nd glands in suspected cases. It will generally be found preferable to embed the latter in celloidin.

Blood Serum Reaction.

The diluted blood serum obtained from a case of plague is said to cause clumping with plague bacilli. Hitherto this has not proved of practical value. The reaction can usually only be obtained late in the disease, and even then it is not always present.

The Bacillus is Non-pyogenic.

The plague bacillus may, I think, be best regarded as belonging to the septicæmic group of pathogenic micro-organisms, that is, one which is present in the blood, and may cause toxæmia or death without local manifestations. It is parallel in some respects with the anthrax bacillus. In plague, however, if local signs are present they occur in the lymphatic glands, whereas in anthrax infections local signs manifest themselves chiefly at the actual site of inoculation. The plague bacillus does not produce pus but it can cause necrosis of tissue. Buboes which contain the bacillus as a pure infection may resolve or slough. If they suppurate it is generally due to staphylococcal and streptococcal infection.

REFERENCES.

¹ Report of the Austrian Commission, 1897. ² Report of the German Commission, 1899. ³ Bombay Government Plague Reports, Condon, 1900. ⁴ *Die Krankheiten der warmen Länder*, von Dr. B. Scheube, 1900. ⁵ *Twentieth Century Practice of Medicine*, vol. xv, Plague by Kitasato. ⁶ Hewlett's *Manual of Bacteriology and Practitioner*, October, 1900. ⁷ Clemow, *BRITISH MEDICAL JOURNAL*, 1900, vol. i, pp. 1141, 1216. ⁸ Nuttall, *ibid.*, 1899, vol. ii, p. 642. ⁹ Manson's *Tropical Diseases*, second edition. ¹⁰ Rees, *Practitioner*, October, 1900.

SOME PRACTICAL NOTES ON THE BACTERIOLOGICAL DIAGNOSIS OF HUMAN PLAGUE (PESTIS HOMINIS).

(With Illustrations on Special Plate.)

By SHERIDAN DELEPINE, M.D.,

Procter Professor of Pathology Owens College, Manchester.

(Extracts from an Address delivered on the occasion of a demonstration given to the Pathological Society of Manchester on October 10th, 1900.)

THE following remarks are based on experiments carried out in my laboratory with a culture of bacillus pestis kindly given to me by Dr. Calmette in April last. This culture was used chiefly for the purpose of repeating experiments of previous observers, Yersin and Kitasato more specially. The culture had been obtained by Dr. Calmette from a case of plague in Oporto (1899), and was so virulent in April last that it was sufficient to prick the skin of a guinea-pig with a needle loaded with bacilli to produce an attack of the disease fatal in about five days. Dr. Calmette also gave me very valuable information in a most generous manner, and I take this opportunity of thanking him cordially.

Through the courtesy of Dr. Brownlee and Dr. McClure I was able to examine 29 of the cases of plague under treatment in the Glasgow Fever Hospital. Medical men have much reason to be grateful to these gentlemen for the very unselfish way in which they sacrificed for the benefit of their colleagues much of the time they could so ill afford to spare.

I have also to acknowledge the kindness of Dr. Buchanan, who supplied me with *post-mortem* material from three of the fatal cases which had then occurred in Glasgow.

THE DISCOVERY OF THE BACILLUS.

It is unnecessary in a communication of this kind to give a systematic account of the bacteriology of plague,¹ as numerous summaries of the subject have appeared of late. Suffice it to say that in 1894 Kitasato and Yersin, working independently in Hong Kong, each isolated a bacillus from the buboes and blood of patients affected with bubonic plague, from the organs of rats and mice which had died of plague, from the dust (Kitasato) and from the soil (Yersin) of houses where patients had died of plague (see Special Plate, Figs. 4 and 5). In addition they had shown that the bacillus could be easily cultivated outside the body, that the disease could be produced experimentally in the lower animals by inoculation, and by feeding on cultures of the

bacillus or on organs containing the plague bacillus. Yersin had also shown that flies might be infected, and become a source of spread of the disease.

The descriptions given by Yersin and Kitasato were very similar, but they disagreed with regard to certain points as to the distribution of the bacillus. Thus Kitasato recognised the bacillus more constantly in the blood than did Yersin, who found it specially in severe cases. Kitasato is also of opinion that the plague microbe may be stained by Gram's method, and causes the coagulation of milk, which is not in agreement with the results obtained by other observers. It is, however, impossible to believe that Kitasato could have examined a number of typical cases of plague without seeing the same organism as that seen by Yersin, for when an unsoftened bubo is examined the bacillus is usually found in great abundance and in a state of purity. From a diagnostic point of view the most important addition which has been made to the discoveries already maintained is due to Childe, who in 1896 established the existence of plague pneumonia by the bacteriological examination of sputa.

Another advance useful in diagnosis was made by the discovery that plague could be produced experimentally by painting the conjunctival mucous membrane with products containing the plague bacillus, as recommended by the Bombay German Commission, or by painting the nostrils of guinea-pigs, rabbits, or monkeys, according to the method introduced by Roux in 1898.

Finally, I should also mention the attempts which have been made to utilise the serum reaction for diagnostic purposes (Wyssokowicz and Zabolotny, and others).

TYPES OR VARIETIES OF PLAGUE.

It is important, from a diagnostic point of view, to be familiar with the various forms which the disease may assume. Like some other observers, I have been able to reproduce these types experimentally in the guinea-pig with the virulent culture I had at my disposal (obtained by Dr. Calmette from one of the cases in Oporto). These varieties depend (1) on the channel of entrance, (2) the virulence, (3) the quantity of the bacilli.

I.—THE BUBONIC TYPE.

The bubonic type is produced in the guinea-pig by infecting slight cutaneous wounds with a virulent culture of the bacillus (or by injecting subcutaneously various quantities of an attenuated culture). After slightly scarifying the skin on the inner side of one of the hind legs of a guinea-pig, I rubbed the part with a loopful of a moderately virulent agar culture of bacillus pestis. Signs of disease became evident at the end of twenty-four hours, and the animal died about five and a half days (138 hours) after inoculation. After death considerable oedema was found near the seat of inoculation, in the corresponding groin, and to a lesser extent over the posterior half of the abdomen. The popliteal and superficial inguinal glands were much enlarged and suppurating, the corresponding lumbar glands were also enlarged and deeply congested. The spleen was enlarged and full of very minute tuberculous-looking lesions (these contained a very large number of plague bacilli). The liver was large and unequally congested. The lungs were intensely congested and contained eight or ten large tuberculous-looking masses, situated chiefly under the pleura. These pseudo-tubercles were surrounded by a wide zone of deeply-congested, partly hæmorrhagic tissue (secondary plague pneumonia). All these lesions resembled those produced in the guinea-pig by the bacillus mallei. The right side of the heart was much distended with dark blood. Most of the lymphatic ganglia other than those already mentioned were swollen and more or less congested. Typical plague bacilli were demonstrated by microscopical examination in a suppurating inguinal glands and the oedematous tissues round them, the spleen, liver, lungs, and blood of the heart. They were most abundant on the spleen and glands. Cultures revealed, in addition to the plague bacillus, the presence of the staphylococcus pyogenes aureus in the inguinal glands but not in the other organs. There were only two colonies of staphylococci amongst a very large number of colonies of the plague bacillus. In one of the agar cultures they were also present in the bouillon cultures. Animals inoculated with these impure cultures all died of plague but in all their organs the plague

bacillus and the staphylococcus were found together, the staphylococcus having apparently multiplied more rapidly than the plague bacillus. Judging by the relative number of colonies obtained, the spread of the staphylococcus had been favoured by the presence of the plague bacillus, but the disease produced by the mixed infection had all the characters of plague.

II.—SEPTICÆMIC PLAGUE.

This was produced by injecting the bacillus under the skin. One-sixth of an agar culture of a slightly attenuated type, which had been kept in the laboratory for about six months, injected deeply into the thigh, produced the death of a guinea-pig in 66 hours. With an agar culture isolated seven days previously from a rapidly fatal case, $\frac{1}{10}$ th of the culture was fatal in less than 48 hours. One mouse was killed in less than 24 hours by $\frac{1}{10}$ th, and another in 45 hours by $\frac{1}{10}$ th part of the same culture. All these cases differed from the first case described in that the enlargement of the glands near the seat of inoculation, though distinct, was less marked; these glands were of a deep red colour, and firm. Nearly all the other lymphatic glands were deeply congested. The spleen was large in all cases, but showed no tuberculous-looking lesions, the lungs were either congested or pale, but generally œdematous. The local œdema was variable, very extensive and hæmorrhagic in one case, but comparatively slight in the others. In all these cases the bacillus was found abundantly in the blood of the heart, spleen, liver, and lymphatic glands.

III.—PULMONARY PLAGUE.

This was produced according to Roux's method by applying the bacilli with a small, soft camel-hair brush to the nostrils of guinea-pigs. In one case I used an agar culture which had been kept for nearly five months in a cupboard at the temperature of the laboratory. For another case I selected an agar culture I had isolated nine days previously from a fatal case. In both cases there was evidence of general discomfort before the end of the third day, and both animals died between the fifth and sixth day after the painting of the nostrils.

There was considerable swelling of the integuments round the nostrils and of the upper lip; the trachea contained mucopurulent exudation, slightly tinged with blood in one case. In both cases the lungs were large, œdematous, generally pale, their surface was slightly uneven owing to collapse of small portions of parenchyma immediately under the pleura; there were also small ecchymoses under the pleura, and in one case several larger hæmorrhagic foci. There was, in fact, well-marked disseminated broncho-pneumonia, and the bronchial and cervical lymphatic ganglia were intensely congested.

Some of the cervical glands contained hæmorrhagic foci; these were almost black; the most affected glands were surrounded by œdematous blood-stained connective tissue. The other organs presented the same appearances as in septicæmic cases. Plague bacilli were easily demonstrated in the tracheal mucus, the lungs, the blood of the heart, the cervical glands, and the spleen. The most interesting feature of plague pneumonia is that it can be produced with bacilli the virulence of which is so attenuated that they do not produce the disease by subcutaneous inoculation.²

IV.—GASTRO-INTESTINAL FORM.

This was introduced in some mice, of which I placed two in a cage which had been occupied by two guinea-pigs that had died of plague, and two others in a clean cage, their food being mixed with some of the excreta of a guinea-pig also dead of plague. The first two mice died in about ninety-six hours; the last two died in about seventy-two hours. They all seemed to have succumbed to some mixed form of infection; I could recover the bacillus in one case only.

The experiments of Yersin and Kitasato showed from the first that infection could be produced by ingestion of infectious products. Kitasato was even able to find the plague bacillus in the contents of the intestine.

It is sometimes argued that animals fed on material containing plague bacillus are not infected through the alimentary tract but through the nose and air passages. Batzaroff has, however, produced the disease by depositing in the mouths of guinea-pigs portions of infected organs, care being

taken that the nostrils were not contaminated or the buccal mucous membrane wounded.

A gastro-intestinal form of plague is not usually recognised as a clinical form of human plague.

V.—CUTANEOUS FORM.

I have not been able yet to produce localised cutaneous lesions not followed by lymphatic extension, giving rise to the bubonic type of the disease. It is said that in the human subject pustules may occasionally occur, without formation of buboes. The contents of such pustules are said to be rich in bacilli. Usually, however, infection through the skin is followed by the production of buboes, and in such cases it is not always possible to recognise any cutaneous lesion at the primary seat of infection.

From the preceding short summary it will be seen that the three chief types of human plague—that is, the bubonic, the pulmonary, and the septicæmic—correspond to three types of infection, the first two being determined by the channel of infection, the last being capable of arising whenever the resistance of the tissues is considerably reduced, or the virulence or quantity of the bacilli very great. It will be noticed that in all cases the lymphatic glands are liable to be affected, but that typical inguinal and axillary buboes are produced chiefly in connection with cutaneous infection of the extremities, whilst cervical buboes may be produced as a result of infection of the skin of the head and neck, or of the nasal or buccal mucous membranes. In the case of infection through the air passages the most characteristic bacteriological feature is the presence of plague bacilli in the bronchial and nasal discharges. Finally, in the rapidly fatal forms without any marked localisation of lesions, the blood contains a large number of bacilli. In the experiments recorded above the blood was invariably found to contain bacilli easily demonstrable by direct microscopical examination and cultivation, whatever mode of infection had been adopted. In the cases of mixed infection which I produced artificially the plague bacillus could be easily separated from the associated cocci.

DIAGNOSIS OF PLAGUE BY EXPERIMENTAL INFECTION.

Two methods of infection may be used for diagnostic purposes:

1. *Subcutaneous inoculation*, for which mice and guinea-pigs are especially suitable.
2. *Infection through the air passages*, obtained by painting the nostrils with the products under investigation. This method is especially suitable when products of low virulence are under investigation—as, for instance, the pus of suppurating buboes. (In suppurating buboes the plague bacilli, when found, are usually in a state of degeneration and frequently of low virulence.) For this method of experimental infection the guinea-pig is especially suitable.

MICROSCOPICAL EXAMINATION.

I have described the experimental methods [in order to make clear the distribution of the bacillus. In practice, however, the first steps to be taken would be to make a microscopical examination:

1. Of material obtained from a bubo or from the œdematous tissue surrounding it.
2. Of blood obtained from the tip of a finger or from the lobe of the ear.
3. Of the expectoration in suspicious cases of pneumonia.

In ordinary cases such an examination presents no difficulty, and can be carried out very rapidly. Almost any of the aniline dyes in common use can be used for the purpose of staining cover-glass preparations of material obtained with the hypodermic syringe from buboes or their neighbourhood. Freshly-prepared aqueous solutions of methyl violet, aqueous solutions of methylene blue, or Loeffler's blue may be used. Carbol-thionin blue or diluted carbol fuchsin have given me the best results. Carbol fuchsin has, however, a tendency to stain too deeply the degenerated cells and products among which the bacilli are found; and, to obtain good preparations showing well the bipolar stain of the bacillus, I treat the film for about five seconds with 15 per cent. solution of sulphuric acid, which is washed off rapidly; carbol fuchsin is then dropped on the film; after a contact not exceeding two or three seconds, the stain is thoroughly washed off. The

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.)

By C. BALFOUR STEWART, M.A., M.B.CANTAB.,
Thompson-Yates Laboratories, University College, Liverpool.

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 micro-organism 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.