cholera vaccine on a constant level of strength.



Intra-peritoneal Inoculation of a Guinea-pig
by Dr. Sasi Bhusan Ghose, Assistant Analyst to the Health Officer of Calcutta.

(*To face p. 70.*)

deaths (0·24 per cent.). (Dr. Arthur Powell, "Results of Haffkine's anti-choleraic inoculations in Cachár," *Indian Medical Gazette*, July 1895, pp. 253-259, and May, June and July 1896, pp. 185-188, 226-228 and 267-269; *The Lancet*, 18th July 1896. *Idem*, "Further results of Haffkine's anti-cholera inoculations," *Journal of Tropical Medicine*, December 1899.) The mode of observation which was instituted on the Tea Estates may be seen from the tables accompanying Dr. Powell's publications in the *Indian Medical Gazette*.

On these Estates, as in the case of the Regiments, the inoculated and non-inoculated lived together, in huts belonging to the Estates. The coolie labourers are registered in the Estate books and receive wages for each working day. They are, therefore, supervised at work daily, and all incidence of disease or of any other cause of absence becomes immediately known.

Valuable material bearing on the effect of inoculation was also gathered by Surgeon-Captain (now Lieutenant Colonel) J. C. Vaughan, while in charge of the Purulia Inoculation Station, in 1896-97 (*vide Indian Medical Gazette*, 1897, pp. 114-119; 157-158; 194-196). The documents at my disposal are, however, not complete. Other references to publications on the subject may be found in the bibliography accompanying the "Revue" in the *Bulletin de l'Institut Pasteur*, Vol. IV, 1906, entitled "Les vaccinations anticholériques aux Indes, par W. M. Haffkine," pp. 697-705 and 737-747.

In the studies described above there is one *desideratum* which a bacteriologist might have wished to see fulfilled to a greater extent than it actually was, and which I would have endeavoured so to fulfil, had there been at the time the necessary requirements at my disposal. I refer to a bacteriological examination of the products of the cholera patients, so as to have that method of diagnosis of the disease added to the clinical diagnosis made by the physicians who observed and investigated the cases. A bacteriological examination was carried out on many patients in his charge by Dr. Arthur Powell of Cachár, and in certain cases by the officials of the Calcutta Health Office; but in most

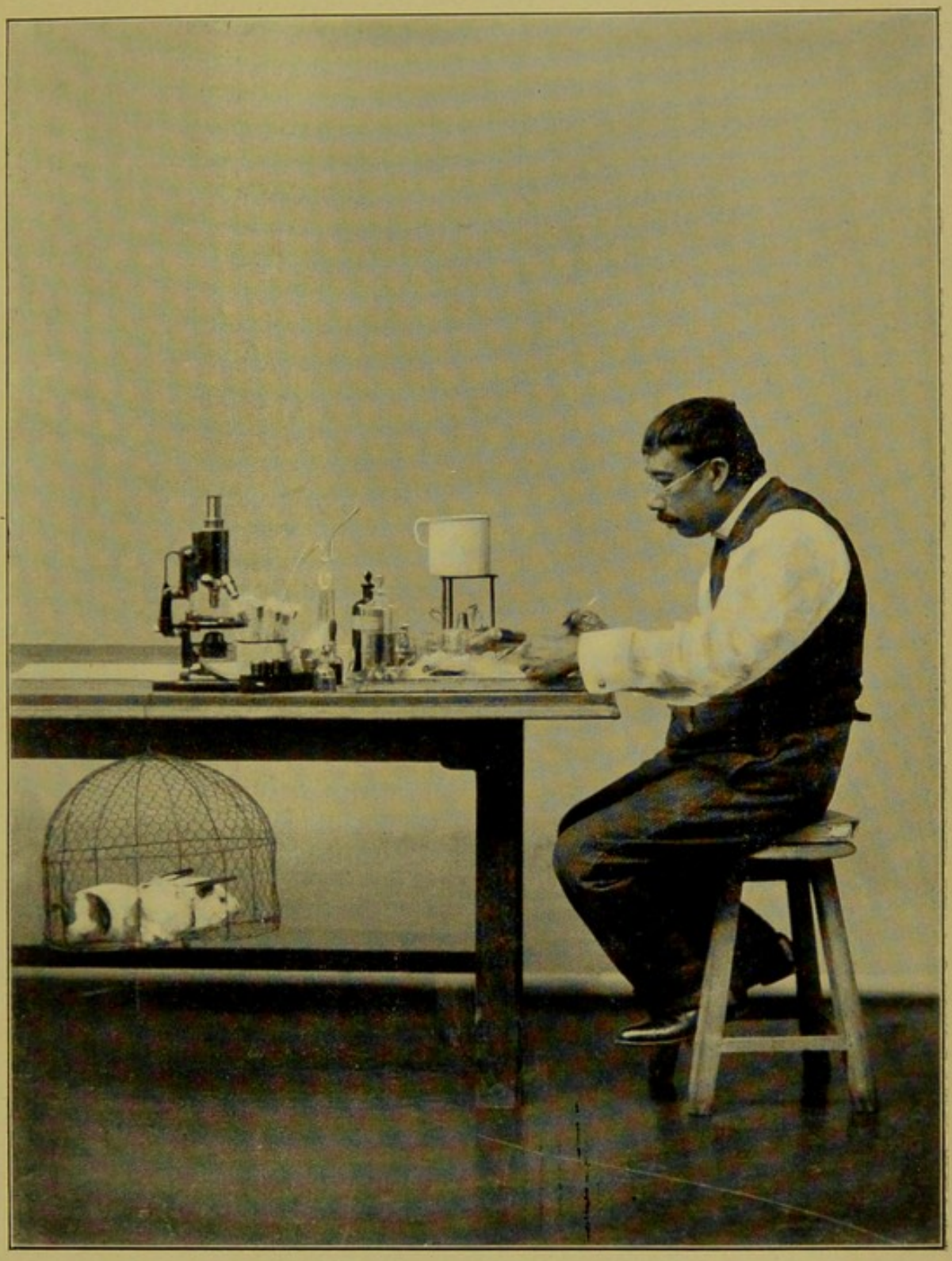
other instances such an examination was not practicable and was not desired by the Regimental or Jail authorities, by the Medical Officers in charge, by the Tea Estate managers and their Medical Officers, or by the sanitary authorities of the country. The reason of this is that the percentage of doubtful cases in an epidemic of cholera is relatively unimportant, as is known to those who have ever been in the midst of an outbreak of this disease and are familiar with its manifestations. As a safeguard, however, against possible mistakes, the rule was adopted, on the occurrence of any uncertainty, always to record doubtful results *against* the cause of inoculation. Thus, if a doubtful illness occurred in an inoculated, it was arranged to record this as a case of failure, *i.e.*, a case of cholera in an inoculated; while if it occurred in a non-inoculated, the doubtful attack was not to be taken into account.

The effect of the anti-cholera inoculation was thus established by carefully studying the history of and comparing inoculated and non-inoculated members of the same households, the same regiments, the same batches of coolies, or inmates of the same prisons. In this way sources of error arising out of privileged social position, sanitary locality, good housing, pure food and water, or any other adventitious cause of immunity in the inoculated were obviated. Students of medicine and sanitation will, I believe, find it not easy to reply, if they are asked to name, in the history of those sciences, a method, whether preventive or curative, the effect of which has been established with a greater degree of precision than in the case of the method under consideration.

Views of various Authorities.

The question as to the possibility of immunizing man against cholera has been judged by many authoritative critics to have received, in the experiments detailed above, a definitely affirmative solution. I might quote a long list of pronouncements on the subject; but one or two will perhaps suffice. The view taken by English scientists was enquired into by Lord George Hamilton, when Secretary of State for India, and was stated by him in letters, dated

Operations at the Health Office, Calcutta, for keeping anti-cholera vaccine on a constant level of strength.



Post-mortem Examination of a Guinea-pig: Collection of Peritoneal Fluid by Dr. Jogendra Nath Dutt, Analyst to the Health Officer of Calcutta.

(*To face p. 72.*)

21st January 1896, to the Earl of Elgin, at the time Viceroy and Governor-General of India, and to Lord Sandhurst, Governor of the Presidency of Bombay. The statement addressed to the Viceroy was as follows :—

"*21st January 1896.*

"DEAR LORD ELGIN,—I have had the pleasure of making Dr. Haffkine's acquaintance during his visit to this country and of hearing from him an account of his work in India. From the inquiries I have made it seems clear that his discovery is regarded as of the highest importance by the best scientific critics ; and I write, therefore, to ask you to be so good as to give directions for Dr. Haffkine to be afforded facilities similar to those which he has already received from Your Excellency's Government.

"H. E.
"THE EARL OF ELGIN,
"etc., etc., etc.

"I am,
Yours sincerely,
GEORGE HAMILTON."

In the letter to the Governor of Bombay the Secretary of State made a suggestion which the appearance of plague in that Presidency, in the autumn of the same year, prevented from being carried out at the time, and to which effect has not yet been given. The following is the text of the letter :—

"*21st January 1896.*

"DEAR LORD SANDHURST,—I have been hearing about Dr. Haffkine's work, both from himself and from experts ; and I imagine there can be no doubt of the importance of his discovery and of the practical good effected by his system of inoculation. I write, therefore, to ask you to be so good as to see that Dr. Haffkine is

given the same facilities as those which he has already received from the Government in India ; and I hope, if the authorities see no practical objection, that it may be found possible to extend the inoculation experiments to the Mecca pilgrims. But the pilgrim traffic is such a delicate matter that I can quite understand that it may be undesirable to take this step, except in a very tentative way.

"I am,
Yours very truly,
GEORGE HAMILTON."

"H. E.
"THE LORD SANDHURST,
"etc., etc., etc.

A scientist whose views on this matter will probably be read with particular interest was the late Robert Koch, who shares with Pasteur the position of founder of the science of bacteriology. It is known that Koch devoted to cholera special studies in France, Egypt and India, in the Hamburg epidemic of 1892 and in a succession of outbreaks which took place in Germany subsequently, and that he was the discoverer of the microbial agent of this disease. Koch's conclusions on the bearing of the Indian studies were more decisive than I dared, in diffidence of my own judgment, to form. He had followed closely the progress of the work and studied the facts as they appeared in periodicals and were summarized in the printed report submitted by me to the Home Department of the Government of India on 25th August 1895 ; and subsequently he allowed his deductions to be cited in the address which I had occasion to deliver, on the 18th December of the same year, at the Conjoint Board of the Royal Colleges of Physicians and Surgeons in London. My statement of his view had been read preliminarily by Koch and approved by him, and was to the following effect (vide *British Medical Journal*, 21st December 1895) :—" When, in recapitulating with Professor Koch the data of my report to the Government of India, I said that, in my idea, the results tended to prove the efficacy of the method, but that I felt it necessary to do all in my power to confirm them by further observation, I was most happy to learn that, for Professor Koch, the demonstration was already complete ; that he believed the protective power of

the method to be established finally by the observations collected in India up to then; that further perfections and simplifications might be possible, but that the main question at issue, the chief part of the problem, was solved by the facts recorded in the above report. Professor Koch gave his kind permission to quote these decisive conclusions in this Hall and to use the terms I have used."

General Results of the above Studies.

The facts of general significance revealed in the course of the studies detailed above were the following:

(1) "active" immunization, realised by means of a purely *bacterial vaccine*, as contrasted with immunization by means of *lymph* or *tissue* of another, previously inoculated, animal, was effective in application to man;

(2) such immunization could be carried out with safety during the progress of acute and fatal outbreaks, as was, *e.g.*, the outbreak in the Durbhanga Jail; and the febrile and other reaction caused by the injection of the vaccine, in the doses used by us, did not increase the susceptibility to infection among the inoculated and did not aggravate the disease when an inoculated happened to become infected during the progress of that reaction or during the days immediately following; and

(3) the development of a rapidly-incubating disease, such as cholera, could be mitigated or entirely averted by applying the same form of immunization to individuals previously infected, in whom the disease was already in the incubation stage. This latter fact, theoretically of a much more paradoxical nature than the others (*vide* p. 92), received in the next two years (1897 and 1898) extensive confirmation in the results of the anti-plague inoculation, which was planned upon the results of the inoculation against cholera; and the principle thus established was adopted for guidance in therapeutic practice and applied in treating diseases actually developed, first tentatively, by some of my co-workers in India, and subsequently by Sir Almroth E. Wright, who learned it during his visit to India as member of the Plague

Commission of 1898-1901. It must be mentioned, however, that, at the time, the Commission did not see their way clear to acquiesce in the validity of the discovery and in their official report pronounced themselves against the applicability of inoculation in the incubation stage of plague. Inoculation as a remedy against a condition of infection developed beyond the incubation stage has since been extensively tried, and its effects and degree of actual utility in such circumstances are studied in many diseases.

Another feature of the anti-cholera inoculation, which was taken into account in devising the plan of the inoculation against plague, was that, while the incidence of cases of cholera and—parallel with this—the incidence of deaths from that disease were powerfully influenced by the inoculation, no effect of any constancy was observed upon the recovery rate of the inoculated attacked, a result which, unfortunately, went against the expectations suggested by *à priori* consideration of the matter (*vide* p. 35). In devising the plan of inoculation against plague endeavours were made to affect favourably also the recovery rate ; and, apparently in answer to the measures adopted, the result proved successful.

Subsequent work by various experimenters and by myself regarding the immunization of man against cholera, typhoid and plague was a continuation and outcome of the studies of 1890-6. I shall have to make later on some references to publications on cholera (*vide* pp. 83-86). As concerns immunization against typhoid, R. Pfeiffer and W. Kolle, shortly after our consultation in 1895 (*vide* p. 13), undertook the first anti-typhoid vaccine operations in Germany. In their original publication on the subject (*Deutsche Medicinische Wochenschrift*, 12th November 1896) the authors quote the Indian researches as opening the way for further application of the method, and state that "as the experiments made to realize artificial immunization of man against cholera gave such an unexpectedly favourable result, the problem which presented itself next was to enquire how man would react to inoculation of typhoid bacilli." The examination of the blood of the persons subjected to such inoculation (*cf.* pp. 84 and 93-97) showed a certain similarity with the blood of individuals who had

recovered from an attack of typhoid fever, and the authors surmized that typhoid inoculation was also likely to be effective. "We are," they state, "the more entitled to this conclusion, as the analogous experiments of Haffkine on preventive inoculation against cholera have already stood the test of many thousands of practical applications." The interest of this statement is, I think, enhanced by the fact, acknowledged in the above passage, that, at the commencement of the Indian investigations, in the memoir of 1893 which I have quoted above (p. 36), Pfeiffer and Wassermann had expressed themselves as not expecting that immunization against intestinal cholera was realizable.—In England, Wright and Semple started anti-typhoid inoculation upon a plan given by me to Professor Wright (*vide* Wright and Semple in the *British Medical Journal* of 30th January 1897). In the paper just cited the writers say: "Mr. Haffkine suggested rather more than twelve months ago to one of us that the method of vaccination which has proved so effectual in combating cholera epidemics in India, might, *mutatis mutandis*, be applied also to the prophylaxis of typhod fever," and refer to the procedure followed by them and to that adopted by Pfeiffer and Kolle in the following terms: "The method of inoculation which these authors have adopted, is exactly similar to the one that we had previously adopted. Like our own method it was based upon the methods which have been so successfully employed by Mr. Haffkine in his anti-cholera inoculation." The latter methods had been previously demonstrated by me to Professor Wright and the probationers of the Army Medical School in a special course of experiments made for them in the pathological laboratory at Netley (*vide* A. E. Wright and D. Bruce, "On Haffkine's method of vaccination against cholera," *British Medical Journal*, 4th February 1893).—Inoculation against plague was similarly a direct outcome of the cholera inoculation in India, and, as already mentioned, was based on the teachings which resulted from that work.—A further application has now been given to the same methods in the preventive inoculation against dysentery.

PART III.

VACCINE "II" AFTER DEVITALIZATION.

PART III.

CONDITION OF THE CHOLERA VACCINE AFTER DEVITALIZATION.

Conservation of essential Properties in devitalized Vaccine. Its reactionary and immunizing Effects.

Some of the most notable properties imparted to vaccine "II" by peritoneal cultivation, namely, the enhancement, up to a certain constant level, of its toxic and immunizing effects, are still observable in that vaccine when it is devitalized by delicate chemical or physical processes. Apparently devitalization in these circumstances leaves uninjured part of the specific substances stored up during life in the bodies of the germs. The preparation of such devitalized vaccine and the results of its injection in animals and man have been described by several authors to whom I had occasion to demonstrate its effects, *viz.*, by Mr. E. H. Hankin (in the above-quoted paper "On Haffkine's method of protective inoculation against cholera," *British Medical Journal*, 10th September 1892); by Dr. Iawein ("Observations sur des cobayes immunisés par les vaccins anticholériques vivants," *Annales de l'Institut Pasteur*, October 1892); by Dr. Tamamcheff ("Expériences sur les vaccins phéniqués de Haffkine," *ibid.*); in the paper on "Vaccination against Asiatic Cholera," in the *Review of Reviews* of 14th January 1893; in A. E. Wright and D. Bruce's memoir "On Haffkine's method of vaccination against cholera," *British Medical Journal*, 4th February 1893; in lectures delivered by me in London, Cambridge and Calcutta (*British Medical Journal* and the *Lancet*, 11th February 1893; *Indian Medical Gazette*, April 1893); in my paper above referred to on "Vaccination against Asiatic Cholera" in the *Fortnightly Review* of 1st March 1893; in an article by Surgeon-Captain (now Colonel) Manifold, I.M.S., entitled: "Report of a case of inoculation with carbolized anti-choleraic vaccine (Haffkine)," *Indian Medical Gazette*, April 1893; and in a note in the *Annual Report of the Sanitary Commissioner with the Government of India for 1900*, p. 91.

In publications issued by other experimentalists results confirmatory of the above were recorded. In 1897 Pfeiffer and Friedberger, working on the cholera virus, and Pfeiffer, working on the virus of plague, ascertained that the immunizing power of devitalized vaccine varied in direct connection with the virulence of the strain of germs of which the vaccine was made (*Berliner Klinische Wochenschrift*, 1902). In 1903 Dr. R. Strong found, in cholera, that the "bactericidal immunity obtained by means of inoculation with dead organisms of different virulence, or their extracts, is proportional to the virulence of the living strain of the bacteria employed." (Manila *Bulletin*, October 1904, p. 35.) Similar conclusions have been arrived at by various other investigators. (*Vide* references on pp. 13-17.) The principles enunciated above in regard to the preparation of the live cholera vaccine, *i.e.*, the necessity of steadying the virulence of the germ on a certain determined level and the procedure by which this may be achieved, hold good, therefore, when the vaccine is intended to be used in a devitalized condition. These principles have been adhered to in the preparation of devitalized cholera and plague vaccine in the Bombay Bacteriological Laboratory, the Manila Biological Laboratory and the Plague Laboratory in Pianosa, Italy ; in the preparation of the cholera vaccine by Dr. W. Kolle, by the Japanese bacteriologists, and by others.

Devitalization by carbolic acid reduces, or altogether suppresses, the mortifying effect which vaccine "II" has on the guinea-pig's skin ; and the effect on man, as described on pp. 30-31, is also correspondingly mitigated. In all cases, however, the degree of fever and of local reaction is in direct connection with the *dose* administered. The desire of lengthening the duration of protection and the uncertainty, which at present cannot be removed, as regards the precise amount of vaccine which will ensure an immunity of a given durability, have often led operators into using exaggerated doses in inoculating man. This circumstance is responsible for the impression that the reaction after the inoculation is severe and stands in the way of a wide application of the method.

As has been stated already, inoculation with devitalized vaccine confers on animals a lesser degree of immunity than inoculation

with the same vaccine in a live condition. It remains possible, however, that the former preparation may still have preserved sufficient potency for immunizing man against natural cholera. The solution of this question in the affirmative would bring about great practical facilities in the application of the method; for devitalized vaccine can be handled as a chemical drug and may be preserved and supplied to distant operators, who thus require no preliminary training in the *technique* of its preparation, such as is necessary in the use of live vaccine.

It is, therefore, desirable that devitalized vaccine "II" —already used on a certain scale in 1892 and 1893 and on various occasions subsequently (*vide* below), and the further investigation of which has been delayed until the question of anti-cholera vaccination was decided in principle by means of the more reliable preparation—should be subjected to a careful study in cholera epidemics.

Expectations of other Observers.

The view just expressed has the endorsement of various authorities, some of whom have recommended the use of the vaccine in question, while others have already been trying it in practice. Among the first may be mentioned Dr. W. Kolle, formerly of Koch's Institute, now Director of the Sero-therapeutic and Vaccinal Institute in Berne. In a paper in the *Centralblatt für Bakteriologie*, I, Vol. XIX, 1896, entitled "Die aktive Immunisierung des Menschen gegen Cholera nach Haffkine's Verfahren in India ausgeführt," Dr. Kolle reviewed the results of the Indian studies and, in his next memoir, in January 1897, recommended the use on a large scale of my devitalized vaccine. Referring to this recommendation Dr. Strong states ("Protective Inoculation against Asiatic cholera," No. 16 of the Manila *Bulletin*, September 1904, p. 9):—

"He (Kolle) recommended the following method of human inoculation: a well grown culture containing about 20 milligrams of growth was suspended in 10 cubic centimeters of physiological salt solution and sterilized for a few minutes at 50 C.; 0·5 per

cent. phenol was added to the preparation without apparently interfering with the effectiveness of the virus . . . Haffkine also sometimes "used dead cultures" (*vide* pp. 37 and 81, and lower down on this page), "but thought that the living organisms gave a greater degree of immunity and a more lasting one. The numerous observations made by Haffkine and others in India speak decidedly for the effectiveness of his own as well as Kolle's vaccine and for the protection which is afforded by them when properly applied."

Unfortunately, however, only the live form of the vaccine has been studied so far in epidemics in India. The modification which Dr. Strong refers to as "Kolle's vaccine" had been prepared by me for experiments on animals and observed in its application to man, but I have not had an occasion of investigating its properties in cholera epidemics. Dr. Kolle recommended its use not because information was available on the latter subject, but because of the results obtained in my experiments with the live vaccine and on account of certain observations which he and other experimentalists had made on the blood of persons inoculated with the live and with the devitalized preparations. I shall refer to the latter observations in some detail lower down. (*Vide* pp. 93-97.)

The plan of inoculating man with the devitalized form of cholera vaccine has, further, the endorsement of a number of bacteriologists who, as stated, have actually tried it, in some cases, on a considerable scale. The first operations on such a scale, with vaccine prepared in the Bombay laboratory, were carried out and studied, under my directions, in August and September 1900, by the late Major G. Lamb, I.M.S., at the time my assistant in Bombay, and Major Buchanan, I.M.S., Superintendent of the Nagpur Central Jail. The vaccine was used on 451 prisoners of that jail, 18 of whom received two injections. No cholera cases occurred in the jail subsequent to the date of inoculation, whether in inoculated or non-inoculated prisoners. (*Vide* above-mentioned reference in the *Annual Report of the Sanitary Commissioner with the Government of India for 1900*, p. 91.)

The beneficial effects obtained, since 1897, from the anti-plague prophylactic, the wide application of which has been facilitated by

the devitalized condition of the virus, have stimulated attempts to apply the same plan for the protection of man from typhoid and cholera and, latterly, from certain forms of dysentery.

Among those who have used the devitalized anti-cholera vaccine subsequent to the inoculations in Nagpur may be cited the bacteriologists of the Japanese Imperial Serum Institute, an account of whose operations is given in Mabry and Gemmil's article "Cholera aboard United States army transport *Sherman*," in the *Journal of the American Medical Association*, 20th December 1902. In the same year, as many as 77,907 persons were inoculated in Japan, in the Hiogo district, under the supervision of Dr. N. Murata (*Centralblatt für Bakteriologie*, I, Orig., Vol. XXXV, No. 5, 15th February 1904, pp. 605-608). Another 1,106 were operated upon by Dr. J. Tsuzuki in North China (*Archive für Schiffs-und Tropen-Hygiene*, Vol. VIII, No. 2, February 1904).

In the following years many other operations with the same vaccine were performed in various parts of the world.

In 1904, a Russian physician, Dr. Zlatogoroff, inoculated in Tauris, Persia, 1,269 individuals (*Roosski Vratch*, 27th November and 4th December 1904).

In Russia, in 1905, Serkowski first performed a few dozen operations at Kharkoff and Lodz (*Centralblatt für Bakteriologie*, I, Orig., XLI, 17th May 1906) ; then, in 1907, Zabolotny reported at the International Congress of Hygiene and Demography, in Berlin, a considerable extension of the work which had taken place in the Volga region and elsewhere in Russia. In that and in the subsequent year, 4,877 people were inoculated in the Southern provinces of that country, while in St. Petersburg the number reached 31,637 (Zabolotny in the Russian edition of the *Archives of Biological Sceinces*, St. Petersburg, 1909, XIV, No. 5, pp. 471-494 ; also Kashkadamoff, on the anti-cholera inoculations in St. Petersburg, in the sanitary report for 1909). In the Ekaterinoslav province, Dr. Lookiantchenko inoculated, in 1907, 11,178 people (*Kharkovsky Medicinsky Journal*, Vol. V, No. 4, 1908), and Dr. Maslakowetz, in the summer of the same year, 4,287 in the town of Astrakhan and its suburbs (*Vratchebnaia Gazetta*, No. 21, 1908).

Between 1906 and 1909, inoculation with devitalized cholera vaccine was carried out also in several districts and provinces of Russian Poland, such as Parczew, Kielce, Tuszyn, Ozorkow, Piotrkow. The vaccine was manufactured in St. Petersburg, in the Imperial Institute of Experimental Medicine; in Warsaw, by Drs. Karwacki and Zurakowski; in Krakau, by Dr. Bujwid, and so on.

In Christiania, Norway, Dr. Aasar inoculated in 1910, 31 individuals. (*Berliner Klinische Wochenschrift*, 22nd August 1910, No. 34.)

In the Dutch East Indies, in Batavia, Dr. Nyland has operated, since 1910, on 31,000 people, including 8,000 Europeans. (*Bulletin de la Société de Pathologie exotique*, Paris, 1912, Vol. V, p. 4.) Van Heel inoculated, in April to October, 1910, in the same town, the inmates of the Chinese Asylum. (*Geneesk. Tijdschr. voor Nederl. Indië*, Feestbundel, 1911.)

The Question of the Degree of Immunity obtainable from devitalized Cholera Vaccine.

The documents which I have been able to examine on this essential matter show, however, that the authors were in a position to obtain only approximate information as to the immunity derivable from the vaccine.

In a paper published in the *Annales de l'Institut Pasteur* of last year, Professor E. Metchnikoff, at present Sub-Director of the Paris Pasteur Institute, makes a somewhat similar statement. Referring to the inoculations which were performed in Persia, in 1904, in various provinces of Russia, in 1907, and in St. Petersburg, in 1908-09, Professor Metchnikoff points out that the inoculated were generally persons not exposed to the same chances of infection as was the average population; that those who availed themselves of the preventive treatment belonged to such classes as observed also precautions of general hygiene; that individuals living in localities already infected, or in any way specially liable to contract the disease were refused inoculation, so as not to discredit the procedure in the

eyes of the public ; and that the incidence of cholera among the inoculated was not accurately known. The official instructions issued at the time by the authorities (*vide* quotation from the *St. Petersburg Medicinishe Wochenschrift* in the *Indian Medical Gazette*, September 1909, p. 346) enjoined, indeed, on the operators certain rules which were reasonable from a general point of view, but which tended to interfere with the subsequent estimation of the results.

The precise circumstances of the operations just referred to are unfortunately unknown to me ; but, I presume, in many cases, the operators, though using the vaccine in a devitalized form, considered that they were applying a method the general protective effects of which had already been established ; and their efforts may have been absorbed mainly in meeting the demands of individuals who applied for inoculation or agreed to avail themselves of the protection which it possibly offered. Under such circumstances, the conditions required for a scientific study are rarely realized. When, at the end of an epidemic, the operators collect information concerning the results and endeavour to extract from it the teaching it offers, they almost invariably find the data incomplete. Not infrequently that incompleteness is clearly perceived by the operator and acknowledged by him. Thus, in the case of the most extensive of the above-mentioned operations, those carried out in Japan in 1902, Dr. N. Murata, in his account of the work, quotes the studies made in India on cholera immunization with live vaccine and states that he introduced inoculation in the Hiogo district, with the devitalized preparation, at the desire of the inhabitants, who were severely tried in the outbreak of that year. In regard to the 77,907 persons who availed themselves of the treatment and to the incidence of cholera among them, he states that he has reason to view the results optimistically, but adds :

" I am unable to say whether the protective inoculation was applied uniformly under all circumstances, or whether the inoculated and the non-inoculated were equally exposed to infection. It is impossible for me to enter into such circumstances with a degree of exactitude, as the inoculations were carried out by many hands. However this may be, one cannot deny that the mortality among

the inoculated was much smaller than among the non-inoculated." (*Centralblatt für Bakteriologie*, I. Orig., Vol. XXXV, 1904, pp., 60 5-608.)

From the details supplied previously in the present Note, it will have been seen that the sources of error alluded to by Professor Metchnikoff were obviated in the Indian studies of 1893-1896 ; but, as mentioned, those studies were made with live, and not with devitalized vaccine.

Experiments with Vaccine Extractions.

The precise effects of inoculation with the devitalized form of the vaccine require, therefore, to be submitted to further investigation. In doing so, the most reliable preparation ought, in my opinion, to be tried first, the actual study being conducted on lines analogous to those which were worked out in India in 1893-6. I will deal first with the question of the form of the vaccine.

A considerable body of bacteriologists (Brieger, Kitasato and Wassermann ; Klebs ; Rosmainski ; Büchner and Hahn ; Lustig and Galeotti ; Emmerich and Löw ; Neisser and Shiga ; Brieger and Schütze ; Brieger and Meyer ; Conradi ; Macfadyen and Rowland ; Bertarelli ; Markl and Dean ; Richard Strong ; Bassenge and Martin Meyer ; Otto Heller ; Friedberger and Carlo Moses ; Stanislas Serkowski ; Karl Smitz ; Besredka ; K. Bischoff ; B. Klein ; Turro ; Eduard Blell ; Citron and Putz ; Fritz Meyer and Peter Bergell ; Levaditi and Muttermilch ; Sydney Rowland ; H. Vincent ; Pietro Rondoni ; Carl Prausnitz, and others) have adopted a favourable view as to the ultimate effects of devitalized vaccine and have recommended for use extractions of such vaccine. The extraction is obtained, in some instances, by merely leaving the vaccine to soak in water, at the temperature of the room or incubator, then filtering off the solids and using the watery solution ; in other cases the process is aided by triturating the bacillary bodies in water or serum, or by freezing them and thus rendering them more brittle ; or by subjecting them to the effect of an alkali, or alcohol, or ether, or common salt, or sulphate of

sodium, etc. Such extractions have been prepared with the germs of cholera, typhoid, bubonic plague, dysentery, chicken cholera, pig-septicæmia, hæmorrhagic septicæmia of cattle, *Bacillus colli*, etc.

The question as to whether vaccine should be used in extraction or in its entirety was dealt with, in reference to plague, by the Indian Plague Commission of 1898-1901 when examining Lustig and Galeotti's proposal of applying "nucleoprotein" (alkali extraction) of plague bacilli for anti-plague inoculation. The Commission did not commend the plan, and so far as I am aware, in the 12 years which have elapsed since then, nucleoprotein has not come into use. I think, this result is not without bearing on the question. As I have already intimated, it is not possible at present to determine accurately the particular constituents of the bacterial body which create in an animal immunity against natural disease, though it is already apparent that in different diseases the effective agents must differ in constitution. For the present, therefore, we place ourselves on surer ground by transforming into vaccine the whole of the substances of the germ which the individual has to deal with in the event of infection. Then, the use of the entire germ, compared with extractions made from it, offers facilities of considerable importance in the matter of manufacture. For the purpose of making the extraction, the scale of preparation would need to be increased to many times its present volume, for only a small amount of specific matter is obtainable in solution from a given volume of vaccine. The larger the scale of preparation, the more complicated are the measures necessary for assuring satisfactory results. Further, the *technique* of effecting the actual extraction, of handling, with all the requisite care, the additional substances necessary for this, of applying the various procedures which are suggested for facilitating the solution, and of filtering off the residual solids, on a scale required for practical purposes, is a far heavier addition to the work than is the extended scale of cultivation.

In this connection I may refer also to the plan which Dr. Besredka, of the Paris Pasteur Institute, has, in a most helpful spirit, suggested with the object partly of mitigating the reactionary symptoms caused by the cholera, plague and typhoid vaccines, partly

of meeting the danger which is believed to result from inoculating persons previously infected or about to become infected with the disease. The plan consists in emulsifying the vaccine, in its entirety, in water, passing it through fine silk, immersing for twelve to twenty-four hours, at a temperature of 68°F., in serum of preliminarily immunized animals, washing it three or four times in saline water and centrifuging after each washing, till the removal of all traces of serum has been assured. This procedure obviously involves a degree of elaboration to which, if carried out on a small scale, there can be little objection, but which becomes of importance when the volume of material is large, and when, at every stage, great precautions are necessary for obviating the possibility of mishap. It may be observed that the reactionary symptoms caused by inoculation are only very marked when exaggerated doses of vaccine are used; while the danger from inoculating persons incubating or about to incubate infection has been definitely proved not to exist (*vide* pp.75-76). The additional exposures involved in Dr. Besredka's plan of manufacture would, therefore, to my mind, not be sufficiently compensated for by the expected advantages. Dr. Besredka favours, further, the use of live vaccine, which he has, indeed, good ground for considering the more effective (*vide* pp. 37-38).

The Methods of Study in Preventive Medicine.

Then, as regards the mode of investigation, research in the case of the devitalized cholera vaccine must, in my opinion, be as strict and scientific as that employed to establish the efficacy of the live vaccine; for the immunizing properties of the two preparations differ sufficiently to render it unsafe to rely upon inferences from the one to the other.

There is, I think, only one way of finding out, as a lasting scientific truth, whether a method such as vaccination against small-pox, cholera, plague or typhoid fever, protects man against those diseases or otherwise; and the way is that of studying the subject in the midst of and in application to the communities which it is

intended to protect. This, indeed, has been the plan which I have applied in India.

The conditions of such a study are obviously very different from those obtaining in a bacteriological laboratory or in an animal out-house; and the responsibilities of the work are, needless to say, widely different also. The methods of obviating experimental illusions and arriving at the right facts and deductions are special and cannot be worked out without prolonged investigation; and the difficulties of execution, every part of which, whether preliminary and tentative, or final, has to be performed in the glare of publicity and under the incessant observation and opposition of critics and opponents, are on a scale not to be measured only by the scale of the studies themselves. The operations alone, besides a vast amount of laboratory work, imply travelling over long distances, combating prejudice, apprehension and obstruction; searching for suitable conditions of enquiry and for volunteers, in their hundreds and thousands, ready to undergo loss of time and temporary illness, for advantages yet unknown; waiting indefinite intervals of time for a concurrence of events necessary for the study of results; carrying out house to house investigations and tracing individuals who have become concerned in the experiment; investigating causes of absence and of disease or death, by conferences with local authorities, relatives and medical attendants, and a variety of other laborious enquiries and proceedings unknown in work on animals. The subsequent part of the investigation, the study of the facts, is an elaborate and technical research. It comprizes a scrutiny of the data collected and the determination of all possible sources of error and of the methods of their elimination; an analysis of the results, and the collection of such supplementary data as have been found to be necessary; and the formation of conclusions and their verification. When the investigator has established the main facts for which he has been searching, and has assured himself of their reality by experiment many times repeated, long years must further elapse before other observers, of an impartial mind, are in a position to verify his findings and to assist in ensuring their acceptance by the world at large.

It is on account of these circumstances that, inevitably, endeavours have always been made to solve questions relating to man by experiments on small animals—guinea-pigs, rabbits, mice, etc.—which could be handled in the laboratory. A vast amount of invaluable information has thus been obtained ; but, unfortunately, differences of organization and of the conditions of life, both in health and disease, make it often impossible to conclude from the effect upon lower animals the effect on man. Thus, for instance, the fact that *active* immunization offers a means of saving individuals who have contracted a rapidly incubating disease (*vide* p. 75) would have remained undiscovered without the studies of cholera and plague in man ; for experiments on laboratory animals gave diametrically opposite results and indicated that, during the first succeeding days, immunization not only did not protect from, but directly predisposed to and aggravated such a disease, and that when infection had been contracted previously, an attempt at immunization would render a fatal issue inevitable. (*Vide* Calmette and Salimbeni's detailed research into the anti-plague inoculation in white mice.) The whole subject of inoculation against cholera offers, of course, an instance of a problem which could not be solved by experiments on animals (*vide* pp. 35-36 ; also Richard Strong, on pp. 96-97 below). An attempt is, therefore, frequently made to arrive at conclusions concerning the immunization of man, by examining samples of blood and serum from individuals operated upon, or else by subjecting inoculated persons to artificial infection, in a manner permissible in human beings.

The last-mentioned plan of investigation was, perhaps, as well and as fully exemplified as it could be in the experiment described by Professor Metchnikoff on the two men inoculated by me in 1892 (*vide* pp. 36-37). The difficulties in arriving at a reliable conclusion by that method are due to the wide divergence between the mode of infection as it takes place in Nature, on the one hand, and as it is done in the Laboratory, on the other ; to the restricted number of individuals on whom the experiment can be attempted and the idiosyncrasies of the patients which, in these circumstances, affect the results ; and to the impossibility of trying on man a decisive mode of infection. Thus, concerning the last-mentioned

point, in the case of the two persons referred to by Professor Metchnikoff, a conclusion had to be formed both from the apparent mitigation of their choleraic symptoms and from the degree of general malaise experienced by them, while the interpretation of the latter symptom was complicated by the fact that it possibly included some manifestation of defence against infection. Professor Metchnikoff, therefore, rightly indicated, in the wording of the conclusions which I have cited above, that he did not view that experiment, or the experiments made on rabbits, and the *à priori* considerations connected with vaccination against poisoning, as containing the data for the final solution of the question.

The information obtainable from the other mode of enquiry, that of examining blood samples, instructive in very many ways as it is, is equally far from being of a decisive character. In view of the importance of the matter involved and of the weight which, on various occasions, has been attributed to such information, it is perhaps desirable to examine with some detail the experience gained in it.

Human blood serum, when injected into a guinea-pig simultaneously with, or a day or so prior to, an injection of a lethal dose of cholera virus, is found in certain cases to have the effect of obviating the fatal action of such virus. In 1892, Lazarus observed this property in the serum of men who had recovered from an attack of cholera (*Berliner Klinische Wochenschrift*, 1892); and in the same year, Klemperer found it in the serum of men artificially inoculated with cholera germs. (*Ibid.*) Drs. R. Pfeiffer and W. Kolle's observations of 1896, made on the blood of persons inoculated with cholera vaccine, both in its live and devitalized conditions (*vide* p. 84), were on analogous, though not identical, lines. The question is how far the appearance in the blood of the above-mentioned property indicates that the individual possesses the necessary immunity to protect him against cholera. The following facts bear on the matter :—

(*a*) Of 12 Europeans examined by Metchnikoff and who were free from any previous history of cholera, 5 were found to have in their serum substances which protected guinea-pigs against a fatal injection of cholera germs, and 7 had no such substances.

(*b*) Of 22 Europeans suffering at the time from cholera and similarly examined, protective substances were found in the serum of 10 and not found in the 12 others.

(*c*) In the bodies of 10 persons who succumbed to cholera, protective substances were found in 5 cases ; and

(*d*) of 24 persons who recovered from a cholera attack, protective substances were found in 14.

Metchnikoff summarizes these observations on the preventive power of human blood thus :—

"It results from our researches on the property of the blood of 68 persons, that that power with reference to the typical vibrio, of Indian origin, is extremely variable. It exists in almost half the men who have not had cholera, and in 58 per cent. of persons who have had an attack. Almost half the cholera patients and half the individuals who died of that affection present equally the preventive property of the blood." (*Annales de l'Institut Pasteur*, VII, 1893, pp. 420-421.) "One may consider as proved that natural recovery in cholera takes place without the appearance of the preventive property of the blood. On the other hand, that property may develop without preventing the man attacked with cholera from dying of the disease, even in the first period of its evolution." (L.c., p. 587.)

Observations of a similar purport have been made in Dr. W. Kolle's Laboratory, where it has been seen that the serum of an inoculated individual, when used on an animal infected with cholera germs, may have the effect of destroying those germs, and yet be inefficient in preventing a fatal issue of the disease. (H. Carrière and E. Tomarkin, "Experimentelle Studien zur Frage der Serumtherapie der cholera asiatica," *Zeitschrift für Immunitätsforsch.*, I, Orig., IV, 16th December 1909.) These results, conjointly with the results mentioned higher up and with others to be quoted presently, unfortunately render uncertain some of the grounds on which Dr. Kolle had formed favourable expectations with regard to my devitalized cholera vaccine (***vide*** pp. 83-84 and 93).

Another illustration of the same nature is the fact that a guinea-pig immunized against the cholera germ contains in its blood

substances by means of which it is possible to protect other guinea pigs against the same germ ; after a time these substances disappear from the blood of the immunized guinea-pig, but the latter remains immune (Pfeiffer in *Zeitschrift für Hygiene*, XVI, 1899).

A parallel case may be seen in typhoid fever. I referred, in my "Epidemiological Notes," dated Calcutta, October 1911, to a statement made by Sir Almroth Wright to the effect that it was the presence of agglutinating properties, shown him by R. Pfeiffer in the blood of persons inoculated in the Berlin Institute against typhoid, that induced him to take up the anti-typhoid inoculation. (*Vide* Sir Almroth E. Wright, "A short Treatise on Anti-typhoid Inoculation," Westminster, Archibald Constable & Co., 1904, p. 19, as compared with A. E. Wright and Surgeon-Major D. Semple's original publication "On vaccination against typhoid fever," *British Medical Journal*, 30th January 1897, p. 256, mentioned on p. 77 above.) It is known that the disparity between various properties of the blood—including the agglutination faculty—on the one hand, and *immunity* of the individual, on the other, is not less in typhoid than in other diseases, inasmuch as patients may die of typhoid while exhibiting in their blood high agglutinative properties, and as the reverse of this paradox proves also true. The blood of convalescents exhibits the property of agglutinating, killing and dissolving typhoid bacilli ; in the course of a few months this property, in a vast number of cases, disappears ; yet the individuals themselves remain immune (Rufus J. Cole, "Experimenteller Beitrag zur Typhusimmunität," *Zeitschrift für Hygiene*, 3rd May 1904 ; Russell, in *Johns Hopkins Hospital Bulletin*, 1910). The particular substances which produce those effects on the bacilli may be removed from the serum artificially, by submerging in it for a time a certain quantity of such bacilli. These use up and absorb the substances in question, and when withdrawn from the fluid, leave it apparently devoid of all anti-bacillary properties ; yet, by means of that exhausted serum, it would seem possible sometimes to protect animals against typhoid (St. Rusznyak, "Untersuchungen uber die Wirkungsweise des Antityphus-serum," *Centralblatt für Bakteriologie*, 1, Orig., LVIII, 22nd March 1911. *Vide*, at the same time, Georg Bessau's results, in

the same magazine, Vol. LIX, 15th July 1911). Neisser and Lubowski, Garbat and Meyer, Besredka and Broughton-Alcock, obtained experimentally anti-typhoid sera which were, from the first, devoid of any property of agglutinating typhoid bacilli or rendering them soluble; yet by means of such serum it was possible to save animals which had been infected with typhoid a few hours before; on the other hand, ordinary anti-typhoid serum, strongly possessed of agglutinating and dissolving properties, has no such curative effect. Besredka rightly quotes these experiments as one of the proofs "that there exists no parallelism between the effects *in vitro* and *in vivo*, and that neither agglutination nor alexine fixation (*vide* p. 18 above) can be used as indices of immunity." (A. Besredka, "De la vaccination par les virus sensibilisés," *Revue* in *Bulletin de l'Institut Pasteur*, 30th June 1912, p. 540.)

In plague, R. Strong, following Kolle and Otto, finds that live virus has greater immunizing powers than any form of devitalized vaccine; yet when he examined the serum of 26 persons inoculated with such virus, he found that it possessed neither the power of agglutinating the plague germ, nor that of protecting an animal against plague infection. (R. Strong, "Studies in Plague Immunity" *Philippine Journal of Science*, II, No. 3, June 1907.)

The above instances could be easily multiplied, but those given should suffice to show that, at present, it is no more possible to infer from blood examination the existence of immunity in an inoculated man or animal, than to infer from the natural or conferred immunity of a lower animal the existence of similar immunity in man. Authors who have worked on the lines under consideration are not unaware of the inevitable uncertainty of the conclusions obtainable in that way. Thus, referring to vaccination against cholera and the effects of the watery extraction of my devitalized vaccine "II," Dr. Strong (Manila *Bulletin* No. 16, September 1904, pp. 45 and 46) states :—

"We have seen that by the subcutaneous injection of the cholera prophylactic" (extraction of vaccine "II") "an excellent cholera immune serum can be obtained in human beings. However, the question naturally arises, whether these individuals are

protected against intestinal infection with the cholera spirillum. In other words, are they really immune to the disease Asiatic cholera? Experiments upon animals cannot satisfactorily answer this query. .... Since animals are not naturally susceptible to intestinal infection, and since it is only through artificial means that such may be produced in them, evidently the answer to our question can be given only by a practical observation of the human beings inoculated with the prophylactic during a severe and general epidemic of the disease. .... It would appear from the numerous statistics of Haffkine in India, and the more recent work of Murata in Japan" (*vide* p. 87), "that simply by the injection of a small amount of the killed organism a certain degree of immunity against the natural mode of infection is acquired. Therefore, judging from what has already been said, it is probable that by the use of our prophylactic, human beings may acquire a good active immunity against the disease."

An analogous indication of the spread of what I believe to be correct views on the methods of study is observable in the various statements embodied in Lieutenant Broughton-Alcock's paper on "Vaccination for typhoid fever by living sensibilised bacilli typhosi," in *The Lancet* of 24th August 1912, p. 507.

GENERAL SUMMARY.

On pages 37-72 of this Note a description has been given of the experiments by means of which the possibility of immunizing man against cholera has been demonstrated in the studies in India with live vaccine "II." The nature and the mode of preparation of that vaccine are described on pp. 25-31.

A number of observers have concluded from this result, and by inference from observations on animals and on human blood serum, that the same vaccine in a devitalized condition, as used tentatively in the author's experiments of 1892-93, and again, on a somewhat larger scale, in 1900, was also likely to be useful. (Pp. 81-88.) The further study of that preparation in India has been delayed by

the advent of the plague; but the above expectations are justifiable. An investigation on the subject, made in the midst of actual outbreaks of cholera, under conditions of accurate scientific research, following the lines which have been described on the preceding pages, is desirable, in view of the advantages which a devitalized prophylactic offers in practical application, and of the great difficulties in the way of using live vaccine on an extensive scale, under the conditions prevailing in many parts of the country.

Just as it was important to obtain first a positive result on the question of cholera immunization in general, and to use, in investigating that matter, the most reliable vaccine preparation, so it is important to avoid in the beginning the possibility of failure in the study of the vaccine in a devitalized form. For this reason it is preferable at present to use the devitalized vaccine in its entirety, as against extractions or other derivations of it. The facilities of manufacture in the case of the entire preparation are also of considerable importance.

In devitalizing vaccine "II," it is essential to employ only the most delicate physical and chemical processes.

Two forms of that vaccine in devitalized condition commend themselves to the author for first investigation:

one, prepared by prolonged cultivation in a fluid medium and devitalized by heat and carbolic acid;

and *another*, prepared by cultivation on a solid medium and devitalized, as soon as developed, by a solution of the same antiseptic compound.

The study of the protection derived from the employment, separately, of these two forms of vaccine, in various doses, should afford information as to the direction in which the plan will require to be modified for further study.

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