PROTECTIVE INOCULATION AGAINST PLAGUE.*

RICHARD P. STRONG, M.D.

(*Chief of the U.S. Government Biological Laboratory Bureau of Science, Manila.*)

The subject of immunization against pest is not only of general scientific interest, but to many tropical and subtropical countries is of great practical importance. One need only recall the mortality in India of nearly a million deaths from this disease during the year 1905 and of over one million during the first six months of the present year to be impressed with the significance of the problem.

It is true that the question of inoculation against plague has received considerable attention during the past few years, and that prophylactics have been recommended by several authors, but while it is admitted that by their use a certain degree of pest immunity can be produced and demonstrated in a number of the more insusceptible animals, and occasionally even in those very susceptible to this infection, nevertheless it has sometimes seemed questionable whether we were able by their inoculation to obtain in man an immunity of such a degree as to be protective against the natural and usual methods of infection of the malady.

In the latter part of the year 1903, when the Board of Health of Manila was practising among the Chinese of that city protective inoculation against plague by the injection of the killed cultures of the pest bacillus, the method at that time carried on in Japan and consisting of the injection of one oese of a twenty-four-hour slant agar culture of Bacillus pestis suspended and killed in one cubic centimeter of .085 saline solution, I decided to investigate whether any immune substances became developed in the serum of the inoculated individuals. It did not seem possible to me that any appreciable degree of immunity could be acquired from these

* Reported at the Fourteenth International Congress for Hygiene Demography Berlin, September, 1907. Received for publication Feb. 24, 1908.

inoculations, owing to the small size of the dose and the very mild local and general reaction which resulted from the injections; I therefore studied the agglutinative and bactericidal reactions of the blood serum of twelve cases, six of which had been inoculated two weeks, and six three weeks previously with one oese of the killed pest culture. The agglutinative reactions were performed by the macroscopic method, and the bactericidal reactions according to the one suggested by Neisser and Wechsberg. No traces of agglutinins or of specific bacteriolysins could be demonstrated in the sera of any of the individuals. (These experiments were undertaken at this time because the statement had previously been made that agglutinins, at least in some cases, had been demonstrated in the blood serum of human beings who had been inoculated against plague a short time before with Haffkine's prophylactic, a conclusion which I have not been able in any manner to confirm.) Obviously, these experiments in themselves were not considered to be conclusive evidence of the fact that no immunity was conferred upon the inoculated, since it was already recognized at this time that these anti-bodies were frequently and indeed, usually, not encountered even in the blood sera of individuals who had recovered from an attack of plague and were immune to this disease. (Experiments demonstrating the fact that animals immune to pest infection may still show no traces of agglutinins in their blood, together with those demonstrating the absence of a true bacteriolytic action of plague immune serum, will be presented later in this paper.) Therefore, experiments in animals were resorted to in order that more information on this subject might be obtained. Guinea-pigs were inoculated subcutaneously, each with the same dose that was being employed in the general human inoculations in this city. After two weeks the immunity of these animals was tested in the following manner: One oese of a virulent pest organism was suspended in one cubic centimeter of .085 saline solution and five loops of this suspension rubbed over a freshly shaved area on the abdomen of the guinea-pig. All of the animals succumbed to acute pest infection,

demonstrating conclusively that an immunity of appreciable degree had not been produced.

A short time after, the important paper of Kolle and Otto[1] was published in which the unfavorable results from the immunization of guinea-pigs with large doses of killed agar cultures of the pest bacillus or with Haffkine's prophylactic were reported. It therefore seemed to me at that time more advisable to experiment further with other methods of immunization against plague before insisting upon the use of larger amounts of more virulent killed pest cultures in the human inoculations being pursued in Manila, further results obtained by Kolle and his associates having demonstrated that in very susceptible animals at least satisfactory immunization could only exceptionally be produced by the methods of human inoculation then in vogue. I recommended at that time to the Commissioner of Health in Manila a suspension of the human inoculations with killed cultures of the pest bacillus.

Methods of immunization studied. — A comparative study of the efficacy of the different methods of protective inoculation against plague was then undertaken and their value investigated not only in guinea-pigs, but particularly in the lower apes.

The argument has been advanced — perhaps justly — that the test of the value of a method of immunization for man against plague should not be judged alone by its action upon such animals as guinea-pigs and mice. Monkeys on the other hand suffer with all forms of pest infection analogous to those observed in man, and moreover are said sometimes to contract the disease naturally. In addition the limit of value of a method for the immunization of man against plague can probably better be studied in monkeys than in any of the lower animals, owing to the fact (as I have been able to demonstrate) that the behavior of these animals in relation to susceptibility and immunity much more closely resembles the behavior observed in man than it does the one seen in other lower animals. It was particularly for these reasons that the lower apes were extensively employed in my experiments in testing the final value of the methods of inoculation which had proved effective in the smaller laboratory animals.

The methods of immunization especially studied consisted of (1st) the inoculation of killed bouillon and killed agar

cultures, (2d) of living attenuated cultures (that is of vaccination), (3d) of filtered cultures and extracts (free receptors of the organism), (4th) of artificial and (5th) of natural plague aggressin, and (6th) of the inoculation according to Klein's method. I may say in passing that in this paper I employ the term "vaccination" as implying exclusively an inoculation with the living attenuated organism.

These procedures comprise all those of importance which have been recommended for active immunization of man against pest, except that one described by Lustig and Galeotti. This method of extraction of the immunizing substances from the plague bacillus I have not employed, because it was believed that the other methods of extraction of these antigens which I used were somewhat more effective. Moreover, Kolle's experiments in immunization with Lustig's method did not lend any encouragement to its further use. The methods described in which serum was added to the killed organism before inoculation were also not extensively studied. Since my earlier experiments in cholera immunization had shown that when an immune serum was added to the killed organisms the immunizing power of the latter became reduced. Only when the living organisms are treated with the immune serum and inoculated does the combined method in plague become more efficacious.

It seemed desirable to experiment further with killed cultures of the pest bacillus for two reasons: First, to compare the immunizing value of the dead organism with that of other methods of inoculation such as those of vaccination, of natural and artificial aggressin injections, etc., and, second, to see whether sufficiently good results could be obtained by experiments on the lower apes to warrant advocating the use of this method in man.

The results of this comparative study of the value of the different methods have been recorded in detail in various papers during the past and present year, most of which have been published in the "Philippine Journal of Science" for 1906 and 1907.

These experiments have demonstrated conclusively and beyond any doubt the great value of vaccination against plague infection, and its evident superiority to the other methods.

Next in value to vaccination as a means of immunization against pest appear to be prophylactic inoculations of natural aggressin. Inoculations with artificial plague aggressin

did not in my experiments prove to be nearly as efficient as those with natural aggressin. A much higher immunity was obtained with the latter prophylactic. However, as I have pointed out previously, there was apparently no difference in the quality of the immunity obtained with the natural aggressin from that produced by the artificial product, and my subsequent experiments, as did my earlier ones,[2] have only further confirmed the views of Wassermann and Citron[3] that the aggressins must be considered to be hypothetical substances and that, so far as their immunizing value is concerned, in these exudates we have to do mainly with the substances extracted from the bacilli themselves. Evidently, in the case of the plague bacillus, the receptors of the organism in the so-called aggressin exudates of animals become liberated in a more efficient manner for immunization and probably exist in a less altered condition than they do in the aqueous suspension of the bacilli obtained in vitro by artificial means. Obviously, in natural plague aggressin no other immunizing substances are existent than are present in the prophylactic against pest recommended by Terni and Bandi. The two methods are practically identical as Bandi[4] has recently pointed out.

However, it must be admitted that Bandi did not originally explain the principle of the action of his prophylactic, as we understand its action to-day after a study of the subjects of free receptors and aggressins.

However, the method of inoculation with natural plague aggressin is not likely to come into general use, because of the great difficulties encountered in the preparation of the prophylactic. Moreover, in my experiments I have not obtained the satisfactory results with it which Hueppe and Kikuchi[5] evidently anticipated. The method of *vaccination*, as already mentioned, gives a much greater degree of protection. Although Klein's method, according to the small number of experiments I have performed, gives about the same results in immunizing guinea-pigs as are obtained by the inoculations with natural aggressin, yet the injection of the former substance in my experiments produced a much

greater local reaction than the latter. Therefore I could not ascertain that this method had any particular advantage over that in which inoculations of natural aggressin were employed.

Evidences of immunity from a study of the blood serum. — After having concluded from the experiments mentioned above that true plague vaccination (that is, inoculation with living attenuated cultures) produced the highest immunity, inoculations were made in human beings with a living culture designated as Pest avirulent. In order to ascertain if any evidence of immunity could be demonstrated in the inoculated, their blood was tested for the presence of agglutinins, anti-infectious substances, and opsonins. This led me to investigate in detail whether, and if so to what extent, these same anti-bodies existed in the blood sera of animals which had been immunized against plague infection.

Agglutinins. — From a large number of experiments I have concluded that the agglutinins are formed slowly and only in very small amounts in animals which are being immunized against pest infection and that they only occur in demonstrable quantities in those which have been very highly immunized. At most, only very minute traces of these substances are encountered after single inoculations of either the killed or the living organisms, no matter how large the dose which is employed. I am convinced that in my earlier experiments with plague agglutination I sometimes mistook pseudo-agglutinations of the pest bacillus for true ones, and from a study of the literature it seems to me probable that other observers have erred in this respect. A study which I have made of the blood sera of guinea-pigs which have been vaccinated against pest infection and which have later shown themselves to be immune to lethal and multiple lethal doses of the pest bacillus, has demonstrated that practically no traces of agglutinins exist in such sera. The same may be said of the sera of other animals immunized in a similar manner. Monkeys which have first been vaccinated with attenuated pest cultures or inoculated with

killed cultures, and which afterwards have been shown to be immune to pest infection by the injection in increasing doses of from one-half oese to nearly one entire agar slant culture of a living virulent strain, still have developed in their sera practically no traces of agglutinins. Indeed, it is very difficult to immunize monkeys to such a high degree that the blood of the animals shows the presence of plague agglutinins. In a series comprising twelve monkeys in which an attempt was made gradually to immunize these animals with a strain designated "Pest Virulent" up to such a degree that the anti-bodies would be demonstrable in their blood, only two were able to survive the inoculations when the immunization had reached the point in which the agglutinins could be detected even in small amounts. The remaining ten animals succumbed either to pest infection or intoxication as a result of the injections, before agglutinins could be shown to exist in their blood. Moreover, notwithstanding the fact that from one-quarter to one-half oese of this strain "Pest virulent" represented a certain lethal dose for normal animals of this species, and although these monkeys were immunized to such an extent that in a number of instances they were able to resist and survive the inoculations of such large amounts as six to eight loops of this organism (over twelve times the maximum fatal dose), agglutinins were still not present in sufficient quantities to be demonstrable in their blood.

Only small quantities of agglutinins could be detected in the examinations of several pest immune sera which were prepared from horses and which were known to possess, in two instances at least, considerable protective (that is, anti-infectious) power.

A horse which was being immunized against pest, and which had acquired a sufficient immunity to withstand the injection of nearly ten agar cultures of a virulent pest strain, gave a serum which at this time showed an agglutinative reaction in dilutions of one to ten, but none above this strength. However, at the same time one cubic centimeter of this serum protected from fatal pest infection about sixty per cent of the white rats inoculated. A pest immune serum obtained from Asia, which protected about seventy-two per cent of the inoculated rats against fatal pest infection in doses of from one to two cubic centimeters, when carefully

tested with the virulent pest strain showed no agglutinative reaction after three hours. However, it must be stated that at the time its anti-infectious and agglutinative reactions were tested this serum had been bottled for about a year. A second pest serum purchased from Asia, which showed a somewhat lower protective power, also revealed no agglutination when tested with the strain "Pest avirulent." This serum had been bottled about nine months. Moreover, a laboratory immune serum, which possessed a higher anti-infectious power and which protected about ninety per cent of the inoculated white rats against plague infection in doses of one cubic centimeter, also showed almost no agglutinative reaction against the virulent strain, giving only a weak reaction in a dilution of one to ten.

A study of the agglutinating properties in the blood of a number of persons who had been vaccinated against plague, as well as of several cases who had suffered with the disease and recovered, was also undertaken. In the majority of the instances no traces of agglutinins could definitely be demonstrated.

In concluding my remarks on agglutination it may again be emphasized that excessive precautions must be taken to distinguish between pseudo and true agglutination in pest, and that apparently only in the organism highly immunized against plague infection do the agglutinins become developed in sufficient quantity to be of any practical importance either in the diagnosis of the infection or in the demonstration of the presence of an immunity. It also would appear from my experiments that the development of the anti-infectious substances in a plague immune serum is quite independent of the agglutinins.

Anti-infectious bodies. — The method employed in investigating the presence of anti-infectious bodies in the serum was as follows: A rat was inoculated intraperitoneally or subcutaneously with the serum to be tested, while at the same time the animal was infected by thrusting beneath the skin near the tail a syringe needle which had been dipped in a suspension of a virulent pest organism. Numerous control animals were inoculated in all the series.

By numerous experiments of this nature it was demonstrated that the anti-infectious substances also become

developed very slowly and in very small quantities in animals immunized against pest. While rabbits which have been given a single, small intravenous inoculation of either living or killed cholera or typhoid bacilli will develop a serum which in high dilutions is protective for guinea-pigs against multiple lethal doses of these organisms, on the other hand, rabbits which have been intravenously inoculated with large amounts of killed virulent or with living attenuated plague cultures yield sera which, when tested on rats, show apparently no protective power whatever against plague infection. Likewise, as large an amount as five cubic centimeters of a serum obtained from a rabbit previously inoculated intravenously with twenty milligrams of artificial plague aggressin proved to possess no anti-infectious power when tested on rats. Monkeys which had been immunized against pest by vaccination, or otherwise by inoculation, and which had been shown to be thoroughly immune by the subcutaneous inoculation of multiple lethal doses of the virulent pest strain, furnished sera which also showed no traces of anti-infectious power when tested on rats. Only in the case of one monkey could a slight anti-infectious power be noted, and this animal had received repeated increasing doses of virulent pest bacilli until it resisted the injection of one and one-half agar slant cultures of the living virulent pest strain. This series of experiments is particularly important because it illustrates that the negative results in the demonstration of an anti-infectious action, obtained with the serum of monkeys less highly immunized against pest, could not be ascribed to the lack of a suitable complement to complete the reaction in the body of the rat.

The sera of thirty-three human beings who had been vaccinated against pest by the inoculation of living attenuated cultures, and of a patient convalescent from plague were also tested, but in no instance showed any demonstrable anti-infectious value. However, since animals which had proved themselves thoroughly immune to pest infection also furnished sera which conferred no greater protection, it would not be reasonable to expect that the human sera would reach

a higher power. Only in the case of horse's serum, where the animal had been finally inoculated with repeated large doses of living pest bacilli, could any marked anti-infectious action be demonstrated, and indeed with some of these sera it required as much as one cubic centimeter to save the rat from fatal pest infection. Therefore, it is unnecessary to emphasize further that the absence of the anti-infectious substances against pest in sufficient quantities to be demonstrated in a serum cannot necessarily be regarded as an evidence of the absence of even a considerable immunity against this disease in the individual in question, since in animals and human beings known to be thoroughly immune to pest infection it has also not been possible to demonstrate these protective substances.

Bactericidal substances. — My experiments relating to the study of the bactericidal action of the serum of animals immune to plague have been considered at length elsewhere, and will be summarized briefly here.

In conformity with the experiments of Kolle and Hetsch,[6] and more recently of Dean,[7] I[8] have found that plague immune serum which is known to possess anti-infectious power in the animal reveals in vitro no bacteriolytic reaction whatever that is similar to that possessed, for example, by typhoid immune serum. I have also been able to show that the pest bacilli are not only not killed after being treated with the immune serum, but that they remain alive and under some conditions are capable of subsequent development. However, that a reaction actually occurs between the plague immune serum and the bacilli is obvious from the further experiments of Kolle and Hetsch; these observers having showed that a binding took place between the amboceptors of the serum and the corresponding receptors of the bacilli, since after contact with the bacilli the serum was found to have lost in immunizing power. I have been able to confirm these experiments and in addition to show (loc. cit.) that in this reaction (binding of plague amboceptor and receptor) if fresh complement is present, it also enters into the reaction

and becomes bound. My further experiments relating to the mechanism of the action of immune serum in vivo have showed in agreement with those of Markl [9] and Löhlein [10] that after the bacilli have been previously acted upon by the serum and prepared for the leucocyte the leucocyte ingests the organisms and plays a further part in their digestion and ultimate destruction, perhaps partly by furnishing the additional amount of complement necessary to complete the reaction. On the other hand, that the phagocytes in non-immunized animals do not to any extent engulf living virulent plague bacilli seems equally clear. Therefore the destruction of the plague bacillus is effected by the immune animal in a manner partly in accord with the humoral theory of Buchner and Ehrlich, and partly in accord with the phagocytic one of Metchnikoff.

Opsonins. — The action of plague serum may also be termed "opsonic" in nature, since it prepares the organism for phagocytosis. However, I have showed that the actual preparation of the bacillus, *i.e.*, the chemical change which it undergoes (in the instance of plague at least), consists of the binding of its receptors by the amboceptors of the serum, and of the union of complement in the serum, though obviously the entire phenomenon of what we have regarded as true bacteriolysis does not take place outside of the leucocyte.

Although the whole literature upon the question of the opsonic reaction cannot be entered into here, it may be mentioned that experiments in connection with this hypothesis have been further confirmed by those of Muir and Martin,[11] who have tested the three chief varieties of immune bodies and have found that in each case the combination of receptors plus immune bodies removes the opsonins at least of normal serum.

The work of Dean [12] and of Leidingham,[13] which has appeared since my experiments on this subject were first reported (March 2, 1907), although Dean had performed earlier work on this question,[14] also supports this hypothesis for opsonins of other sera. While the experimental work of Neufeld and Hüne [15] and the still more recent of Muir and Martin [16] on opsonins of immune serum is not in opposition to it in its most important features, the conclusions of these authors oppose somewhat this view. Neufeld believes that the action of the opsonin of normal serum is

different from that of the bacteriotropin of immune serum. That the former consists of the action of amboceptor and complement, while the latter consists of the action of a thermostable substance, "Bakteriotropine Stoffe" which does not require the action of complement. However, to me it seems probable that in both instances the essential substance which prepares the bacilli for phagocytosis is a thermostable amboceptor. If a normal serum which contains but a small quantity of thermostable amboceptor is heated, obviously the complement is destroyed or converted into complementoid. If now the bacteria are treated with such serum and if the amount of amboceptor present in the serum is very small, the organisms remain unsensitized for phagocytosis, unless fresh serum containing complement is added to start the reaction. If on the other hand an immune serum which contains a large amount of thermostable amboceptor is heated and the complement largely destroyed and the bacteria then treated with it, the organisms become, nevertheless, sensitized for phagocytosis. For in the presence of a large amount of amboceptor but a small quantity of complement is necessary for the reaction, such as remains present, though altered, or which may be drawn from and furnished by the leucocytes. The question is particularly one of the quantitative relationship between complement and amboceptor, and particularly one of affinity of the amboceptor. This well-known phenomena was pointed out by Ehrlich, Morgenroth and Sachs several years ago.

Further, if certain immune sera, such as cholera and typhoid, which have earlier possessed bacteriolytic properties, and in which the complement has been converted into complementoid or destroyed, are added to their respective organisms, bacteriolysis obviously no longer occurs, unless fresh complement is added, but the organisms are, nevertheless, sensitized for phagocytosis. With such bacteriolytic sera it is either by means of a destruction or alteration of the complement or by diluting the amount of the amboceptor of the sera that the entire phenomena of lysis is prevented from occurring. The first stages in the reaction of bacteriolysis, nevertheless, take place, and the organisms are taken up by the leucocytes and later undergo destruction. The fresh complement when present in sufficient quantities quickens the rate of the reaction.

In plague serum both amboceptor and complement enter into the reaction of sensitizing the bacillus, for whose ultimate destruction usually the leucocyte is necessary. It seems probable that the morphological structure of this organism — its cellular membrane, capsule, etc., — afford it a certain protection against bacteriolysis, while free in the serum before ingestion by the leucocytes has taken place.

However, leaving aside the discussion of what this opsonic reaction in plague immunity exactly consists of, and whether

it depends upon the action of new bodies or not, it may be stated that this reaction — at least sometimes — affords us a means of determining the existence of such an immunity, since I have been able to show that the blood serum of both human beings and animals which have been vaccinated and immunized against pest, frequently revealed an increased opsonic index, even though this was not invariably the case. The phagocytic power apparently varied somewhat according to the virulence of the organism employed in the test, the index usually being higher with the avirulent strain.

The technic which I have come to employ in determining the opsonic power of the serum is somewhat different from that which Wright recommends. In my experiments the leucocytes are always used in a perfectly fresh condition and obtained from the abdominal cavity of a normal guinea-pig after an intraperitoneal injection of aleuronat. They are kept at a temperature of 37° C. until the smears on the slides are prepared. Only very virulent strains of the pest bacillus were employed in testing the comparative value of the opsonic power between a plague immune and a normal serum.

In my earlier experiments the determination of the phagocytic power of the sera was arrived at in the usual way by attempting to count the number of bacteria in a certain number of cells and to divide the total number of bacteria by the number of cells counted. Later the errors which resulted in employing this method, in my hands, caused me to abandon it and to adopt the method of determining the highest dilution in which a given immune serum will give rise to a marked phagocytosis, and of then comparing its action with that of a normal serum in this same dilution. For example, when the organisms which have been treated with a certain serum in a fixed dilution undergo a marked phagocytosis, while the organisms of the same strain of bacteria treated with a normal serum in the same dilution undergo practically no phagocytosis, the opsonic reaction may be said to be increased in the former serum. While in the beginning of the work it was thought that the value of certain immune

sera might be more or less accurately compared by this manner, it was soon found that the reaction was not sufficiently delicate for this purpose, and that only in those cases where one serum in a certain dilution gave rise to a very marked phagocytosis, and the other in the same dilution to practically no phagocytosis, could the opsonic reaction of the first serum be said to be increased.

Personally, I have not had any experience in attempting to determine the opsonic value of the serum in furunculosis and tuberculosis and of other infections where the nature of the resulting immunity is obscure. We have no satisfactory method of testing the immunizing power of antitubercular and staphylococcus sera.

The work of Moss [17] and of Fitzgerrard, Whiteman and Strangeways, [18] which has recently been published, demonstrates conclusively the errors which may occur in determining by the counting method usually employed, the opsonic index in the same serum, and in other sera of practically the same immunizing power. Moss further showed that the sera obtained in rabbits by the repeated inoculation of killed and living cultures of Staphylococcus aureus possessed no greater opsonic power than normal rabbit's sera when tested by this method; while Fitzgerrard, Whiteman and Strangeways by their extensive work showed that with the usual technic no difference in the opsonic index could be shown to exist between the sera of healthy and of tuberculous human beings.

It seems to me regrettable that these same authors after having so carefully studied this technic with certain sera (staphylococcus and tubercular) did not pursue its study further in relation to those sera whose immunizing power and value have been definitely demonstrated by animal experiment.

Neufeld and Hüne have recently demonstrated the bacteriotropic reaction with typhoid and cholera immune sera, though the technic in determining what constituted an increased phagocytic power of the blood was different from that employed by Moss, Fitzgerrard and Strangeways.

Personally, I believe that the serum of the average tuberculous patient contains no immunizing power of demonstrable value; moreover, I believe it is not practicable to increase the immunizing power of such a serum to a demonstrable extent by a single small injection of killed tubercle bacilli, and certainly we have no proof that we can do so. I am inclined to believe it is only with living attenuated cultures that a valuable immunity against tuberculosis may be produced. However, the method of treatment with killed cultures is having an extensive trial, and the results obtained will doubtless shed further light upon this subject.

In connection with this work on immunization, one must not lose sight of the fact that it is largely through Wright's efforts, and the confidence in England and America, at least, which his work has aroused in the

general public, that opportunity has and is being given to extensively and thoroughly test the value of inoculation with killed organisms in relation to the production of immunity in the treatment of various bacterial infections in human beings. While it seems probable that in many of these diseases no favorable results will ultimately be obtained, nevertheless the question may be decided definitely for each infection, provided that the experiments are sufficiently, extensively, and carefully performed.

Although some of us feel, and have shown that there are certain errors and limitations in the technic in determining the opsonic index as it has been employed, yet effort must be made to improve and place this upon a more satisfactory basis. While with some infections this may be difficult or impossible, nevertheless this need not be a reason in itself for the abolishment of the treatment of these various infections by inoculative measures. Care must be exercised not to bring discredit too hastily upon this method of immunization for the various infections, before it has been carefully and completely tested.

Deflection of complement. — Finally I have found that by means of the phenomenon of the deflection of the complement of Bordet and Gengou, we are also able to demonstrate in the serum the existence of an immunity against pest. This reaction has been carried on with the blood serum of vaccinated human beings and guinea-pigs and with horses' pest immune serum, as well as with normal human, guinea-pig, and horse serum. In each instance with the normal serum no deflection of the complement took place, while with the serum from the vaccinated individual and animals and with the horse immune serum, the deflection regularly occurred. All the necessary controls were performed and all the precautions in the technic of the experiments were taken. In spite of its many abuses, and all the objections raised against the method, in the hands of a competent bacteriologist it is the most delicate test we possess for the demonstration of the deflection of the complement.

In concluding the discussion of this portion of the subject, it may be stated that only by means of the deflection of the complement, and sometimes by the opsonic reaction, are we usually able to discover the existence of immune bodies in the blood serum of an individual or animal immunized to plague. The examination of the serum for agglutinative bactericidal or anti-infectious substances is usually of no

practicable value in determining the existence of an immunity against pest infection, since agglutinins and anti-infectious substances are not present in sufficient quantities to be demonstrable even in animals which have been shown to be highly immunized against this disease and which have resisted multiple lethal doses of the organism, while the existence of true bacteriolytic substances cannot be shown to exist even in animals most highly immunized against plague.

The most satisfactory demonstration of the existence of plague immunity can be obtained from a study of the resistance of the animal to artificial infection, but such a test can obviously not be employed on human beings.

Human vaccinations. — The superiority of vaccination against plague having been shown conclusively by animal experiment, and other experiments[19] having demonstrated the entire safety of the inoculation of human beings with large amounts of the strain designated "Pest avirulent," more extensive vaccinations were made in man with this culture. The size of the dose employed for an adult was always one twenty-four-hour agar slant culture and for children from one-third to one-half of this quantity. Surprising as it may seem, the injection of these large amounts of the living plague organism have not given rise to any very severe reactions. A few hours after the inoculation the temperature of the individual usually begins to rise. When the injections have been made in the morning the fever may in the evening of the first day reach from 38.5° to 39° C. Only in a few cases has it touched 40° C. The temperature gradually declines on the following day, and by the third or fourth one has become normal. Occasionally, the cases showed a moderate leucocytosis after the injection. As in the earlier experiments, the organisms were always suspended in one cubic centimeter of .085 saline solution and the inoculations were made deeply into the deltoid muscle. Intramuscular instead of subcutaneous injections were performed on account of the quicker absorption which

occurs from the former method, and because Meltzer and Auer found that intramuscular injections as regards absorption in general stand in value very near that of a direct injection into the circulation. On the day after the vaccination there is usually distinct induration and redness about the point of the injection, with soreness on pressure, but these symptoms, together with the febrile reaction, subside in from one to three days. No visible suppuration of the tissues has ever occurred.

In order to observe the length of time during which the organisms remained alive in the tissues, similar inoculations were made in monkeys and the tissues near the point of injection incised at varying intervals after the vaccination and microscopical preparations and cultures made from the drops of lymph and blood which escaped from the wound. In the different series of cultures made from the animals inoculated with the strain "Pest avirulent" it was found that from six to eight hours after the time of the inoculation the organisms were still very numerous in the tissues, after which time they gradually diminished, and, usually after twenty-four hours, they were no longer reclaimable in cultures. However, in several instances a few colonies developed in the cultures made after this period of time. In immunized animals the organism was destroyed in a short period, the cultures made after twelve hours frequently remaining sterile. In these instances smears from the tissues three or four hours after the inoculation showed very extensive phagocytosis of the bacteria. Therefore, the process of immunization occurs as in a true vaccination, the organism reproducing itself in the tissues for probably one hundred or more generations and its successive groups of receptors stimulating the production of corresponding groups of amboceptors in the animal body. It therefore is not difficult to understand why the immunity derived from vaccination in plague is greater than that obtained from the injection of the killed organism.

Although abundant evidence had been obtained of the resistance against plague produced in animals even more

susceptible to infection than man (that is, guinea-pigs) by vaccination with these attenuated cultures, yet it was interesting to observe what evidence could be discovered, from a study of the blood serum, of the immunity resulting in human beings from such vaccinations.

The results of these investigations have already been referred to, and the evidences of the immunity obtained by a study of the opsonic reaction and the power of the serum to deflect complement emphasized.

Since my first report made upon the method of vaccination against plague in man, such inoculations have been made from time to time, and during the past year two hundred vaccinations have been performed. The cultures with which I have worked have so far proved themselves to be entirely safe for human beings, and I have observed no unfavorable results in the inoculated. However, as emphasized in my previous communication, vaccinations against plague should not be made in man unless the investigator can guarantee the particular organism which he is working to be sufficiently attenuated to be no longer dangerous for human beings. Strains of the bacilli which in extensive series of experiments, carried over long periods of time, invariably no longer kill guinea-pigs of two hundred and fifty grams weight upon subcutaneous inoculation, in amounts of one agar slant culture, are probably safe in small amounts for human beings. However, obviously each strain must be carefully and thoroughly tested before extensive inoculations in man are made with it. Moreover, one must always bear in mind that because a particular pest culture inoculated in a certain dose may have been demonstrated to be harmless for a small group of healthy and robust human beings, the conclusion must not be drawn that this same culture will always prove to be non-dangerous for individuals in poor health, and for those possessing a greater natural susceptibility to pest infection. The susceptibility to infection is so variable in different human beings that unless extreme caution is used in the selection of a proper culture, disastrous results are sure to follow. On the other hand, care must be taken to employ a

culture which has been shown to be capable of immunizing against pest infection a high percentage of the guinea-pigs vaccinated with it.

Owing to the great stability in virulence of the plague bacillus under certain conditions, its use in vaccination is much simplified. During the past year and a half I have been able to detect no change either in virulence, toxicity, or immunizing power in the strains with which I have performed my vaccinations in man and in animals. The cultures apparently possess the same immunizing power to-day as they did at the commencement of the experiments. However, it is probable that they could be further attenuated by artificial means.

Douglas and Bullock (Allbutt and Rolleston's System of Medicine), in commenting upon my work in vaccination against plague, state that naturally very great care would be necessary in recommending a method like this on a large scale in plague-stricken communities, as from unforeseen circumstances the virulence might increase and plague be induced.

As I have pointed out, I have no evidence to support this statement and my cultures, which for nearly two years have been used at intervals in human beings, are as safe for use in man to-day as they were at the time of my first inoculations.

Nevertheless, cultures which are to be employed for human vaccination must be continually tested on animals to guard against the possibility of any such occurrence as that which has been suggested by Douglas and Bullock.

In discussing this question of vaccination in man against plague, with suitable cultures, I wish to emphasize the fact that the method is not infallible, and that very brilliant results may not always be obtained by it. In the vaccination of large numbers of human beings, owing to individual variations in susceptibility to plague infection, and natural resistance, just as in numerous experiments performed with monkeys, certainly all of the individuals will not be protected against the usual lethal infection by a single vaccination with one agar culture, but we have proof that a good proportion of them may be immunized by this method, and an appreciable

degree of immunity may be retained for at least ten or eleven months. The population in a plague-stricken district may gradually be immunized against this disease by the employment of vaccination.

In plague, as in small-pox vaccinations, it may frequently be necessary to repeat the vaccination, and perhaps with a larger dose, in order to secure a satisfactory immunity.

As yet we know of no practical method for the detection of those cases which would require a second plague vaccination to protect them from the natural manner of infection of the disease, and this subject requires further investigation.

In closing my remarks upon this subject, I shall summarize the work on protective inoculation against plague in the following conclusions:

1st. The usual hygienic methods which have hitherto been undertaken in combating plague in certain of its endemic centers, and from which there is continuous danger of invasion by the pestilence into other countries, have not always proved themselves to be effective. This fact is clear because the disease is not markedly decreasing in these centers. For example, India is at present suffering from an epidemic of plague larger than the one which occurred there in 1905. The official monthly returns from that country for the present year up to June, 1907, show 1,062,908 deaths from this disease. (These official figures have been kindly supplied me by Dr. Martin and Dr. Boycott of the Lister Institute, London.) The British Commission under the direction of Martin and Lamb by their recent important studies[20] have, moreover, demonstrated why the ordinary hygienic measures have failed and why they must continue to do so in the suppression of the epidemic.

2d. Following the earlier principles of Jenner in vaccination against small-pox, elaborated and extended by Pasteur to rabies, and certain other infectious diseases of bacterial origin, the German Pest Commission, consisting of Gaffky, Pfeiffer, Sticker and Dieudonnè performed experiments in the immunization of animals with living pest cultures, but owing to the

difficulty of obtaining strains of sufficient attenuation, the method was abandoned. The Austrian Commission, Albrecht and Gohn, and the French investigators, Yersin and Carré, also employed pest cultures of moderate attenuation in the immunization of rats, guinea-pigs, and monkeys, and Yersin vaccinated himself cutaneously with a small amount of an attenuated culture. Kolla first conclusively demonstrated that in animals a higher immunity is obtainable by the use of attenuated plague cultures than by the inoculation of either the killed pest organisms or by Lustig's prophylactic. Finally, I have shown that by vaccination of animals with attenuated cultures not dangerous for inoculation in human beings a higher immunity can be obtained than by the inoculation of either the killed pest cultures or their extracts, including among them natural aggressin.

3d. While it is obvious for several reasons that for immunization in man in general, the inoculation of killed cultures or their extracts is far preferable and safer than the method of bacterial vaccination, nevertheless it would appear that in pest only by vaccination can a satisfactory immunity be obtained.

4th. While the attenuated cultures which I have employed in the vaccination of human beings have so far shown that their use in man is without danger, my work must nevertheless be considered as experimental, and more extensive experiments must be performed with different races and classes of people and under various conditions before the method can be recommended for general use.

The question which confronts us is whether we shall continue to combat plague by these measures which have shown themselves by ten years' experience to be entirely inadequate in the suppression of this malady in certain endemic centers, or whether we shall experiment further with a method which appears to offer us a greater hope of success in the suppression of this disease. Personally, I have demonstrated the value of the method of vaccination as far as it is possible for me to do so.

REFERENCES.

1. Zeitschr. f. Hyg. u Infektionskrankh. Leipz., 1903, xlv, 507.
2. Philippine Journal of Science, 1906, i, 512.
3. Deutsche Med. Wchnschr., 1905, xxxi, 1101.
4. Centralbl. f. Bakt., 1906, xlii, 448.
5. Centralbl. f. Bakt., Orig., 1905, xxxix, 610.
6. Zeitschr. f. Hyg. u Infectionskrankh, 1904, xlviii, 371.
7. Studies in Pathology, Aberdeen, 1906.
8. Phil. Jour. of Science, 1907, ii, 256.
9. Zeitschr. f. Hyg. u Infectionskrankh, 1903, xlii, 244.
10. Centralblat. f. Bakt., Abt. i, 1906, xxxviii, 32.
11. British Medical Journal, 1906, 1783.
12. Proceedings Royal Society, 1907, lxxix, May 30, 1907.
13. Ibid. June 13, 1907.
14. Proceedings Royal Society, 1905, lxxvi.
15. Arbeiten aus dem Kaiserliches Gesundheitsamte, 1907, xxv, heft. 1.
16. Proceedings Royal Society, 1907, lxxix.
17. Johns Hopkins Hospital Bulletin, Baltimore, xviii, June–July, 1907.
18. Bulletin of the Committee for Study of Special Diseases. Cambridge, i, Nos. 3–9, August, 1907.
19. Philippine Journal of Science, i, No. 1, 1906.
20. Journal of Hygiene, 1906 and 1907.