bacilli do not stain by Gram's method, but a little of the material surrounding them remains slightly stained.

Cultivation of the Bacillus.

This presents usually no difficulty except in the case of sputa. Ordinary alkaline peptone bouillon agar seems to me the best medium to use in ordinary cases, at any rate I have not found that with the blood or tissues of animals affected with plague it was possible to obtain typical cultures more rapidly on any other medium. On agar at the temperature of the body minute colonies are already visible eight or ten hours after inoculation of the medium. Neither blood serum, glycerine agar, or gelatine agar present any advantage. (In separating the plague bacillus from other microbes in sputum, advantage may be taken of the fact that the plague bacillus grows quicker at a low temperature than other pathogenic bacteria; this method of cultivation has been recommended to me by Dr. Calmette.) Growth is also rapid in ordinary alkaline peptone bouillon, if care be taken not to disturb the bouillon the growth in that medium is characteristic, the bacillus grows near the surface, and adheres to the sides of the tube near the surface of the fluid, forming a whitish flocculent ring. The fluid remains clear so long as the fluid is not disturbed, but the slightest motion causes flakes of bacilli to fall, and for a time the fluid seems cloudy; this probably accounts for the turbidity noticed by Kitasato. The production of involution forms by cultivation on salted agar, as recommended by Hankin and Leumann, is not always sufficiently rapid to be very helpful when rapid diagnosis is wanted. The same objection may be offered to Haffkine's method of cultivation on buttered bouillon; the appearances of a stalactite culture are, however, very striking. I have not found that milk was coagulated by this bacillus.

Precautions against Laboratory Plague.

I cannot conclude these very cursory remarks on the bacteriological diagnosis of plague without pointing out the dangers of the slightest carelessness in such work.

Animals under observation should be kept in a separate room, in glass jars covered with fine gauze weighted lids. For additional security the jars should be placed in a larger case, constructed very much like a meat safe, but entirely made of metal and glass (iron frame, sides entirely closed by fine wire gauze, glass doors, with well-fitting frame). This outer case should entirely prevent rats, mice, or even insects, from having access to the animals or their food.

As a further precaution this case should be placed above a large metal tray easily disinfected, and supported above the floor by a metallic stand. These precautions are necessary to prevent accidental infection of rats and mice, and of various parasites which usually find their way wherever animals are kept.

The skin of animals which have to be dissected should be well soaked with an acid solution of perchloride of mercury (1 in 500) before the animal is opened, and the dissection should be made over a tray containing some of the same solution. The body and organs which are not preserved in suitable preservative fluids should at once be destroyed by fire. Laboratory attendants should not be allowed to touch any infected animal or products before these have been thoroughly disinfected. The bacteriologist may take the risks of his own work, and being well acquainted with them is in a position to avoid them, at any rate it is his duty to face them; the case is not the same with regard to his assistants, and more especially to those that are young and untrained. The comparatively great number of deaths attributable to plague infection in the laboratory or the *post-mortem* room, which have occurred during the last three or four years, clearly indicates that an exceptional amount of care is needed in work of this kind.

In these short notes I have not attempted to give a complete account of methods; I have simply tried to give such information as is not already easily accessible to all through the many excellent summaries of the subject which have appeared of late.

References.

[1] An excellent review of the subject will be found in Netter's short but masterly treatise on *La Peste et son Microbe*, Paris, 1900. [2] Batzaroff, *Annales de l'Institut Pasteur*, 1899, p. 385.

ON THE METHODS OF MAKING ANTITOXIC AND PREVENTIVE FLUIDS,

WITH SPECIAL REFERENCE TO THOSE OF PLAGUE.

(With Illustration on Special Plate.)

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It is unnecessary here to enter upon a discussion of the nature of immunity or of the action which takes place between toxin and antitoxin, a subject which we know very little about, but we may with advantage consider the means by which the human or animal body may be rendered immune to an infectious disease. It has long been known that immunity is in general obtained to the virulent disease by passing through a modified form.

Immunity Produced by the Attenuated Virus (Living Microbes).

The first experiments in this direction with artificial cultures were made in 1880 by Pasteur, who showed that immunity may be induced by inoculation with the attenuated virus—that is, with cultures of attenuated, but living, microbes. The outcome of these experiments was the protection of animals from anthrax and protective inoculation against rabies, although in this disease the specific microorganism has not yet been found. In 1884 Ferran first protected guinea-pigs against cholera by the same means; and later Haffkine, in 1893-94, started protective inoculations against cholera in man on a large scale in India.

Immunity Produced by Microbes Killed by Heat.

The method of inoculating with living microbes has its obvious disadvantages when carried out with the cause of a disease which may take a septicæmic form; and this fact led Haffkine, when he came to experiment with plague, to try to immunise animals by inoculating them with microbes killed by heat. Haffkine had observed with respect to cholera,[1] and others before with respect to several other diseases, that it was possible to confer immunity by inoculating animals with the dead bodies of the microbes of certain diseases. An animal inoculated with either the attenuated living or with the dead microbe undergoes a reaction lasting several days.

The tissue cells of the animals, stimulated by the specific substance contained in the body of the microbe, are induced to elaborate an antitoxin or immunising substance; and the animal acquires an active immunity. An animal that has been at the trouble of manufacturing its own antitoxin has a more lasting and efficient protection than one that has been injected with an antitoxic serum obtained second hand, as it were, from an immunised animal. The antitoxic serum confers immunity almost directly, but its effects are fleeting, a fact which one would expect, considering the serum is a foreign body and is soon got rid of.

The Active Bodies in Haffkine's Prophylactic Fluid.

With respect to plague prophylactics, it is the intracellular poisons of the dead microbes that are mostly concerned in conferring protection, but Haffkine injects the broth in which they have grown as well. He was led to adopt this measure from previous observation on cholera. The object is to counteract an attack of plague in an inoculated person—supposing he became infected—by previously accustoming the tissues to the poison given out by the microbe in the cultivation medium.

This theory assumes that the intracellular poisons induce a bactericidal power in the tissues, and that the extracellular poisons induce antitoxic properties which would come into play supposing the bactericidal power were insufficient to prevent infection, that is to say, if the person did contract plague.

I showed by experiments on rabbits that the broth in which the microbes had grown apart from the bodies of the microbes was of use as a means of causing protection.[2]

I have touched upon these points because it has often been asked why the broth is used at all, and not the dead microbes alone, which would make the technique of manufacture simpler.

Since a dead culture only is used the prophylactic is in no sense a serum. The false use of the word serum for what is really a vaccine is to be deprecated, for it gives a wrong notion of what the fluid really is. The word serum is constantly used in describing the prophylactic, even in Government reports. The very misleading, not to say alarming, statement that inoculation with the prophylactic causes a mild attack of plague is not what one would expect from a scientific physician.[3] A small definite quantity of plague poison is injected which is easily dealt with by the body and it cannot increase. There is the same distinction as would be between a case of mild septic intoxication and a case of septicæmia.

Preparation of Haffkine's Prophylactic Fluid or Vaccine.

The vaccine is a sterilised culture of the plague microbe in broth. The microbe should be obtained fresh from a case of plague, or from a culture in which the virulence has been kept up.

In India, where the religious prejudices of Hindu and Mohammedan have to be allowed for, the broth is made with goat's flesh in a way that is unnecessary to describe here. Ordinary broth as usually made in the laboratory is really better.

In order to ensure sufficient aëration of the microbe which it would not obtain at the bottom of a flask of broth, Haffkine first placed a few drops of oil or butter on the surface of the broth. The microbes attach themselves to the butter, and grow down into the broth in long threads like stalactites, forming a very characteristic and beautiful appearance (see Fig. 3 of special plate). If the flask is moved the "stalactites" break and fall to the bottom, to be succeeded by a fresh crop in a few days.

The stalactite formation not only serves its primary purpose, but is also a very important test in the bacteriological diagnosis of plague.[4] The oil on the surface acts as a convenient substance for the microbes to attach themselves to. The microbe has a great affinity for any substance it can grow on, it prefers to grow up the side of a flask by continuity than to grow in the depths of the broth. Having attached themselves to the under surface of the particles of butter they grow down into the broth. The microbe does not require a large supply of oxygen. I have grown it anaërobically and found it to be quite as virulent as a corresponding culture grown aërobically.

The flasks of broth are incubated at 70° to 80° F. At this temperature the microbe will have finished growing in about four to six weeks. This may be known by the fact that no fresh "stalactite" growth is obtained after shaking the flask and allowing it to remain stationary for a few days.

After the culture has done growing, its purity from contamination is tested by taking a small quantity out of the flask and inoculating an agar tube. If the agar is dry and the culture spread evenly over the surface, the growth after one to two days' inoculation is seen as a thin, translucent, colourless film with a ground-glass appearance when the underneath surface is viewed through the substance of the agar. Any stray colonies of a foreign microbe are easily seen. The broth culture which has been tested and found pure is then sterilised by heating it to 65° C. for one hour, and a small quantity (0.5 per cent.) of carbolic acid is added. The fluid is then drawn off into small bottles by means of a siphon.

Antitoxic Serums.

The antitoxic serum of Yersin is used as a curative agent for plague. It also acts as a prophylactic agent, but the immunity conferred is very short—only about fifteen days.[5] The principle of this serum is the same as that of antidiphtherial serum and others of like nature. An animal—preferably a horse—is immunised by inoculation at intervals with dead or living cultures of the specific microbe; after the first or second inoculation the quantities are increased and the intervals between shortened. The animal, which takes the place of the human being who has been inoculated with a vaccine, acquires an active immunity; and, if the serum of such an immunised horse be inoculated in considerable quantities into a human being suffering from the specific disease, the disease is ameliorated or cured.

Bactericidal and Antitoxic Action of Serums.

A serum may act in either of two ways: it may be bactericidal—that is to say, it can prevent the growth of the microbe in the body; or it may be antitoxic, in which case it exerts no bactericidal action, but neutralises the poisonous products given out by the microbe in growing, or it may be both bactericidal and antitoxic. The serum having bactericidal properties alone will suffice to prevent a disease if inoculated before infection; but, if the serum be used as a curative agent, it must have antitoxic properties to counteract the poison already formed by the microbe, as well as bactericidal properties to arrest the growth.

With respect to antiplague serum, Roux asserts that the serums, however they are made, are always antitoxic, but the antitoxic property is more marked in some serums than in others; those made by injecting living cultures are more antitoxic than those made by injecting dead cultures.[6] This fact, according to Metchnikoff, explains the difference in results obtained with Yersin's serum.

Preparation of Yersin's Serum.

Having shown the possibility of preparing a curative serum in smaller animals, Yersin, Calmette, and Borrel started to immunise a horse in 1895 at the Institut Pasteur. Living cultures of the plague bacillus obtained from Hong Kong were used. Since injection of these cultures under the skin was found to cause long-standing induration, the method of intravenous injection was adopted, as much as a whole culture on an agar tube being used. The feverish reaction lasted for a week. After waiting twenty days a second inoculation was made; the reaction in this case was intense, but of shorter duration. From this time larger quantities were injected at shorter intervals. The serum was found to be preventive and curative for laboratory animals after six weeks' treatment.[7] It was from this horse after a year's treatment that the best results were obtained in Canton, and Amoy in the summer of 1896.[8]

Yersin afterwards started making serum in his laboratory at Nha-Trang, using living cultures. Roux started a stable of twenty-five horses at Garches, near Paris, but he considered it inadvisable to use living cultures for so many horses, which could not be kept under observation in the laboratory. He therefore used cultures killed by heat, and toxins formed in the cultivation media. The serums made both at Garches and at Nha-Trang were used by Yersin in Bombay and Cutch Mandvi in 1897. The results were distinctly inferior to those obtained the year before at Canton. The results with the serum made at Garches with killed cultures gave the least good results.[9]

On account of the inferior results of the Garches serum, and considering the progressive encroachment of plague, the Pasteur Institute decided to prepare several horses vaccinated first with cultures killed by heat and afterwards with living cultures, as was done in the first instance. This serum was used at Oporto in 1899, and gave somewhat better results.[10]

Vaccine and Serum of Lustig.

Lustig's method of preparing vaccine is based on the observation that a nucleo-proteid which he succeeded in separating from the bodies of plague microbes is a substance which induces immunising properties when injected; neither the metabolic products of growth nor toxins are used. The plague microbe is cultivated on agar plates and the growth is scraped off and dissolved in 1 per cent. sterilised solution of caustic potash. This solution is then rendered slightly acid with hydrochloric or acetic acid, and the resulting precipitate collected on filter paper; after washing it is dried *in vacuo*. This substance, which gives the chemical tests of a nucleo-proteid, is easily soluble in a weak solution of carbonate of soda.[11]

Lustig proposes to use this solution as a prophylactic.[12] He also immunises horses with it by repeated inoculations during a period of three or four months. The serum has been prepared in Bombay and tried at the Arthur Road Hospital.

So far, there has been no report of the use of the nucleo-proteid used as a prophylactic if it has been tried. The results of the curative serum were not at first very satisfactory, but lately better results seem to have been obtained. Dr. Polverini, in a report to the Bombay Municipal Committee,

states that in 475 cases treated a recovery-percentage of 39 was obtained. From the comparatively small number of trials of serum inoculations hitherto reported, neither serum seems to come up to what might be expected from a specific treatment. Lustig's serum does not appear to be so efficacious as that of Yersin. This is possibly accounted for in a way similar to Metchnikoff's explanation of the difference in Yersin's serums referred to above. A horse treated with a nucleo-proteid extracted from the dead microbes would be immunised with even less efficiency than one treated with the killed microbes together with their metabolic products, and a horse immunised in this way was shown to give a less powerful serum than one immunised with the living microbes. Compared in this way with Haffkine's prophylactic, the latter is the greater success as a scientific preparation.

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A NOTE ON THE METHOD OF USING HAFFKINE'S PROPHYLACTIC.

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A short description of the method of inoculating with Haffkine's prophylactic, and of the immediate clinical effect, may be of use at the present time. While I was in Calcutta in 1898 Dr. Nield Cook (the Medical Officer of Health) and I inoculated some 2,490 cases between April and July, none of whom died of plague during that year's epidemic. A stock of Haffkine's prophylactic was obtained from the Bombay Research Laboratory in January, 1898, and when the first fatal case of plague occurred at the European General Hospital, 10 members of the staff were inoculated at the request of Colonel Ranking, the Senior Medical Officer.

Roux's syringes, with a capacity of 10 c.cm., were employed. At different times the fluid varied in strength; some bottles were marked "double strength," in which case only half the dose was given. The dose has varied from 5 c.cm. to 20 c.cm. The ordinary dose for an adult male of the fluid then provided was 5 c.cm.; I used to give a woman 4 c.cm., girls aged from 10 to 14 years 3 c.cm., and children from 2 to 10 years from 1½ to 3 c.cm. according to age. To save time the whole of the syringe was filled, and used to inoculate several people, the graduations on the cylinder making it possible to gauge the dose accurately for each person; the needle was well rubbed with a cotton wool sponge dipped in 1 in 20 carbolic in the interval between two inoculations. Strict aseptic precautions were observed throughout. Carbolic was used, as it has been proved to have no action on the prophylactic. The neck of the sealed bottle of prophylactic was sterilised in the flame of a spirit lamp. The cork was then extracted with sterilised dissecting forceps. The bottle was covered with a pad of absorbent cotton just squeezed out of carbolic lotion until the syringe was quite ready, and in filling the syringe the bottle was held on the slant. The syringe was carefully taken to pieces each day after it had been used, washed with boiling water, then with carbolic 1 in 20, and carefully dried. Just before using the needle it was boiled in a test tube and inserted into the syringe with sterilised forceps.

I invariably selected the back and outer side of the arm midway between shoulder and elbow as the site of inoculation: in men I believe the flank was sometimes used. The skin was well rubbed with a sponge dipped in 1 in 20 carbolic; if any dirty patient presented herself I made her wash the arm herself first with water and, when procurable, soap; but the numbers were so large that as a rule the rubbing with carbolic was the only measure employed, and I never had an abscess or other untoward result. The needle was run into the superficial fascia for quite half or three-quarters of an inch, and the fluid slowly injected; the needle was quickly withdrawn, the skin being pinched up as it emerged. If the needle be run in towards the elbow it quite prevents any of the fluid emerging with it. The pain was of course trifling. I let the patient press a small sponge squeezed out of carbolic over the spot to stop any bleeding. I only remember one case where more than one or two drops of blood flowed, and usually there was none at all. In view of the subsequent inflammation and itching, I avoided the application of collodion or strapping. It is not necessary to apply any dressing at all.

The reaction in many cases began at once with pricking at the site of inoculation and the gradual formation of a hard tender red swelling for a few inches round. The temperature rose, in the cases in which I was able to take it, within six hours of the inoculation: it was highest on the night of inoculation. The height of the fever varied a great deal. In a few cases it only reached 99.6° or 100° F. In most it rose to 102° or or 103°. It was rarely possible to reinoculate immediately any who manifested no reaction, though I believe Mr. Haffkine considers it more satisfactory to reinoculate in all cases which do not present a rise of temperature to 103°. The fever rarely lasted more than two days, though the arm remained more or less painful and tender for a week or ten days, and a small hard nodule often remained for a few weeks.

I was not able to make a practice of seeing all inoculated persons the next day. Those I did see presented a red, hot, hard swelling of the skin and subcutaneous tissue of the arm rarely spreading above the shoulder, but often extending to the elbow or even to the wrist. The margin was as defined in many cases as in erysipelas; the pseudo-erysipelatous condition was not, I am told, noticed when the flank was chosen. There seemed to be no relation between the degree of fever and the amount of local reaction. The axillary and cervical glands were sometimes swollen and tender, and this occurred at times when the arm itself was only slightly inflamed. Many patients complained of shooting "rheumatic" pains in the shoulder and elbow-joints. One said she felt as if the arm were so heavy that it was dragging itself out of the socket. The general symptoms varied with the individual. Some people vomited or had diarrhœa, others suffered from nausea, syncope, or shivering. Two cases had only slight fever and local reaction but were intensely sleepy, for twenty-four hours in one case and thirty-six hours in another. I never saw a case of delirium but have heard of one or two. Some patients felt slightly out of health for a week or two after inoculation.

Neither Dr. Cook nor myself had any untoward results. There should be no suppuration if cocci are excluded by aseptic proceedings. We used to advise the patients to keep quiet for a few days, to drink chiefly milk, to take a simple aperient the next morning, and to apply cold water or lead lotion to the arm and keep it in a sling. It was our practice to refuse to inoculate any who were already ill (especially if they had any increase of temperature), aged and feeble persons, nursing mothers, pregnant women, and children under two years. I never saw any ill-effects in those cases in which old women urged me to inoculate them, or mothers insisted on my inoculating babies under two; I never injected any under a year. We had to take special care to refuse any cases that were at all likely to be ill from causes apart from inoculation, as any serious or fatal illness would have been at once attributed to the inoculation.

During the first two months, reports were constantly spread that inoculated persons had died within a few hours or days; one newspaper was only induced to withdraw such statements by a letter from the health officer's solicitors threatening legal action. As it was, a young Austrian, who from the fact that he carried a handbag, was supposed to be an inoculator, was beaten to death; and an attack, intended for Dr. Nield Cook and myself as inoculators, was made on Dr. Laing in a quarter where we really had been inoculating the day before, but where he was only inspecting a house suitable for a hospital.

Certificates were granted to every inoculated person, and particulars inserted in the register; these certificates were highly valued in Calcutta, as segregation of contacts was not enforced where the whole household had been inoculated.