89 plus

STUDIES IN THE BACTERIOLOGY AND ETIOLOGY OF ORIENTAL PLAGUE

STUDIES

IN THE

BACTERIOLOGY & ETIOLOGY

OF

ORIENTAL PLAGUE

BY

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TO

W. H. POWER, Esq.,

C.B., F.R.S., F.R.C.S.,

MEDICAL OFFICER OF THE LOCAL GOVERNMENT BOARD,

THIS VOLUME

IS RESPECTFULLY DEDICATED

BY

THE AUTHOR

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INTRODUCTION

It is admitted on all sides that the *Bacillus pestis* is the real and essential cause of Oriental or bubonic plague, and consequently that the presence of this microbe in any material derived from a human or animal being denotes the disease plague in such a being. It is likewise admitted that a patient, although exhibiting one or more symptoms suspicious of the disease plague — *e.g.* fever with swollen and inflamed subcutaneous lymph glands in one or the other region of the body, cervical, axillary, inguinal, or femoral,—need not necessarily be affected with bubonic plague, notwithstanding that such person might have been indirectly exposed to plague infection. Should, however, in such swollen inflamed glands the *B. pestis* be demonstrated, epidemiologists and physicians would accept such a case unquestionably as true plague. It is obvious that should this be the case the relation of such a patient towards his surroundings would at once be vastly different from that in which a negative bacteriological result showed that the patient is not affected with that infectious disease, but is suffering from some other malady not requiring those stringent and costly measures that a case of plague requires.

No greater misfortune, from a public health point of

view, could befall a community than an epidemic outbreak of bubonic plague in a large and crowded city. An epidemic might originate from a patient or patients who, while not exhibiting symptoms clinically typical of plague, nevertheless harbour the *B. pestis*, and this being overlooked might become a focus of further infection. If such be the case, the blame that an important step in the diagnosis of the disease, namely, the bacteriological evidence, had been omitted would rightly fall on those who relied solely on clinical evidence. The bacteriological examination of cases which, for one reason or another, are under suspicion of being affected with plague, and if undetected may be the means of introducing the disease into a new locality, is therefore of the greatest importance, since the clinician cannot venture to pronounce on the case with certainty. The same applies to rats in a ship coming from a plague-infected locality. It is established that ships have harboured plague rats without any one on board contracting the disease, but nevertheless such a ship if left to itself remains a real source of danger, not only to those who afterwards use it—as has actually occurred—but to the port of landing, where its plague-sick rats may carry infection to rats on shore, and further carry infection to human beings. The bacteriological diagnosis of plague in rats—the only examination that is of scientific value—is from a public health point not less important than the examination of suspected human cases.

In the following pages we shall have opportunity of giving an account of cases which, from a clinician's and epidemiologist's point of view, were under suspicion of being plague, and on bacteriological analyses were

actually found to be so, while other similar cases were proved bacteriologically not to be cases of plague. Such cases, although clinically suspected to be plague, were, in confirmation of the negative bacteriological evidence, not followed by any further cases of the disease. The same applies to rats; for while, on the one hand, bacteriological analysis confirmed the preliminary diagnosis made by the sanitary authority, viz. that mortality amongst rats on certain ships coming from infected ports was due to plague, it has, on the other hand, shown that mortality of rats on ships need not necessarily be due to plague, because rats are subject also to several forms of acute infectious maladies other than plague.

During the last ten years I have had a good many opportunities of investigating bacteriologically materials of suspected and real cases of plague of human beings and of rats; I have also made special studies of the *B. pestis* in its morphological, cultural, and physiological characters, and in the manner of its conveyance and action. Some of these studies have been published in the Annual Reports of the Medical Officer of the Local Government Board, and are here reproduced, partially or wholly, by permission of the Controller of H.M. Stationery Office. It seems not out of place to collect the results of all these studies, carried on now for a succession of years, in a connected and easily accessible form. The following pages are devoted to this purpose.[1]

[1] With the exception of Fig. 1, all the illustrations are photograms made for me by Mr. Albert Norman, M.R.C.S., London.

CHAPTER I

THE *BACILLUS PESTIS* THE ESSENTIAL CAUSE OF ORIENTAL PLAGUE

THE literature of Plague, from the earliest historical periods, when the disease was recognised to be a communicable disease, down to 1894, *i.e.* down to the outbreak of plague in Hong-Kong, contains a number of suggestions and assumptions as to the causation of the disease. But as is the case with other communicable diseases, before the discovery of the actual contagium no scientific distinction was or could be made between the primary or essential cause, *i.e.* the *causa causans*, and those secondary conditions which contribute to, and which favour infection: terrestrial influences, peculiar atmospheric states, social defects, famine and want, crowded and ill-ventilated habitations, decomposing corpses, decomposed, insufficient, and unclean food-stuffs, and a number of other conditions which are apt to weaken and to influence in an unfavourable sense the resistance of the individual; that is to say, all conditions which in most infectious diseases play a part in facilitating and enhancing infection were formerly considered as being of the nature of essentials. The discovery of the *Bacillus pestis*, however, as the true essence of the

contagion, relegated all the above states to their proper place, viz. as being of the nature of secondary causes, and therefore of secondary importance.

Yersin (*Annales de l'Institute Pasteur*, viii. p. 662), and Kitasato (*Lancet*, 1894, ii. p. 428) showed that in all cases of bubonic plague the buboes, the spleen (and also the blood) contain in very large numbers minute bacilli, which in morphological and cultural respects possess definite characters; that a trace of culture of the microbe inoculated into a rodent causes invariably the typical acute fatal disease, bubonic plague, with the same copious multiplication of the same bacillus. These statements are easy of verification, and there can therefore remain no doubt that all postulates which a microbe has to fulfil in order to be regarded as specifically the *causa causans* has been complied with in the case of *B. pestis*. Moreover, the numerous cases of plague in man which, from time to time, have come under the notice of a large number of observers in various countries, in which plague has appeared since 1894, and the numerous rats dead in various plague-stricken countries that have been subjected to bacteriological examination, have all yielded the same result, viz. the inflamed lymph glands, the spleen, and other organs teemed with the same *B. pestis*. This bacillus, as will be shown, is a well-characterised species—well characterised in morphology, in culture, and by experiment—so much so, that it has become an established fact that the identification in the glands, spleen, or other organs of any body is proof positive that that body is subject to and affected with Oriental plague.

Kitasato's earlier and later accounts of the *B. pestis* differ somewhat from the description given by Yersin,

inasmuch as Yersin's account is all throughout consistent, whereas Kitasato's earlier account appears somewhat at variance with his later statement, and it is for these reasons that an undesirable confusion has arisen in the minds of some later observers. Thus Aoyama (*Centralblatt f. Bakter. und Parasit.* xix. 481), while confirming Yersin's *B. pestis* as the microbe of plague, denies to Kitasato's bacillus this same claim. We shall show that Kitasato's bacillus of his later account is the *B. pestis*, but under a modification, being a different type of the plague microbe.

As mentioned just now, bacteriologists in all countries who have had the opportunity of becoming acquainted with, and who have carefully investigated the nature and character of *B. pestis* in man, and in the rat, affected with plague, have confirmed Yersin's and Kitasato's discovery, that the *B. pestis* is the specific microbe of Oriental plague.

The distribution of the *B. pestis* in the affected body, both of man and the rat, under natural conditions, is subject to certain slight variations, which are dependent, in a large measure, on the various forms under which plague declares itself. We proceed to consider in detail these variations.

I. In Man

(A) *Pestis bubonica.*—The most common form, as admitted on all sides, is the bubonic form, *i.e.* the one associated with conspicuous swelling and inflammation of the lymph glands, with œdema, hæmorrhage, and inflammation of the subcutaneous tissue around the glands; both together forming a painful, large, more or less soft "bubo."

Whether the bubo occurs in the neck, the axilla, the inguinal or femoral region, in all instances the glands show hæmorrhage in their substance with partially necrotised foci. A droplet of the gland juice, as also, but to a less degree, of the surrounding inflamed cellular tissue, shows in film specimens a conspicuously large number of *B. pestis.* The great number of bacilli, possessing the same characters, aspect, staining power, and size, is alone sufficient to make diagnosis of plague highly probable, for there is no other acute disease known except plague which so affects the lymph glands, containing such vast numbers of this kind of bacilli, viz. non-motile, Gram-negative and bipolar in staining, *i.e.* a vacuole in the centre, or possessing a vacuole at one or both ends, and then appearing as if gnawed out at one or both ends. Unfortunately there are cases of bubonic plague in which a film specimen made of a droplet of the juice of the bubo of the living does not show great numbers of the *B. pestis.* This is, however, the exception, and is, in most instances, explicable by the fact that the material is not really derived from the interior of the gland, for when such glands after post mortem are dissected out and examined in film specimens, there is no difficulty in ascertaining that the *B. pestis* are really present in enormous numbers in the gland tissue; in fact, such film specimens show that the gland tissue is densely packed with them. I have seen several such instances, in which the juice of the bubo, obtained by puncture of the bubo during life, showed in film specimens a comparatively limited number of bacilli—limited, that is, inasmuch as each field of the microscope under a magnifying power of 300 did not show more than a dozen or so; whereas the same bubo, having been removed after post mortem,

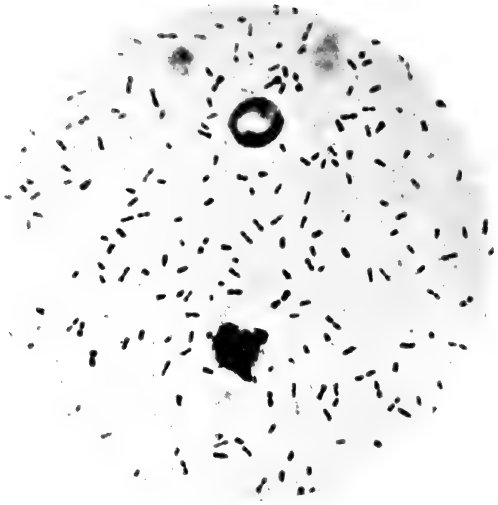

Fig. 1.

Fig. 2.

FIG. 1.

Stained film of the juice of a bubo—bubonic plague of man. Magnification × 1000.

FIG. 2.

Stained film of the lung juice in fatal pneumonic plague of man. × 1000.

FIG. 3.

Stained film of the juice of a bubo—fatal bubonic plague of an Indian native, s.s. *City of Perth.* × 1000.

FIG. 4.

Stained film of spleen juice of a rat, dead of natural plague, Cardiff. × 1000.

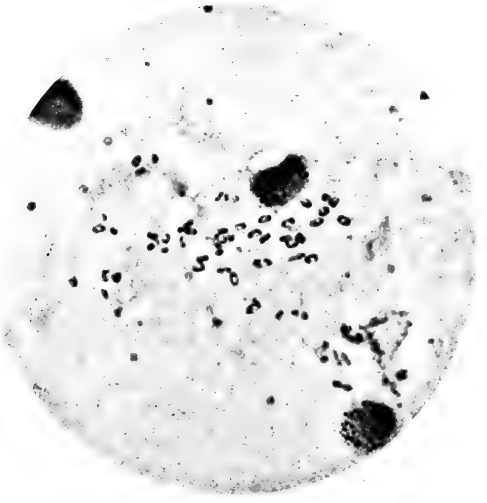

FIG. 3.

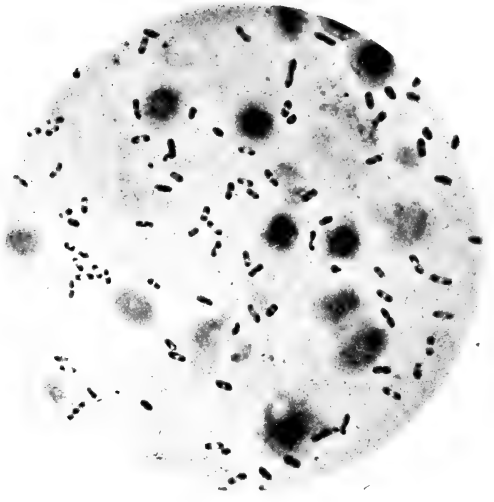

FIG. 4.

when examined by film specimens, showed each field of the microscope literally crowded with the bacilli.

While, then, in the acute cases, the microscopic examination of the bubo material enables one to make the preliminary diagnosis "plague most probable," it is not so in the chronic cases, *i.e.* in *pestis minor*, or in ambulating plague, when the buboes suppurate and become converted into open discharging sores, for here the material in microscopic specimens may, and generally does, show a variety of microbes. (*a*) Foremost among these one finds cocci, which by culture are shown to be either *Staphylococcus albus* or *aureus*; they are Gram-positive; (*b*) next one finds streptococci; they are Gram-positive; these are less common; (*c*) here and there a diphtheroid bacillus, Gram-positive, which by culture is shown to belong to the xerosis group; (*d*) bacilli with round ends, and showing on staining more or less distinct bipolar character; these may be *B. proteus*, *B. coli*, or *B. pestis*, all being Gram-negative. The culture test, however—surface agar plates at 37° C. incubated,—shows already in twenty-four hours the differentiation in a marked manner; these differences will be dealt with fully later in a special chapter.

I have in the course of the last nine years examined a large amount of material of inguinal, femoral, axillary, and cervical swellings which had been associated with febrile symptoms, and there being, on epidemiological grounds, a possibility of such disease being plague—the persons being either in a port or had arrived in a ship which had come from or had touched an infected port—the material was subjected to careful bacteriological analysis, with the result that plague was negatived, and, as the further history proved,

the disease was not plague. Such materials in several instances showed in films no microbes, agar plates made with relatively large amounts remained quite free of growth. In other instances the microscopic examination showed abundance of cocci, chiefly diplococci in clusters in and amongst the leucocytes, and, as culture test proved, they were *Staphylococcus pyogenes aureus*; in a few instances the microscopic examination, and particularly the culture test, showed streptococci—Gram-positive *Streptococcus pyogenes*; in one case, in which suspicion was justified on clinical, less on epidemiological grounds, the suppurating bubo contained crowds of bipolar bacilli, which by culture were shown to be *Proteus vulgaris*.

From all these facts it follows, that in cases of real bubonic plague great abundance of *B. pestis*, *i.e.* of Gram-negative, non-motile bacilli of the same aspect and size and staining power, in the tissue of the inflamed gland, and less so, but still sufficiently conspicuously, in the hæmorrhagic and œdematous tissues around the swollen lymph gland, is a fact, and therefore from the abundance of such bacilli in the gland juice of a person affected with symptoms resembling those of plague—fever and bubo—the preliminary diagnosis of bubonic plague may be justifiably ventured upon. The bacteriologist need not, and generally does not in his preliminary diagnosis, *i.e.* microscopic examination, rely on or know of epidemiological evidence, if any, such as would point to plague. Moreover, there are cases, and I have had several such, where at first neither the epidemiologist nor the clinician knew of all the facts concerning the case; in such instances the bacteriologist has, of necessity, to rely entirely on his own analysis. In such cases the micro-

scopic examination alone of the gland juice is sufficient to assert that the analysis is pointing in a distinct manner to plague, viz. great abundance of Gram-negative, bipolar-stained bacilli all of the same aspect; subsequent culture and animal experiment should complement the analysis and definitely prove the case to be one of plague. Further inquiry by the epidemiologist leaves no doubt about the correctness of the diagnosis.

Sections through the inflamed glands show, on microscopic examination, the following condition:—The tissue around the gland is highly œdematous, some lymph vessels containing leucocytes, red blood corpuscles, and numerous *B. pestis*; the veins and capillaries are distended and filled with coagulated blood; amongst the blood corpuscles are numerous *B. pestis*. The afferent lymph vessels are filled with coagulated lymph—leucocytes and fibrin—and crowds of *B. pestis*, some of the vessels showing thrombi almost entirely composed of *B. pestis*. The cortical sinuses are distended and densely packed with *B. pestis*, so are many of the medullary lymph sinuses, besides containing blood corpuscles; the lymphatic tissues of the cortical and medullary portions contain a large amount of extravasated blood; in many parts the lymph tissue is broken down, necrotic, and is not easily stained; numerous *B. pestis* are found in it. The efferent lymph vessels are distended by, and filled with, coagulated lymph, numerous blood corpuscles, and masses of *B. pestis*. In a subsequent chapter we shall reproduce a photogram of a section through the inflamed lymph gland of a rat inoculated with plague bacilli cutaneously at the root of the tail; the illustration may be taken likewise as a faithful representation of the bubo as it occurs in man.

The blood of the peripheral circulation in an acute case of bubonic plague contains very few *B. pestis.* A few hours before death they can be recognised already in film specimens, though on making a culture (plate or tube) with a drop of the blood at this stage, numerous colonies appear; but in the early stages they are difficult to find either in films or by culture, unless the blood is derived from the skin over or about the bubo.

The lungs and kidneys, after post mortem, show the plague bacilli, in proportion to their distribution and presence in the general circulation.

The liver, and particularly the spleen, are the organs which, next to the bubo, contain *B. pestis* in great numbers. Sections show that they are chiefly present in the spleen pulp; in this the blood spaces are distended by blood in stasis with crowds of *B. pestis*; in many places these occur in continuous masses, and are arranged more or less in reticulated fashion corresponding to the blood spaces and small veins; the pulp tissue itself around and between these masses is in a state of necrosis. Fig. 24, taken from a section through a guinea-pig's spleen, gives a good representation of the state and condition of the spleen in bubonic plague in man.

(B) *Septicæmic Plague.*—Just as in the acute form of plague caused by inoculation of animals—which is almost always of the septicæmic type—so also in the septicæmic plague in man, the *B. pestis* are copiously distributed throughout all the organs, notably in the blood-vessels. Amongst these organs, the spleen, lungs, kidneys, and suprarenals, as also the liver and intestines, show conspicuous changes. These consist essentially in capillary hæmorrhages with crowds of *B. pestis* in the capillaries and

veins. In the lungs there occur lobular patches of consolidation, due to fibrinous exudation into the alveoli and infundibula, the capillaries being distended by blood; hæmorrhages occur in the peribronchial tissue with numerous *B. pestis*. In the kidney the capillaries of the glomeruli are distended by and filled with blood and *B. pestis*; the blood-vessels in the cortex next the boundary layer are in many places surrounded by extravasated blood with numerous *B. pestis*. The vessels of the Malpighian pyramids are distended with blood, and some contain *B. pestis*. Plague bacilli can be recognised in the space of the Malpighian capsules, in the connective tissue around the extravasated blood, and also in some of the uriniferous tubules of the boundary layer. It is obvious that the blood of the general circulation readily yields positive result *quâ B. pestis* on microscopic examination and in culture.

The intestine shows, both in its small and large division, occasionally numerous punctiform hæmorrhages, the contents being bloody mucus; such mucus, even on microscopic examination, and, better, on culture and experiment on animals, reveals the presence of *B. pestis* in it. The spleen is literally packed with *B. pestis*; it is enlarged, and on section shows all parts of the pulp permeated by continuous masses of *B. pestis*. The number of *B. pestis* in the blood of the general circulation after death is, in some cases, so great that their number is not much inferior to that of the blood corpuscles.

In the liver many interlobular capillaries show masses of *B. pestis*; the liver cells around them in some places are full of fat globules, in other places they show coagulation necrosis. In the suprarenals the blood-vessels are dis-

tended by blood : in some of these vessels are seen continuous masses of *B. pestis* ; particularly is this the case in the medullary part, in which extravasated blood is not uncommon.

The description given here is taken from the examination of the organs of animals (guinea-pig, rat, mouse) dead of acute plague, but on comparing with them sections through the hardened organs derived from a human subject dead of septicæmic plague, I have no doubt the above description is also correct for the human subject in general.

(C) *Pneumonic Plague.*—I have had the opportunity of examining cases of acute pneumonic plague—two sailors who died in the port of Hull in 1902. Pieces of lung and spleen were submitted to bacteriological examination ; the microscopic examination of the juice of the inflamed lung was alone sufficient to make diagnosis certain. Figure 2 shows a film specimen of the lung of one case ; in the other case the appearances were exactly the same. The photo shows an almost uniform mass of beautifully stained bipolar bacilli amongst blood corpuscles, and as there exists, except in plague, no pathological condition — no acute inflammation of the lung—in which a film specimen of the juice shows crowds of uniform bipolar bacilli, the diagnosis could be made at once. Culture and experiment fully confirmed the diagnosis. Also the spleen, in film specimens, showed abundance of bipolar bacilli like *B. pestis*, but they were not anything like so numerous as in the film specimens of the lung.

The greater portion of the lung in these cases was in a state of red hepatisation—deeply red, almost purple,—and bits of it sank in water.

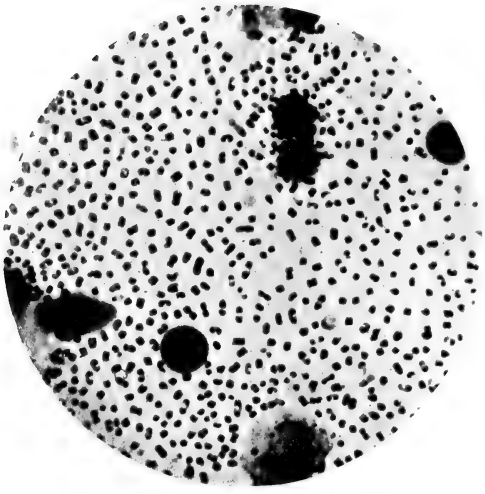

FIG. 5.

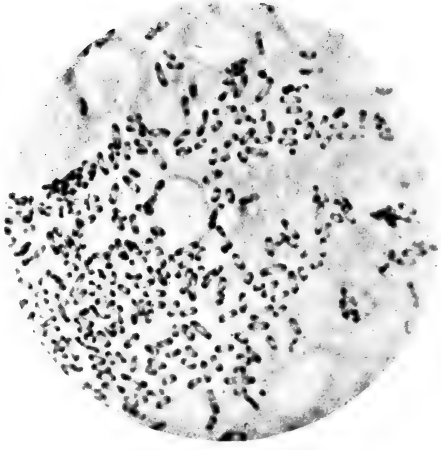

FIG. 6.

FIG. 5.

Stained film of the inguinal bubo of a rat, cutaneously infected with a trace of culture of *B. pestis*. × 1000.

FIG. 6.

Stained film of inguinal bubo of a rat, after being inoculated cutaneously at the root of tail with pharyngo-laryngeal mucus of a rat dead of plague with extensive pneumonia. × 1000.

FIG. 7.

Stained film of lung juice of a monkey dead of pneumonic plague after inoculation. × 1000.

FIG. 8.

Stained film of liver juice of same monkey. × 1000.

Fig. 7.

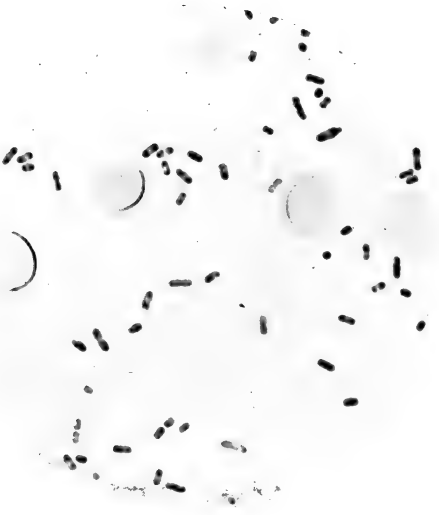

Fig. 8.

Sections were made and stained; examined under the microscope the hepatised portions showed uniform and dense infiltration with leucocytes and red blood corpuscles, the alveolar septa indistinct, the infundibula and bronchi distended by and filled with red blood corpuscles, leucocytes, and fibrin. Everywhere in these parts the walls of the infundibula, bronchi, and alveoli were indistinct and more or less broken, and everywhere between the blood corpuscles and leucocytes there were *B. pestis* in continuous streaks, so much so that they filled all spaces left by the cells; some of the bronchioles were plugged with continuous masses of *B. pestis*. Hæmorrhage was present in the peribronchial tissue.

The rusty hæmorrhagic sputum of cases of plague pneumonia during life is described, by some observers, as showing, besides numerous normal bipolar plague bacilli, also some spindle-shaped and other swollen involution forms. I have not come across these either in the lung juice or lung sections of the above two cases, nor in the bronchial contents of the affected lungs of animals which had died of subacute plague, in which the lungs showed larger or smaller, more or less necrotic patches, or were in the condition of red hepatisation; whether these spindle forms occur naturally in the sputum or are artefacts, made when preparing the film specimens, I am unable to say. I have paid particular attention to this point in connection with the exudation which is invariably present in the larynx, trachea, and bronchi of animals (guinea-pigs and rats) which are affected with subacute plague, and in which the lungs are the seat of severe inflammation and necrosis (pneumonic plague), but I have not been able to meet with these spindle forms, and, as mentioned above, I

have failed to find them either in the numerous film specimens or in the numerous sections which I have examined of the above two Hull cases of pneumonic plague; all these showed everywhere only oval or cylindrical bipolar bacilli.

II. Plague in Animals

The chief animal to be considered in this connection is, of course, the rat, and of this species I have had the opportunity to examine in several instances rats naturally dead of plague, while in other instances dead rats were found to have been affected with another microbe, not *B. pestis* (see below).

I will describe here the appearances in rats that had died of plague in docks and in warehouses in Cardiff.

P.M.—The most strikingly affected organ was the spleen, this organ being dark red, firm, and at least twice its normal size; when cut into, it showed a fairly dry cut surface; cover-glass impressions made of the cut surface, stained and mounted, showed everywhere abundance of cylindrical bacilli with rounded ends and marked bipolar staining; the liver, kidneys, and lungs were more or less congested, so were almost all lymph glands—film specimens of all these organs showing abundance of typical cylindrical bipolar *B. pestis.* The heart's blood contained likewise abundance of *B. pestis.* The small intestine—particularly the ileum—was relaxed and contained sanguineous mucus, in which numerous bipolar plague-like bacilli could be recognised. The bladder was in one case distended, and filled with blood-tinged urine; in others it was collapsed and empty. In the blood-tinged urine,

kept for some minutes in a watch-glass, bipolar bacilli could be found in the sediment.

Cultures made of the spleen, lymph (inguinal) gland, kidney, liver, and lung, all produced a crop of typical colonies of *B. pestis*, most abundant in the case of the spleen and heart's blood.

From this it follows that the form of plague of which these rats had died was the septicæmic form, viz. congestion of the viscera—particularly the spleen, lymph glands, and lungs—with general distribution of *B. pestis* in the circulation.

The distribution in and effects of *B. pestis* on rodents infected with materials containing *B. pestis* are subject to certain variations, which will be considered fully later in connection with the experimental production in various ways of plague in these animals; we proceed now to consider the morphology, cultural characters, and experimental effects of the *B. pestis*.

CHAPTER II

CHARACTERS OF THE *BACILLUS PESTIS*

(A) *Morphology.*—The plague bacillus is a non-motile rod, of a short oval to cylindrical shape, possessed of rounded ends, and measuring in dried or stained film specimens of the tissues on an average from 0·8 μ to 1·6 μ in length, 0·4 μ to 0·8 μ in thickness. Measurements which I made of various races show that the longest bacilli occur in the bubo of the rat spontaneously dead of the plague; these bacilli are at the same time well rounded, almost slightly tapering at the ends. Next in size are those of the bubo of man affected with plague, presumably through the rat.

The *B. pestis* stains readily with all the aniline dyes usually employed for staining bacteria; the only special feature about the staining of the *B. pestis* is that when taken from tissues—film specimens—and subjected to particular staining, most individuals show within a delicately stained sheath a marked bipolar arrangement of the stained or chromatic substance, *i.e.* rods, short or long, with rounded ends, and showing a stained mass at each end, whereas the middle part is clear and not stained; that is to say, the chromatic substance is limited to the

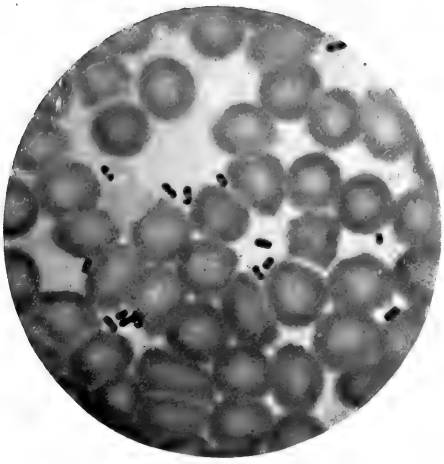

FIG. 9.

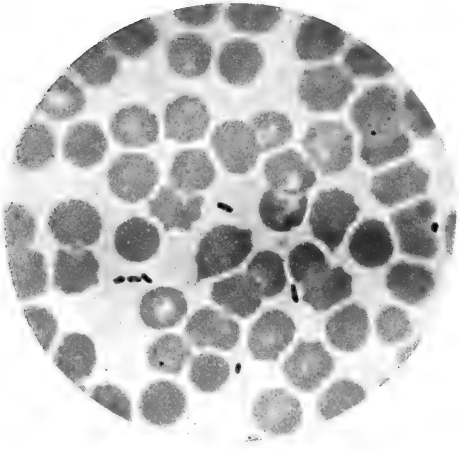

FIG. 10.

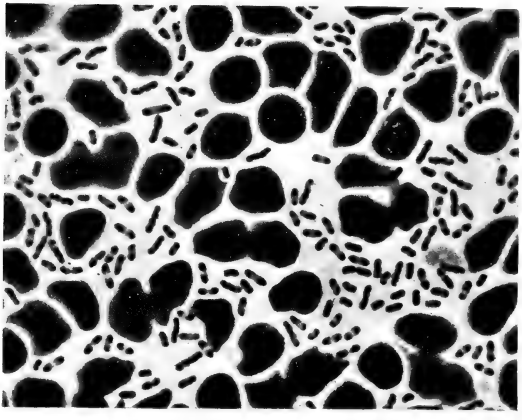

FIG. 11.

FIG. 9.

Stained film of blood of a guinea-pig dead of plague after inoculation with *B. pestis*. Bipolar plague bacilli amongst blood discs. × 1000.

FIG. 10.

Stained film of the blood of another guinea-pig dead of inoculated plague. × 1000.

FIG. 11.

Stained film of blood of a mouse dead of acute plague after cutaneous inoculation. × 1000.

FIG. 12.

From a stained section through the medullary part of the inflamed inguinal lymph gland of a rat dead after cutaneous inoculation at the root of tail, same rat as Fig. 6. A mass of plague bacilli (dark) in the lymph space—the lighter part—filled with effused blood. × 300.

FIG. 13.

Same preparation as in Fig. 12. The lighter parts are the medullary lymph sinuses filled with effused blood; the darker parts are the masses of adenoid tissue, of which only the densely aggregated nuclei are seen. × 300.

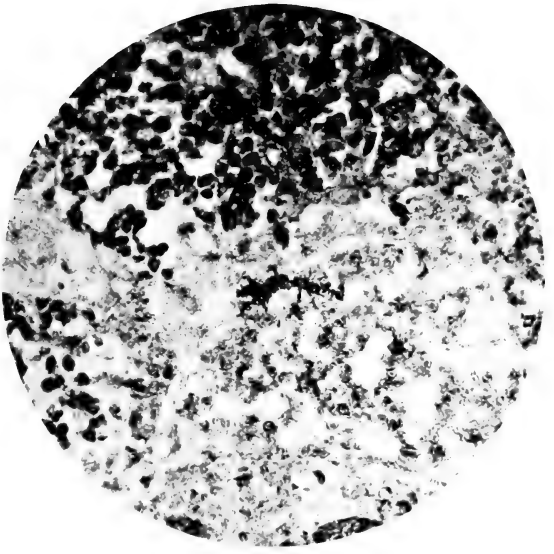

FIG. 12.

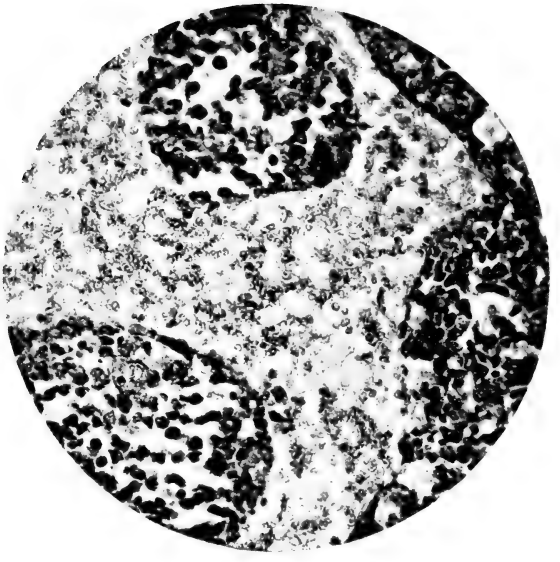

FIG. 13.

ends of the rods, whereas the middle portion is occupied by a vacuole. Some observers (Fisher) maintain that this peculiar staining is observed only in dried (film) specimens; but I cannot accept this interpretation as entirely satisfactory, because I find, on careful examination of *B. pestis* in the hanging drop, that some bacilli show a central vacuole already in the living and fresh state; moreover, such a vacuole is also observed in many other bacteria in the fresh condition, as also in stained and dried specimens —*e.g. B. coli*, *B. typhosus*, *Proteus vulgaris*,—with this difference, however, that *B. pestis* shows the bipolar staining, both in dried film specimens of tissues as also of culture, in a more conspicuous manner and more constant than other bacteria. A further point to be mentioned in this connection is that in *B. pestis* taken from the tissues —and this applies notably to *B. pestis* in the bubo—a fair number of individuals show an unstained vacuole either at one or both ends, whereas the rest contains the stained chromatic substance. Plague bacilli of this kind appear at first sight to exhibit one or both ends truncated or concave, the former if the vacuole is at one end only, the latter if each end has a vacuole. Such forms do not occur in culture.

The bipolar condition is always marked and easily demonstrated both in *B. pestis* of the tissues as also of recent culture, provided the film specimens are well stained and then well washed; if the specimen is overstained and insufficiently washed, the whole bacillus appears either uniformly stained, or it shows in places slight differences in depth of colour.

The best methods of staining to show the bipolar character are these:—

(*a*) Methylene blue and eosine, and

(*b*) Dilute alcoholic fuchsin.

As to (*a*): a mixture is made according to Czinzinski's formula. It is this :—

Concentrated aqueous solution of methylene blue, Grübler,	50 cc.
Eosine (soluble in alcohol)	0·5 gram.
Absolute alcohol	70 cc.
Distilled water	130 cc.

This stain, if correctly made and properly used, is far preferable to any double stain that I know; it is most useful for tissues (film specimens, as also sections of hardened tissues) not only containing bacteria or fungi, but for all histological and pathological purposes. Nuclei of cells, certain granules and bacteria assume a deep-blue colour; cell substances, eosinophyle granules, red blood corpuscles appear bright pink.

Film specimens are fixed, as usual, in the flame, then placed in absolute alcohol for about half a minute, dried, and placed, film downwards, over the stain contained in a watch-glass; here they are heated, the watch-glass being held by forceps high over a small flame till the dye shows distinct steaming. Wash well in tap water, then in distilled water, dry, and mount in balsam. The plague bacilli appear deep blue (blue black) and distinctly bipolarly stained, the red blood corpuscles are pink, the nuclei of leucocytes and other cells more or less deep blue.

Sections are placed first in absolute alcohol for several minutes, are then kept in the cold stain for several (six to twelve) hours, are then well washed in water, and passed in the usual manner through absolute alcohol, xylol, and finally mounted in balsam. In these sections the contrast

between blood corpuscles and connective tissues—pink,—bacteria and nuclei—blue,—is very striking.

I have extensively used this mixture both for film specimens of tissues and for sections for the last thirteen years, and do not consider any other mixture comparable to it in effecting strong contrasts and bringing out the different parts of the tissues, bacteria, fungi, etc., with admirable distinctness.

(*b*) The ordinary Ziehl's carbolfuchsin is used; a small quantity of it is diluted with an equal volume of absolute alcohol; over this fluid in a watch-glass is placed the cover film specimen, having previously been kept for half a minute in absolute alcohol as above; the film specimen remains in the dilute fuchsin for $\frac{1}{2}$-$\frac{3}{4}$-1 minute, then it is well washed in tap water, then in distilled water, dried, and mounted in balsam. The plague bacilli both of tissues and of recent culture show polar staining very distinctly; but it is essential that the films after staining should be well washed—that is, until no further discharge to the water of pink colour is noticed.

These two methods suffice for all purposes; in fact, the first-named double stain will practically be found quite efficient. *B. pestis* do not retain the stain after Gram solution, that is they are Gram-negative, and share therefore this character with the microbes of the coli-typhoid and with those of the proteus group; it is important to remember this, because it is just with these two groups (*B. coli*, *B. Gaertner*, proteus) that error in diagnosis may be, and as a matter of fact has been, in some instances, committed. Judging from the accounts given by different observers, "Gram staining" appears as a sort of variable quantity; I will therefore state here the manner which

from very long experience I consider as perfectly reliable for obtaining the necessary result.

Film specimens fixed in the flame are placed in absolute alcohol for ½ to 1 minute, then are placed, film downwards, on gentian violet aniline water contained in a watch-glass; after one minute they are removed, well drained with blotting-paper, and then transferred to Gram solution (iodine dissolved in iodide of potassium), where they remain for two minutes; they are then transferred to two successive lots of absolute alcohol, in each being washed for a few (two to five) seconds, then well washed in water, dried, and mounted. Gram-positive bacteria, like *B. anthracis*, *B. diphtheriæ*, *Staphylococcus aureus*, and others, appear of a deep-violet—almost black-violet colour; Gram-negative bacteria, like those of the coli-typhoid group, proteus, gonococcus, *B. pestis*, are discoloured, in fact are not stained. A good result is obtained by using Gordon's modification, particularly when the material contains, or is supposed to contain, a mixture of Gram-positive and Gram-negative bacteria.

This method is as follows:—The Gram staining is made in the same manner as above, with this difference, that the specimens remain in the gentian violet ¾ to 1 minute, in the Gram solution for the same time (¾ to 1 minute), and after washing in alcohol, and then in water, are placed in 0·5 p.c. watery fuchsin solution for five to ten seconds, then washed in water, dried, and mounted. The result is that the Gram-positive bacteria, having retained the gentian violet stain, appear violet black, whereas the Gram-negative bacteria, having lost the first stain by the Gram solution, have taken up the second, viz. the fuchsin stain, and therefore appear bright pink. Film specimens of pus of a

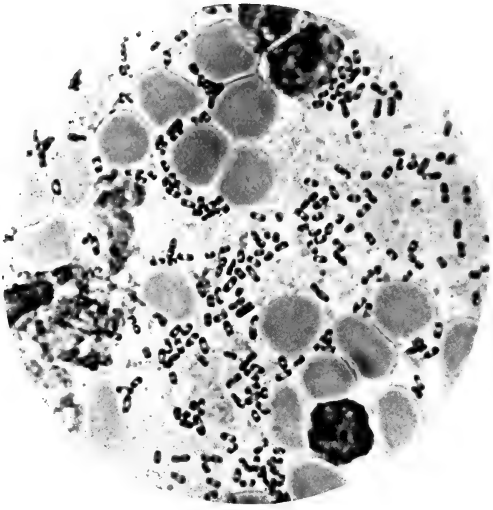

Fig. 14.

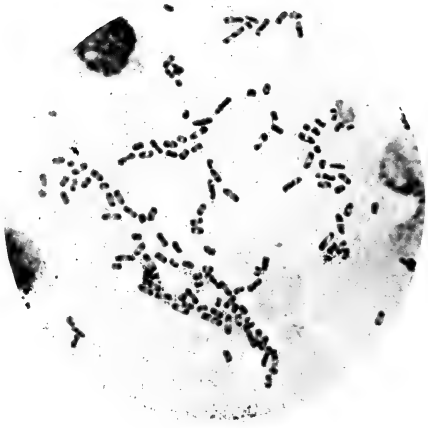

Fig. 15.

FIG. 14.

Stained film of spleen juice of a guinea-pig dead of acute plague. Crowds of bipolar *B. pestis* amongst red blood discs and nuclei of leucocytes. × 1000.

FIG. 15.

Stained film of spleen juice of a rat dead of acute plague after inoculation. × 1000.

FIG. 16.

Section through the consolidated lung of a rat dead twelve days after cutaneous inoculation with juice of bubo. The photogram shows on the upper left the consolidated lobule; the rest is interlobular tissue containing on the right a bronchus in cross section and filled with exudation, on the left a blood-vessel in cross-section and filled with blood. × 30.

FIG. 17.

Part of the contents of the bronchus of previous figure, showing crowds of *B. pestis*. × 1000.

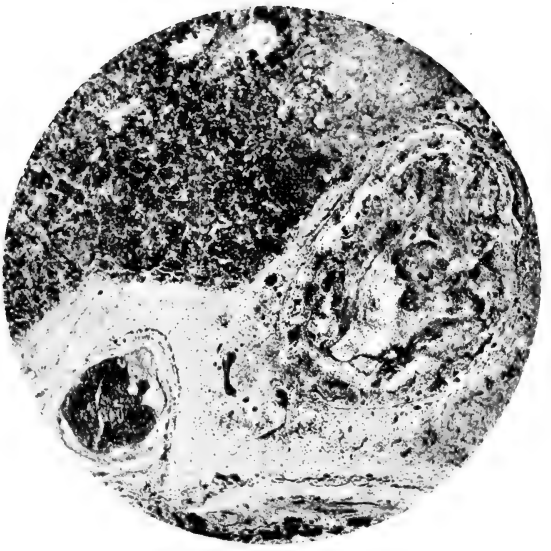

FIG. 16.

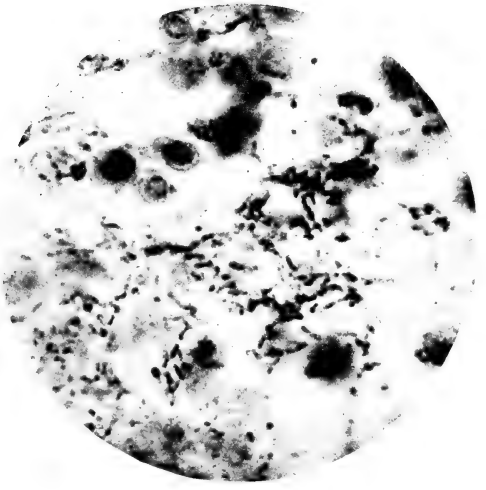

FIG. 17.

suppurating bubo stained after this method have, in several instances, yielded instructive specimens: *Staphylococcus aureus*, *Streptococcus pyogenes*, diphtheroid (xerosis) bacilli appeared deep violet; *B. pestis*, proteus, coli-like microbes, bright pink.

Plague bacilli are described by some observers as being possessed of a capsule. This I think is based on a misunderstanding: when film specimens, say of bubo or other diseased tissues, are fixed and then stained, and if the ground substance, owing to the thickness of the film, happens to be present in too thick a layer, or if the film has been insufficiently heated, or if the specimen is insufficiently washed after staining, the deeply stained bacilli or cocci, as the case may be, appear surrounded by a clear space; this is owing to the fact that the ground substance, which is also more or less stained, has during the drying process shrunk away from the bacilli, therefore the latter appear surrounded by an unstained clear area, which might be thus taken for a capsule surrounding each bacillus. But this is, of course, not a real capsule, such as surrounds the pneumococcus for instance, for this capsule can be actually stained; whereas the other is merely a space, and cannot be stained. When the film specimen is thin, well heated, and the preparation after staining well washed, no capsule can be observed on the plague bacilli. I have paid particular attention to this point, and can speak confidently about film specimens made of bubo materials, of the spleen, the lung, and blood of human plague cases or of plague animals. Nor is there any trace of a capsule around the individual bacilli or the chains of bacilli which make up the characteristic powdery or floccular sediment of broth-cultures of *B. pestis*.

When a guinea-pig is injected intraperitoneally with a trace of virulent *B. pestis*, the animal is found dead within twenty-four to thirty hours; the peritoneal cavity contains a quantity of grey slimy exudation, which, under the microscope, is made up of a sticky, viscid ground substance in which are embedded densely packed *B. pestis*, singly, in dumb-bells, and short chains. In stained film specimens the bacilli appear surrounded, though somewhat irregularly, by a faintly stained broader or narrower capsule; this is the gelatinous ground in which the bacilli are suspended (see Fig. 26), but it is by no means comparable to a typical capsule such as surrounds the *Diplococcus pneumoniæ*. As will be mentioned later, the *B. pestis* forms on the surface of solidified agar a slimy translucent layer; when a particle of it is removed with a platinum needle it is viscid and is easily drawn out into threads; it does not emulsify readily, because the plague bacilli are agglutinated together into larger or smaller masses by a viscid hyaline intercellular secretion; stained film specimens show this intercellular substance faintly stained, but this is not comparable to a real capsule. In gelatine-surface cultures the growth of *B. pestis* is devoid of this slimy character, the plague bacilli emulsify more readily in salt solution, and there is in film specimens of such emulsion no capsule recognisable.

From all this it follows that the *B. pestis* does not possess a real capsule such as is possessed by *Diplococcus pneumoniæ*.

(B) *Cultural Characters.*—The plague bacillus grows well at 37° C.; it grows also well, but slower, below 37° C. down to 20° C. and less. There seems a misunderstanding on the part of Professor Simpson in saying, in his *Treatise*

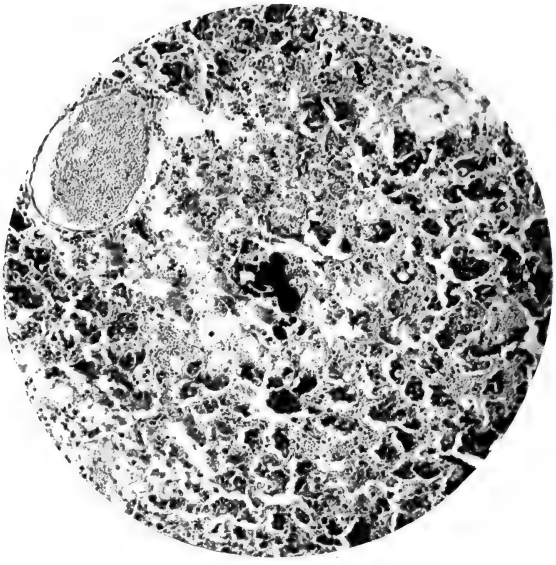

Fig. 18.

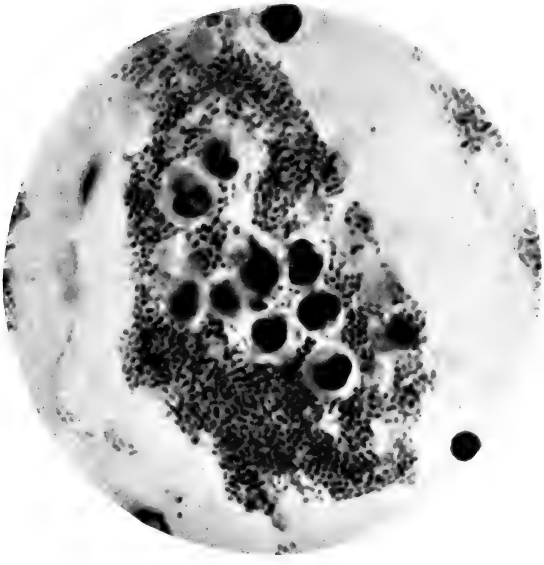

Fig. 19.

FIG. 18.

Section through a lobule of the lung in pneumonic plague of a guinea-pig. Most of infundibula and alveoli are filled with exudation; in the centre several vessels filled with plague bacilli—dark in figure. × 85.

FIG. 19.

The exudation in one of the infundibula of previous figure more highly magnified (× 1000), showing that the exudation is practically a continuous mass of *B. pestis*. × 1000.

FIG. 20.

From a section through the lung of a guinea-pig dead of subacute plague, showing a nodule with masses of plague bacilli (dark). × 85.

FIG. 21.

From a section through the lung of a guinea-pig dead of subacute plague, showing a nodule the centre of which is the alveolar duct and corresponding alveoli, all filled with plague bacilli (dark). × 100.

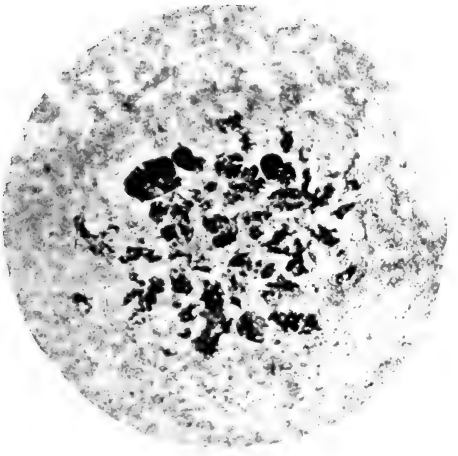

FIG. 20.

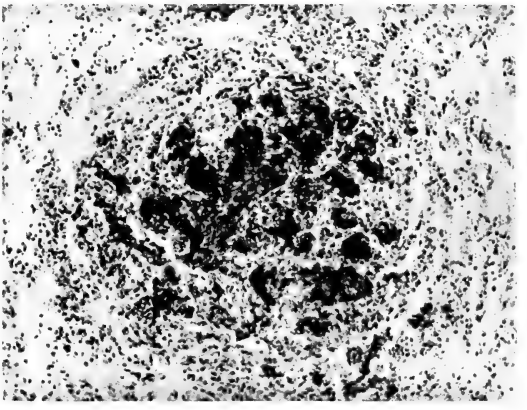

FIG. 21.

on Plague, that the plague bacillus grows better at the lower than at the higher temperature. I am confident it would considerably delay diagnosis in a given analysis of suspected material if the cultures were kept at a temperature lower than 37° C. Plague bacilli planted, for instance, on agar kept at 37° C. show their colonies well developed to the aided and unaided eye already after twenty-four hours, whereas when kept at the temperature of 20° to 25° C. for forty-eight hours would be only just discernible with a glass. I believe the statement of Simpson may be explained in the following manner:—As will presently be shown, plague bacilli grow well, but somewhat slow, in broth even at 37° C. In preparing Haffkine's prophylactic (see later) a broth is used, of which the top is more or less covered with clarified butter (ghee); now this was introduced by Haffkine in order to obtain copious masses of plague bacilli, for he made the observation that in connection with the ghee drops of the surface a rich crop of plague bacilli is continuously reproduced (stalactites), and the culture flask being shaken every twenty-four hours or so, these masses fall to the bottom, forming and accumulating here as a copious whitish, powdery, flaky sediment. Now, the most abundant crops of bacillary masses sprouting downwards from the surface or ghee layer are developed if the ghee layer is in a solid state—they are far less abundant if the ghee layer is in a fluid state. From this it follows that such a ghee broth flask shows far less abundance of masses (granules and flakes) of plague bacilli if incubated at 37° C. —at which temperature the ghee is fluid—than at 25° C. —at which temperature it is solidified. In order to obtain in such ghee broth the most abundant masses of plague

bacilli, the flasks are incubated at 25° C. or even down to 20° C. This seems to me the origin of Simpson's statement, viz. that *B. pestis* grows better at lower temperatures than at 37° C. I have made series of comparative experiments with *B. pestis* of different races derived from various sources, planting them on the surface of various solid media such as are generally used for culture of bacteria in the laboratory, *e.g.* nutrient gelatine (streak and stab), nutrient agar (streak and stab), solidified blood serum (streak), ascites agar (streak and stab), nutrose ascites agar (streak and stab), and there never was any difference in regard to the growth of the different races, for all showed the quickest and most abundant growth when the culture tubes were kept at 37° C.

According to my experience, the best way to obtain rapid and reliable evidence of the growth of *B. pestis* is to plant the suspected material on the surface of the ordinary, *i.e.* faintly alkaline agar (beef broth, peptone, agar), set in a plate dish and kept at 37° C. Next day the colonies are visible already to the unaided eye—better, of course, with a magnifying-glass—as small, rounded, grey, translucent, watery, slightly raised droplets. Examining these carefully with a magnifying-glass, it is noticed that the edge of the colonies is not quite rounded, showing already now slight irregularities; these become more pronounced after a further twenty-four hours. At this time the colony is thinner at the margin than in the centre, is therefore slightly conical; this also becomes more pronounced later. In transmitted light, and viewed under a glass, the substance of the colony—particularly in the central portion—is finely granular.

Now, I wish already here to state that a difference

can be noticed between the colonies of *B. pestis* derived from a virulent source (human or rat) and those derived from a slightly virulent source (human or rat), this difference being that the former, as incubation proceeds, show a gradually more pronounced angular margin, and are more conically raised in the centre than the latter.

What, however, is very characteristic of colonies of *B. pestis* growing on the surface of an agar plate is the fact that when attempting to remove a particle of the growth with a platinum needle it is found that the substance of the colony is very viscid, so much so that either the whole colony adheres to the point of the needle or that it is difficult to take up any part of the colony; further, when the growth is deposited in a drop of fluid —*e.g.* saline solution, water, or broth—it is difficult to emulsify it, the growth remains in coherent threads and flakes; under the microscope, therefore, connected masses of bacilli—small and large, according to the amount of mechanical force used in the attempt to emulsify—are met with, some of these masses drawn out into longer or shorter strings.

On the surface of gelatine the colonies of the typical *B. pestis* as soon as they are discernible, *i.e.* soon after forty-eight hours, show themselves as grey, in transmitted light as somewhat opaque, distinctly granular, rounded or slightly angular points. Later on the distinction between an angular thin marginal and a conically raised thick middle part becomes very marked; so also the granular character of the colony, so much so that when examining in transmitted light, with a magnifying-glass, a well-developed colony, *i.e.* after four to six days incubation at 20° to 21° C., its substance, particularly in the middle parts,

is coarsely granular, almost filamentous, and its margin irregular and much fringed, such as is shown in Figs. 34 and 36. Seen in reflected light under a glass it may truly be compared to a limpet-like mass stuck to the surface of the gelatine (Fig. 28).

Removing a particle of a colony from the gelatine surface it will be found to be coherent, but less so than from agar, and further, that it emulsifies better in saline solution than one from agar, but there are nevertheless present larger or smaller aggregations of bacilli.

When viewing a surface gelatine plate, *i.e.* one containing numerous surface colonies, it will be found that, while all are more or less angular with filmy margin and conically raised centre, there is a distinction to be drawn if of a recent plate, *e.g.* one only a few days old (21° C.), an impression preparation is made. When after staining and mounting they are examined under the microscope with a low magnifying power, say 60 to 90, two kinds of colonies will be noticed: (*a*) the great majority are typical colonies—angular patches composed of rod-shaped bacilli fairly well separated from one another by clear interstitial substance; (*b*) a small number of irregular colonies, which, however, are composed chiefly of more or less thready or long cylindrical bacilli; these latter colonies represent what I have called atypical colonies, and both sets are well shown in Figs. 41 and 42.[1]

We shall have the opportunity of showing that as regards virulence two types of *B. pestis* may be distinguished: the first type (type 1)—human type—is the one that is the more virulent, and is found in typical

[1] As to the filamentous modification of *B. pestis* in salted media, see a later page.

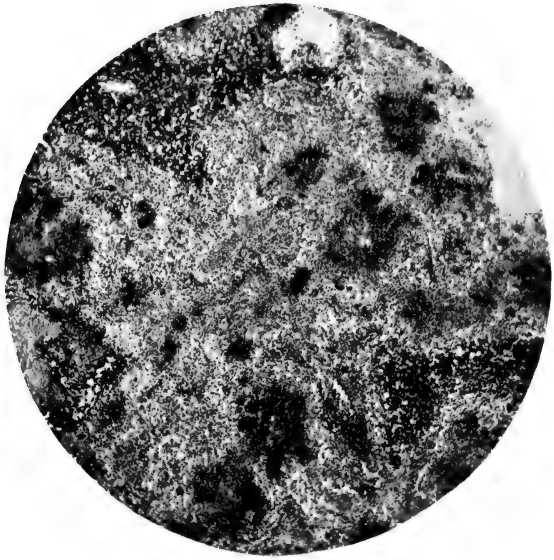

Fig. 22.

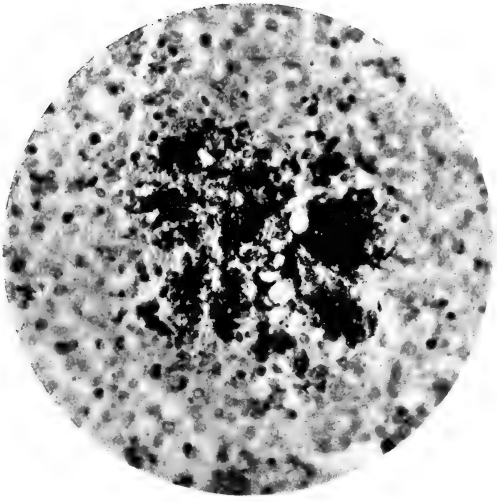

Fig. 23.

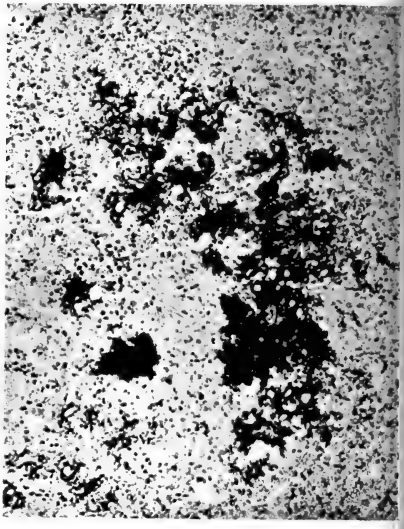

Fig. 24.

FIG. 22.

From a section through the hardened spleen of the same guinea-pig as Fig. 21, showing above and below in the figure masses of Malpighian corpuscles; the rest—the main part of the figure—is spleen pulp with numerous necrotic nodules; in these nodules are masses (dark) of bacilli. × 40.

FIG. 23.

One of the bacillary masses of the previous figure more highly magnified. × 300.

FIG. 24.

One of the bacillary (dark) masses of a necrotic nodule of a guinea-pig dead of subacute plague; the bacillary masses show a reticulated arrangement, being contained in the blood spaces of the pulp. × 100.

FIG. 25.

Film specimen of peritoneal exudation of a guinea-pig dead after peritoneal injection with *B. pestis* of attenuated type 2. Chains of bipolar bacilli in a gelatinous matrix. × 1000.

FIG. 26.

Stained film of the peritoneal exudation of a guinea-pig dead after intraperitoneal injection with *B. pestis*, showing the bipolar bacilli embedded in a gelatinous matrix, and simulating capsules. × 1000.

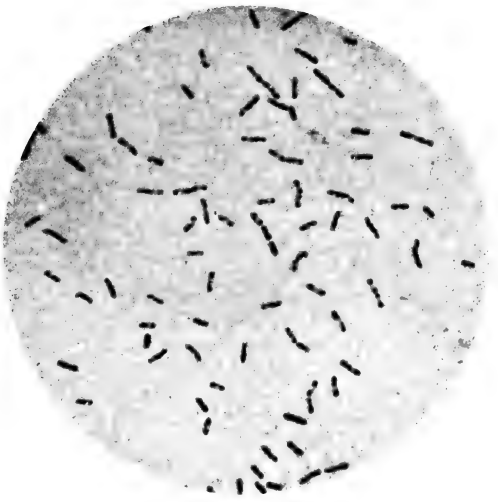

FIG. 25.

FIG. 26.

human cases of plague; the second type (type 2)—rat type—is the one which is less virulent, and which is characteristic for the rat, that is when it has been propagated through rats. These two types differ in the nature and aspect of their colonies during the early period of their growth on the surface of nutrient gelatine; type 2 forming colonies distinctly more translucent and less granular than those of type 1, and at the same time those of the former are less angular, more rounded, than those of the latter or type 1. It will be understood that these differences, although distinct and easily ascertained in the early phases—two to five days at 20° to 21° C.,—become lost as development proceeds; after ten days or more the angular thinned margin, the granular opaque raised centre, appear the same in both sets.

Microscopic specimens of the two types of colonies during the earlier phases show also a marked distinction, inasmuch as those of type 1 are distinctly more cylindrical than those of type 2 (see Figs. 38 and 39).

In stab culture of *B. pestis*, both in gelatine and in agar, the stab becomes marked as a series of opaque granules, and after some progress has been made each granule shows a filmy projection at one side or the other (Fig. 33), at the same time the upper or free end of the stab being marked by a greyish-white filmy expansion of growth, which in cultures (agar) of some standing (two to three weeks) has the character of a rounded shield, showing distinct concentric and radial markings as shown in Fig. 30. This surface growth possesses the viscid nature of plague growth in a marked manner.

Streak cultures of *B. pestis* on agar show a filmy, grey, translucent growth, which, as mentioned on a former page,

is of a characteristic viscid character. Streak cultures on serum and other agar compounds are of the same character. Later on the growth becomes thicker, less translucent, in reflected light of a slightly brownish tint, with numerous raised droplike round thickenings.

On gelatine surface streak culture the streak becomes marked as an at first grey, then whitish, dry band, gradually thickening, and becoming more opaque and granular; the centre of the streak is thicker than the margin, which latter is crenate, or, more correctly speaking, knobbed and with fine projections. The gelatine is not liquefied at any time. A gelatine streak culture of *B. pestis*, as also a gelatine surface culture containing isolated colonies of *B. pestis*, is, after several weeks' incubation, extremely characteristic: whitish, dry, thick, granular, opaque, thicker in centre than at margin; in streak, knobbed margin; in isolated colonies with filmy irregular margin and conically raised centre.

In milk *B. pestis* grows well at 20° to 37° C. without causing any change of the milk either in aspect or its fluid character. In litmus milk a slow and gradual change of the at first blue colour into less blue, then violet, and ultimately slight red colour takes place, thus showing that the *B. pestis* is a slow acid producer. This can be proved also in this way, that if of a culture of *B. pestis* in alkaline glucose broth, incubated at 37° C. for two or three days, a few drops be added to a few cc. of a watery solution of litmus, this at once turns distinctly red.

B. pestis grows on steamed potato but feebly, forming thereon a transparent film; the growth is therefore invisible to the unaided eye, and only by taking a particle of the inoculated surface and examining it in a

drop of fluid under the microscope can the actual presence of growth be ascertained.

In neutral-red beef broth the growth is of the same character as in ordinary alkaline broth (see below), the normal cherry colour of the neutral-red broth remaining unaltered.

B. pestis does not thrive in glucose taurocholate peptone water (MacConkey fluid), nor in lactose peptone water, nor in peptone salt water.

B. pestis grows well at 37° C. in faintly alkaline beef-broth peptone; the broth remains clear, but along the glass wall and at the bottom there appear whitish granules and flocculi, these being masses of the bacilli, many forming longer or shorter chains;[1] as growth proceeds the amount of floccular sediment increases, but is not at any time very copious. Pakes and Joseph (*Transactions of the Pathological Society of London*, vol. lvi. p. 135) show that *B. pestis* grows in slightly acid broth, and by this means can be readily differentiated from the pneumococcus present in the sputum of pneumonic plague.

For the production in broth of greater amounts of bacillary masses, Haffkine hit upon the plan of covering the top of the broth with a layer of clarified butter or ghee. As mentioned above, when incubation takes place at a temperature at which the ghee remains solid, *i.e.* temperatures at or below 25° C., its (the ghee's) under surface becomes covered with masses of growth hanging down like shorter or longer whitish fringes ("stalactites"); these, on disturbing the fluid, *e.g.* by shaking, become detached and

[1] The formation and the arrangement in chains is noticed in *B. pestis* whenever it grows in fluid media—broth, fluid serum, condensation fluid of agar or serum and the like. The same is observed in the peritoneal fluid. These chains at first sight resemble streptococcus chains.

sink to the bottom of the fluid. This falling of copious flakes of growth from the surface to the bottom is graphically compared by Haffkine to a fall of snowflakes. On further incubation new stalactites are developed, which in their turn, on shaking the culture, become detached, and fall to the bottom to increase the deposit. In this way—by rest and then shaking—in the course of four to six weeks a continuous re-formation of bacillary masses (stalactites) can be ensured, so that by this time considerable amounts of bacillary sediment are obtained. A flask of slightly alkaline ghee broth infected with *B. pestis*, and kept for some weeks at 25° C., shows an almost complete pellicle of growth on the top of the fluid, and from it projects downwards into the fluid a dense forest of threads or stalactites, which on slight disturbance rapidly fall to the bottom (Figs. 44 and 45).

The formation of stalactites in ghee broth kept in a perfectly quiet place is very characteristic for *B. pestis*; but it has to be added that unless the culture is kept in a place where absence of all disturbance, vibration, etc., can be ensured, no visible stalactites are formed. I have shown that a strain of *B. pestis* after many transferences in artificial culture may lose the power to form stalactites altogether, but regains this power when passed through an animal. *B. pestis* obtained from a case of pneumonic plague in 1896, and kept up in the laboratory in subculture, failed completely to form stalactites in ghee broth in 1899, but at once regained this power when cultures were again started from the spleen and bubo of a guinea-pig dead of acute plague after subcutaneous injection with this same strain. For diagnostic purposes the formation of stalactites in ghee broth is of value, although in practice

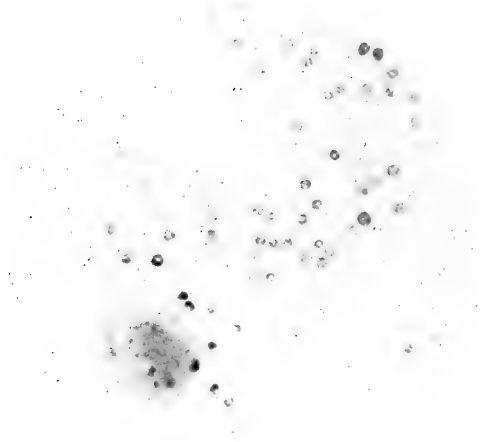

Fig. 27.

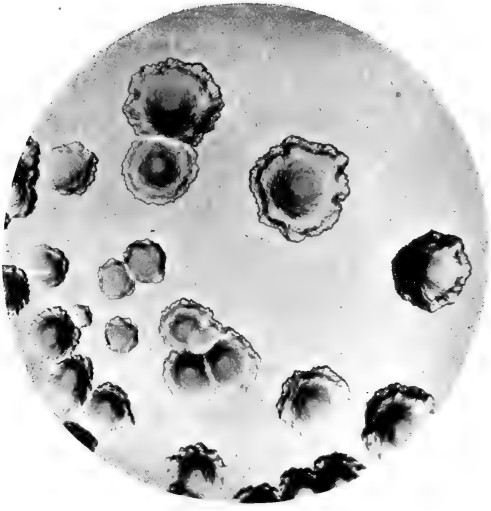

Fig. 28.

FIG. 27.

Stained film specimen of the bubo fluid of a protected guinea-pig, the plague bacilli as involution forms. × 1000.

FIG. 28.

Young colonies of *B. pestis* on the surface of a gelatine plate, after several days' incubation; the colonies are raised, conical in the centre, filmy and irregular at the margin, the centre granular or even filamentous. × 17.

FIG. 29.

Young colonies of *B. pestis* on the surface of an agar plate; the colonies are thicker and granular in the centre, filmy and slightly irregular at their margin. × 50.

FIG. 30.

Characteristic colonies of *B. pestis* on agar after several weeks' incubation; the contrast between thickened centre and filmy crenate margin is striking. × $2\frac{1}{2}$.

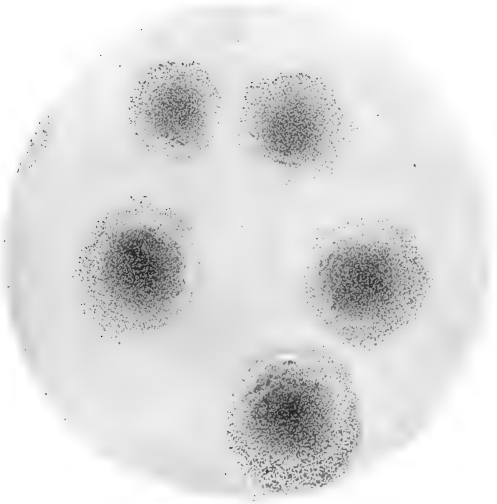

FIG. 29.

FIG. 30.

it need not, on account of the delay and the above difficulties, be considered as essential, and also because there are other quicker means of establishing positive diagnosis.

Hankin describes a modification of the *B. pestis* when growing in salted media, viz. that the bacilli grow out into long filaments. I have many years ago[1] described such filamentous modification occurring in many microbes (coli-typhoid group and others) when growing in media to which more than the normal amount of salt is added, and I cannot therefore accept either Hankin's priority (generally attributed to him in this matter) nor that such filamentous change is characteristic for *B. pestis*.

(C) *Experimental.*—*B. pestis* produces in rodents after cutaneous or subcutaneous injection definite disease. This will be considered in detail later on, here we wish merely to give a general account of its action.

A loopful, or even a part of a loop, of a 24 h. culture on agar at 37° C., or a trace of plague material (bubo, spleen) containing a large number of *B. pestis*, injected into the peritoneal cavity of a young or half-grown guinea-pig, causes fatal results in twenty to thirty-six hours, if the *B. pestis* is of normal virulence; if the dose is too small or the virulence subnormal, death may be considerably delayed. In the former case the abdominal organs are found much congested, with petechiæ on the serous covering of the intestine and on the parietal peritoneum; in the peritoneal cavity is copious, sticky, viscid grey exudation; under the microscope, besides red blood corpuscles and a few leucocytes—the number of the latter

[1] Klein, *Grouse Disease and Fowl Enteritis*, 1892, p. 31; *Micro-organisms and Disease*, new edition, 1896, p. 93.

inversely proportional to the duration of the disease—the fluid is densely packed with *B. pestis* embedded in a gelatinous ground substance, many of the *B. pestis* forming shorter or longer chains. Staining film specimens, the bipolar nature of the individual bacilli is well brought out, as also the gelatinous ground substance, which here and there may be mistaken for a special capsule enveloping the bacilli (see a former page).

The viscid nature of the exudation together with the dense aggregation of bipolar bacilli, many of them in longer or shorter chains, may be considered as fairly characteristic for plague; but it is not absolutely so, since we shall mention some other microbes which, under similar conditions, produce similar results.

Cutaneous inoculation of mice, rats, and guinea-pigs causes the disease with certainty. The great value of cutaneous inoculation, which was practised by the Austrian Plague Commission, lies principally in this, that very small amounts can be used for infection with positive results; leaving out malignant anthrax, and one or two others, *B. pestis* is one of the few microbes which cause acute disease in rodents when applied in minute doses to a scratch or a surface incision of the cutis, or is merely rubbed into the cutis. This shows that the *B. pestis* readily and rapidly multiplies in the skin; at the same time, as far as mice, rats, and guinea-pigs are concerned, when inoculated into the skin the *B. pestis* does not lose anything of its virulence.

As regards mice and rats I proceed in the following manner:—The hairs are cut off with scissors from the skin of the dorsum at the root of the tail; with the point of a scalpel which is previously dipped into plague material

(bubo, blood, spleen) or into an emulsion of a recent culture of *B. pestis* (twenty-four hours' agar culture at 37° C.) a few scratches or superficial scarifications are made, or the surface cuticle is scraped off from the place bared of the hairs and slightly scratched with the point of a knife; then a particle of growth from an agar culture (twenty-four hours at 37° C.), having been removed from the culture with a platinum loop, or a particle of plague tissue, is directly rubbed in by the platinum loop or the scalpel respectively into the scratched skin. Provided the culture be of medium virulence, I have in a very considerable number of experiments never failed by this method to obtain positive results in mice and rats—that is, acute plague with fatal issue. According to the virulence of the culture, fatal issue, both in mice and in rats, follows in as short a time as 30-40-48 hours; or, working with less virulent culture, death may be delayed up to four or five days. In mice such delay is, however, rare even with less virulent culture, since mice are highly susceptible and promptly answer even to plague materials which, when tested on rats, and particularly on guinea-pigs, appear only of moderate virulence.

Subcutaneous injection of mice and rats with doses much larger than are required for cutaneous inoculation does not cause quicker or more virulent results than cutaneous inoculation. It is otherwise with guinea-pigs: subcutaneous injection, while permitting the application of larger doses than does the cutaneous inoculation, causes —if the virulence is not abnormally low—fatal issue in between two and three days; material which acts thus may be considered of normal virulence. Death in less than forty-eight hours in a guinea-pig after subcutaneous injec-

tion even of large doses is extremely rare. Death after three or even four days would not necessarily indicate virulence greatly inferior than normal. Cutaneous inoculation of guinea-pigs with virulent and moderately virulent material is followed by death *after* four days up to seven, nine, or even more days; and this mode of inoculation distinguishes in a marked manner the guinea-pig from the rat, for in this latter animal the result of cutaneous inoculation with plague material of great or moderate virulence always causes acute fatal illness. In the guinea-pig, on the other hand, material which on subcutaneous injection in moderate doses causes death in two to three days, and which material is therefore of normal virulence, when administered cutaneously causes fatal issue always much later, and with pathological symptoms different from those found in animals dead in two or three days. When death ensues later (four to seven days or more) the bubo and spleen, also the liver and lungs, exhibit more or less necrotic changes in the form of nodules and patches of various sizes and in varying numbers. Such is generally the case with guinea-pigs inoculated cutaneously even with virulent material; it is the same also in guinea-pigs injected subcutaneously with material of less than normal virulence. Cutaneous inoculation of guinea-pigs with exceptionally virulent material may cause death occasionally, but not invariably, in two to three days with symptoms of acute plague—that is to say, before necrotic changes in the bubo or spleen or lung had had time to develop and to make their appearance. The necrotic changes—viz. whitish nodules in bubo, spleen, liver, and lung,—which require at least four days for their development, follow cutaneous inoculation of the guinea-pig with

FIG. 31.

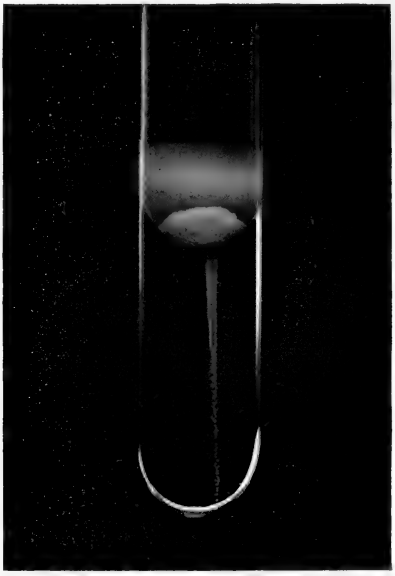

FIG. 32.

FIG. 31.

Streak culture of *B. pestis* on gelatine after a few weeks' incubation. Natural size.

FIG. 32.

Stab culture of *B. pestis* in gelatine after a few weeks' incubation. Natural size.

FIG. 33.

The deeper part of the stab of previous figure magnified. × 4.

FIG. 34.

Colonies of plague bacilli on gelatine after some days' incubation. Virulent type 1 (human). × 8.

FIG. 33.

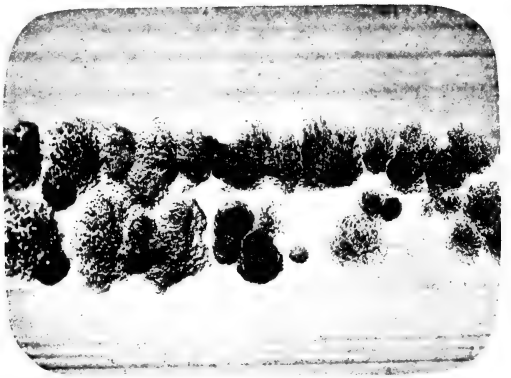

FIG. 34.

normally virulent material, or subcutaneous injection with material of less than normal virulence. This form of plague with delayed death and necrotic changes in the bubo, spleen, liver, and lung, I have termed the "subacute form."

The rabbit is not so well suited for plague experiments, because its susceptibility is not sufficiently high and not sufficiently constant, and therefore a fatal result with a given material cannot be depended on. Body weight seems to make no difference, since a subcutaneous dose may be productive of fatal issue in one rabbit, while the same dose of exactly the same material may cause nothing more than transitory illness in a rabbit of lesser body weight.

Amongst rats the tame or white rat is the most susceptible, the common brown sewer rat is considerably less so, and therefore the former is for experimental purposes far preferable. Another reason which enables us to dispense with the sewer rat for laboratory purposes is the fact that between 25 to 30 per cent die when kept in captivity, and, further, the fact that they are unpleasant and unsafe to handle; whereas the tame or white rat (all white, white-black, white-brown, white and plum-coloured) is born and bred in captivity, lives well in cages, and is easily and safely handled.

Next in plague susceptibility to the white rat is the brown dock and ship rat; about the same, but a little less perhaps, is the black or plum-coloured ship rat, and still lower in the scale is the brown rat with yellowish cream-coloured belly—the Norwegian rat (which we get from ships coming from Norway). The common sewer rat seems to compare as regards susceptibility with the Norway rat.

Statements have been made (Calmette) according to which the virulence of a strain of *B. pestis* may become enhanced by its passage through a *series* of animals of the same species, but that while thus increasing in virulence for this species it does not follow that this same strain of *B. pestis* possesses also increased virulence for another species of animals—in fact the contrary generally is said to take place. Quite a different statement is made by Hankin and Colonel Skinner, viz. that, on the contrary, the virulence of *B. pestis* decreases on its passage through a series of animals of the same species, including man.

I have had a considerable experience in testing these propositions and cannot agree with either. I have tested *B. pestis* derived from acute fatal human cases by injecting the plague material directly into guinea-pigs and rats, in the former subcutaneously, in the latter cutaneously, and have proceeded to do so from guinea-pig to guinea-pig and from rat to rat, both by using the material directly from the spleen of a previous animal, as also by using twenty-four hours' agar culture of the spleen of the previous animal, and as a result I have not noticed anything of an increase in virulence of the *B. pestis* in its passage through these series of animals. True, *B. pestis*, like other pathogenic microbes, lose in virulence by transmission in the laboratory through series of subcultures on artificial media, and, unless this decrease has become very great, become generally enhanced in virulence by passage through the suitable animal body; but this has nothing to do with the above statement that a race of *B. pestis* derived from a particular source (human or animal) undergoes increase in virulence by passage through a

series of animals. Nor have I seen anything of a decrease in virulence either in the animals of the same species or when plague material (bubo or spleen of the dead animal) is taken from one species (guinea-pig or rat) and is used for infection of the other species (rat or guinea-pig).

What I found in a very large number of experiments made for this object is that it does not matter whether the animal which yields or receives the material is a guinea-pig or a rat, so long as the bubo, or spleen, or blood of the plague giver contains the typical *B. pestis* in considerable numbers, the result of cutaneous inoculation of the rat or of the guinea-pig is followed by fatal plague, acute in the rat, subacute in the guinea-pig. A difference, however, which I have noticed to occur and which denotes some kind of difference in virulence, refers to an altogether different circumstance, viz. to the race of the *B. pestis* itself. With this we shall have to deal in a future chapter. Another difference which is to be mentioned in connection with the above is the fact that *B. pestis* when passed through the mouse (dead of plague after inoculation) is as a rule of lesser virulence towards the other rodents than when the same race of *B. pestis* is passed through the rat or the guinea-pig. All races of *B. pestis* act virulently on the mouse, this animal being highly susceptible and easily affected with acute fatal effect by cutaneous inoculation of almost any *B. pestis*.

CHAPTER III

ANALYSIS OF PLAGUE MATERIALS

SINCE Oriental plague, which appeared in Hong-Kong 1894, in India 1896, had become pandemic, amongst the many ships—passenger and cargo—constantly arriving at English ports, there must be, as a matter of course, always a considerable number which have started from one or another plague-infected country—India, the Argentine, the Levant, Egypt, and China; it therefore behoves all port sanitary officers to be continually on the alert, both as regards possible infection of the crew as also of the cargo and rats of such vessels. While in several instances such ships have, on arrival, been found to have had on their voyage cases of plague in man or in rats, or in both; others were, according to the captain's statement, free of suspicious illness both in man and the rat, but on closer inspection were found to harbour one or other of the crew affected with inguinal bubo; further, ships which have started from an infected locality, although free of suspicious illness on the voyage, have, soon after arrival into a port, been found to harbour one or other of the crew affected with, or rather developing, suspicious illness; in a further set of ships coming from infected localities the presence of dead

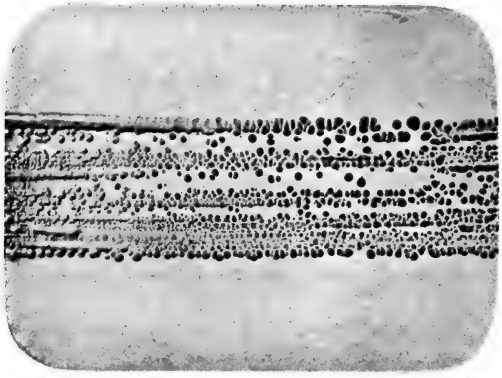

Fig. 35.

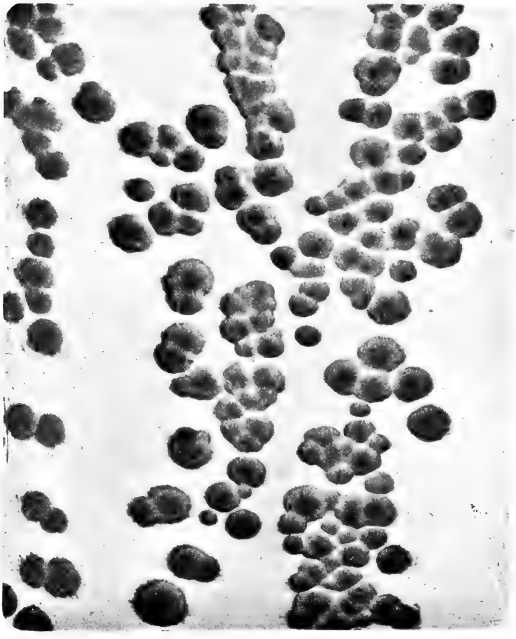

Fig. 36.

FIG. 35.

Colonies of plague bacilli on gelatine after some days' incubation.
Attenuated type 2 (rat type). × 8.

FIG. 36.

Colonies of *B. pestis* on gelatine after many weeks' incubation.
Attenuated type (rat type 2). × 5.

FIG. 37.

Same colonies as in previous figure, more magnified. × 10.

FIG. 38.

Film specimen of the growth of *B. pestis* from agar culture, twenty-four hours' incubation. Virulent human type 1. × 1000.

Fig. 37.

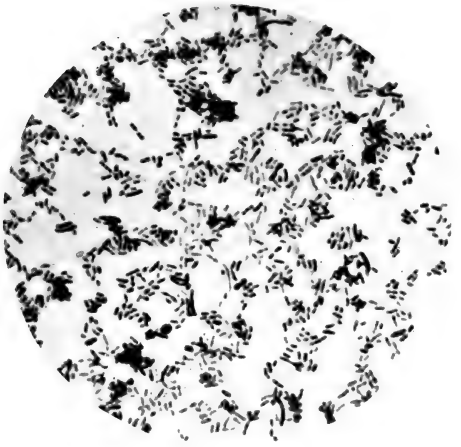

Fig. 38.

rats, nay even highly suspicious illness amongst one or other member of the crew, had been, owing to the master's negatory statements or from some other cause, either overlooked or had been discovered only some time after landing crew or cargo, or both; and finally, ships have come into port which during the voyage and on landing had on board cases of suspicious illness, presumably plague, in man or in rats, or in both. Dr. Bruce Low, in *Report and Papers on Bubonic Plague* (Loc. Gov. Board, 1902), has dealt in detail with all such cases, and it is not my object to repeat what Dr. Low has already ably and fully described, but I wish merely to point out briefly some of the many occasions when the aid of the bacteriologist is called in to help to make, or to verify, diagnosis. In addition to these occasions—that is, when bacteriological diagnosis is wanted of cases directly connected with a ship arriving from an infected country—there are a good many other occasions when in times of threatened invasion cases of suspicious illness are brought to the notice of medical officers of health in different inland parts, which cases *per se* might be cases of Oriental plague, and which might be such on account of their having been in some indirect way in contact or in relation with men or goods coming from a ship that had been some time previously in contact with an infected country; that is to say, now and again isolated indigenous cases of suspected or real plague may occur, and as a fact have occurred, *e.g.* such as suddenly appeared in an hotel in Glasgow in 1902, in Liverpool in 1904. It is unnecessary to emphasise the importance of early recognition of such indigenous cases in order to take at once the necessary preventive measures. It is equally obvious that in

times of pandemics such as we have had now for several years, cases simulating indigenous cases of plague may occur which are clinically indistinguishable from real plague, but on bacteriological analysis turn out not to be plague; wherefore the bacteriological analysis of such cases is obviously of the first importance.

During the last nine or ten years I have analysed for the medical department of the Local Government Board materials derived from a good many ports and also from inland places in considerable numbers. These materials were taken from cases—man or rat—which were either *prima facie*, *i.e.* epidemiologically, probably cases of plague, or which might have been plague both on clinical and on epidemiological grounds. Without wishing to give the details of these analyses I will restrict myself to giving a few instances of different categories only, in order to show on what lines these analyses were carried out:—

1. The s.s. *Simla*.[1]—"The *Simla* is a hospital ship, which had been conveying invalids from South Africa to Southampton. This vessel arrived at the latter port on March 13th, 1901, having touched at Plymouth on March 12th, where she had been given pratique by the Customs after all the usual questions had been satisfactorily answered. A case of enteric fever was landed at Plymouth and another at Southampton. It appears that two days after leaving Cape Town a Lascar member of the crew came under the ship's surgeon's care with a bubo in the groin. Though a natural suspicion of plague arose at once the idea was dismissed, and when the ship was boarded at Plymouth, and again at Southampton, and the usual questions were put as to plague, cholera, etc., no mention of this case was made in their replies by the master or the surgeon of the ship. On landing, the Lascar obtained admission to the South Hants Hospital for treatment of a large abscess pointing in his groin. Some time prior to this Dr. Wellesley Harris, the port medical

[1] Dr. R. Bruce Low's *Report and Papers on Bubonic Plague*, p. 18.

officer of health, in view of the importation of plague by anomalous, mild, or unrecognised cases, had requested the authorities of the hospital to inform him when any cases of glandular swelling were admitted for treatment. On March 17th the resident surgeon sent Dr. Harris intimation of the Lascar's case. The medical officer of health at once took material from the abscess in the groin and sent it to the Board for bacterioscopic examination."

The pus sent was examined in stained film specimens; it showed numerous microbes: cocci, singly and in clusters; streptococcus chains; some diphtheroid bacilli, and some oval rods showing distinct bipolar staining. Agar plates (surface inoculation) were made, and a guinea-pig was injected subcutaneously in the groin with a fair amount of the pus. Next day the plates showed, besides colonies of cocci (albus and aureus), a few colonies of streptococci, and a sprinkling of colonies resembling those of *B. pestis*, viz. small, grey, watery, raised in centre, with slightly irregular margin. When taken up with the point of a platinum needle they were found viscid, and placed in saline solution emulsified with difficulty. Examined in the hanging drop they were found non-motile rods in clumps and in streaks; they showed in fuchsin staining (see above) distinct bipolar character and were Gram-negative. Subculture on gelatine and on agar produced typical growth of *B. pestis*. Patient recovered.

The guinea-pig, injected in the groin, was quiet and slightly off feed after twenty-four hours; it had distinct soft swelling in the groin, seemingly painful to the touch. With a pointed glass capillary pipette a drop of sanguineous fluid from the subcutaneous swelling was obtained; examined under the microscope in stained film specimens it exhibited, besides a few leucocytes, a fair number of red blood corpuscles and a very large number of oval to cylindrical bacilli showing very marked bipolar staining; they were non-motile and gave negative Gram staining.

On account of the typical character of the plague-like colonies in the agar plates, and on account of the microscopic character of the bubo fluid of the guinea-pig, the diagnosis was made: *Pestis bubonica.*

The subsequent subcultures on agar and gelatine of the bubo fluid of the guinea-pig and the death of the guinea-pig on the third to fourth day with all the typical appearances of plague in the groin and spleen—which organs were crowded with *B. pestis*—confirmed the correctness of the early diagnosis.

The subcultures obtained from the primary agar cultures, *i.e.* direct from the pus of the patient, as also those obtained from the bubo fluid of the guinea-pig during life, and those of the spleen of the animal after death, were, for some time afterwards, on several occasions used for experimental purposes on guinea-pigs and rats and found highly virulent.

2. "The s.s. *Friary*,[1] from Alexandria, arrived at Hull on January 10th, 1901, having called at only one port on the return voyage, namely, Algiers. The usual inquiries on arrival were made by the Customs, who informed the port medical officer that on board there was the body of a sailor who died on January 10th, about twelve hours before the vessel reached Hull, from an illness then regarded as 'influenza.' The corpse was removed to the mortuary and afterwards buried. At the time of arrival no cases of sickness existed on board, but on January 12th two sailors sickened. They were seen by a private medical man as well as by Dr. Mason, the port medical officer of health, who made a provisional diagnosis of 'influenza with lung complications.' On January 15th two more members of the crew sickened, and subsequently two others also were attacked. All of these six cases proved fatal, and in all the diagnosis of plague was confirmed by bacterioscopic investigation."

The materials submitted to me for bacterioscopic analysis were pieces of lung and spleen of two of the dead sailors.

In both cases the lung pieces were deep purple red, solid, and sank in water. A film specimen (impression of a cut surface) made of the lung showed, besides a few leucocytes and red blood corpuscles, an almost continuous layer of oval bacilli with marked bipolar staining in pure culture (Fig. 2); these bacilli were Gram-negative; film specimens of the spleen likewise exhibited abundance of the same oval bipolar bacilli. Since these appearances of the lung juice do not occur in any other acute disease than pneumonic plague, the diagnosis of "plague" could be at once made. Cultures in agar plates and agar tubes, injection of guinea-pigs with lung juice and spleen juice of both cases, fully confirmed the diagnosis.

3. *Manchester Case.*—The second cook of a steamer which arrived at Middlesbrough had with other seamen been passed by the port sanitary authorities. He travelled to Manchester, where soon after arrival he was taken ill with fever and inguinal bubo, and admitted (June 10, 1905) to hospital. His illness was diagnosed as (?) *pestis*

[1] Dr. R. Bruce Low's Report, p. 14.

bubonica. He died on June 12. Capillary pipettes containing sanguineous fluid which had been taken by puncture from the inguinal bubo soon after the death of the patient were submitted for analysis. Film specimens showed numerous bipolar bacilli in size like *B. pestis*, Gram-negative.

Diagnosis was made: "Probably plague."

Agar surface plates were made with the material; these brought forth in twenty-four hours (37° C.) pure cultures of colonies of typical *B. pestis*. A guinea-pig injected subcutaneously at the same time with a few drops of the original material showed, after twenty-four hours, a big soft swelling. A drop of the fluid was withdrawn from the swelling; in stained film specimens it showed crowds of bipolar bacilli like *B. pestis*, Gram-negative, non-motile. Diagnosis was made: *Pestis bubonica*. Subcultures and further inoculations of guinea-pigs and rats, both from the original agar plate as also from the above guinea-pig, fully confirmed diagnosis of virulent plague.

4. *A case of Plague at Tynemouth, September* 1904.—German seaman of s.s. *Bishopsgate*, ill with bubo, suspicions of plague; recovered.

Juice of bubo, obtained by aspiration, was received in sealed capillary tubes. Film specimens showed no definite bacilli. With the bubo fluid agar surface plates were made, and a guinea-pig was injected subcutaneously in groin.

After twenty-four hours one agar plate showed one, the other two colonies, which in aspect, character, and constitution (staining characters) were identical with those of *B. pestis*. The guinea-pig exhibited after twenty-four hours a soft distinct swelling, and was quiet and a little off feed. A drop of fluid was withdrawn from the swelling by means of a pointed capillary pipette. This fluid in film specimens showed crowds of non-motile bipolar *B. pestis*. Diagnosis was now justified: "Plague."

Inoculation of a further guinea-pig (subcutaneously) and a rat (cutaneously) with a colony from the agar plate, and a similar set with the juice of the bubo of the guinea-pig, caused fatal plague in all the four animals inoculated. The two guinea-pigs as also the first guinea-pig (injected with the original material from the patient) died on the sixth day with post-mortem appearances of subacute plague: necrotic bubo, necrotic nodules of spleen. The cultures obtained from the spleen and bubo of the guinea-pig and of the rat inoculated with colonies from the original agar plate were subsequently used in the laboratory for experiments and study, and it was found that this

race of *B. pestis* exhibited the characters of our second or rat type, *i.e.* shorter rods than those of the human type, colonies more translucent in gelatine, and of less virulence than the first or human type.

It subsequently was elicited by Dr. Buchanan that this steamer had arrived from Hamburg, where a number of rats had been found on it, which rats had been diagnosed to have died of plague; the ship had been disinfected in Hamburg, and the seamen on leaving Hamburg were all well. But soon after arrival the seaman was taken ill and removed by the port medical officer to hospital as "being clinically very suspicious."

5. The notes of this case (5) are as follows:—"Purulent discharge from a suppurating bubo, most other groups of glands are enlarged" (May 28, 1904). "The patient is a sailor on a steamer just arrived from Rosario. One of the crew died suddenly on the voyage." The medical officer of health who forwarded the material added: "I think it is syphilitic, but I am sending the discharge in case it might by any chance happen to be plague." It has to be remembered that Rosario is an infected place, and several instances of plague on ships arriving from Rosario in English ports had been previously recorded.

Film specimens showed amongst leucocytes and red blood discs numerous cocci, as diplococci, in fours and in small and large masses; no bacilli. Agar surface plate was inoculated with the pus; it showed, after twenty-four hours, crowds of colonies of typical *Staphylococcus aureus* in pure state. The guinea-pig that had been injected subcutaneously with a fair amount of the purulent matter showed no tumour twenty-four hours afterwards, nor later, and remained quite well. The diagnosis was therefore negative *qua* plague. Patient recovered.

6. *Case at Plymouth.*[1]—Towards the end of February, Dr. Williams, the medical officer of health, reported to the Local Government Board a case as to which the medical attendant had been entertaining suspicion that the illness might be plague. The patient, a Jewish pawnbroker, became ill on February 16, with rigors, headache, and fever, attended with prostration. Painful glandular swellings developed in his armpit and groin. Material taken from this patient was submitted to bacterioscopic examination.

What had added to the suspicion of the medical attendant was the fact that the pawnbroker had in the course of his business to handle a considerable amount of clothing that had already been

[1] From Dr. R. Bruce Low's Report, p. 32.

FIG. 39.

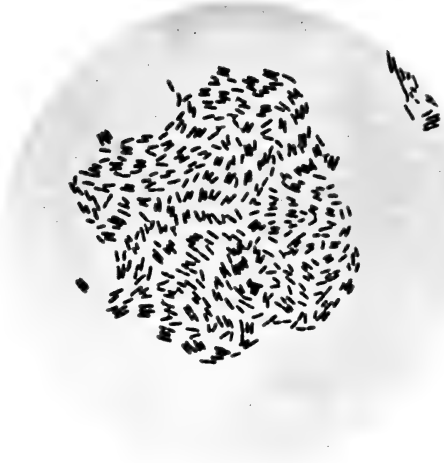

FIG. 40.

FIG. 39.

Film specimen from a recent agar culture of the attenuated *B. pestis* (rat type or type 2). × 1000.

FIG. 40.

Stained impression of a young colony of *B. pestis* (virulent type) on gelatine. × 800.

FIG. 41.

Stained impression of an atypical colony—filamentous bacilli. × 800.

FIG. 42.

Impression of a number of young colonies of *B. pestis* on the surface of gelatine. × 80. One of these colonies was shown in Fig. 40 more magnified. Near the lower right margin and isolated the atypical colony shown in Fig. 41.

FIG 41.

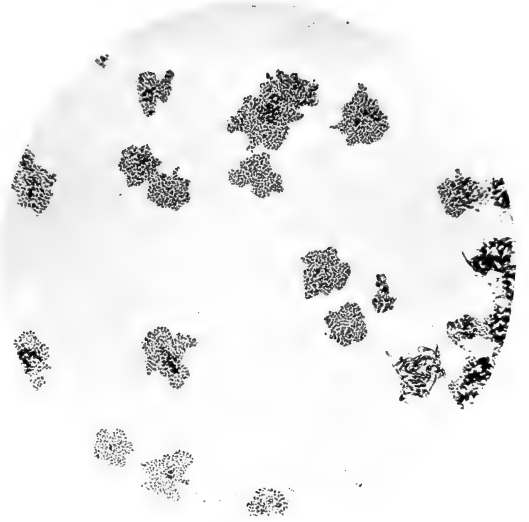

FIG. 42.

worn, and it was thought that possibly plague infection had been contracted through the instrumentality of infected clothing from some unknown or concealed case.

Sanguineous fluid taken from the glandular swelling of the armpit was submitted for examination; film specimens showed amongst the red blood discs and pus cells numerous cocci (Gram-positive), either as diplococci or in small clusters; no bacilli. Agar surface plates yielded pure cultures of *Staphylococcus pyogenes aureus*; a guinea-pig injected subcutaneously with a fair amount of the material showed no illness. Diagnosis: Infection with *Staphylococcus pyogenes aureus*. Case recovered.

7. Dr. Williams, port medical officer (port of London), informed the medical officer of the Local Government Board that two Chinamen (sailors, s.s. *Dundas* and s.s. *Haselder*) had died in the Seamen's Hospital at Greenwich of pneumonia. They had before admission been living at a lodging-house in Limehouse Causeway. One was admitted to hospital on the 19th December 1903, and died the same day; the other was admitted on the 24th December at 1 P.M., and died at 4 P.M. on the same day. The house physician informed that "the cases were very suspicious," he believed them to be plague, and he was supported in this opinion by the visiting physician. At the post-mortem of the second patient material was obtained: pieces of lung and pieces of spleen. The lung was highly congested, in parts almost consolidated. Film specimens made of the spleen showed no bacteria. Film specimens of the juice of the inflamed lung showed abundance of very minute bacilli, singly, in chains, and particularly in large connected masses and streaks; they resembled in staining and arrangement the typical influenza bacillus. Several drops of the sanguineous juice of the lung were rubbed over the surface of agar solidified in plates; the same kind of plates were made of the sanguineous juice of the spleen. Guinea-pigs were injected subcutaneously with large amounts of the lung juice, as also of the spleen juice, one animal for each set. After twenty-four hours the agar plates of the spleen showed no colonies, those of the lung juice showed abundance of typical colonies of *B. influenzæ*. Both guinea-pigs showed no tumour and remained unaffected. Diagnosis: Influenza pneumonia.

From the above cases, which represent types of cases, of which materials were submitted for bacterioscopic

examination, the following conclusions may be drawn. According to this analysis we may group the cases in—

(1) Cases which already on microscopic examination of stained film specimens alone may be pronounced to be most probably plague, and therefore the microscopic examination is able to justify the epidemiologist's and clinician's preliminary diagnosis of plague, or at any rate make it highly probable that the cases are true plague; such are Case 2 (pneumonic plague) and Case 3 (bubonic plague). Culture in surface agar plates and experiment on guinea-pigs clinched the diagnosis in twenty-four hours.

(2) Cases in which the microscopic examination of stained film specimens could not venture to make any diagnosis, but in which culture in agar surface plates and animal experiment enabled diagnosis of *B. pestis* to be made after twenty-four hours or later; such were Case 1 (abscess in groin) and Case 4 (bubo in groin).

(3) Cases in which film specimens and twenty-four hours' agar culture could negative plague; in some of these the glandular swelling, or the suppurating bubo, was clearly caused by infection with *Staphylococcus pyogenes aureus*, and in others (Case 7) the fatal pneumonia was caused by *B. influenzæ*.

(4) There is a fourth category of cases, however, in which the microscopic examination and the culture of material taken from a glandular swelling (associated with fever) yielded no bacteria of any kind: cases, that is, which already on this evidence could be negatived as due to plague. I have had several such instances; materials of these were submitted for analysis because, notwithstanding the want of epidemiological evidence, the medical officers of health quite laudably did not wish to incur any risk of

possibly overlooking a case of plague at a time when occurrence of plague in their respective districts—after the numerous cases of plague that had landed in ports of these Islands—could not be excluded.

Several points which ought to be here mentioned in connection with the culture test I consider of particular importance. The first point is this: surface plates on agar ought in all instances to be made with the materials submitted. A fair amount of the material, several drops of the juice of bubo or inflamed gland, of the lung, of the spleen when available, are rubbed over the surface of nutrient agar (beef broth, peptone, salt, agar), solidified in a plate dish of good size (three inches diameter). If *B. pestis* are contained in the submitted material they are sure to be discovered in twenty-four to thirty-six hours at 37° C. incubation. I have never failed to find them even in cases in which film specimens failed to give clear indication of their presence. If film specimens already show *B. pestis* or bacilli like them in great numbers, agar tubes (slanting surface) will bring them forth, but such cases are not often submitted; the materials generally submitted are in the great majority of instances such as contain few *B. pestis* amongst other microbes, or contain few *B. pestis* only. In the former instance, if the inoculation has been made in a tube, the few colonies of *B. pestis* become generally overgrown or crowded out by other microbes (cocci, bacilli); in the latter, not enough material is used for development on the limited surface of the agar tube.

The great importance of using at once plate cultures in preference to tube cultures of solid or fluid media cannot be sufficiently emphasised. If *B. pestis* is mixed in any

material with other microbes, the only way, in my experience, to identify it by culture is by plate cultures. And if it cannot be identified in this way, it is hardly to be expected that it will be identified by tube culture. Of course it is supposed that the observer is familiar with the appearances and nature of the colonies of *B. pestis* in their early stages. This conceded, I am confident, from a large experience, that there need be no difficulty in identifying *B. pestis* in a mixture, such, for instance, as would occur in the sputum of some pneumonic cases, or in the purulent discharge of a suppurating bubo. The plates should be, of course, sufficiently large (three inches diameter), and several plates should be inoculated with the material. As regards the sputum, it should be remembered that the *Diplococcus pneumoniæ* is a frequent microbe in expectorations, and except in acute cases of croupous pneumonia, in which this microbe occurs very numerously, it is present generally only in moderate numbers. But however scarce or however frequent, there ought to be no difficulty in identifying the *B. pestis* by plates, if this latter should be present in such sputum. Drs. Pakes and Joseph of Johannesburg have recommended to make the cultures of sputum of suspected lung cases in slightly acid broth, for in this medium the *B. pestis* grows well, whereas the *Diplococcus pneumoniæ* does not, and consequently the former is secured from being crowded out by the latter, which, according to Pakes and Joseph, does happen if the culture medium is ordinary broth. Without for a moment doubting that this modification, viz. using slightly acid broth as culture medium, may be very useful, I have likewise no doubt that plate cultivation with proper nutrient beef-broth agar, such as I have

FIG. 43.

FIG. 44.

FIG. 43.

Stained impression of a recent colony of *B. pestis* on gelatine. × 1000.

FIGS. 44 and 45.

Two ghee broth cultures of *B. pestis*, each holding 1500 cc. of culture fluid, showing developing surface pellicle with stalactites; the broth is clear; at the bottom masses of granular sediment—former stalactites that had become detached. Reduced size.

FIG. 46.

Bacterium Bristolense.—Growth on potato, showing numerous gas bubbles in the growth. × 4.

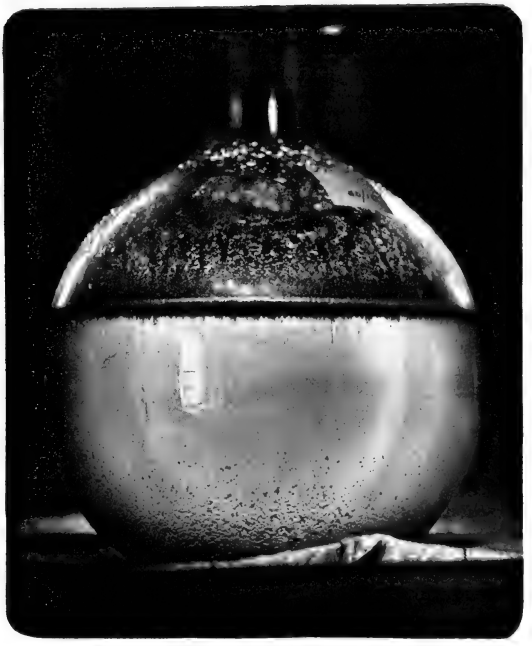

FIG. 45.

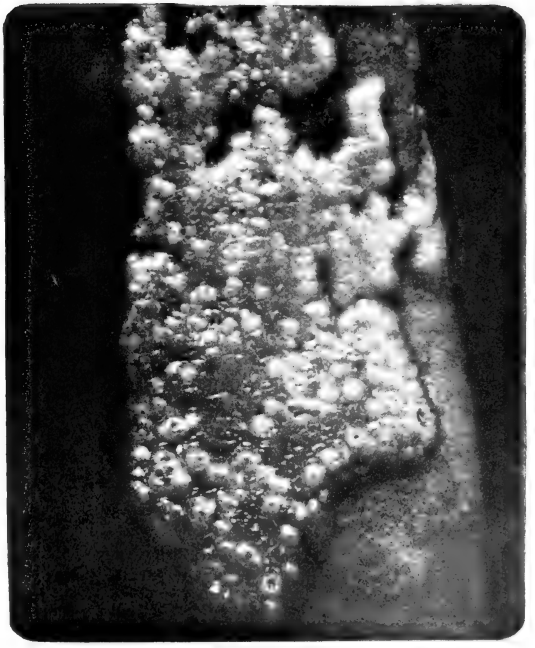

FIG. 46.

mentioned above, is for all practical purposes perfectly sufficient. Moreover, my experience leads me to suspect that failure by Dr. Pakes and his colleague in identifying the *B. pestis* in the earlier cases of pneumonia in Johannesburg might have been due to the cultures not having been made by plates, but in tubes.

The second point I wish to point out, and to insist on, is this: it ought to be remembered that in sputum of acute lung affections, besides the *Diplococcus pneumoniæ* there may be, and occasionally are present, coli-like microbes, or microbes belonging to the proteus group; both these microbes in film specimens of the sputum, well stained and well washed, appear as bipolar oval to cylindrical rods not unlike *B. pestis.* In these cases to make diagnosis "plague" or even "probably plague" from the microscopic examination alone would be quite unjustifiable, and might lead to extremely embarrassing results. Some observers appear to place, with Hankin, a certain diagnostic reliance on the capability of the plague microbe to grow into long threads in salted media. I have shown many years ago, *i.e.* long before Hankin, that a number of different bacteria (including *B. coli* and those of the coli group) when grown in media containing excess of salt grow into long filaments; it is therefore clear that this modification can be of no diagnostic help.

Moreover, these lung *B. coli* and lung proteus, when injected into the groin of the guinea-pig in sufficient amounts, cause acute septicæmia: hæmorrhagic effusions extending over the thigh, groin, abdomen, and even the chest wall; the effusion is crowded with the bacilli, and these in film specimens show bipolar staining and are Gram-negative. Owing to this morphological and experimental

result, to conclude that the sputum which caused this state in the guinea-pig contained the *B. pestis*, and that the disease of the lung is due to plague, would be a grave error. The culture (plate) test with the sputum made at the same time as the animal experiment would at once check this possible error; and, further, the examination of the hæmorrhagic exudation in the fresh and living state would show at once that the microbe, being motile, cannot be *B. pestis*. The culture of the exudation would also soon show that the microbe is not *B. pestis*. There occurred actually such a case of pneumonia, which was said to be due to *B. pestis*, but which was not due to *B. pestis* at all, but to a motile, highly pathogenic (for the guinea-pig), coli-like microbe. The premature diagnosis of plague on insufficient or on erroneous data is not only apt to discredit the great value and great importance of bacterioscopic analysis in respect of suspected cases—which of all others are those in which the bacteriologist's assistance is most needed,—but is liable to cause considerable embarrassment and actual damage to the locality in which the case has occurred. For it has to be remembered that by international convention (Venice and Paris) the Foreign Office are bound to notify to the foreign Consuls the occurrence of any case of plague, and that our Foreign Office have always carried out this obligation both in the spirit and in the letter—different from some continental Foreign Offices.

The loss liable to be inflicted on commerce and the damage done in other respects to the inhabitants and the locality in whose midst an indigenous case of plague suddenly occurs—or rather is said to have occurred—can easily be imagined. The diagnosis of such an indigenous

case of plague rests practically on the bacterioscopic diagnosis, and it therefore behoves the bacteriologist to have his data resting on a firm basis: the morphological, cultural, and experimental evidence must be clear and decisive. Such evidence is not difficult to obtain if all the steps above detailed are taken with care and circumspection, and not the least important part of the analysis is, or should be, the culture test and the cutaneous or subcutaneous inoculation of the mouse or rat or guinea-pig. A single colony which, in a plate cultivation, complies both in general aspect and constitution with the characters of a colony of the true *B. pestis*, and which, inoculated *cutaneously* into the mouse or rat, *subcutaneously* into the guinea-pig, causes, as it should, an acutely fatal disease with the presence in great numbers of typical *B. pestis* in the bubo and spleen, is perfectly sufficient to ensure correct diagnosis of "plague."

A third point which ought to be here mentioned is this: when plate cultivations are made of material which is obtained by puncture from a bubo, *i.e.* from a subcutaneous inflamed swelling, there occur not infrequently one or more colonies which appear as small, grey, translucent, flat discs, with a central slight thickening due to an opaque granule; these colonies are somewhat slow in their growth, and have a crenated or slightly angular margin. When touched with the point of a platinum needle they are found to be composed of a somewhat cohesive material, they do not well emulsify in saline solution, and when looked at under the microscope are composed of non-motile bacilli more or less connected in strings and masses. When stained they do not show bipolar character, moreover are in shape diphtheroid, *i.e.*

conical, and some of them club-shaped; they are *Gram-positive.* In fact, they are non-pathogenic diphtheroid bacilli belonging to the group of *B. xerosis.* I am inclined to think that the "modified plague bacilli" which Dr. Cantley and Professor Hewlett described (Pathological Society, 1904), and which were found in a culture made from the juice of a punctured "climatic bubo" (Cantley), are nothing more or less than such xerosis bacilli belonging to the pathological skin over the "climatic bubo," and that therefore Dr. Cantley's contention that these climatic buboes which, according to him, are "not yet plague" but "may lead to plague," and which he wishes us to term "pestis minor," receives no confirmation from the above bacteriological fact, *i.e.* the presence of the xerosis bacilli. I have seen such xerosis colonies in several instances in plate cultivations made with material derived by puncture from buboes associated with fever, which buboes were connected not with plague, of which there was no history, and which led to no further occurrence of plague, but which were due to infection with *Staphylococcus aureus* or other microbe.

CHAPTER IV

MICROBES SIMULATING IN ONE OR ANOTHER RESPECT THE *B. PESTIS*

ALTHOUGH the *B. pestis* is, in respect of its morphological, cultural, and experimental characters, a well-defined species, and not easily overlooked or mistaken, yet there are in the nature of things occasions when either in cases of man under suspicion of plague, or in cases of rats dead under suspicious circumstances — in docks, on board ship, in houses and localities open to invasion or actually invaded by plague,—correct bacterioscopic diagnosis might be for one reason or another difficult, or, owing to inexperience of the analyst, might not be forthcoming. And for this reason we shall presently show there are microbic species which, in their pathogenic effect on some test animals, *e.g.* the guinea-pig, simulate plague; or in their morphological and staining characters are not easy to distinguish from the true *B. pestis*, e.g. *B. proteus* or *B. coli* and other similar microbes; or which in morphology and certain cultural characters bear a certain resemblance to *B. pestis*. Add to this that these simulating species may be found in man or in the rat under suspicious circumstances—that is, under circumstances which do not exclude plague—and no

apology is required to enter more fully into a description of these microbes.

1. *Proteus vulgaris.*—First in importance of frequency is the *proteus vulgaris*, and its varieties or races.

Why I consider this microbe of first importance will be apparent if I describe two instances.

In a large city situate at a port on the East Coast, in which port a case of plague had been landed the previous year, a woman, the owner of a small shop in the town, was suddenly taken ill with fever (T. 101 F.), swelling and inflammation of axillary and femoral lymph glands. After four days' illness the temperature fell and soon became normal. While the temperature was still abnormal, a small amount of the sanguineous juice of the swollen axillary gland was obtained by puncture, and submitted to bacterioscopic analysis. The suspicion of plague was justified, first, by the fact that without any visible external wound the woman suddenly showed clinically symptoms resembling plague, and, secondly, that this case occurred in a port in which a case of plague had occurred. According to the inquiry, instituted by the medical officer of health, a coloured man (sailor), himself quite well, had visited the shop, but besides having shaken hands with the woman, no other channel of possible infection could be established. The woman recovered.

Microscopic examination of stained film specimens revealed a very small number of oval rods, some of which showed more or less distinctly bipolar staining. Agar plates were made, and a guinea-pig was injected subcutaneously with the whole of the remaining material. The agar plates showed next day a filmy, translucent, viscid growth, which, when examined under the microscope,

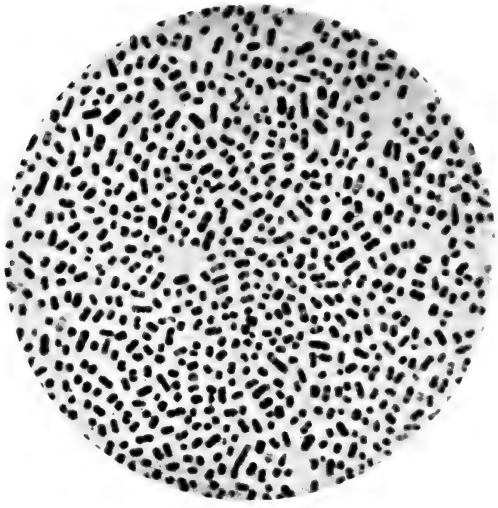

Fig. 47.

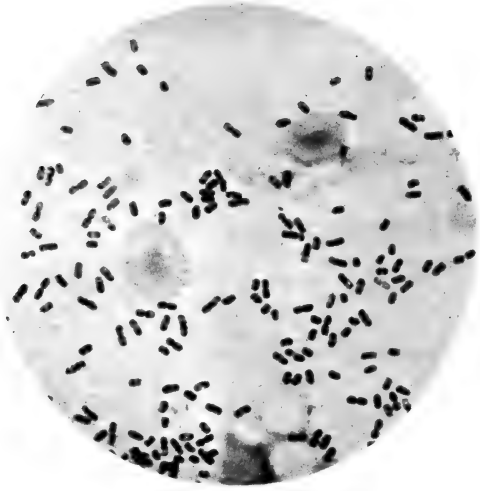

Fig. 48.

FIG. 47.

Bacterium Bristolense.—Peritoneal exudation of a rat dead after subcutaneous injection with culture. The stained film specimen here shown exhibits bipolarly-stained bacilli embedded in a gelatinous matrix like a zooglœa. × 1000.

FIG. 48.

Bacterium Bristolense.—Film specimen of the spleen juice of a guinea-pig dead after subcutaneous injection with culture, showing numerous bipolar-stained bacilli. × 1000.

FIG. 49.

Bacterium Bristolense.—Stained film specimen of the swollen inguinal lymph gland of a guinea-pig injected into the groin with culture of the microbe; some of the bacilli are bipolar and oval, but many others seem swollen up like involution forms. × 1000.

FIG. 50.

Stained film specimen of the subcutaneous local exudation of a mouse injected with culture, showing numerous bipolar bacilli, some thicker than others. × 1000.

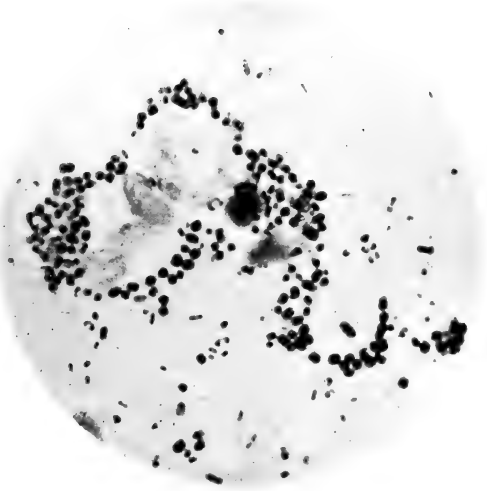

FIG. 49.

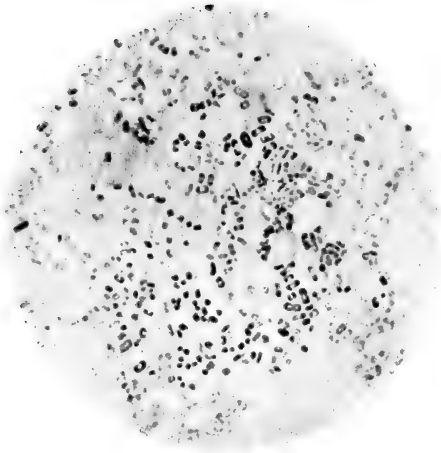

FIG. 50.

in the hanging drop, was seen to consist of motile bacilli. The extent of the filmy growth—covering the greater part of the surface of the plate after twenty-four hours' incubation—was alone sufficient to negative *B. pestis*; add to this that the bacilli were motile. But a bacteriologist of insufficient experience—and the number of these who perform bacteriological analytical work has of late years greatly increased—might have been misled by the translucent viscid growth (ground-glass-like); further, omitting to examine the growth in the living state—an omission not infrequent—and therefore not noticing the motility of the component bacilli, might (and generally does) at once proceed to prepare stained film specimens. In these he would notice that most of the bacilli show bipolar staining, just like *B. pestis*; moreover, he would ascertain that they are Gram-negative, and he might well sustain and express what appears to him a justifiable suspicion, that he is dealing with *B. pestis*. The guinea-pig injected with the original material did not show after twenty-four hours any noticeable bubo, nor did it after forty-eight hours, but this might perhaps be thought to be due to the material containing only very few of the incriminated bacilli; he therefore would proceed to inject subcutaneously a fresh guinea-pig in the groin with a larger—considerable—amount of the filmy growth from the agar plate. This second guinea-pig shows next day a decided gelatinous swelling about the groin, and the animal is quiet and a little off feed. If the guinea-pig is killed, the subcutaneous tissue of the thigh, groin, and flanks appears greatly congested and infiltrated with sanguineous fluid; when a drop of this is examined in the fresh state it shows numerous *actively motile* bacilli; but if, as not infrequently

happens, the analyst proceeds at once, without examination of fresh specimens, to make stained film specimens, he would notice that the bacilli, at any rate some of them, show distinct bipolar staining, that they are Gram-negative, and that in shape and size they resemble the *B. pestis.* Diagnosis of "plague" under the conditions of analysis just detailed might seem justified, but all the time the analyst was working with the common *Proteus vulgaris.* A gelatine plate or gelatine tube subcultured from the agar plate or from the subcutaneous exudation of the second guinea-pig would produce the typically liquefying *Proteus vulgaris.*

I have in the foregoing described the results, not of an hypothetical, but of the actual analysis of the case. This proteus, as is highly probable, might have been picked up from the surface of the (dirty) skin of the axilla while gland juice was taken from the gland, or it might have been in the gland itself; at any rate the microbe was undoubtedly *Proteus vulgaris.*

Or take other instances. Repeatedly it occurred that dead rats found in the hold of a ship were sent for examination. These ships had touched at or came from an infected country. Although no cases of plague had occurred on board ship, nor had any diseased or dead rats been found during the voyage, nevertheless when discharging cargo one or other dead rat had been found. In other cases, the ship having had a case of plague during the voyage, or a case of plague having occurred on landing, and the ship having been subjected to disinfection by the Clayton or other process, dead rats in numbers would then of course be found, and some submitted to analysis.

Rats are peculiar in this respect, that even a short time after death—in the warm part of the year, in less than twelve hours—the spleen may swarm with proteus, obviously derived from the intestine. Stained film specimens of such a spleen always show more or less numerously—particularly in the outer portions of the organ—oval bacilli with bipolar appearance. These bacilli under culture prove to be *Proteus vulgaris*: an agar surface plate shows already, after twenty-four hours and less, a filmy, translucent, rapidly spreading growth; when examined in the hanging drop the growth is composed of actively motile bacilli; when stained, many exhibit bipolar staining; gelatine is liquefied by the growth. The bacilli of this growth are virulent for the guinea-pig: when an emulsion of the rat spleen—if containing abundance of these bacilli—or when a portion of the agar growth is injected subcutaneously into the groin of a guinea-pig, it will be found that the animal shows after twenty-four hours extensive hæmorrhagic infiltration of the subcutaneous tissue, the exudation being crowded with *Proteus vulgaris*—actively motile rods, Gram-negative, bipolar in staining. The animal may be dead within thirty-six to forty-eight hours; no bacilli are found in the blood or spleen, but they abound in the local infiltration. It is therefore obvious that, judging by mere stained film specimens of the original spleen, or by the fatal effect on the experimental guinea-pig, showing hæmorrhagic tumour about the seat of injection, containing abundance of bacilli which in stained films exhibit bipolar staining, an error in diagnosis may be easily committed. If a rat really had died of plague the bipolar bacilli seen in the film specimens of its spleen will yield on

agar plates the characteristic circumscribed small watery raised colonies, composed of non-motile bacilli, and when inoculated *cutaneously* into mice, rats, or guinea-pigs, will produce the appearances and fatal results of plague. Subcutaneously injected into the guinea-pig the *B. pestis* produces marked swelling and inflammation of the lymphatic gland themselves, not merely a subcutaneous infiltration; after death the spleen is crowded with the non-motile, Gram-negative, bipolar bacilli. *Proteus vulgaris*, however virulent otherwise, when inoculated *cutaneously* into guinea-pigs, mice, or rats, produces no effect.

The frequent occurrence of virulent *Proteus vulgaris*—that is, virulent on subcutaneous injection of fair amounts into the groin of the guinea-pig—particularly in the spleen of rats, in the lung of man some time after death (in the warm season in less than twenty-four hours), as also in the fluid of the mouth, and therefore in the expectoration, has to be carefully borne in mind. These microbes show, in properly stained film specimens, more or less marked bipolar staining; wherefore, to make diagnosis by a mere stained film specimen is to be seriously avoided in such cases.

2. *B. coli.*—Next in importance are the pathogenic varieties of *B. coli*, or at any rate the microbic species which, by their general characters, belong to the group of *B. coli* and allies—that is, bacillary species which do not liquefy gelatine, which are Gram-negative, which ferment glucose (and some other sugars), which in surface plate cultivations (gelatine, agar) exhibit the character of colonies of *B. coli* and coli-like microbes, viz. rapidly growing, flat, dry, angular, grey expansions on gelatine, their bacilli motile. Obtained from diseased tissues—sputum, cystitis, perito-

FIG. 51.

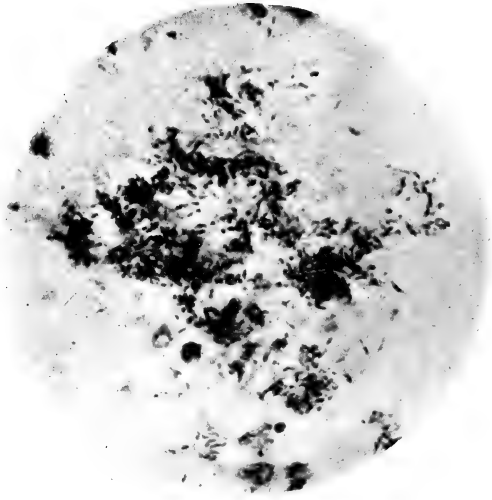

FIG. 52.

FIG. 51.

From a section through the consolidated portion of the lung of a rat spontaneously dead, showing the alveoli filled with exudation in which are masses (deeply stained) of the bacilli. × 85.

FIG. 52.

The exudation of an alveolus of the lung as in previous figure, but more highly magnified, showing the bacilli. × 1000.

FIG. 53.

A gelatine plate culture of the heart's blood of the rat spontaneously dead with lung disease. The culture is some few weeks old. × 30.

FIG. 54.

Film specimen of a gelatine culture of the tissue of lung of rat; methylene-blue staining, showing distinct metachromatismus. × 1000.

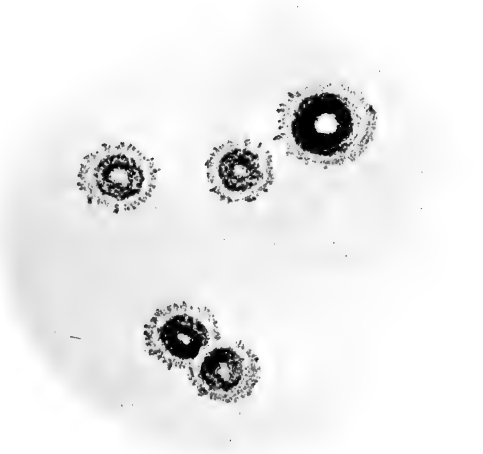

Fig. 53.

Fig. 54.

nitis, suppurations—they prove virulent to the guinea-pig. There is one character by which these pathogenic coli-like microbes can be at once distinguished, viz. a particle of a colony examined in the hanging drop shows motile bacilli. Further, inoculated cutaneously into mice or rats or guinea-pigs they cause no effect, also subcutaneously into rats they are non-pathogenic; but subcutaneously injected in fair amounts into the groin of guinea-pigs they cause gelatinous, often sanguineous, infiltration, leading in some instances to death of the animal within thirty to forty-eight hours. The bacilli of the culture as also of the subcutaneous infiltration show motility, and, after suitable staining in film specimens, more or less marked bipolar staining.

I will here give an instance in which coli-like microbes present in diseased tissues (of man) had, on account of their bipolar staining in film specimens, and on account of their virulence for the guinea-pig, been erroneously declared as *B. pestis.*

A case of acute broncho-pneumonia occurred in hospital in a certain city—A. In the routine work of the pathologist of the hospital the sputum of the case showed in stained film specimens a large number of bacilli with marked bipolar appearance. This occurred at a time when not only in several ports of England single cases of plague had been imported, but there had also occurred several cases of plague in a port B. in fairly close and frequent communication with A. Cultures of the sputum and also injection of a guinea-pig were made. The guinea-pig died after a few days with sanguineous tumour. In this sanguineous exudation were crowds of bacilli, which in film specimens showed bipolar staining. Diagnosis was made: Plague pneumonia.

Now, the above sputum, as also its cultures, and the cultures obtained from the injected dead guinea-pig, were submitted through the Local Government Board to analysis, and it was at once seen that there was no justification for the above diagnosis, because the cultures all showed that the bacilli, when examined in the hanging drop, were actively motile. Subcultures and experiment proved them to be *B. coli* of a pathogenic kind. The above case of pneumonia recovered, and no further cases occurred.

Unfortunately the case had been already notified as plague at A. on the basis of the local evidence, and it required a considerable amount of effort, not of the most desirable kind, to negative the first impression and to retract the erroneous diagnosis of plague.

Another instance: a ship coming into port was at first thought probably to harbour plague rats owing to mortality of these rodents on board; but on careful bacterioscopic analysis the mortality was shown not to be due to *B. pestis*, but to an altogether different microbe, as the following description will show:—

3. *Bacterium Bristolense.*[1] — In a cargo vessel, s.s. *George Royle*, which was being unloaded at Bristol, a number of dead rats were found. One of these was forwarded by Dr. Davies, medical officer of health, to the Board for bacterioscopic examination.

Owing to the steamer having come from Smyrna, which was then plague-infected, it was thought probable that the rats in question had died of plague. No case of plague in man had occurred on board the vessel.

[1] Report of the Medical Officer of the Local Government Board, 1902-1903, p. 414 and *passim*.

The post-mortem examination of the above rat showed the following condition:—The inguinal glands were not enlarged. The spleen was enlarged, dark, and very juicy. Both lungs were very congested, showing in addition patches of consolidation. In the spleen and in the inflamed parts of the lung a variety of microbes were found in stained film specimens, and amongst them some which owing to bipolar staining might have passed for *B. pestis*. In the film specimens of the spleen in particular the great majority of the microbes were shorter or longer oval bacilli showing bipolar staining.

Subcutaneous injection of a few drops of the inflamed lung juice into a guinea-pig (No. 1) caused illness within twenty-four hours, viz. the animal became quiet and developed local tumour. It was found dead on the second day. The post-mortem examination showed the following condition:—The subcutaneous tissue of the groin, abdomen, and chest of the injected side—the injection having been made in the groin—was much infiltrated with sanguineous fluid. In this fluid crowds of bacilli were present, of the same size and staining as those mentioned above as present in the lung juice of the rat. The inguinal glands on the injected side were swollen and firm; and in them were much blood and numerous bacilli of the same character as those already mentioned. Both lungs were hyperæmic; sanguineous exudation was present in the pericardium; the spleen was not enlarged; the suprarenals were deeply congested.

Cultures were made, not only from this animal, but also from a large number of other animals, both guinea-pigs and rats, inoculated in series from it; so that the morphological, cultural, and physiological characters of the microbe

could be studied in every detail. As a result it was definitely ascertained that the microbe was not *B. pestis*, but a microbe related partly to *Bacillus coli* and partly to the *Bacterium lactis aerogenes*. In some of its physiological and also in its morphological characters it resembles to a considerable degree *B. pestis*; indeed, if the microbe were insufficiently examined, *i.e.* by these tests alone, the mistake that it was *B. pestis* might easily have been made, as will appear from the details of my observations.

From the first experimental guinea-pig, No. 1, a guinea-pig, No. 2, was injected intraperitoneally with a few drops of the sanguineous subcutaneous exudation, while another guinea-pig, No. 3, received a similar dose subcutaneously. The latter (No. 3) died within forty-eight hours; the former (No. 2), which in forty-eight hours was in a dying condition, was killed. The post-mortem of the subcutaneously injected guinea-pig (No. 3) was exactly similar to that of guinea-pig No. 1. Guinea-pig No. 2 showed copious exudation in the peritoneum, and in the exudation purulent flocculi and pseudomembranes. These materials contained dense crowds of the same bipolar-stained bacilli as in guinea-pig No. 1; the bacilli appeared embedded in a viscid, gelatinous matrix.

Of a single colony of an agar plate (twenty-four hours old) of the peritoneal exudation of guinea-pig No. 2, a small amount was injected intraperitoneally into a guinea-pig No. 4. This animal was found dead in twenty hours. Its post-mortem appearances showed copious viscid grey exudation in the peritoneal cavity; the spleen was small; the liver was covered with grey pseudomembranes; both lungs appeared injected; the pleural cavity contained

Fig. 55.

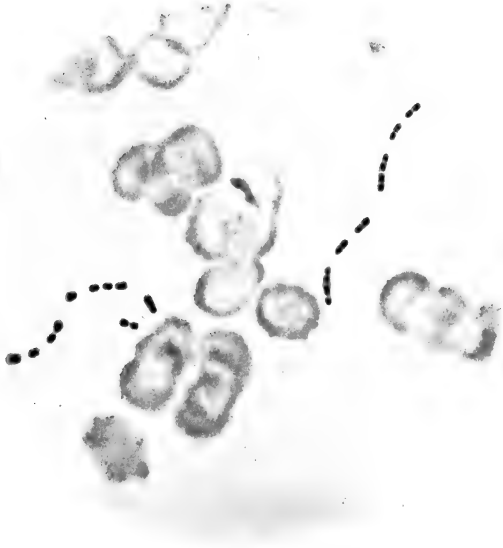

Fig. 56.

FIG. 55.

Film specimen of same culture as in previous figure, stained after Gram. × 1000.

FIG. 56.

Film specimen of blood from the right ventricle of Nurse T., dead December 9, showing numerous capsulated diplococci. × 1000.

FIG. 57.

Exudation at the seat of inoculation in a mouse dead after subcutaneous inoculation with culture of the above capsulated diplococcus. × 1000.

FIG. 58.

Stained film of agar plate of blood of Nurse T., showing the *Diplococcus pneumoniæ*, chiefly in chains, as also the bipolar-stained *B. myxoides*. × 1000.

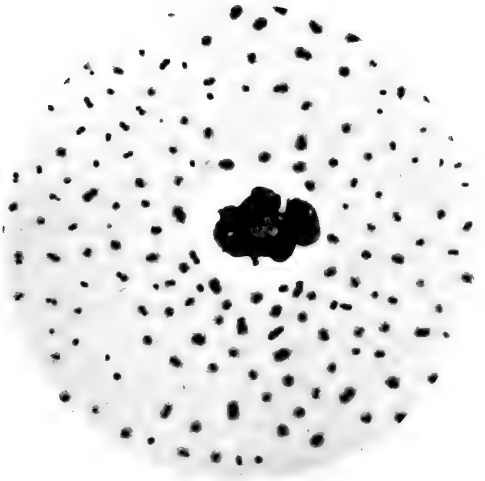

Fig. 57.

Fig. 58.

viscid grey exudation. Both the peritoneal and the pleural exudations contained in a gelatinous matrix crowds of beautifully bipolar-stained oval bacilli.

The result of the injection, the nature of the peritoneal exudation, and the aspect and staining of the bacilli in this exudation, were such that a mistake for *B. pestis* could easily have been made.

One further experiment only need be here detailed. Of the viscid peritoneal exudation of guinea-pig 4 a few drops were injected into the groin of two rats (Nos. 5 and 6) on April 12. On April 14 both animals seemed affected; they were quiet, their coats rough, their breathing accelerated, and they did not feed normally.

On April 15 the animals seemed a little better, though not quite well. On April 16 one of the two rats appeared much worse, and on April 17 it was found dead. Post mortem there was in the groin a large tumour which involved the subcutaneons tissue and the inguinal lymph glands, which were infiltrated with sanguineous fluid. In this fluid were crowds of bacilli, which in size and bipolar staining resembled *B. pestis*. The peritoneum contained slimy grey pseudomembranes covering the surface of the liver, the spleen, and the parietes. These pseudomembranes consisted of an interstitial gelatinous matrix, embedded in which were bacilli of the size of *B. pestis*, showing (in stained films) exquisite bipolar staining. In the omentum were hæmorrhagic spots. The small intestines were found much congested, relaxed, and containing sanguineous mucus. Both lungs were much inflamed.

From this post-mortem examination, therefore, a diagnosis of "plague" might have been justified. But cultures of the peritoneal exudation, of the tissue and

juices of the bubo, and of the heart-blood, proved conclusively that the microbe was not *B. pestis.* All these cultures were pure growths of one and the same species—which was *not B. pestis.* Numerous other experiments with the same material gave like positive results.

To sum up the facts so far with reference to the *George Royle* rat: there was obtained by experiment on the guinea-pig, as also by culture, with the inflamed lung of the rat in question, a microbe which acted pathogenically in the guinea-pig, both subcutaneously and intraperitoneally, and on the rat when injected subcutaneously. The result of the inoculation of these animals was production in them of a hæmorrhagic septicæmia, with the copious presence, in the exudations of the inflamed parts and tissues, of a single definite species of bacillus. The post-mortem appearances were in their character not unlike those of plague, except that the spleen was not much enlarged, or at least not so distinctly as in plague. The microbe recovered from the tissues of the dead animals showed in its general morphology and staining power a remarkable similarity to *B. pestis*; so much so that film specimens, such as are shown, would no doubt justify the provisional diagnosis of *B. pestis.* I refer here particularly to the stained film specimen of the peritoneal exudation of a guinea-pig dead after intraperitoneal injection, shown, and to the film specimen of the spleen juice of a guinea-pig dead after injection subcutaneously with culture (Figs. 47 and 48).

Another point which it is necessary to insist on is the important one that neither in the guinea-pig nor in the rat have I been able to produce with this bacillus, whether using the animal tissues or culture, any positive result

by *cutaneous* inoculation, though that method ensures always characteristic and positive result with *B. pestis* and with plague material.

In order the more effectively to discuss the nature of the microbe in question, I propose to describe it under the name of *Bacterium Bristolense*; "bacterium" because following Migula's nomenclature the microbe is non-motile, and "Bristolense" because it was derived from a vessel in the Port of Bristol and was not *B. pestis.*

The *Bacterium Bristolense* is a non-motile rod, varying in length just as does *B. pestis*; similarly it varies in shape from a short oval to a cylindrical bacillus, and is of about the same thickness as *B. pestis.* The character of the microbe on gelatine, on agar, on serum, and in broth is altogether similar to that of some varieties of *B. coli communis*, and different therefore from that of *B. pestis.* The colonies on gelatine are round, raised, and moist-looking. *B. Bristolense*, like *B. coli communis*, produces indol in broth; it gives positive neutral-red test; it "bubbles" (*i.e.* forms gas) in glucose gelatine shake culture; it forms acid in milk, and curdles this medium in a few days. In these characters the microbe therefore corresponds closely with *B. coli*, and in particular to a definite variety of *B. coli communis.* But it differs from *B. coli* by the more rounded, thicker, and whiter aspect of its colonies in gelatine plates, and its thicker, whiter, and less expansive growth in gelatine streak culture. After some weeks' culture on gelatine there is just an indication of liquefaction, but it never becomes marked. In these respects the microbe resembles more the *B. lactis aerogenes.* *B. lactis aerogenes*, however, does not produce indol in broth, and does not give

a positive reaction in neutral-red broth. On potato at 37° C. the microbe forms a thick, moist, whitish cream-coloured growth in which copious gas bubbles appear: this condition obtains when the surface of potato of some age is inoculated; on recently prepared potato the gas bubbles are not evident. Fig. 46 is an accurate illustration of this appearance, and the character in question conclusively eliminates this microbe from the *B. coli communis*, and places it amongst the group of *B. lactis aerogenes.* Culturally, then, the *B. Bristolense* stands somewhere between *B. coli communis* and *B. lactis aerogenes.*

As already mentioned, *B. Bristolense* is pathogenic to rats and to guinea-pigs both by subcutaneous and by intraperitoneal injection; and, further, the pathological appearances induced by it in the infected animals—particularly the distribution, aspect, and staining power of the microbe in the tissues of these animals—might be easily mistaken for those of *pestis bubonica.* In this connection it is necessary to insist on what has been already said as to the nature of the tumour at the seat of injection and as to the bipolar-stained numerous bacilli in the tissue of the tumour, in the peritoneal exudation, and in the spleen of the experimental animals; for a diagnosis made from the morphological appearances alone or from the results of intraperitoneal and subcutaneous injections of rats and guinea-pigs might lead to serious mistakes. The culture test is, however, in this instance decisive against *B. pestis.* But, as already noted, neither a culture nor the pathological products of the experimental animals can reproduce the disease in other animals by *cutaneous* inoculation alone; and this fact is an important help towards a correct

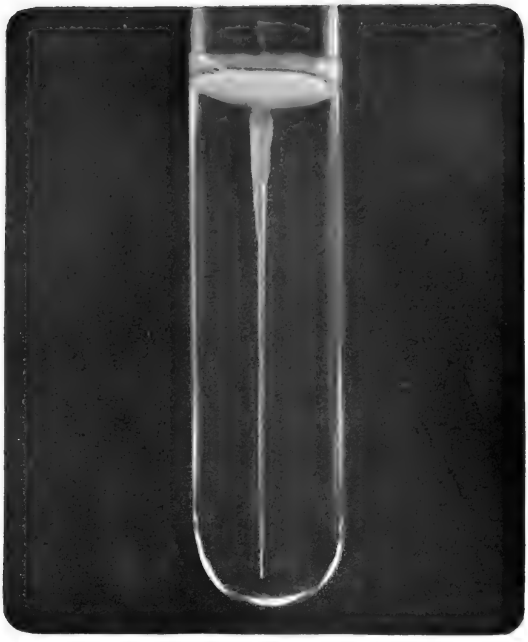

FIG. 59.

FIG. 60.

FIG. 59.

Stab culture in gelatine of *B. myxoides* after a week's incubation. Natural size.

FIG. 60.

From a pure culture of the *B. myxoides* (from Nurse T.'s blood), showing a zooglœa of the (bipolar) plague-like bacilli. × 1000.

FIG. 61.

Film specimen of the viscid peritoneal exudation of a guinea-pig dead after intraperitoneal injection with *B. myxoides*. × 1000.

FIG. 62.

Specimen of heart's blood of a guinea-pig dead after intraperitoneal injection with *B. myxoides*, showing great numbers of the bipolar bacilli. × 1000.

FIG. 61

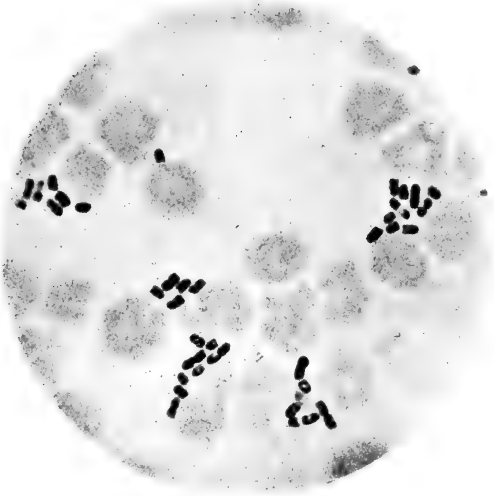

FIG. 62.

diagnosis, since the plague bacillus reacts positively, *caeteris paribus*, by cutaneous inoculation.

Though *B. Bristolense* possesses, as already shown, pathogenic action for rodents, the statement requires certain qualifications. Rabbits, for instance, appear insusceptible. Moreover, the virulence of the subcultures rapidly decreases even for the susceptible animals; a comparatively small number of transferences in culture suffice to diminish its pathogenicity. Feeding with culture produces no effect either in guinea-pigs or in rats. Mice are killed by the culture in thirty to forty-eight hours on subcutaneous injection. They show œdema at the seat of injection; the bacilli which are numerously present in their spleens and blood exhibit good polar staining, but are decidedly thicker than *B. pestis*.

Dunbar and Kister (*Centralblatt f. Bakt. und Parasitenk.* Bd. xxxvi. p. 127) found in the organs of some rats dead on board ship, which were submitted to them, bacteria of various kinds, not *B. pestis*. Some of those figured by them, showing bipolar staining, clearly belong to the tribe of proteus, others may have been *B. Bristolense*.

4. *Bacterium myxoides*.[1] — A particular case of a hæmorrhagic acute febrile disease which by most clinicians would be, and as a matter of fact has been, classed as hæmorrhagic small-pox may be here quoted. This is a case of a nurse, T., who was under the care of Sir Hugh Beevor. She was taken ill on December 4, and she died on December 9. The post mortem was ordered by the medical officer of the London County Council, Sir Shirley Murphy, to whom one of the attending physicians had notified the case as possibly one of septicæmic plague.

[1] Report of the Medical Officer, 1901-1902, p. 549 and *passim*.

"Post mortem on 9th. On exposing the surface of the abdomen, the lower part was found to be covered with a purpuric eruption, consisting of thickly-set pin-point petechiæ with larger blotches, some of which were the size of a split pea. The eruption was especially marked, roughly speaking, in a triangular area included between a transverse line drawn through the umbilicus and bounded below by two lines drawn across the front of the thighs parallel to and a few inches below Poupart's ligaments. Two chains of more scattered petechiæ extended from the main area of eruption upwards towards the armpits. On the arms and lower legs there were purpuric blotches here and there, but the main development of the eruption was in the situation already defined. There were conjunctival hæmorrhages and minute petechiæ on the pericardium. The pericardial sac contained several ounces of fluid. The appearances of the eruption and of the conjunctivæ were those characteristic of hæmorrhagic smallpox. The lungs had a few petechiæ, but otherwise seemed healthy."

Material was obtained from this body: blood which had been withdrawn aseptically from the right ventricle by means of freshly drawn-out glass capillary pipettes, afterwards sealed; and pieces of lung and pieces of skin of the abdominal and femoral region, which had been cut out under all necessary precautions. In the laboratory these samples were used for cultivation and experiment, pieces of the lung and of the skin being also placed into Müller's fluid for hardening.

The heart's blood was used thus:—

1. Film specimens were made and stained;
2. Agar surface plates were inoculated and incubated at 37° C.;
3. Guinea-pigs were subcutaneously injected.

The above proceedings were undertaken for demonstration of the presence of plague bacilli, the case having, as already mentioned, been notified as possibly plague.

Examination of specimens of the heart's blood yielded the following results:—

Microscopic film specimens, stained, showed numerous capsulated diplococci—each diplococcus consisting of two demilunes closely facing each other and invested in a distinct capsule. They were arranged either as single diplococci or more commonly as short (two diplococci) and longer chains (four diplococci). Fig. 56 shows a specimen of this kind. Not every microscopic field of the specimen contained these capsulated diplococci in such numbers as is shown in Fig. 56; never-

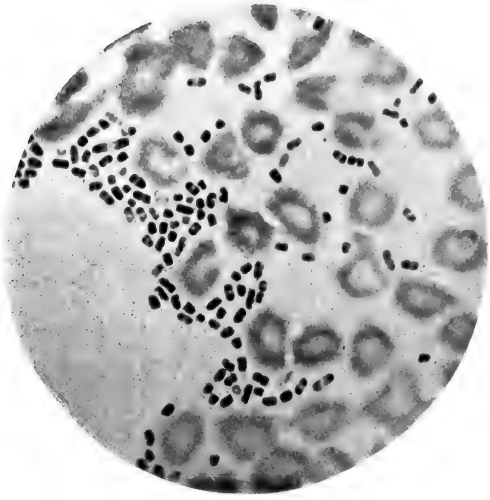

FIG. 63.

FIG. 64.

FIG. 63.

Specimen of heart's blood of a guinea-pig dead after intraperitoneal injection with *B. myxoides*, showing great numbers of the bipolar bacilli. × 1000.

FIG. 64.

From a section through the liver of a guinea-pig dead after subcutaneous injection with *B. pseudo-tuberculosis*. The liver was pervaded by nodules, some in the early stage (round cell masses), others already necrotised, near the surface of the liver (upper part of the figure); one such necrotic mass is shown, in which large and small aggregations (deeply stained masses) of the bacilli are recognisable. × 50.

FIG. 65.

From a section of a similar liver, showing the liver tissue pervaded by the nodules. × 23.

FIG. 66.

Section through a swollen Peyer's patch of the ileum of a guinea-pig infected by feeding with culture of *B. pseudo-tuberculosis*. The lymph follicles of the Peyer's patch are enlarged and necrotic. × 18.

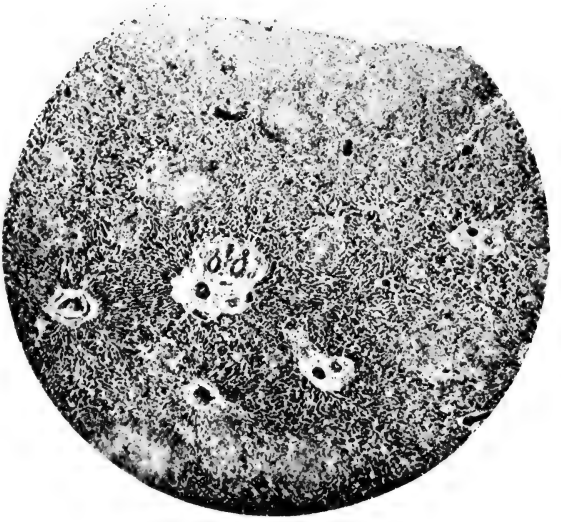

FIG. 65.

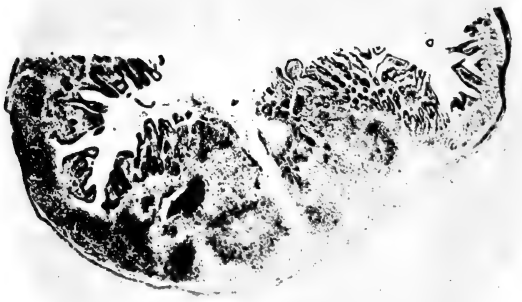

FIG. 66.

theless the microbe was on the whole very abundant, and it was obvious from microscopic examination alone that the case was distinctly one of blood infection—infection due, that is, to a *Diplococcus capsulatus*, the exact nature of which could, of course, only be determined by culture and by animal experiment.

The agar surface plate made from the original blood showed next day (but better still after forty-eight hours) an uncountable number of translucent grey colonies, in many places confluent into a grey filmy layer. These colonies in their general aspect were identical with those of *Diplococcus pneumoniæ*, and, like the latter, were made up of diplococci, either altogether wanting in capsule or possessed only of an indication of it. So, too, they were chiefly arranged as chains, some of these chains being composed of as many as eighteen to twenty diplococci. A small amount of the growth, a few colonies only, was used for making an emulsion, and with this emulsion mice and guinea-pigs were injected subcutaneously. The result was perfectly distinct and uniform; the guinea-pigs showed no illness, while the mice died. The post-mortem appearances in the latter case were those found in mice after infection with the *Diplococcus pneumoniæ*; the œdematous fluid of the seat of inoculation was found crowded with the capsulated diplococcus, either as single diplococci or as short chains of two elements. Fig. 57 is a characteristic specimen of this kind, the capsules being shown with great distinctness.

There can, then, be no question as to the identity of this microbe. It was the *Diplococcus pneumoniæ*, and it was present in the blood of the patient T. in very large numbers. As already mentioned, this patient had no pneumonia, while at the post-mortem examination only a few petechiæ were found in the lung. The case, therefore, was undoubtedly one of general blood infection with the *Diplococcus pneumoniæ*. That such a general infection—viz. copious presence of the microbe in the blood—existed in this case ante mortem may be taken as certain: cold weather prevailed at the time (December 9); moreover, the blood was obtained within twelve hours of death. It is altogether improbable, therefore, that an appreciable multiplication of the microbe had taken place after death, since the *Diplococcus pneumoniæ* requires for its growth and multiplication higher temperatures than could have obtained here. Below 25° C. the growth of this microbe in the laboratory is either nil or very delayed and slow.

The abundant presence in the circulation of a virulent microbe like the *Diplococcus pneumoniæ* must needs be of considerable importance, and in the particular instance may well have caused the severe constitutional illness and death. As will presently be seen, the microbe was present also in the skin in the hæmorrhagic spots, and although it may have been so present as a result of blood effusion, it might, on the other hand, by its intravascular toxin have been the primary cause of the destructive (chemical) vascular change leading to the hæmorrhage. It is well known that many microbes have such an (vascular) angiolytic action; as, for instance, all the pathogenic microbes which grow and multiply within the circulation, such as the whole group of bacilli causing hæmorrhagic septicæmia. To these must be added the *Bacillus pestis*, the *B. anthracis*, the *Diplococcus* (*lanceolatus*) *pneumoniæ* (when injected into the vascular system [1]), and as well some species of streptococcus and some virulent species of *Bacillus coli* (*e.g.* bacillus of aerobic malignant œdema, bacillus of Gærtner, bacillus of Danysz, and others). The destruction (chemical solution) of the wall of minute blood-vessels by the toxins of many microbes growing and multiplying within the circulation is indeed a well-known fact, and accordingly the hæmorrhage in the skin of Nurse T. may have been due to the copious presence of the *Diplococcus pneumoniæ* within the circulation.

The questions, therefore, that arise in connection with this case are these: If this was really a case of small-pox, was the presence of the *Diplococcus pneumoniæ* the cause of the hæmorrhagic condition? and is it also the cause of the hæmorrhagic condition in other cases of small-pox?

Before proceeding to seek for an answer to these questions it is necessary first to supplement the bacterioscopic analysis of the case of Nurse T.

The *Diplococcus pneumoniæ* present in this case in the blood (see film specimens and culture plate) in enormous numbers was not the only microbe found therein. In the film specimens of the blood now and again, but on the whole very sparsely, there were found single microbes without capsule, which on more careful examination were recognised as oval bacilli. On the agar plate, too, above mentioned, which had uncountable translucent small grey colonies

[1] The *Diplococcus pneumoniæ* seems to be capable of causing vascular disruption, even when growing outside, but close to, capillaries, *e.g.* the hæmorrhage into the exudation of the alveoli of the lung in acute croupous pneumonia causing the "rusty sputum."

of the *Diplococcus pneumoniæ*, there were present in addition, after twenty-four hours' incubation, eight to twelve colonies of an altogether different character. These were heaped-up whitish-grey round to irregular colonies several times larger than those of the *Diplococcus pneumoniæ*. Such colonies were composed of a viscid slimy material which under the microscope was seen to be made up of a hyaline transparent viscid interstitial or ground substance in which were embedded in close juxtaposition oval to cylindrical non-motile rods, thus representing a typical zoogloœa mass. In stained films the bipolar staining of the rods was very distinct; so that these bacilli are not dissimilar to those of *Bacillus pestis*. Subcultures were made from the primary colonies in the different media, and in these the characters of the microbe were found similar to those of microbes constituting the group of bacillus of Friedländer, but with this difference, namely, that the growth of the microbe in question was quicker, and that it formed more pronounced viscid slimy masses. Another point which distinguishes it from the *B. Friedländer* is that in culture it formed distinct zoogloœa; that is to say, the individual bacilli are barren of a separate capsule like the typical bacillus of Friedländer, but, unlike that microbe, are embedded in, and held together by, a slimy interstitial substance. The character, aspect, and rapid growth of the colonies, as well as their mode of growth in the different media, seem in general to distinguish this microbe from the *B. pestis*, which it closely resembles as regards polar staining, and also as regards length and thickness. Owing to the conspicuously slimy character of the growth of this microbe I propose calling it *Bacterium myxoides*. For the rest, in a general way it presents the characters of the microbes belonging to the group of *Bacillus Friedländer*; like these, it does not stain by Gram's method, and does not liquefy gelatine.

A point of constant difference between this *Bacterium myxoides* and *Bacillus Friedländer* is that the former, taken from the animal tissues, does not under any circumstances show a capsule. This, of course, can be demonstrated readily in the tissues after inoculation into animals.

Guinea-pigs and rats were, with the pure culture of *B. myxoides*, injected cutaneously and subcutaneously. But no effect whatever was hereby produced; the animals remained well. This of course proved also experimentally that the microbe was not *B. pestis*.

Intraperitoneal injection of the microbe into guinea-pigs, how-

ever, even in small doses, caused invariably a fatal result in twenty to thirty hours. The post-mortem appearances were as follows:—Copious grey viscid peritoneal exudation; intestines much inflamed; liver, spleen, and kidneys highly congested.

The peritoneal exudation of these guinea-pigs in stained film specimens showed crowds of oval to cylindrical bacilli, often in couples end to end. The conspicuous thing about them was their exquisite bipolar staining; no leucocytes and no other microbes were present. Plate cultures proved that the exudation was a pure culture of *Bacterium myxoides*. There was nowhere any indication of a capsule around individual bacilli, but the matrix in which the bacilli were embedded was a homogeneous viscid stainable substance, similar to that mentioned in regard of the zoogloœa matrix of the culture.

The blood of these guinea-pigs contained a very large number of the same microbes, viz. the *B. myxoides*; so much so that in a dried and stained film specimen of the heart's blood every field of the microscope contained great numbers of the bacilli; some fields, indeed, appeared crowded. The bacilli showed no trace of a capsule, but they all showed very distinct bipolar staining. The majority of the bacilli in the blood were rounded at both ends, but some showed one end, seldom both, as if cut away. The bacilli in the blood were distinctly thicker than those of plague.

The subcutaneous injection of culture of the microbe into mice always produced acute disease and death in three to four days. At the seat of inoculation inflammation and gangrene were apparent; the spleen was found enlarged, congested; the peritoneum inflamed; all the viscera were hyperæmic. The bacilli (*B. myxoides*) were readily demonstrated by film specimens and in culture, being very numerously present at the seat of injection, in the blood, in the spleen, and particularly in the peritoneal exudation; the latter appeared densely crowded with them. Rabbits are unsusceptible alike to subcutaneous and intravenous injection of large doses of culture.

It has been shown, then, that in the blood of Nurse T. there were present two virulent species of microbes,—the one, the *Diplococcus pneumoniæ*, in enormous numbers, so much so that its presence in the blood would be quite sufficient to account for the severe illness and death with hæmorrhages; the other microbe, the *Bacterium myxoides*, although not present in large numbers, is nevertheless a

microbe pathogenic for certain rodents. This *B. myxoides*, which morphologically and in staining presents a certain resemblance to *Bacillus pestis*, has cultural characters sufficiently distinct to differentiate it from the plague bacillus.

5. *Bacterium muris.*[1]—For some time back I have kept white rats—half-grown and adult—in my laboratory. They are generally in couples in clean and specially made cages.

On July 21 one of a couple of such rats was found dead. It had been observed to be ailing some days previously: its coat being rough, its breathing rapid, and the animal showing somnolence.

On post-mortem examination the following condition was found:—A portion of the left and the whole of the right lung were dark red. These portions were consolidated and sank in water; when cut into they appeared solid; there was caseous matter in the bronchi. The spleen was not enlarged. No swollen lymph glands could be found. The small intestine was congested, relaxed, and contained mucus and numerous gas bubbles. Film specimens made from the inflamed portions of the lungs showed numerous large and small masses of cylindrical and even filamentous bacilli. These when stained in methylene-blue exhibited pronounced metachromatism just like the diphtheria bacilli. Sections through the hardened lungs showed that the consolidation was due to distension of the alveoli, infundibula, and bronchi by fibrinous exudation; the latter containing numerous leucocytes and coloured blood corpuscles, and, as well, connected masses of the above bacilli. Cultivations made of the inflamed lung tissue yielded pure

[1] Report of Medical Officer, 1902-1903, p. 418 and *passim*.

cultures of one and the same bacillus, as also did the blood of the heart.

The companion rat died eighteen days later, and on post mortem showed precisely the same pathological condition and the same bacteria.

As far as the morphological characters were concerned, the bacterium in question appeared identical with Klebs-Löffler's *Bacillus diphtheriæ*, not only in regard of shape and non-motility, but also as regards staining characters. The microbe stains well by Gram's method; indeed gives positive Neisser staining; and shows, as mentioned already, distinct metachromatism in methyl-blue staining. Culturally, also, it resembles the diphtheria bacillus: on agar, on gelatine, on serum, and in broth the character of the growth is very much the same in both cases; like the diphtheria bacillus this microbe produces acid reaction in glucose broth. As regards its effect on animals, it is distinctly pathogenic for rats and for guinea-pigs. In both these animals it causes on subcutaneous injection a local tumour. Subcutaneous injection into the groin of a small or large dose of culture causes the formation of a gradually enlarging firm tumour, which in the course of ten to twelve days attains a very considerable size. The fate of the tumour is either a gradual resolution and absorption, or it becomes converted into an abscess which contains thick pus. In either case complete recovery takes place. The tissue of the tumour, and the pus of the abscess at all stages, contain the bacilli in abundance. I have not been able hitherto to produce any but a local result, be the injected dose small or large. Several other rats dead since with the same lung disease due to the same

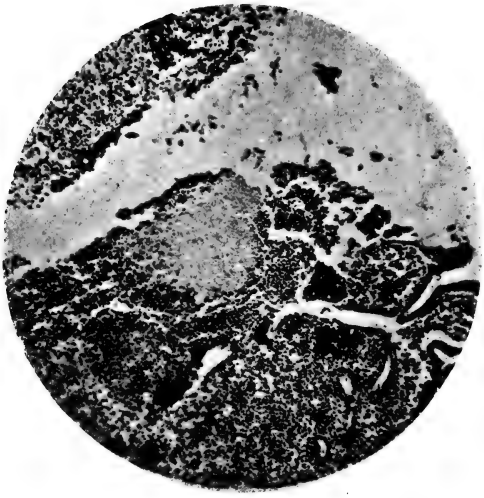

FIG. 67.

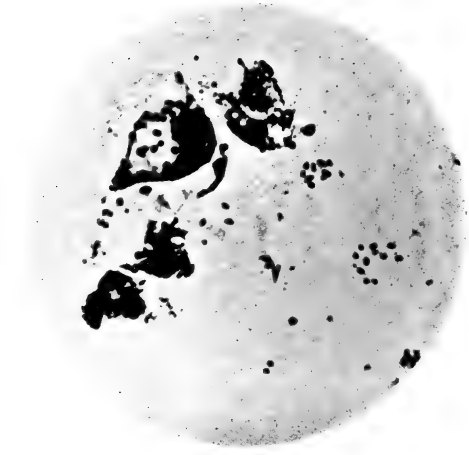

FIG. 68.

FIG. 67.

A portion of necrotic lymph follicle of a similar Peyer's patch as preceding figure, more magnified, showing deeply stained masses of the bacilli around it. × 65.

FIG. 68.

Film specimen of purulent caseous matter of inguinal lymph gland of a guinea-pig dead of pseudo-tuberculosis after injection into the groin. Leucocytes swollen and disintegrating, containing the bacilli. × 1000.

FIG. 69.

Gelatine surface plate showing colonies of *B. pseudo-tuberculosis*, after forty-eight hours' incubation. × 18.

FIG. 70.

Same colonies on agar plate, less magnified. × 5.

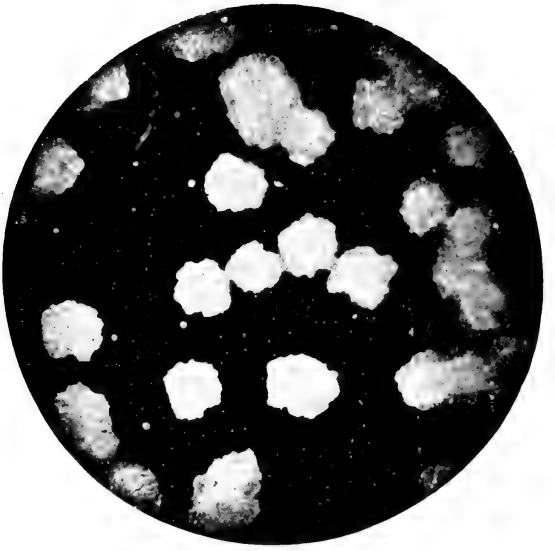

FIG. 69.

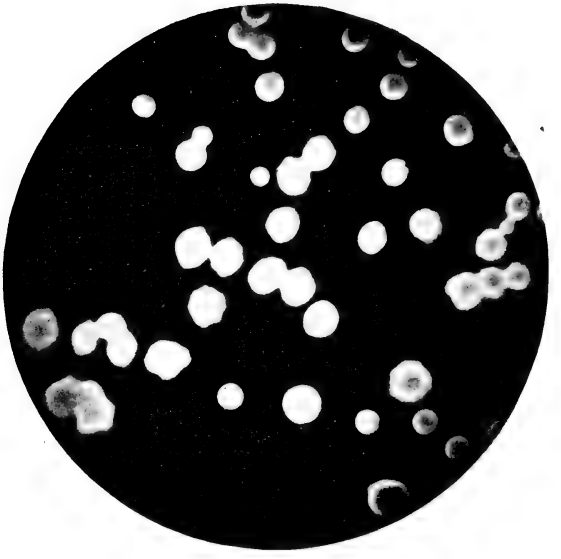

FIG. 70.

microbe, would suggest that the disease is naturally contracted by way of the respiratory organs.

It might be inferred from the foregoing description that this microbe is the diphtheria bacillus in an attenuated state of virulence. Against such conclusion there are, however, some very valid reasons: (*a*) the microbe acts well on adult rats, animals which are refractory towards even virulent diphtheria cultures; (*b*) diphtheria anti-toxin has no effect whatever in neutralising the action of even a small dose of this microbe. In this direction I have made a number of experiments. A dose in each instance of a recent culture of the microbe was subcutaneously injected into certain control guinea-pigs, while a similar dose of the same culture mixed with 400, 500, and even 600 units of diphtheria anti-toxin (obtained of Burroughs and Wellcome, as also of Allen and Hanbury) was injected into each of several other guinea-pigs. At the same time a dose of diphtheria anti-toxin (100 and 200 units) mixed with a lethal dose of culture of the true diphtheria bacilli was injected into each of another series of guinea-pigs. These latter remained without any disease, but the control animals and those injected with mixture of culture of the microbe which is in question and diphtheria anti-toxin developed the characteristic tumour. It is quite clear, then, that this microbe is in no way affected by the diphtheria anti-toxin, and that it is, therefore, not the true diphtheria bacillus, though it is a pathogenic microbe belonging to the group of diphtheroid bacilli. On account of its having been found in the rat I have named it *Bacterium muris*.

During the last twelve months I have had the oppor-

tunity of experimenting for purposes of studying the new plague prophylactic (which I described in a preliminary report to the Local Government Board, December 19, 1905) on a large number of white rats. Amongst these (two hundred odd), I have seen at different times spontaneous deaths in about half-a-dozen; on post-mortem examination they exhibited the caseous necrotic patches in the lungs (chiefly of the upper lobes), due to the presence of masses of the *B. muris*.

6. *Bacterium diphtheroides of Mice.*—For completeness' sake I add here the description of a microbe which distinctly belongs to the group of diphtheroid bacilli, is indeed closely related, if not identical, with the preceding *B. muris*, and therefore in morphology and by positive Gram staining can be readily distinguished from *B. pestis*; as on several occasions I have met with it in mice, it might not be out of place to mention this microbe here, as it is pathogenic to mice, and as these animals are very useful and often used in experimental and diagnostic work for plague.

The history of this pathogenic diphtheroid microbe is as follows:—

A guinea-pig had been inoculated cutaneously on February 18, 1905, with a trace of an agar culture (forty-eight hours old) of *B. pestis* derived originally from the spleen of a rat dead of plague. The guinea-pig was found dead on February 22 (fifth day). On post-mortem examination it showed large hæmorrhagic bubo crowded with *B. pestis*; almost the whole of the intestine showed numerous petechiæ in the serous coat; both testes showed the superficial lymphatics injected with blood; the pelvic lymph gland was enlarged and hæmorrhagic; the spleen was large, mottled with grey nodules and crowded with *B. pestis*; the liver was pervaded by whitish punctiform nodules; the suprarenals were hæmorrhagic. From this it follows that the guinea-pig had died of typical subacute plague. The spleen and the bubo of this guinea-pig were finely minced, and a small amount of this material was placed on bits of cloth, which were then kept under a bell jar and left to dry at the temperature of the laboratory. On March 8, *i.e.* after fourteen days' drying, some of the dry particles were taken off the cloth and rubbed down in sterile distilled water, and from this emulsion

a few minims were injected subcutaneously into the dorsum of two mice.

One of these mice was found dead next morning, *i.e.* within twenty hours. The second mouse was very ill—lumpy, coat rough, eyes closed, breathing very rapid; it was found dead the next morning, *i.e.* within forty hours.

At the post-mortem examination both mice showed the following appearances:—At the seat of inoculation the subcutaneous tissue was inflamed, œdematous, and with hæmorrhagic spots; the intestines and lungs were very hyperæmic and showed hæmorrhagic spots; the spleen was large and dark. Neither in the subcutaneous exudation nor in the spleen or lungs could any *B. pestis* be discovered, either in stained film specimens or by culture.[1] But in the subcutaneous exudation were numerous bacilli in clumps and in streaks which could at once be recognised as diphtheroid: small bacilli pointed at one end, some with clubs and showing segregation of their chromatic substance; they stained positive in Gram. Cultures on agar surface and on agar plate were made, and in these came up in pure state numerous colonies of the same diphtheroid bacilli. Subcutaneous injections with these cultures were made into mice and guinea-pigs, and thereby it was shown that the microbe was possessed of distinct pathogenic action: in the guinea-pigs the subcutaneous injection into the groin of a moderate dose of culture—several drops of turbid emulsion—caused after forty-eight hours slight but firm enlargement of the inguinal glands; this enlargement gradually increased, till in about ten days the tumour was of the size of a filbert, changing at the same time from a firm tumour into a tumour containing thick pus. The pus contained crowds of the same diphtheroid bacilli, many in small and large masses. None of the guinea-pigs died, and the animals remained the whole time apparently lively and feeding. The mice showed constitutional disturbance for two or three days, being quiet and off feed, but they soon recovered and showed tumour at the seat of injection. This tumour about the end of a week or ten days began to ulcerate, and became covered with a dry scab about $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter. The mice recovered, and after about three weeks the place was healed up. Several other experiments were made with the culture in guinea-pigs and mice with the same result.

[1] In a subsequent chapter it will be shown that death was due to plague toxin present in the dried plague tissues.

The microbe in question shows the morphological characters of the *B. diphtheriæ* of the short variety, it gives but feeble Neisser granules, and it does not act fatally on guinea-pigs or mice, be the injected dose large or small. The microbe, as stated just now, is strongly Gram-positive, it produces acid in glucose broth after forty-eight hours' incubation at 37° C. On gelatine at 21° C. its growth is extremely retarded, not before the fourth day is there a noticeable growth to be detected, and when it has started, the colonies are and remain very minute and very translucent. In this respect it differs both from the true *B. diphtheriæ* and from the *B. muris*. On account of its action on the guinea-pig being similar to that of *B. muris*—causing the gradual formation of abscess—and on account of its having been obtained from the mouse, I conclude that it is the *B. muris*, or a variety of the microbe obtained from the lung of rats (see previous pages); but the diphtheroid microbe of the mouse is shorter, gives but feeble Neisser, and grows much more slowly on gelatine than the typical *B. muris* of the rat. I do not think the two are quite identical, although it must be inferred that they are clearly different varieties closely related to one another. I am confirmed in this by the result of their culture in different sugar media.

Dr. Gordon informs me that both the *B. muris* of the rat and the diphtheroid bacillus of the mouse exhibit exactly the same reactions in the different sugared media—reactions which not only prove their mutual relation, but distinguish them from the *B. diphtheriæ*.

	B. diphtheriæ.	*B. muris.*	Bacillus of Mouse.
Glucose . .	+	+	+
Saccharose . .	−	+	+
Salicin . .	−	+	+

+ means acid in two days.
− means no acid in two days.

The question of interest is, Whence is derived the diphtheroid microbe—*B. muris*—of the mouse? Was it present in the organs (bubo and spleen) of the guinea-pig dead of subacute plague; was it present in the cloth on which the particles of those plague organs had been dried; or was it present in the skin of the experimented mice, and carried during the subcutaneous injection of the emulsion into the subcutaneous tissue, where, owing to the inflammatory effect of the plague toxin, it multiplied?

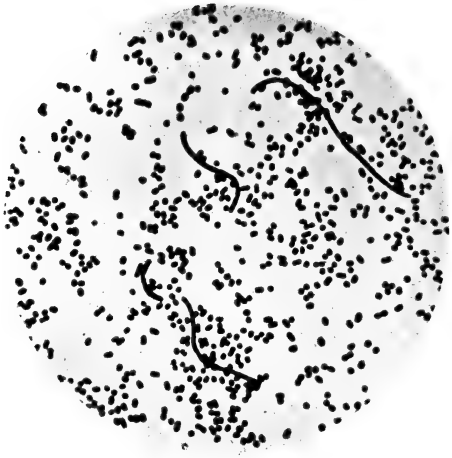

FIG. 71.

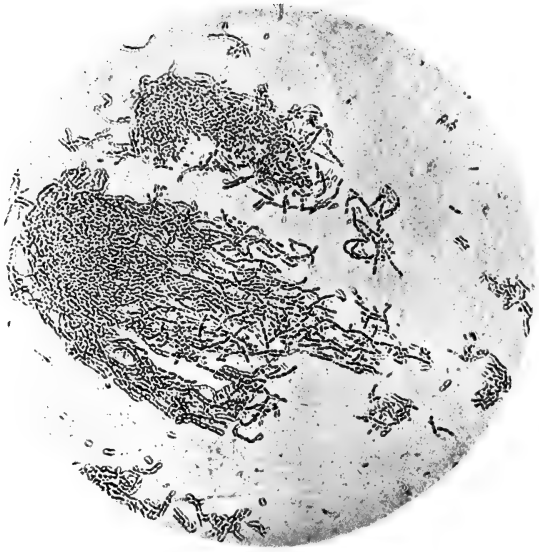

FIG. 72.

FIG. 71.

Film specimen (stained) of *B. pseudo-tuberculosis*, from agar colonies twenty-four hours old. × 1000.

FIG. 72.

From an unstained broth culture of *B. pseudo-tuberculosis*, incubated twenty-four hours at 37° C.; showing a "granule" or flocculus composed of chains of the bacilli. × 400.

FIG. 73.

From an impression (film) specimen of a young colony on a gelatine plate of *B. pseudo-tuberculosis.* × 1000.

FIG. 74.

Stained blood film of *B. equi* from rabbit's heart blood. × 1000.

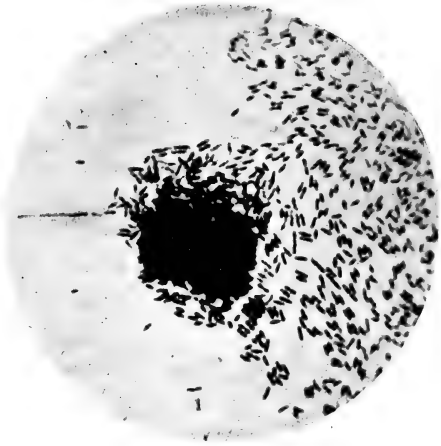

FIG. 73.

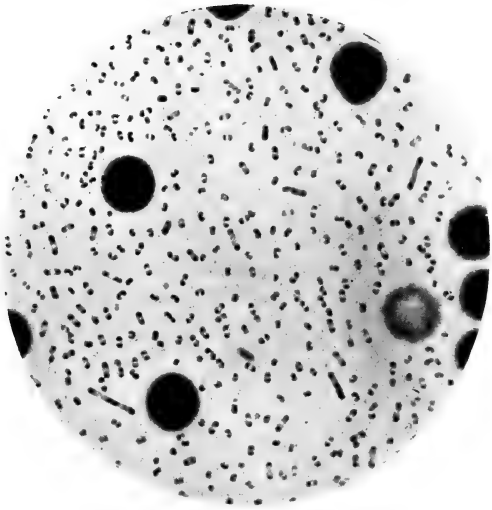

FIG. 74.

The first and second source can, I think, be left out as being highly improbable; the third appears the more probable source—viz. that this microbe inhabited the skin of the mouse, and under conditions of inflammation is capable of rapidly multiplying. Diphtheroid bacilli of the skin in and over inflamed and pathological areas occur both in man and the rat, but they are without any pathogenic action—are, in fact, *B. xerosis*. I have mentioned the occurrence of *B. xerosis* in the skin over the inflamed bubos; Dr. Dean has described this microbe (*Journal of Hygiene*, 1905) in connection with the lepra-like nodules in rats; but in all these latter instances the microbe was quite without any pathogenic action.

As regards the *B. muris* of the rat, on account of its being associated generally with the natural lung disease, it appears feasible to assume that in this animal it enters by way of the respiratory organs, and causes here the local inflammation and necrotic change of the lungs.

7. *B. Danysz and B. Gaertner.*—The *B. Danysz*, first isolated by Danysz, is a microbe which belongs to the coli-typhoid group. As I have previously shown (*Transactions of the Pathological Society*, London, 1902, p. 342), and as I have recently been able to confirm, the *B. Danysz* is in all and every respect identical with the *B. enteritidis Gaertner*.

According to Danysz, the microbe causes acute septicæmic infection and death of rats by subcutaneous or intraperitoneal injection, as also in a certain percentage of the animals by feeding, and therefore, says Danysz, cultures of this microbe, if of the proper virulence, can be used with good effect in the destruction of rats, for the rat dead after feeding serves as infective material for rats eating its body. That the Danysz bacillus causes acute septicæmic infection of the rat after subcutaneous and intraperitoneal injection with copious distribution of the bacilli in the blood and in the viscera, is a matter easy of demonstration; but it is more difficult to confirm the statement that the *B. Danysz* does not act on other rodents, or that it can be relied upon to infect an appreciable number of rats with the fatal disease by feeding them either on culture or on animals dead of the disease. In the first place, the microbe injected subcutaneously or intraperitoneally is as virulent to the guinea-pig and the mouse as it is to the rat, and, in the second place, its efficacy by feeding is somewhat uncertain. Dr.

Williams and myself have published in the *Lancet* (1902) the results that have been obtained in one of the London docks with a large number (60) of cultures of the microbe (bought directly of Danysz), as also with the bodies of guinea-pigs, mice, and rats dead after subcutaneous injection with the microbe. The cultures—bread soaked with broth emulsion of the microbe—as also the infected bodies had been removed and eaten by the dock rats, but neither have any dead rats been found in consequence, nor was the number of the dock rats noticeably reduced. Other observers who have experimented in the same manner have also had purely negative results. In some instances undoubtedly positive results, *i.e.* destruction of rats by placing infected materials (bread soaked in culture), have been achieved; while in still other instances it was shown that not only is the breed of rats an important factor, but that even the same breed of rats coming from different localities are affected differently.

Quite recently, owing to an advertisement that had appeared in the *Times* and in the *Meat Trade Journal*, I have bought a tube of Danysz culture at the offices of the "Danysz Rat Microbe Company" in Leadenhall Street, London. The experiment of feeding rats was strictly carried out according to the printed directions: viz. the whole of the contents of the agar slope—the growth, as also the agar itself—were emulsified in and well mixed with broth, and in this emulsion bread was soaked. This was then given as food to three white rats. The animals had eaten up the whole within the next few hours. The result was this: one rat died after two weeks, the other two survived. With a fair dose of the same culture a rat was subcutaneously injected at the same time that the food was being prepared; this animal died after four days, thus showing that the culture sold to me was not very virulent. If the culture had been really of normal virulence, the animal would have been dead within two days. But the fact remains that this culture was sold by the Danysz Company as capable of destroying rats by feeding, which character it only partially possessed, as was proved by the experiment.

The Danysz bacillus behaves in gelatine and agar surface plates and in gelatine and agar streaks exactly as the microbes of the coli group, and these need not be further detailed. It produces gas in glucose gelatine shake culture, the colonies extending all through the gelatine; it causes acid and gas production in MacConkey fluid, but less than *B. coli communis* or *B. Gaertner*;

it bleaches litmus lactose peptone without gas production, just like *B. Gaertner*; it forms a colourless filmy growth on steamed potato, like *B. Gaertner*; it produces in litmus milk, like *B. Gaertner*, at first very slight redness, this soon gives way to alkali production, the litmus milk becoming blue till it is of a dark-blue slate colour, the milk remaining fluid; it grows well in phenol broth, causing uniform turbidity like *B. Gaertner*; it produces slight indol in broth after a week, like *B. Gaertner*; it forms "blue" colonies on Drigalski-Conradi medium at 37° C. like *B. Gaertner*; the colonies are smaller than those of *B. typhosus*, but, like these latter, are at first more or less round, later they are angular and at all times are raised in the centre, flat at the margin, their substance finely granular: characters, in short, identical with those of *B. Gaertner*. The bacilli are oval to short cylindrical rods, actively motile in the hanging drop, and Gram-negative; they are provided with many flagella all round. Of precisely the same characters, morphological, cultural, and experimental—at any rate as regards the guinea-pig and mouse—is the typical *B. enteritidis Gaertner*; and it is not necessary to specially enumerate them, having described them in full just now of the *B. Danysz*.

The identity of the two microbes—*B. Danysz* and *B. Gaertner*—has been confirmed also by the following experiments (*Transactions Pathological Society*, 1902, p. 243):—(*a*) Guinea-pigs immunised by *B. Danysz* were found immune against *B. Gaertner*; (*b*) guinea-pigs immunised by *B. Gaertner* were found immune against *B. Danysz*; (*c*) blood serum of guinea-pigs immunised by *B. Danysz* agglutinates equally well an emulsion of *B. Danysz* and of *B. Gaertner*; (*d*) blood serum of guinea-pigs immunised by *B. Gaertner* agglutinates equally well an emulsion of *B. Gaertner* and of *B. Danysz*. Recently I have carried out the same experiments with rabbits. A rabbit was immunised by repeated injections of sub-fatal doses of living *B. Danysz*, a second rabbit by repeated injections of sub-fatal doses of living *B. Gaertner*. The blood serum of both animals was then tested on emulsions of *B. Danysz* and of *B. Gaertner* and was found to agglutinate emulsions of both microbes equally well.

I therefore consider that both microbes are identical.

Considering that acute gastro-enteritis or some forms of it have been shown to have been caused by the eating of meat (beef, pork) contaminated with the *B. Gaertner*, it seems rather a risky thing to follow the recommendation of the Danysz Company, printed on their

advertisements, viz. to spread about in meat stores and the like, cultures of the *B. Danysz*, with the object of destroying rats; for it might happen that while the intended rat destruction fails, the meat pollution and subsequent human infection might succeed.

The rapid growth on gelatine and on agar, the motility of the microbe, the production of acid and gas in glucose gelatine, and in MacConkey fluid, and the inefficacy of cutaneous inoculation of guinea-pigs, mice, and rats, would at once guard against the microbe (*B. Danysz* and *B. Gaertner*) being mistaken for *B. pestis*.

8. *Bacillus pseudo-tuberculosis*.—This microbe causes in the guinea-pig, when injected subcutaneously or intraperitoneally, or by feeding, the well known sub-acute process which in a marked manner resembles, as I have fully described and illustrated in a Report to the Local Government Board (1899-1900), the chronic process of "inoculation-tuberculosis." The microbe, first discovered by A. Pfeiffer, was in that Report fully described in its morphological, cultural, and experimental characters. It was shown that the microbe differed markedly in these respects from all others. I would not introduce it here, were it not that in its effects on the guinea-pig it bears a resemblance to subacute plague in the guinea-pig, only in this that like the *B. pestis* of decreased virulence it causes necrotic nodules in the spleen, liver, and lung, and since the guinea-pig is generally, or at any rate often, used for diagnostic purposes as regards plague, it may not be out of place to consider the subject here. The production of necrotic nodules in the spleen, liver, and lung by the *B. pseudo-tuberculosis* is the only ground for considering this microbe in connection with plague, the two microbes being in other respects totally unlike. On account of the above effect on the guinea-pig, Galli Valerio and others have taken the trouble to make a comparison between the two microbes, but in my opinion he would be a very superficial observer who could make a confusion between the two microbes.

In the first place as regards the effect on the animal:—

The *B. pseudo-tuberculosis* is without any effect when inoculated cutaneously; neither the mouse, nor the guinea-pig, nor the rat shows any symptoms of abnormal change when thus inoculated with it. The rat is quite unsusceptible even when injected subcutaneously. As regards the guinea-pig, the *B. pseudo-tuberculosis* injected subcutaneously in the groin causes a comparatively slow process, consisting in the gradual enlargement of the inguinal gland, but it

causes no hæmorrhage or œdema in the surrounding tissue; the enlargement commences in two or three days, and by the end of the week—at the earliest—the gland has been converted into an abscess. Injected in small doses intraperitoneally it does not cause acute peritonitis and death in a day or two with viscid copious exudation as *B. pestis* does; for with *B. pseudo-tuberculosis* death occurs not earlier than seven or eight days, generally later; on post-mortem examination the omentum and the pancreas are thickened, and contain smaller and larger firm nodules with purulent caseous centre. The necrotic nodules of the spleen, liver, and lung are typical in their occurrence in guinea-pigs injected subcutaneously with *B. pseudo-tuberculosis*; they are found after death of the animal, *i.e.* after seven days, generally between nine and sixteen days' duration of the illness. I have repeatedly pointed out that necrotic nodules in the spleen, liver, and lung of guinea-pigs occur also when these animals are injected subcutaneously with *B. pestis* of subnormal virulence, and that they constantly occur in them when inoculated cutaneously, but the time in which they appear is very much shorter than with *B. pseudo-tuberculosis.*

The morphological and cultural characters of *B. pseudo-tuberculosis* I have described in detail in the Reports of the Medical Officer of the Local Government Board, 1899-1900; those of *B. pestis* on a previous page; and I will here summarise the essential differences only:—

(1) In the affected organs the *B. pseudo-tuberculosis* occurs generally within cells, *e.g.* in those of the nodules of the omentum and pancreas; it is shorter than the *B. pestis,* does not stain so readily as the latter, and does not show the typical bipolar staining.

(2) Taken from culture *B. pseudo-tuberculosis* shows feeble Gram staining.

(3) *B. pseudo-tuberculosis* grows much faster on agar and its colonies are decidedly less translucent, being more whitish than those of *B. pestis.*

(4) *B. pseudo-tuberculosis* on gelatine forms colonies decidedly more translucent than *B. pestis,* and they are not granular, or distinctly less so than those of *B. pestis.*

(5) Particles of agar culture of *B. pseudo-tuberculosis* emulsify readily in saline solution or bouillon; those of *B. pestis,* as mentioned in a former chapter, are viscid and do not emulsify readily.

(6) In broth *B. pseudo-tuberculosis* forms in a week a distinct pellicle, at any rate at the rim of the surface. *B. pestis* does not do so.

(7) *B. pseudo-tuberculosis* in litmus milk has a tendency to form alkali; *B. pestis*, on the other hand, has a slight tendency to form acid.

(8) *B. pseudo-tuberculosis* on potato forms a straw-coloured, pale-yellow growth; *B. pestis* forms no visible growth on potato.

It is therefore obvious that, apart from these last points, the different staining characters, the rapid growth on agar, the slow and different action on the guinea-pig both subcutaneously and peritoneally, and the absence of pathogenic action by cutaneous inoculation are quite sufficient to enable even the inexperienced readily to distinguish the two microbes.

Bacillus of Fowl Cholera and Allied Microbes.—I add this microbe, because it has the well-known character of staining bipolarly, of being Gram-negative, of producing on agar a translucent growth, which, like that of *B. pestis*, does not emulsify well and is more or less tenacious. I do not think that this group of microbes can be easily mistaken for *B. pestis*; but it has to be remembered that, although of rare occurrence generally, in some places it is fairly widely distributed, and further, that the subcutaneous injection into rodents of materials containing this microbe causes hæmorrhagic infiltration with crowds of bipolarly stained bacilli. This character of showing bipolar staining, and of yielding on agar surface round, translucent, cohesive colonies, might lead one, not fully acquainted with this group, to assume that he is dealing with *B. pestis*. I have quite recently come across a microbe which is a close relation to the bacillus of fowl cholera, and which at first sight might be still more easily mistaken for *B. pestis*; this microbe (*B. equi*) had been obtained from the blood of a horse spontaneously dead.

The bacillus of fowl cholera, although giving distinct bipolar appearance on staining, is smaller than the *B. pestis*, but the *B. equi*, which also conspicuously shows bipolar staining, is larger than the bacillus of fowl cholera and nearly as large as the *B. pestis*. The photo shown (Fig. 74) is the *B. equi* of the blood of a rabbit dead within twenty hours after intravenous injection with *B. equi*. It will be seen that except for the enormous number of the microbes present in the blood—in plague the bacilli, even under the most favourable conditions, are never present in the blood in such numbers—the aspect of the bacilli both as to size and staining is remarkably like that of *B. pestis*.

The members of the group of bacillus of fowl cholera do not act

on the rat, do not cause in the guinea-pig or in the mouse the large firm spleen characteristic of plague; they do not form the "granular" irregular conical colonies on gelatine as *B. pestis* does. On agar they form more or less flat colonies. They make broth turbid and form indol. Injected into the veins of rabbits the members of this group cause death within twenty hours; *B. pestis* in this way administered does not cause death before two or three days or later. As is also well known, bacillus of fowl cholera subcutaneously injected causes death of rabbits and pigeons in less than two days. The *B. equi*, however, takes much longer in the rabbit (four days), causing pseudo-membranes on the viscera, and is non-pathogenic to the pigeon. From this it is evident that a diagnosis would have to take account of the morphological and cultural, as well as the experimental data. A mistake would, however, be possible if diagnosis were made, for instance, on morphological grounds alone, *e.g.* non-motility, bipolar staining, and being Gram-negative; or say, on the ground of the nature of an agar culture, forming a translucent cohesive film on the surface of the agar; or, say, by its subcutaneous action on rodents.

Kister and Schmidt (*Centralbl. f. Bakt. und Parasit.* vol. xxxvi. p. 454) found in some rats dead on board ship a microbe which showed bipolar staining and which belonged to the group of bacteria causing hæmorrhagic septicæmia. The peculiarity of the microbe of these observers was, that it proved virulent even for rats and cats. It was highly virulent for other rodents.

CHAPTER V

PLAGUE IN THE RAT

In the 33rd Annual Report of the Medical Officer of the Local Government Board (1903-1904), Dr. Theodore Thomson fully considered (pp. 317-329) the question of the relation of plague in the rat to plague in man. He has carefully on the basis of official data collected the facts bearing on (1) the danger of transmission of infection from shore to ship by plague-infected rats, (2) the danger of transmission of infection from plague-infected rats to man on board ship, and (3) the danger of transmission of infection from ship to shore by plague-infected rats.

Dr. Thomson arrives (p. 320) at the same conclusions which I have expressed in the Medical Officer's Report for 1902-1903, viz.: that in considering mortality among rats on board ship, it is not necessary that this should be due to plague, "but may be caused by other maladies in no way related to that disease." "And we further know that one of these maladies is characterised by the presence in the rat of a micro-organism that cannot be clearly differentiated from that of plague by microscopic examination alone, but must have culture and experiment." "So that it is open to serious doubt

whether the numbers I have given of ships that have had plague among rats on board them should not be materially reduced"—that is to say, the instances in which the diagnosis relied only on microscopic examination, and was not supplemented by culture and animal experiment.

Dr. Thomson, in respect of danger (1) takes (pp. 318-319) "the four years 1898-1901, and finds that in 95 ships plague appeared, whether in rat or man; on 58 of these vessels plague was observed in man only; on 28 it was observed in both rats and man; while on 9 it was observed in rats only."

We quote here some of the passages of Dr. Thomson's Report, p. 319 and *passim*:—

"In all, therefore, plague among rats was observed on 37 ships during the four years in question. As regards the 28, out of these 37, on which both man and rats were affected, it may, in some cases at least, be questioned whether the primary source of infection from shore was the rat or the human subject. To this point reference will be made later. Be this as it may, however, these data seem to indicate that transference of infection from shore to ship is more likely to occur through man than by the rat. Nor do they point to a serious degree of danger of this sort, since only some nine vessels per year are recorded as having developed plague among rats on board.

In this connection, it is of interest to consider the number of vessels leaving Bombay, a plague-infected port, for ports out of India, during the period 1898-1901, on which plague, whether among rats or men, is known to have made its appearance after departure from Bombay. The measures taken at Bombay as regards these vessels prior to their departure were medical examination, by day and on shore, of every person proposing to sail by them; disinfection of clothing, bedding, and effects of native crew, third-class and deck passengers; attention to the sanitary condition of the vessels, leading to such action as cleansing and disinfection of crew's quarters, cleansing of bilges, and, in the case of ships in ballast, the cleansing of 'tween decks and holds.

In the four years, 1898-1901, 3408 vessels left Bombay for ports out of India. On the voyage plague appeared on board 20 of these vessels. It was observed in man alone in 16 of the 20; in both man and rats in 3; and in 1 in rats alone. In detail, the figures are as follows:—

Years.	In Man alone.	In Rats alone.	In both Man and Rats.
1898 . . .	5	...	2
1899 . . .	4		
1900 . . .	3	...	1
1901 . . .	4	1	
1898-1901 . .	16	1	3

[I note here, in fairness to Bombay, that it is not clear in some of the above instances that the infection had been derived from Bombay.]

These figures as regards Bombay shipping suggest, it will be seen, the same inference as do those concerning shipping in general. And they possess a special interest, since the measures I have quoted as in force at Bombay in respect of ships outward bound for ports out of India comprise nothing specially intended to secure destruction of rats on board ship. This notwithstanding, known appearance of plague among rats on board these vessels was extremely rare.

There is another consideration to be borne in mind, the effect of which is to minimise still further, in relation with alleged appearances of plague among rats on board ship, the apprehended degree of danger of transmission of this disease by rats from shore to ship. In not a few of the instances which I have elected to class as 'Plague among rats,' because narrators of the circumstances chose to so regard it, the evidence of this depends wholly upon the existence of notable mortality among rats, or, at best, upon microscopical examination alone; while, in other instances, it is not clear that adequate bacteriological examination, physiologically confirmed, had been made. But we know, from experience in this country, that notable mortality among rats on board ship is not necessarily due to plague, but may be caused by other maladies in no

way related to that disease. And we further know that one of these maladies is characterised by the presence in the rat of a micro-organism that cannot be clearly differentiated from that of plague by microscopic examination alone; and that, indeed, definite differentiation cannot be satisfactorily established without physiological experiment in addition to microscopical and cultural examination. So that it is open to serious doubt whether the numbers I have given of ships that have had plague among rats on board them should not be materially reduced.

But, on the other hand, it is claimed that plague may affect rats on board and yet remain unrecognised, since only a notable mortality among them is likely to attract attention, while the presence of plague among rats may not necessarily set up such a mortality. Certain observations that point in this direction will be cited at a later stage of this memorandum."

(2) *The danger of transmission of infection from plague-infected rats to man on board ship.*

"The number of ships on which plague is known to have occurred, among man or rats or both man and rats, during the period 1898-1901, according to the records I have consulted, is, as already stated, 95. The yearly distribution is as follows:—

Years.	In Man alone.	In Rats alone.	In both Man and Rats.
1898 . . .	12	1	4
1899 . . .	13	...	5
1900 . . .	16	...	8
1901 . . .	17	8	11
1898-1901 . .	58	9	28

These figures are subject to two criticisms of opposite intention. On the one hand, it may be contended that some of the instances classed as 'man alone' were in reality instances in which there was unrecognised plague among rats on board. On the other, it may be claimed that in some instances the disease observed among rats was not plague.

In support of the first of these criticisms it might be pointed out that the proportion of vessels alleged to have had plague among rats is larger in 1901 than in any previous year, due, it may be alleged, to more careful search for suspicious sickness among these rodents. It is difficult to appreciate the just value of this criticism by reason of there being in many instances entire absence in the accounts given, of any statement as to whether or not there had been sickness among rats. In the 86 vessels (out of a total of 95) on which plague appeared in man or in both man and rats, the proportion in which definite statement is recorded as to the 'health' of rats on board appears from the following table :—

Years.	Definite Statement.	No Statement.
1898 . . .	4	12
1899 . . .	10	8
1900 . . .	9	15
1901 . . .	14	14
1898-1901 . .	37	49

There is nothing in the figures for the last three years of the period 1898-1901 which appears to justify belief that increasing watchfulness had led to detection of rat-sickness in an increasing proportion of vessels on which there was human plague; the figures for 1898, however, are more open to doubt. On the whole, it seems reasonable to assume that, in most cases, no statement as to the health of rats implies that no sickness had been observed among them. This, however, does not exclude the possibility, already referred to, of plague-sickness having been present among rats in a form not readily recognisable. It is therefore possible that there may have been instances among the 58 vessels where only human plague was recognised, in which rats also were affected.

In regard of the second criticism, that the disease observed among rats may not have been plague in all instances, it is to be noted that in the 28 ships in which both man and rats were affected, positive bacteriological evidence as to its nature in these rodents is recorded in seven instances only. As regards the 9 vessels in which the disease affected rats alone, there is positive

bacteriological evidence in six instances, and in two instances microscopical evidence only. I do not think, however, that mere absence of positive evidence of this sort can properly be regarded as excluding presence of plague among rats; but such absence must be held to have weight when other circumstances tend to cast doubt upon the assertion that a particular rat sickness was plague.

Subject to such allowance as may be made for these considerations, I proceed to examine in detail the figures I have given under this head.

As regards the 58 vessels on which plague is not known to have made its appearance save in man, these instances seem to point the moral, at present overlooked by many, that, whatever the degree of danger to persons on board ship from infected rats, the latter condition is not the greatest danger. So marked is the difference between the number of vessels (58) in which human plague only is recorded and the number (28) in which plague is stated to have occurred in both man and rat, that this proposition must, I think, stand, even if some transference had to be made from the first class to the second. These figures certainly do not support the contention that the rat is the chief agency by which plague is transmitted to man. That such view is erroneous is further indicated by consideration of the circumstances in which plague made its appearance on the 28 vessels in which both man and rat are stated to have been affected. As regards these vessels it has been too readily assumed that it was in all cases the rat that gave the disease to man; to the exclusion of the consideration that in reality man may have infected the rat. Again, the mere fact that one or two dead rats had been found on board a ship with human plague has been taken, without further evidence, as proof of there having been rat plague on board—with inference that here was the source of the human plague. . . ."

(3) *The danger of transmission of infection from ship to shore by plague-infected rats.*

"Under this head I propose to consider, in the first instance, how far plague is known to have spread to places on shore in 1898-1901 from the 28 ships stated to have had both human plague and rat plague on board, and from the 9 ships stated to have had rat plague only.

From 4 of the 28 vessels above referred to, plague is stated

to have spread to places on shore. But as regards two of these, viz. the *Friary* in 1901 and the *Niger* in 1900, the spread on shore is not alleged to have taken place through the medium of infected rats, but occurred from direct contact with sick persons. Indeed, as regards the *Friary*, bacteriological examination of rats on board at the port of arrival gave negative results; while, in the case of the *Niger*, it is not clear that, on the particular voyage in question, rats on board had plague. The *Centauro*, however, which reached Ascension on 26th April 1899, is stated to have set up plague there through plague-infected rats on board making their way on shore. Four of the *Centauro's* crew were attacked by plague on the 27th and 28th April; they were taken ashore and three of them died. In the *Centauro's* hold, while cargo was being discharged, some thirty dead rats were found. A fortnight after her arrival an epidemic was observed among rats on the Custom House premises. There was not at the time any suspicion as to the disease, among either rats or men, being plague. Plague was not, in fact, recognised in Ascension until the following September. Other ways in which the disease may have reached Ascension have been suggested; and it is not clear whether the *Centauro* was responsible or not for its introduction to this place.

The *Gironde*, also, is credited by one authority (Dr. Borel) with having infected Lorenço Marques in 1898. She reached Lorenço Marques on 21st October, and when her holds were opened there large numbers of dead rats were found. After a week's stay she left, and five days later human plague appeared on board. It is affirmed that during discharge of the *Gironde's* cargo at Lorenço Marques infection was conveyed ashore by rats. Plague was recognised in this place towards the end of the following November. On the other hand, it has also been alleged that there probably was plague, in unrecognised form, in Lorenço Marques at the time the *Gironde* called there, and that the *Gironde* was infected from Lorenço Marques.

It must be observed, however, that most of the 28 vessels under consideration had been fumigated at the port of arrival with a view to secure destruction of rats on board; which may be taken to have diminished the risk of infected rats making their way ashore. Among exceptions were the *Marienburg* and the *South Garth*, in 1900, in which fumigation was not resorted to until after discharge of the whole cargo; seemingly also the *Dundee* in the same year; and

perhaps two or three others, in respect of which it is not clear what measures were taken.

As regards the nine vessels in which rat plague alone was observed, the disease is alleged to have spread to places on shore in one instance, viz. the *Y.*, account of which has been given on a previous page. So far as these places—Port Louis, Réunion, and Tamatave—are concerned, it seems probable that plague appeared at Port Louis within a month of the *Y.* having called there. Tamatave seems to have been plague-infected about the time of that vessel's arrival—Réunion not for several months later. The *Y.*, it will be remembered, never had any suspicious human sickness on board during her long voyage of some three months; and it may be doubted whether the rat disease really was plague. One of the crew attributed the rat mortality on the *Y.* to poison which he had laid down for them. In the remaining eight vessels no spread took place from ship to shore; and this notwithstanding that, in at least five of these vessels, the cargo had been partly or wholly discharged before the infected rats were discovered, and therefore before any measures had been taken to secure destruction of rats. Indeed, in the case of the *Chios*, at Hamburg, part of the cargo had been conveyed into the interior. In none of these eight vessels, however, did plague attack the persons unloading or handling the cargo, nor is plague known to have made its appearance among persons or rats ashore.

Our own experience at home is not without bearing on this question as to what degree of risk there may be of conveyance of plague infection from ship to shore by means of rats. In the earlier days of the present recurrence of plague in epidemic form, no measures whatever were taken as regards rats in our home ports; and, indeed, even to-day such measures are, in most of our ports, limited to ships known to have plague on board. Yet, notwithstanding our enormous mercantile traffic with all parts of the world, extension of plague on shore has taken place in four instances only, viz. twice at Glasgow, once at Liverpool, and once at Cardiff. It is further to be noted that in the first outbreak of plague in Glasgow (in 1900), notwithstanding careful bacteriological examination of large numbers of rats, no single instance of rat infection was discovered; while in the Liverpool outbreak in 1901 there was like absence of rat infection. In Cardiff, however, plague made its appearance, in 1901, among rats on shore, but it was accompanied by only one human case. These facts, like others already cited, tend to suggest

that, so far from the rat being the chief source of transmission of plague to man, it is of minor importance among the agencies, known or unknown, by which such transmission takes place.

Review of all the facts set out in the foregoing pages leads, in my judgment, to the conclusion that the part played by the rat in transmission of plague to man, although real, falls far short of the importance which has generally been attributed to it. That it should be so over-estimated is a matter of serious practical moment, since such a view may lead to comparative neglect of other risks of infection and to the imposition of measures, in relation with rats, of unwarrantable stringency.

That the rat should have attained this position in the minds of many, as an agency in the propagation of plague, is easily comprehensible. The tracing of the causation of disease is ever present with the medical investigator; the rat suffers readily from plague, and is capable of transmitting this disease to man; and wherever man is, there the rat abounds. When, therefore, other cause of plague in man is not readily apparent, the tendency is to blame the rat, always there and notoriously liable to this malady. But we know, from the study of other infectious diseases, that, in this class of malady generally, infection is wont to spread in ways beyond our comprehension; in this respect plague has taught us nothing new. It is only by impartial and thorough consideration of all the circumstances that the degree of importance of a particular agency of infection can be correctly estimated. My view as regards those who believe the rat to be the chief agency whereby plague is transmitted to man is that they have paid too much attention to individual instances, of an impressive character, in which the rat has given plague to man, and, thus unduly influenced, have failed to weigh the whole evidence, negative as well as positive."

There is one further point not included in Dr. Thomson's pages; it is in my opinion of great importance in estimating the amount of danger of infection of man with plague from the rat. I have shown (Medical Officer's Report, 1902-1903 and 1903-1904) that *B. pestis* bred in the rat is of decidedly lesser virulence than that bred in the human subject; moreover, the former is liable, outside the animal body, to a much greater extent to

rapidly lose its virulence, while that of the human type is capable to retain it for a much longer period.

These two types will be fully discussed presently, but it is not out of place to mention here the greater danger to the human species of infection with the human plague type, and the much lesser danger of infection with the rat plague type.

We proceed now to consider plague in the rat—the natural disease, as also the disease induced by artificial means.

Plague in Rats[1]

1. *Pestis bubonica occurring naturally in rats.*—I have had the opportunity of examining several rats which had died of true plague; some obtained from Cardiff, others from Bristol. The former had been found dead, amongst many others, in and about particular docks, a workman in connection with which actually contracted plague (see Dr. Low's Report, p. 30). How the Cardiff rats became smitten with the disease has not been explained, but the rats received from Bristol were ship rats from the cargo steamer *Rembrandt*, coming from Smyrna —a plague-infected place.

All the above rats presented, on post-mortem examination, the same appearances. The lymph glands in the groin could be felt as small nodules, those of the neck could be seen as dark-red spots after removal of the skin. The inguinal glands were congested and showed hæmorrhages. Film specimens made from a particle of either these or of the cervical glands showed abundance of bipolar-stained oval rods corresponding in size and

[1] L.G.B. Report, 1902-1903, p. 400 and *passim*.

shape to *B. pestis.* Cultures and animal experiments confirmed this diagnosis. The spleen and liver were enlarged, dark, and firm; when cut into, the organs were not found juicy. Film specimens of these organs yielded abundance of plague bacilli; they invariably and readily showed the bipolar staining. Inoculation of these samples and of cultures from them on guinea-pigs and on rats confirmed the diagnosis of *B. pestis.* The small intestine of these experimental animals was congested, relaxed, and contained more or less blood-stained mucus. Film specimens of this mucus showed, amongst many other microbes, fairly numerous oval bacilli showing readily the bipolar staining. The lungs showed general congestion; in the pleural cavity there was a small amount of sanguineous exudation. Film specimens of the lung juice, as also of the pleural exudation, exhibited numerous plague-like bacilli. Examined under a glass, small hæmorrhagic spots on the surface of the lung were readily recognisable. Film specimens of the heart blood showed fairly numerous bacilli which, in size and aspect and staining power, corresponded to *B. pestis.* Cultures left no doubt as to the real character of these blood bacilli; in all respects they were true *B. pestis.* So also cultures made of the intestinal mucus and lung juice.

There is, then, ample evidence that these rats died of Oriental plague, and that they exhibited the characters of a general infection; that is to say, they suffered the hæmorrhagic type of plague with the habitual general distribution throughout the body of the plague bacilli. These results are in perfect accord with those observed in other countries—India, Sydney, and Cape Town.

The Bristol rat bacillus differed, however, from that of

the Cardiff rat in—(*a*) lesser virulence towards the guinea-pig; (*b*) rapid loss of virulence on cultivation in the laboratory; and (*c*) that in cultural respects it was more of the rat *B. pestis*.

Before giving a further detailed account of the cultural and experimental results obtained with the above rat-plague bacillus, I wish to introduce here some observations bearing on the same subject made with cultures sent to me by Dr. Edington of the Cape of Good Hope.

Dr. Edington, it will be remembered, not only doubted, but actually denied, that the mortality which had occurred amongst the rats of Cape Town and Simon's Bay early in the occurrence of human plague in these places was real plague. He described it as a separate specific disease of the rat, and placed it along with hæmorrhagic septicæmias such as swine fever, fowl cholera, etc. According to Edington, the microbe which caused this rat disease was distinctly different both morphologically and culturally from *B. pestis*; moreover, the action of cultures, as also of original morbid tissues, on rodents differed from, in that it was considerably less effective than, that of true plague. Dr. Edington was kind enough to send me on two different occasions agar cultures of the microbe in question, as also stained film specimens. I have with these cultures made a considerable number of observations—by culture and by experiment—and I am of the opinion that the microbe in question is no other than *B. pestis*. It has, however, to be added that in the state in which it reached me this microbe certainly presented both in morphological and cultural respects those peculiarities which Dr. Edington had seen and described; and further, as mentioned by

Dr. Edington, it was possessed of a considerably diminished virulence. Nevertheless, it was endowed in various cultures and under other conditions, presently to be described, with characters which I think can leave no doubt about its being the true *B. pestis*. In the stained cover film specimens sent by Dr. Edington there were to be seen numerous bacilli which were undergoing changes that are generally and justly considered as indicating degenerative forms; all gradations from oval rods showing something of a polar staining to large and small globular or irregular bodies deeply stained could be observed. These same kinds of degenerative forms are to be seen in some old agar cultures of the plague bacillus, and especially in the tissues of animals in which the result of plague infection is on the point of aborting; that is to say, in animals—guinea-pig or rat—which, having been infected with a weakened plague culture, or having been previously partially protected, are inoculated with virulent plague culture. In such case, although there exists some indication, *e.g.* some swelling at the seat of inoculation and bubo, that the infection has taken some slight effect, it nevertheless appears probable that the animal will recover. When such an animal is killed, and the swelling at or about the seat of inoculation (likewise the spleen) is examined in stained film specimens, large and small distorted and globular stained bodies will be found, as to which there can be little doubt that they are derivations of plague bacilli: some few bipolar bacilli may still be found, and between these two extremes some intermediate forms.

Such observations I have, in common with other observers, repeatedly recorded in regard of both guinea-

pigs and rats which had been injected with plague cultures which in the course of many transferences on gelatine or agar had become naturally attenuated. This natural attenuation by continued subculturing of *B. pestis* is a well-known fact. It does not, however, involve all races to an equal degree. Thus, for instance, the strain of *B. pestis* derived from the fatal case in London Docks in October 1896, which has been kept up in my laboratory on gelatine subcultures, was in 1903 still possessed of a fair degree of virulence; likewise the strain of *B. pestis* derived from the Cardiff rat when taken from gelatine culture is still lethally active when injected in small doses into guinea-pigs and rats. It is different with a strain of *B. pestis* derived from the lung of a plague case dead at Hull (s.s. *Friary*). Of this strain a considerable dose of gelatine culture has to be injected in order to produce general infection and death. With a gelatine culture of the *B. pestis* of the Bristol rat, or with a strain derived from the Oporto outbreak, not even half a culture injected intraperitoneally can be relied upon to cause fatal issue in the guinea-pig. A similar remarkable and rapid loss of virulence was observed in regard of a strain (L.P. No. II.) derived last year from a case of human plague in a London dock, from an Indian native. It follows therefore that conspicuously degenerative forms observed in culture or in animals abortively infected afford no indication of a radical difference between bacilli which are and those which are not *B. pestis*.

The cultures already referred to as forwarded by Dr. Edington were at once, on receipt, used for plate cultures on agar and for injection into the peritoneum of guinea-pigs.

The plate cultures yielded colonies which in their aspect differed in no way from those of *B. pestis*. Under the microscope, however, the young colonies were composed of bacilli which were less cylindrical than the typical plague bacilli, most of them being more like oval cocci. Subcultures were made on agar surface, on gelatine, and in broth. These subcultures yielded growths indistinguishable from those of *B. pestis*, except that on gelatine the colonies were more translucent and less granular than those of the typical *B. pestis*; on agar the growth was of the typical grey, filmy, viscid character. In staining power no difference between Dr. Edington's bacilli and those of typical *B. pestis* could be detected.

Intraperitoneal injection of the guinea-pig with the culture obtained direct from Dr. Edington yielded no result. But injection of a large dose ($\frac{1}{6}$th–$\frac{1}{7}$th) of a fresh subculture of this Cape bacillus, on agar or on gelatine, into the peritoneum of a guinea-pig caused within twenty-four hours death of the animal with typical plague manifestations. The peritoneal cavity contained grey, thick, viscid exudation, and the serous covering of the intestines was much inflamed. This exudation was one mass of oval bacilli, some arranged as chains. Film specimens stained in dilute fuchsin showed the bacilli with the characteristic bipolar appearance. Subcutaneous injection into guinea-pigs and rats of a fairly large dose of the peritoneal exudation ($\frac{1}{4}$–$\frac{1}{2}$ c.c.) produced in them the typical symptoms of plague with the characteristic bubo.

I have, therefore, no hesitation in concluding that the cultures sent by Dr. Edington were those of *B. pestis* of somewhat attenuated and of atypical character. The atypical nature of the microbe was indicated chiefly in the

greater transparency of the colonies on gelatine, in the shorter size of the individual bacilli (causing them to resemble oval cocci), and in rapid loss by the microbe of infective power when carried on in subculture. In this instance, as in others, I have regularly subcultured the bacillus on gelatine once a month. This Cape rat *B. pestis*, after repeated subculture for some months—six months at the least—has practically lost all, or almost all, pathogenic action; a whole agar surface culture is not now able to kill a guinea-pig when injected intraperitoneally. Similarly, I have injected subcutaneously into the groins of guinea-pigs and rats as much in each instance as 1 c.c. of a strongly turbid emulsion of these later subcultures, with the result that the animals remained alive, although they developed temporary tumour in the groin. Also, I have inoculated cutaneously —which is the most effective method (see later) of infection—rats by rubbing into an abrasion of their skin a solid particle of the growth of this later subculture, but apparently without result. But there was a striking and twofold result observed in connection with rats and guinea-pigs which had been repeatedly injected subcutaneously with large amounts of these later subcultures: (*a*) the blood serum of animals thus "protected" against *B. pestis* was proved to be capable of agglutinating an emulsion of Edington bacillus; (*b*) rats "prepared" with Edington subcultures were found to be immunised against infection with virulent *B. pestis*. These two observations place it, I think, beyond doubt that the Edington cultures were really those of *B. pestis*.

As to the minor virulence exhibited by it, this is an occurrence which has been noted by many observers, and

is a natural sequence with most races of *B. pestis* under continued subculture; as a matter of fact, it is impossible to be quite certain about the action of any race of *B. pestis* that has been kept in artificial culture for many removes. Only quite recently I have found unexpectedly that gelatine culture of even a race that hitherto had not failed in any way, viz. that obtained from the London dock case of 1896, had abated somewhat in its virulence; so that it was necessary to pass it again, starting with a big dose, through a series of animals, in order to restore it to a fair degree of virulence.

With reference to the slight alteration of the morphological character exhibited by Edington's bacillus, viz. its being shorter and more like oval cocci, this has been observed in the cultures of various other races of *B. pestis* having attenuated virulence. For instance, I find this to be the case with greatly attenuated plague culture of a Yeddah case, of an Oporto case, and of a case of an Indian native from s.s. *City of Perth.*

As regards the different character of the appearance of colonies on the surface of nutrient gelatine, it is to be noted that there certainly exists a marked difference between the aspect of the colonies on gelatine of the *B. pestis* derived from different cases. It seems to me that two definite types of these colonies can be recognised :—

(*a*) Typical virulent *B. pestis* forms on gelatine, in twenty-four to forty-eight hours, angular colonies, grey, translucent, heaped up in the centre, or slightly excentrical, and exhibiting granulation under a glass in transmitted light. All these features become more pronounced as incubation proceeds. Fig. 28 shows such a typical

colony after about a week's incubation on gelatine, in reflected light. The colony in question looks like a limpet with a heaped-up thickened central, and a thinned-out filmy peripheral irregular part. In transmitted light the colonies are opaque and granular.

(*b*) *B. pestis* of another type shows, on the other hand, on gelatine culture, the young colonies round and distinctly translucent; in fact, alike in its early and late phases, the growth—both the separate colonies on the surface, as also the surface streak culture—is markedly translucent, so much so that the colonies in this respect look not unlike those of the *B. coli*. They increase, however, more slowly than the latter, and of course are easily distinguished from them by the nature of the growth in other media. Of course, in old cultures, *i.e.* many weeks old, even the colonies of this (*b*) type become opaque and thick in the centre. When examined in film specimens the individual bacilli are distinctly shorter (less cylindrical) than the (*a*) type, the majority being more like oval cocci or short oval bacilli.

Now, it is this (*b*) type of *B. pestis*, which at starting is of a distinctly low virulence, that rapidly loses its virulence. I have myself obtained this type from the animal body in two instances, *i.e.* (1) from Bristol rats, and (2) from an Indian native suffering from plague on board the s.s. *City of Perth* in the port of London in 1902. The *B. pestis* last referred to I have designated in my laboratory "L.P. No. II." In both instances the *B. pestis* was from the outset of a distinctly attenuated kind, and showed in a marked degree the above-mentioned character of translucent colonies on gelatine. On subculture, attenuation of its virulence proceeded so rapidly

that after about five or six months, *i.e.* five or six transferences, not even half to the whole of a surface gelatine culture injected peritoneally was capable of producing a fatal result in the guinea-pig.

It is interesting in this connection to note that there is presumption that the plague cases that occurred on board the steamer *City of Perth* (L.P. II.) were caused by rats[1]; for it may be that this particular (*b*) variety of *B. pestis* is in a sense proper to the rat, and that it is moreover normally of comparatively minor virulence. Such assumption does not, of course, exclude the possibility[2] that the typical and virulent or (*a*) race of *B. pestis* is also capable of transmission through the rat; that animal, indeed, is found to be highly susceptible, experimentally, to both varieties of *B. pestis*. However this may be, there is strong suggestion that the second type of *B. pestis*, in so far as it is derived directly from the rat, is less virulent to start with, and may be therefore much less dangerous to man than the first type, for the reason that only highly susceptible persons can take the infection.[3]

[1] The *City of Perth* left Calcutta on May 2, all well. On June 5 the first human case of plague occurred; later two further cases followed. One of these later cases died at the Denton Hospital, and the lung and swollen inguinal glands yielded the material for investigation. Before the first case occurred on June 5 some mortality amongst rats had been observed on board the vessel, and as a matter of fact both the first and second patients had handled and thrown overboard dead rats found on the ship.

[2] As a matter of fact various instances of rat plague on ships are recorded in which several persons who handled sick or dead rats took the disease and died. The Cardiff plague rat, which was described at the commencement of this report, had died of the virulent first (*a*) type, and both from this rat and from the man presumably infected from similar rats *B. pestis* of the first or virulent type was obtained.

[3] In this way may be explained the repeatedly observed fact that in some localities mortality and disease of rats had been occurring for a comparatively long time before plague (if any) broke out in man. This is consistent with highly susceptible human individuals, capable of taking and transmitting the

In connection with these later experiences I am disposed to consider that what Kitasato first described as *B. pestis* was after all *B. pestis*, though of the (*b*) type. I myself once received from Dr. Atkinson of Hong-Kong a culture, originally received from Kitasato, which belonged to this type. It was a coccus-like oval bacillus of very slight virulence, practically not fatal to rodents, and its colonies were very translucent or colon-like. Kitasato's coccus-like bacillus was, it will be remembered, denied the rank of *B. pestis* on account of its low pathogenicity; but for the above reasons I believe it to stand on the same footing as Edington's rat bacillus.

2. *Bubonic Plague artificially induced in rats.*—The white rat is just as, in fact more, susceptible to infection with plague as the brown or wild rat. For this reason I use for experiment the former in preference to the latter. It is easier kept in the laboratory, it is easier handled, and, unlike the wild rat, of which when in captivity a large percentage die spontaneously, it thrives and breeds well in captivity. All my experiments on the rat therefore invariably, unless otherwise stated, refer to the white or tame rat.

The rat being, as has been said, highly susceptible of plague, is easily infected in a variety of ways; the simplest is by subcutaneous injection or by cutaneous inoculation. This latter mode is undoubtedly that by which plague bacilli can be differentiated with certainty from any other similar microbes. It was first introduced by Albrecht and Gohn (Austrian Plague Commission), and it is a method by which infection with plague can be

rat plague, being few and far between; plague imported by rats seldom makes progress as an epidemic.

produced (both in the guinea-pig and rat) with the smallest trace of plague material, and moreover that by which the virulence best asserts itself in the rat. The plan which I employ is to scrape off the superficial epidermis, in the guinea-pig at the inguinal mammary teat, in the rat at the root of the tail, and, after slightly and superficially scarifying the exposed surface, to rub in lightly the material—culture or organ juice, as the case may be. Subcutaneous injection, when adopted, is of course performed in the groin. Whether cutaneously or subcutaneously injected, the result, if positive, consists in the formation of a tumour in the groin, swelling of the inguinal glands, and hæmorrhagic œdema of the tissues around. If the rat be cutaneously inoculated at the root of the tail, the site of the resulting inguinal bubo depends whether the inoculation is lateral or median. In the former case the bubo develops on the side of inoculation, in the latter the bubo develops in both groins. A lethal dose of normal virulent *B. pestis* is the one which suffices to kill a rat in thirty-six to seventy-two hours.

The shortest duration of the fatal illness in rats occurred in thirty-six hours; it was observed after cutaneous inoculation at the root of the tail, with a twenty-four hours' agar surface culture; a turbid emulsion was made, and of this a droplet was rubbed into a few superficial scratches. In most of the cases of control rats used by me in connection with the experiments on my new plague prophylactic, the fatal illness occurred between thirty-six and forty-eight hours, so that the culture used must be pronounced of great, *i.e.* normal, virulence.

On post-mortem examination of the infected rat the inguinal glands are found enlarged and deep red, and, as

sections of the hardened material show, the gland tissue contains effused blood in both the cortical and medullary lymph sinuses, while larger or smaller groups of *B. pestis* are detectable in this effused blood. The juice of the gland shows also crowds of the typical plague bacilli readily stainable bipolarly in fuchsin (see Fig. 6). The spleen is much enlarged, dark, and firm; it is not juicy. Stained film specimens from it show crowds of bipolar-stained *B. pestis* (see Fig. 4). The lungs are more or less congested and show occasionally petechiæ. The heart-blood contains sometimes very numerous, at other times only fairly numerous *B. pestis*, as shown by cultures. The intestines and mesenteric glands are congested, the small intestines being relaxed and containing blood-stained mucus. In this mucus *B. pestis* can be demonstrated by stained film specimens, as also by culture of a particle of the mucus distributed in sterile salt solution and then employed for making agar surface plates, or, and this is a method which is equally certain of positive result in a case in which the above condition of the intestine obtains, by injecting a little of the intestinal mucus subcutaneously, or, better still, by inoculating it cutaneously into guinea-pigs or into rats.

It is necessary to emphasise an important fact, viz. that in whatever way (subcutaneously or cutaneously) the rat is infected, the abdominal viscera, including the intestines and mesenteric glands, generally participate in the disease. For this reason the presence of the lesions in the intestines and mesenteric glands may not be interpreted as due to infection by food.

The above is the condition in the rat which generally obtains at the end of three days when the animal has been

inoculated (subcutaneously or cutaneously) with virulent plague material. In some cases, however, owing either to the lessened virulence of material, to the smaller dose, or to peculiarity of a given animal, death does not take place within three or four days—the animal survives up to five, six, seven, or more days. In these subacute cases the inflamed inguinal glands show more or less advanced necrosis in their centres, the necrotic material containing abundantly masses of plague bacilli. The spleen is enlarged, so is the liver. There exists an interesting difference with regard to the condition of these two organs in the guinea-pig and in the rat in the subacute forms of plague. The difference is this: while the spleen and the liver in the rat do not (with few exceptions) in general appearance differ from the condition of these organs found in the same animal dead from acute plague, it is otherwise with the guinea-pig. In this animal the two organs, as also the lungs, are pervaded by small whitish necrotic nodules and patches. Only in a small percentage of rats dead of the subacute form of plague are necrotic punctiform nodules in the spleen and liver found. The necrotic nodules in the spleen and liver of guinea-pigs dead of the subacute form of inoculated plague are similar in appearance to those met with in inoculated pseudo-tuberculosis in the guinea-pig (see my Report 1900-1901). But this latter disease is much slower in progress, and its microbe is totally different from *B. pestis* (see a former page).

The above necrotic nodules of the spleen involve chiefly the pulp tissue, in which numerous small and large vessels are to be observed filled with masses of *B. pestis*. The necrotic nodules in the liver involve both the

interlobular connective tissue as well as the liver cells of the acini. The lungs both in the rat and in the guinea-pig, besides showing petechiæ, are also permeated by few or many grey consolidations (some small and more or less circumscribed, others large and irregular), the surrounding lobules being much congested and showing more or less red hepatisation. In the lung, both in the red hepatised parts as also in the grey patches, vast numbers of *B. pestis* can be demonstrated. Sections through the diseased parts show the bronchi, infundibula, and alveoli distended by and filled with fibrinous exudation—red and white cells and continuous masses of *B. pestis*. Figs. 16 and 17 show this condition well. Fig. 16 is from a section of the lung of a rat showing extensive grey hepatisation. This rat (*l*) had died nine days after cutaneous inoculation with the sanguineous mucus of the intestine of a previous plague-infected rat (*k*). The muco-purulent matter which was found, on careful dissection and opening, in the pharyngeal cavity of the rat (*l*), was inoculated cutaneously into a rat (*m*). Rat (*m*) developed the typical inguinal bubo and died in three days of plague. A film specimen of this inguinal bubo of rat (*m*) is shown in Fig. 6. I shall return later to the application of these observations; at present I am content to record them, and to add that the muco-purulent matter that was present in the oral and pharyngeal cavity of the rat (*l*) was examined by stained film specimens and by culture, and that the abundant presence in it of typical *B. pestis* was in this way demonstrated. Looking at Fig. 17, which represents a section through a bronchus, it will be readily understood how it comes that the oral and pharyngeal mucus of this animal contained *B. pestis*

in large numbers. I should add here the important observation that from the pharyngeal and oral mucus of rats dead from the *acute* disease, that is to say within three days of inoculation, and in which the lungs do not show any disease beyond a general slight hyperæmia, no plague bacilli at all have been recovered by culture, nor have I as yet been able to infect with such mucus either rats or guinea-pigs. In this connection it is necessary to bear in mind that in collecting mucus from the oral and pharyngeal cavity great care must be taken not to allow any admixture with even traces of blood; rats dead of the acute disease have in the great majority of instances fairly numerous *B. pestis* in their blood, and admixture therefore of blood with mucus would vitiate the observation. I note here also that in a small minority of rats, infected subcutaneously or cutaneously, and as a result dead with the *acute* disease, the heart blood, as also the spleen pulp, shows few *B. pestis* whether in film specimens or in culture. But even in these cases the swollen and hæmorrhagic inguinal glands contain always an abundance of *B. pestis*.

The following set of observations on rats and guinea-pigs seems to have an important bearing on the etiology of pneumonic plague. I have had repeatedly opportunity to test the plague prophylactic, which I described in a preliminary publication to the Local Government Board in December 1905. In the experiments which I made in order to ascertain the protective dose of the various plague organs (for rats as also for guinea-pigs), it was repeatedly noticed that amongst a number of rats injected at the same time with a certain amount of a particular sample of prophylactic, considered to be fully protective,

one or the other of these animals on subsequent testing (by cutaneous inoculation) with virulent *B. pestis* succumbed to plague, while the remainder proved themselves fully protected. Those insufficiently protected rats died generally on the ninth or tenth day or even later. The animals seemed lively till almost the evening preceding death. On post-mortem examination it was invariably found that they showed no bubo, the spleen was not markedly enlarged and contained very few (if any) *B. pestis*. The liver appeared unaltered, the heart's blood contained no *B. pestis* in film specimens. But the lungs always showed profound changes, varying, however, in the different animals; between red hepatisation of one or more lobes in one or both lungs, and extensive white necrotic patches, all intermediate conditions were found. In all cases, however, the altered parts were literally packed with *B. pestis*. Stained film specimens of the red hepatised portions looked exactly like similar film specimens of the juice of the lung in pneumonic plague of man, described and illustrated in Chapter I. A similar condition obtains also in the guinea-pig when insufficiently protected by the plague prophylactic and afterwards inoculated with virulent *B. pestis*. Here also the lungs chiefly were found affected, showing all stages between red hepatisation and extensive necrotic patches. The affected parts are also literally filled with *B. pestis*. I may add here that an experiment performed on monkeys shows that this holds good also for the monkey, inasmuch as one of the insufficiently protected monkeys, which succumbed to plague on the eleventh day, showed distinct evidence of pneumonia, patches of lung being in the state of red hepatisation, others already showing commencing

necrosis, the affected portions being literally crammed with *B. pestis* (see Fig. 7). Now, returning to the rat, I think these experiments suggest that also under natural conditions there may occur in rats cases of pneumonic plague, due to a lesser susceptibility of the individual rat; cases, that is, which run a much longer course, and in which the chief organs affected are the lungs. Such animals would obviously be more dangerous in an epidemiological sense, and for two reasons: (1) they harbour plague for many days without showing distinct signs of illness, and (2) their bronchial secretion, pharyngeal and laryngeal mucus (see a former page), being crowded with *B. pestis*, would represent highly infective contagium, which by the breath, by the saliva, or by a bite might, and probably would, be readily and widely spread amongst the surroundings. Moreover, these observations would also suggest that the disease may linger on for considerable periods amongst rats on board ship or on land if one or the other of them, being less susceptible, is afflicted with this pneumonic type of plague. And it would further suggest that also as regards pneumonic plague in man a "first" case of this kind might in reality be a case in a person who was naturally not endowed with the normal susceptibility, and on account of such subnormal susceptibility became after infection (cutaneously or by mucous membrane) affected with the pneumonic type. Such a person, as is well known, on account of the abundance of *B. pestis* in the excretion from the lung, is highly infective for and readily communicates plague to others, in whom, owing to the easy access of the *B. pestis* to the air-passages (by the breath), it is capable of causing pneumonic plague.

An important point noticeable in the *B. pestis* of the pneumonic plague in the above rats was that its virulence was distinctly of a subnormal character; this appeared to follow from the experimental fact that when tested on the guinea-pig by subcutaneous injection of even considerable doses (of a twenty-four hours agar culture an emulsion having been made, and several drops of the emulsion being used for injection) it was found that in no case did it cause the acute form of plague, but always the subacute form leading to death in five to nine days. The same applies also to other instances in which natural or artificial attenuation of a strain of *B. pestis* had been produced, viz. that even fairly large doses injected subcutaneously into the guinea-pig cause the subacute form of the disease (see later).

In connection with the subject of attenuated type of *B. pestis*, I now proceed to record experiments which indicate that some strains of *B. pestis*, however virulent originally, may in the laboratory, *i.e.* artificially, be made to breed true as the attenuated or rat type, and these experiments would suggest that occasions may arise also in nature when a strain of *B. pestis*, originally virulent, may become attenuated, and may then persist in this form through many generations. I have above already indicated two such occasions—

(1) By passing through a succession of rats by which the *B. pestis* becomes of the nature of the rat type (2); and

(2) By the bacillus being allowed to breed in a rat of lesser or subnormal susceptibility.

I proceed now to record a number of experiments in further support of these propositions.

On several occasions I have tested for the Board[1] the efficacy of the Haffkine plague prophylactic. This was done on rats, for the reason that Professor Haffkine and I had ascertained that subcutaneous injection of 10 cc. of his prophylactic fluid is uniformly efficacious in protecting a full-grown rat against a subsequent injection of a large (multiple fatal) dose of virulent culture of *B. pestis.* The samples of prophylactic fluid tested comprised a batch prepared by Professor Haffkine in Bombay and a batch prepared by me in London.

A number (ten) of rats[2] were injected subcutaneously in the groin with plague prophylactic, 10 cc. per animal.

All these animals appeared on next and subsequent days lively and fed well; but all exhibited tumour in the groin. After three weeks, during which time the animals remained quite well, the initial tumour having for some days completely disappeared, all of them were inoculated cutaneously with the tissue of the bubo of a rat that had died in three days of typical acute plague after inoculation with culture of L.P. I.[3] The juice of this bubo was crammed with *B. pestis.* At the same time, and with the same material, one control rat was inoculated also cutaneously. This control rat died within three days with typical plague; but all the "protected" rats appeared lively and feeding well, and they remained so. At the end of the week these animals, being seemingly normal, were killed. On post-mortem examina-

[1] Report of the Medical Officer of the Local Government Board, 1903-1904, p. 371 and *passim.*

[2] In all these experiments tame (white) rats were used, for reasons which were stated on a former page, viz. that white rats are highly susceptible to plague, are easier to be kept in cages, and easier also to handle.

[3] London Plague of 1896.

tion all but two were found quite normal in every respect. All had perfectly normal viscera; but one rat had a small gland in the groin at the side on which the inoculation (cutaneous at root of tail) had been performed, and a second rat had a swollen gland in the groin, about the size of a filbert, also on the inoculated side.

An incision into the swollen gland, especially in the case of the second rat, showed the interior necrotic; film specimens made of the gland in both cases showed numerous particles representing bacilli in various stages of degeneration, from rods more or less granular to spherical small and large globules. Cultures on gelatine were made in both cases with relatively large amounts of the gland tissue, and in each instance a fair number of colonies of *B. pestis* was obtained. The number of *B. pestis* colonies was, however, incomparably smaller than that induced by a similar proceeding in an unprotected animal when a much smaller particle of a typical plague bubo is used. The colonies (on gelatine) did not on inspection with a glass present any noticeable difference from those of the typical *B. pestis* that had been initially employed (L.P. I.), except that during the first few days after their appearance they might be considered somewhat less granular and more translucent than usual. In film specimens examined under the microscope the bacilli appeared markedly short, certainly shorter than in a similar preparation of the typical L.P. I. The most striking characteristic of this culture and subsequent subcultures was, however, the fact that their virulence was decidedly of a lower degree than that of the *B. pestis* (L.P. I.) with which the series had been started.

The following experiments illustrate this :—

Series A

Experiment 1.—A rat (*a*) was injected cutaneously with a three days old gelatine culture of "L.P. I."

A second rat (*b*) was at the same time injected with a three days old gelatine culture of the swollen gland of one of the Haffkine protected rats just referred to.

In both cases, therefore, the *B. pestis* was originally the same strain, viz. L.P. I. ; but, as just described, the culture for rat (*b*) was derived from the gland of a protected rat.

Rat (*a*) died in less than three days: post mortem there was a big bubo on the inoculated side crowded with *B. pestis* ; the spleen was large, dark, full of *B. pestis* ; the intestine was inflamed, containing sanguineous mucus ; the heart's blood contained numerous *B. pestis.*

Rat (*b*) had a big bubo on the inoculated side ; but it remained lively and fed well up to the seventh day, when it was killed. Post-mortem examination showed the inguinal glands much enlarged, and necrotic in their interior ; few bacilli noticeable. The viscera, including the spleen, appeared unaltered.

Experiment 2.—One rat, 1A, was inoculated subcutaneously with (a large dose) two loops of a four days old agar subculture (second transfer) of the swollen gland of the protected rat. This rat died on the fourth day. On post-mortem examination it showed: big bubo, crowded with short *B. pestis* ; spleen enlarged and containing some (bipolar) *B. pestis* ; intestine, liver, and

lung showed no perceptible change; the heart's blood did not contain bacilli.

Experiment 3.—One rat, 1B, was injected cutaneously with a fair dose (several drops being well rubbed into scarifications of the skin at the root of the tail) of sanguineous turbid juice (crowded with *B. pestis*) from the bubo of rat 1A. This rat was found dead on the fourth day. Post mortem :—Hæmorrhagic inguinal bubo on the side corresponding to the site of the cutaneous inoculation. The juice of this bubo was crowded with short (bipolar) *B. pestis.* Spleen not enlarged, no bacilli; heart's blood no bacilli; other viscera showed no noticeable change. The culture of material from the bubo produced translucent growth on gelatine, the bacilli very short.

Experiment 4.—One rat, 1C, was inoculated cutaneously with a good-sized loop of a subculture on agar of the above bubo of rat 1B. The animal was found dead on the fourth day. The inguinal gland corresponding to the side of inoculation was found enlarged, and contained numerous short *B. pestis*; most of them in a state of degeneration (granular), some swollen up into spherical masses. The spleen was slightly enlarged and contained a fair number of *B. pestis*, showing bipolar staining. Heart's blood no bacilli.

From this series of experiments it appears that typical virulent *B. pestis* (L.P. I.) by passage through a Haffkine protected rat had distinctly become attenuated in virulence when employed, whether as bubo juice or as culture therefrom, in the inoculation of a succession of rats. Under ordinary conditions, be it remembered, the most virulent material available is the juice of the bubo

of an animal (of the same species) dead of acute plague. Nevertheless in the experiments in question such material proved barren of a high degree of virulence, the chief manifestation of disease induced being a local bubo, with delay of fatal result. Furthermore, this attenuated *B. pestis* presented the morphological and cultural characters (conspicuous shortness of the bacilli in culture, transparency of growth on gelatine in the earlier phases) of the type of *B. pestis* which I have characterised as type 2.

Series B

A considerable number of experiments was made with virulent *B. pestis* that had been obtained on January 31, 1901, from the bubo of a man dead of plague who had worked in a plague-infected flour-mill in Cardiff. The subculture of this strain (as also of a rat—strain from the same flour-mill) is at present—end of 1903 and beginning of 1904—of normal high virulence: a trace of a forty-eight hours old growth on agar inoculated cutaneously into a rat causes (fatal within seventy-two hours) acute septicæmic plague of the typical character.

Experiment 5.—With a trace of a forty-eight hours old agar growth of *B. pestis* from the Cardiff bubo, a rat was inoculated cutaneously on left side of its tail-root. The animal was found dead in about sixty to sixty-six hours. Post-mortem examination showed the following condition:—Scab on site of inoculation; left inguinal glands much enlarged and deeply congested, in parts purple, hæmorrhagic; spleen much enlarged, dark, firm; intestines congested, relaxed and full of sanguineous mucus; kidneys and liver much congested; both lungs

deeply congested. The juice of the swollen hæmorrhagic inguinal gland and the juice of the spleen were crowded with typical *B. pestis.* Droplets of heart's blood and of spleen juice gave rise to crowds of colonies of *B. pestis* in pure culture.

With a trace of the spleen juice of this rat another rat was inoculated cutaneously by way of superficial abrasions at the tail-root. The animal was dead within seventy hours. Post mortem :—Right inguinal glands enlarged, hæmorrhagic; spleen much enlarged, dark, firm; kidneys and liver congested; lungs congested and showing numerous punctiform hæmorrhages. Droplets of heart's blood and spleen juice yielded crowds of colonies of *B. pestis* in pure culture.

One further set of experiments with this strain of the Cardiff bubo *B. pestis* deserve to be mentioned.

Experiment 6.—With a trace of the growth of a forty-eight hours old agar subculture (directly descended from the original culture), one rat (*a*) and two mice (*b*) and (*c*) were inoculated cutaneously, each at the root of the tail.

One mouse (*b*) was found dead within forty-four hours, the other mouse died within sixty to sixty-five hours. In both animals the result of post-mortem examination was the same :—Bubo in left groin crowded with *B. pestis*, the tissue surrounding it very œdematous; spleen much enlarged, dark, firm, and crowded with *B. pestis*; lungs much congested; liver pale; kidneys large, dark red. Heart's blood yielded copious growth of *B. pestis.*

The rat was found dead within seventy hours. Post-mortem appearances :—Big inguinal glands with hæmorrhage showing crowds of *B. pestis*; spleen enlarged, dark,

firm, and containing crowds of *B. pestis*; kidney and liver large and much congested; both lungs much congested. Heart's blood yielded crowds of colonies of *B. pestis* in pure culture.

It is clear from the above that this strain of *B. pestis* (Cardiff bubo) is of normal and high virulence; and I may add that colonies of the microbe in their early phases of culture on gelatine were of the typical angular, granular, "opaque" kind, and that the bacilli from them were of the typical cylindrical form; that is to say, this strain is in respect of virulence and in all its morphological and cultural characters a good representative of the type No. 1 (human).

In contrast with the *B. pestis* (type No. 1) of experiments 5 and 6, I pass to further experiments with the attenuated *B. pestis* of the second or rat type, derived from the inguinal gland of the Haffkine protected rat already referred to earlier. *B. pestis* of this type is in culture shorter than that of type No. 1, and it forms in the earlier phases of its growth on gelatine less angular and more rounded colonies, the growth on the gelatine being at the same time more translucent and less granular. These characters have now become permanent through many subcultures. The difference between the two types here dealt with — viz. the strain of Cardiff bubo (human type) and the strain of protected rat (rat type)—is so distinct that, on looking at streak as also stab cultures, or at plate cultures on gelatine of the two strains under exactly the same conditions, the distinction during the first three or four days cannot be missed. I have repeatedly asked colleagues to inspect with a glass a number of parallel cultures of the two strains, and

they invariably, and without difficulty, picked out and grouped quite correctly the cultures of the two strains.

The parallel experiments made with the strain from the protected rat (type No. 2) are these :—

Experiment 7. — A rat (rat 1K) was inoculated cutaneously with a trace of the growth of a two days old agar subculture of the bubo of the rat 1B mentioned in Series A. (experiments with strain of *B. pestis* derived from protected rat). This rat was found dead on the seventh day. Post mortem :—The inguinal glands were found swollen and hæmorrhagic ; small intestine and lungs congested ; spleen not enlarged. The inguinal glands were crowded with *B. pestis*, which by culture was proved to be of the type No. 2. The heart's blood did not yield colonies of *B. pestis*. The culture was quite liquefied in forty-eight hours, owing to contamination.

The result of this experiment is, then, quite confirmatory of experiments mentioned in Series A., viz. that *B. pestis* of type No. 2 does not cause the pathological changes typical of *B. pestis* type No. 1, its effects being chiefly bubo with crowds of *B. pestis* (type 2) in it.

Experiment 8.—A rat, 1L, was inoculated cutaneously with a two days old agar subculture of material from the bubo of rat 1C of Series A., which had itself been inoculated with a subculture of the bubo of rat 1B.

Rat 1L was found dead on the fifth day—it died in the night between the fourth and fifth days. The post-mortem examination showed the inguinal glands corresponding to the inoculated side swollen and hæmorrhagic ; small intestine relaxed, containing sanguineous mucus ;

spleen enlarged; upper lobe of right lung deeply congested. Inguinal glands and spleen contained abundance of *B. pestis* of type No. 2.

This, then, was a case of distinct septicæmic plague, differing from the typical form (virulent type 1) only in the fact that death was delayed, and that the colonies derived from both the bubo and spleen were of the same transparent variety as those of the *B. pestis* used for inoculation.

Experiment 9.—A further rat, 1M, was inoculated cutaneously with a fair amount of the (transparent) growth of a culture from the spleen of rat 1L. This animal died on the fifth day. On post-mortem examination the inguinal glands of the inoculated side were found slightly enlarged, the spleen very slightly enlarged. Cultures of the spleen and heart's blood yielded no growth.

Experiment 10.—A further rat, 1N, was inoculated cutaneously with the growth of a two days old agar subculture (second remove) of the same stock (spleen of rat 1L) as in the preceding experiment. This animal was distinctly ill on the third and fourth days, being quiet, not feeding, its coat slightly rough. The control rat inoculated on same day as 1N with culture of the same date (two days agar) of the Cardiff bubo type was found dead in sixty to sixty-six hours with severe plague. But rat 1N on the sixth day, though still ill, was alive. On the fifteenth day it was still not feeding well and had a slightly rough coat, but it was better in respect of moving about freely. The animal was now killed and showed the following appearances: no bubo; spleen not enlarged; ileum congested; Peyer's glands marked; liver normal;

kidneys large, congested. No cultures were obtained from either the inguinal glands, the spleen, or the heart's blood.

This experiment shows conclusively the attenuated, less virulent condition of the type 2, and contrasts strongly with the action of a parallel culture of the type 1. Be it remembered that rat 1N was inoculated with a subculture (second remove) from the spleen of a rat (1L), which rat had died of acute plague, caused by inoculation with the *B. pestis* of the second type.

It follows from these experiments that after passing through a succession of rats *B. pestis* of the type 2 retained its attenuated action unimpaired.

The above are not by any means the only experiments which I have made on rats with active cultures, twenty-four hours and forty-eight hours old agar cultures, of the (transparent) strain (type 2). Thus, I have records of rats 1O, 1P, 1Q, 1R.

Experiment 11.—Rat 1O was inoculated cutaneously with the growth of a forty-eight hours agar subculture from rat 1L.

Experiment 12.—Rat 1P with forty-eight hours culture from the spleen of a mouse dead within forty-eight hours after cutaneous inoculation with same strain (see later), cutaneously.

Experiment 13.—Rat 1Q with a twenty-four hours old agar subculture from rat 1L, cutaneously.

Experiment 14.—Rat 1R with a twenty-four hours old agar subculture (second remove) from spleen of rat 1Q, subcutaneously.

In all the cases where inoculation was practised cutaneously a fair-sized particle of the growth was well

rubbed into several crossed incisions. In the case of rat 1R a large dose of culture was injected subcutaneously into the groin.

The subsequent history of these animals is as follows :—

Rat 1O died between fifth and sixth days.

Rat 1P, which appeared normal, was killed on the eighteenth day ; completely negative result.

Rat 1Q, which died on the fourth day, showed post mortem : swollen lymph glands, with hæmorrhage, in both inguinal regions ; spleen large ; lungs congested. Spleen, bubo, and heart's blood yielded crowds of colonies of *B. pestis* of the type 2.

Rat 1R died on the seventh day. As mentioned above, this rat had been injected subcutaneously. The post-mortem examination showed : bubo in groin at injected side ; spleen slightly enlarged ; intestines congested. Cultures of the spleen brought forth a fair number of colonies of *B. pestis* (type 2) ; cultures of heart's blood yielded a small number of colonies of the same type (2).

In all these instances there are seen, therefore, the same features not only as regards virulence and pathological result, but also as regards the preservation of type 2.

Experiment 15. — This same type 2 of *B. pestis* (derived from rat 1L) was used for cutaneous inoculation of mice, and at the same time other mice were cutaneously inoculated with *B. pestis* of type 1 (Cardiff bubo), with result as follows :—

Two mice (1 and 2) were inoculated cutaneously at the root of the tail with a forty-eight hours agar sub-culture from spleen of rat 1L.

Two mice (3 and 4) were cutaneously inoculated with

forty-eight hours agar subculture of the bacillus of Cardiff bubo.

Mouse 3 found dead in 40 to 44 hours.
Mouse 4 „ „ 60 to 66 „
Mouse 1 died in 48 hours.
Mouse 2 found dead in 60 to 66 hours.

The post-mortem examination was in all four animals the same: Swollen hæmorrhagic inguinal glands with surrounding œdema on the inoculated side; spleen large, dark, firm; liver pale; kidneys large, deep red. The inguinal lymph glands and the spleen contained crowds of *B. pestis*. The lymph glands were especially remarkable in this respect, and later a description will be given of sections made of these hardened glands.

But though the two sets of mice showed no difference as to virulence and pathology corresponding to the two types of *B. pestis* from which the infecting material was derived, the morphological and cultural distinctions between *B. pestis* of the two types were nevertheless maintained in the cultures obtained from their organs. Moreover, although mice 1 and 2 died as rapidly as mice 3 and 4, a culture from mouse 1 when tested on a rat (1P) proved of very little pathogenicity, as was described above, experiment 12.

It might of course be contended that this attenuated action of the culture of the spleen of mouse 1 when inoculated into rat 1P was due to the fact that possibly *B. pestis* having passed through the mouse had as a consequence become less virulent for an animal of a different species, to wit the rat; an opinion to this effect has indeed been actually expressed by Calmette. I must confess that I was rather surprised at Calmette's state-

ment, for I have not found any marked attenuation taking place of the really virulent *B. pestis*—*e.g.* type 1—when this is transferred from an animal such as the mouse dead of typical acute plague to an animal of a different species, *e.g.* the rat or the guinea-pig. Experiments which I have made in this direction (and as to which I need not enter into details) with virulent *B. pestis* (Cardiff bubo) show that no such general rule obtains as is implied in Calmette's statement; that, indeed, general experience is to the contrary.

On the other hand, attenuation of *B. pestis* once having become as it were fixed, I have not been able to notably modify it. For instance, I have not found that, starting with attenuated *B. pestis*, passage of it through a succession of *guinea-pigs* causes any enhancement of its virulence.

As I have already pointed out, inoculation of a guinea-pig *cutaneously* with plague from whatever source (virulent or less virulent) results invariably in a subacute disease, fatal after six, seven, and more days; the animal showing post mortem necrotic bubo and small necrotic white nodules in the spleen, liver, and often also the lung. This is, as I have said, invariably the result of inoculating the animal by rubbing in the infective material into cutaneous abrasions or superficial incisions. Also I have shown that in the case of *subcutaneous* injection with ordinary small dose of virulent material the disease is fatal in from forty-eight to seventy-two hours, but without necrotic changes being found in the bubo, spleen, liver, or lung. And further, I have shown that in the case of attenuated virus (or of very small doses of more virulent material) even *subcutaneous* injection will cause the subacute disease with

the above necrotic nodules in spleen, liver, and often lung.

Experiment 16.—Several experiments were made on successive guinea-pigs with the strain of *B. pestis* of type No. 2 (derived from the Haffkine protected rat). Always, however, this retained its initial attenuated or less virulent character even through a succession of six guinea-pigs. Every one of the animals died of the subacute disease (six to twelve days) with the necrotic change in the bubo, necrotic nodules in the spleen, liver, and lung. These guinea-pigs had been inoculated, some cutaneously, some subcutaneously, with considerable doses of the necrotic tissue of the bubo (teeming with *B. pestis*) of a preceding guinea-pig. The last of the series received subcutaneously in the groin a cubic centimetre of a thick turbid emulsion of the necrotic bubo of the preceding guinea-pig; but, as before, the result was the subacute disease, showing necrotic bubo, and necrotic nodules in spleen, liver, and lung.

Experiment 17.—The following experiment demonstrates the same result from another point of view. As already mentioned, mouse 1 (*B. pestis*, type No. 2) died in forty-eight hours from typical acute plague; the spleen was found large and crowded with *B. pestis* of type No. 2. With a good dose—about two loops in each instance—of recent agar culture of the spleen of this mouse two other mice (5 and 6) were inoculated, the culture being well rubbed in into several crossed incisions of the skin at the roots of their tails.

One of these mice, No. 5, died on the fifth day, the other, No. 6, died on the seventh day. Both on post-mortem examination showed big inguinal glands and big

dark spleen. Bubo, spleen, and heart's blood yielded numerous *B. pestis* of type 2.

There is distinct evidence, then, that the *B. pestis* taken from mouse 1 was also for mice 5 and 6 of a distinctly minor virulent character, since death ensued in one case on the fifth day, in the other on the seventh. This is altogether different from what took place in the case of mice inoculated cutaneously with the *B. pestis* of the Cardiff bubo type (experiment 15).

It is justifiable, therefore, to conclude that the *B. pestis* secured through the medium of the Haffkine protected rat is of a different type ("rat" type) to the *B. pestis* derived from the Cardiff bubo ("human" type), seeing that *B. pestis* in this phase maintained its particular and minor virulence, as also its morphological and cultural characters, unimpaired, however many times it was subcultured or was passed through the animal body. Further, there is in these experiments confirmation of previous observations as to type No. 1 (or human type) being of a more virulent character than type No. 2 (rat type) in the fact that type No. 2 has been now derived from the swollen gland of a protected rat—a rat, namely, that had by previous injection with plague prophylactic been furnished with a high degree of resistance.

A strain of *B. pestis* which possesses and which maintains in its passage through successive rats its attenuated virulence might obviously very well be bred in nature. If, for instance, in a ship sailing from an infected port plague brought on board by rats spread epidemically amongst the ship rats in the course of a long voyage, the most susceptible of these ship rats would of course be the first to die off; the last to remain

would be those infected with the chronic form of plague, *i.e.* with *B. pestis* which had passed through a series of rats less susceptible to the disease. As a result the *B. pestis* passed on from the remaining rats, after arrival of the vessel at her destination, to rats on shore would tend to be of no great consequence, for the reason that by the passage of a weakened strain of plague through a succession of rats the acquired attenuation of virulence would be likely to have become permanent. Further, plague of this sort communicated by the shore rats to the human community ashore would in all probability be little diffused and of a non-virulent type; so that only amongst the more highly susceptible inhabitants would there occur recognisable plague cases and deaths. It is probable that for some such reason many outbreaks of plague presumably originating in plague of rats in Occidental countries have proved, both as to incidence and mortality, of a less severe type than in Oriental countries where the virulent or human type is the predominating character of the *B. pestis*, and where also rats are constantly being infected directly from the human subject.

While this view of an attenuated type of plague bred in the rat appears indicated by these experiments, it does not necessarily follow that, when after its passage through a succession of human beings the *B. pestis* again enters the rat, virulence in the sense of type No. 1 will not again be restored to it, and with the result that, for a time at any rate, the virulent or human type of *B. pestis* might prevail among the local rats, until, indeed, by a succession of passages through the rat, attenuation of type was again brought about. Further observations on this point will need to be made.

That the rat is highly susceptible to plague, more susceptible than the human being, seems a generally accepted fact, and this would accord with the views above expressed, viz. that while the rat is fatally amenable to both types of plague, the human being is less susceptible to type No. 2 (the rat type) than to type No. 1 (the human type).

CHAPTER VI

PLAGUE INDUCED IN OTHER RODENTS

In the preceding chapters (Chapters III. and V.) we have had occasion to describe the result of inoculation of mice with *B. pestis*, and it is therefore not necessary to further dwell on this subject except to state that the inoculation of both the wild as also the tame mouse subcutaneously or cutaneously with either the virulent type (type 1, human) or the less virulent type (type 2, rat) is followed by acute fatal plague, the *B. pestis* distributed very copiously in the blood and in all viscera.

We now proceed to describe the results of the microscopic examination of the organs of rats and mice dead of plague.

Description of the Pathology of the Organs in Rats and Mice infected with Plague[1]

In all instances the organs and tissues were hardened in Müller's fluid in the usual manner, and fine sections of them were stained in methylene-blue and eosin, the former dye picking out (blue) nuclei and bacilli, the latter (red) the blood discs.

I.—*Rats inoculated cutaneously with the Virulent Type of Plague*

Rats thus dealt with—*i.e.* inoculated with plague of London Port No. I. and Cardiff bubo type—exhibit, with very few exceptions, the following conditions :—

[1] Report 1903-1904, p. 380 and *passim*.

1. *The Inguinal Lymph Glands.*—As already mentioned, cutaneous inoculation of rats in these experiments was in all cases made by rubbing a trace of the plague material on to the abraded surface (on which several superficial crossed incisions had been made) of the skin at the root of the tail, the hairs of this place having been previously removed with scissors. Whenever this inoculation was performed in the middle line the inguinal glands became subsequently involved in both groins, but when the inoculation was not in the middle line only the inguinal glands corresponding to the inoculated side were found affected. The change in the glands consisted of enlargement, one or another gland showing at the same time great congestion and even hæmorrhage to a greater or smaller extent. The loose connective tissue around the gland was always congested, and sometimes, though not always, œdematous. Sections through the glands and through the surrounding tissue showed invariably extensive necrosis of the lymphatic or adenoid tissue, with a large amount of blood diffused in it and in the medullary sinuses. The large blood-vessels of the medulla were greatly distended with blood. Both in the cortex and in the medulla the lymph sinuses in places—particularly in the cortical portion—were filled with continuous masses of *B. pestis* forming definite plugs. It was easily recognised that the individual bacilli were imbedded in a hyaline ground substance, thus forming true zoologea ; the lymphatics both going to and coming from the gland—*i.e.* the afferent and efferent vessels—were distended by and filled with leucocytes and numerous masses of *B. pestis*, or they were almost entirely injected with masses of the latter. Similarly, the lymph spaces of the surrounding fat tissue were in some instances literally filled with the bacilli. That the lymph vessels passing to the glands should be found containing abundance of *B. pestis* was to be expected, since the latter would readily be carried from the inoculated spot of the skin of the tail to the lymph glands. Also it was to be anticipated that *B. pestis* would readily multiply in these lymph vessels, in the lymph spaces of the groin, and in those of the gland itself, and thus produce continuous masses filling these lymphatics. But what was not looked for, and which is a point of great interest, is the fact that all the venous blood-vessels, including many capillaries of the tissue surrounding the gland, likewise contained large and small continuous streaks and masses of *B. pestis.* Such a condition is not or is only very rarely present in other organs. It is not observed either in the kidney or intestine or

lung—organs, that is, which are always found more or less congested; and only in few instances have I seen the intralobular capillary blood-vessels of the liver containing groups and streaks of plague bacilli. It follows, therefore, that the state of the blood-vessels around the inguinal glands is entirely different from the state of the blood-vessels obtaining in other organs; and further, that this peculiar state of the venous vessels around the gland—viz. their containing large and small masses of *B. pestis*—is due to the *B. pestis* being absorbed and carried from the seat of inoculation directly by way of the veins. The cutaneous injury, above described, would no doubt involve many superficial veins, and owing to the state of the inguinal glands—viz. great swelling and necrosis—the circulation of blood in them would become impeded. This in its turn would affect and impede the circulation also of the surrounding tissue, hence in many of them stasis of blood and multiplication of the *B. pestis* would be the result.

2. The next organ in importance is *the spleen.* This organ is generally considerably enlarged, dark in colour, and firm; on a cut being made into its substance no fluid (blood) oozes out. This latter condition of the organ is in contrast with that in septicæmia and other diseases in which, the spleen being large and dark owing to hyperæmia and active congestion, there is always a quantity of blood oozing out from the spleen's cut surface. On examining microscopic sections through the "plague spleen" such as is in question, the blood-vessels and blood spaces of the pulp are found greatly distended by blood which mostly is not in a fluid but in a clotted state; in fact, there is extensive infarct. In many instances there is indication of necrosis and breaking down of pulp tissue of the spleen, but only in small microscopic foci. As regards the distribution of *B. pestis* in the spleen, these micro-organisms are found almost everywhere and in great abundance — in a scattered manner, but particularly in smaller and larger connected masses, in streaks and clumps in the blood-vessels (stasis), and in amongst the pulp tissue in which breaking-down process is observed. On careful inspection the streaks and clumps of *B. pestis* are seen to be really contained within the blood spaces of the pulp tissue where this reaches an extensive degree, as, for instance, in the necrotic nodules of the spleen in the subacute form in guinea-pigs. The blood spaces show in places an almost perfect natural injection with *B. pestis.*

3. *The lungs* are greatly congested either uniformly through lobes

or limited to one or another lobule; in the latter case hæmorrhages into the infundibula and alveoli are not infrequently to be met with. The large and small vessels, *i.e.* capillaries of the alveolar wall, are distended, much coiled and twisted, and filled with blood. In many instances some lobules and even lobes show consolidation of two kinds :—

(*a*) Patches in which all vessels are filled with blood in stasis, the central point being a bronchus distended by and filled with débris in which numerous *B. pestis* in masses are recognisable.

(*b*) Irregular patches in which a number of alveoli are filled and distended by leucocytes; plague bacilli are not numerous in this second form of consolidation.

In a former report I have described these changes in a more advanced stage of the subacute form.

4. *The liver* shows extensive areas in which the capillary blood-vessels of the lobules are distended and filled with blood; in some parts the blood is in stasis, the central vein being greatly distended and filled with coagulated blood. This is always associated with extensive breaking-down and coagulation-necrosis of the parenchyma. It is for this reason that such parts appear opaque and more or less grey as compared with other neighbouring congested (red) parts. Plague bacilli are numerously present, particularly in the intra-lobular blood capillaries of the infarcted parts, which appear as if injected with plague bacilli.

5. *The kidneys* are generally enlarged and much congested. Sections of them show the blood-vessels of the cortex, including the glomeruli, and of the medulla distended and filled with blood; in some portions of the cortex capillary hæmorrhages may also be noticed. *B. pestis* in groups are found in the glomeruli and particularly in the larger vessels of the cortex and medulla, where they form connected streaks and clumps. The epithelium lining the convoluted tubes is generally in a state of more or less distinct granular degeneration. What is of great interest is that plague bacilli can be found in the space of the Malpighian corpuscles, in some convoluted tubes, and in some uriniferous tubes of the medulla. It is therefore clear that plague bacilli may appear also in the urine of the bladder.

6. As already mentioned, the *small intestine* is congested and contains sanguineous mucus. Sections of the gut show that the

vessels of the mucosa are distended and filled with blood, with capillary hæmorrhages into the tissue; the epithelium of the surface is detached and wanting in many parts, and here the tissue of the mucosa is breaking down; the epithelium lining the crypts of Lieberkühn is detached and disintegrating. Plague bacilli are found in great numbers and masses in the superficial parts of the broken-down mucosa; where this is denuded of its epithelium there may be a continuous layer of these bacilli, all showing beautiful bipolar staining. It is clear, therefore, that the bowel discharges of such an animal would be containing plenty of *B. pestis.*

From these descriptions it is seen that in the acute and typical form of plague such as in rats follows the cutaneous inoculation of small doses of virulent *B. pestis* the changes in the different organs are generally severe and very pronounced, and that the distribution and multiplication of the *B. pestis* in these organs is of a very intensive nature.

II.—*Mice inoculated cutaneously with the Virulent Type of B. pestis*

The conditions of the organs in the mouse are of a character which may briefly be described as an exaggeration in intensity of those observed in like circumstances in the rat. In all organs distension of the blood-vessels with hæmorrhages is a dominant feature; and besides, the inguinal lymph glands and the spleen may be said to be practically crowded with masses of *B. pestis*, which are in all parts in continuous masses and in some places may almost entirely obscure the tissue.

1. *The inguinal glands* corresponding to the inoculated side, the cortical as well as the medullary lymph sinuses, are practically injected with *B. pestis.* Also in the lymphatic tissue of the cortex and medulla masses of *B. pestis* can be seen everywhere. What is left of the lymph tissue is necrotic in many parts. Not only the lymph vessels and lymph spaces of the gland itself, but those also of the surrounding tissue, show distension and almost complete injection with *B. pestis.*

2. In *the spleen pulp* there exists a continuous network of streaks and irregular masses of plague bacilli, the blood-vessels of the pulp being greatly distended and filled with blood.

3. *The lungs* show uniform distension and filling of blood-vessels with blood; in many places within them are found continuous masses

of *B. pestis*; some capillaries and small veins appear almost injected, *i.e.* completely filled, with these bacilli.

4. The like is the case with *the liver*, where the capillaries between the columns of liver cells are in many parts continuous masses of plague bacilli, the liver cells at the same time showing granular degeneration.

5. In *the kidney* all parts are uniformly congested, with many capillary hæmorrhages; many vessels of the cortex as also of the medulla are injected with plague bacilli. This is very strikingly shown in some of the Malpighian tufts and in their afferent and efferent vessels. The masses of bacilli being stained blue—methylene-blue—the specimen now looks, under moderate magnification, as if some of these vessels had been actually injected with blue colouring matter. Granular degeneration and breaking down of the epithelium is seen almost everywhere in the convoluted tubes. Plague bacilli are seen in the uriniferous tubules both of the cortex and the medulla.

In respect of the rapid absorption and multiplication of plague bacilli in the inguinal glands after cutaneous inoculation, their rapid distribution all through the viscera and their enormous multiplication therein, the mouse is undoubtedly the animal offering the best nidus for the growth and multiplication of *B. pestis*. The above description applies equally to mice dead within forty hours and to those dead within sixty or sixty-six hours.

III.—*Rats inoculated cutaneously with Attenuated Plague Type No.* 2

As was mentioned in the experiments already described, death of such animals was delayed, and, except as regards the inguinal bubo and spleen, they showed comparatively few changes in their viscera. The changes consisted of a more or less general congestion, less in intensity than in the animals dead of the virulent type. The inguinal glands presented very much the same character as after cutaneous inoculation with plague of the virulent type, and also the spleen was in some instances found enlarged, dark, and firm, in others only slightly or not appreciably so.

Examination of sections through the hardened organs of rats dead after cutaneous inoculation with plague of type No. 2 was undertaken in a number of instances. The rats thus minutely examined were :—

Rat 1K, experiment 7, p. 119.
Rat 1L, experiment 8, p. 119.
Rat 1O, experiment 11, p. 121.
Rat 1Q, experiment 13, p. 121.
Rat 1R, experiment 14, p. 121.

RAT 1K.—(1) The *bubo* showed in the lymph gland hæmorrhage, and the blood-vessels generally distended with coagulated blood; cortical lymph follicles necrotic in many points; cortical sinuses full of *B. pestis*; surrounding lymph vessels either filled with *B. pestis* or with leucocytes and *B. pestis* together; surrounding blood-vessels greatly distended with blood.

(2) *Spleen.*—Large blood-vessels filled with coagulated blood; pulp spaces distended by blood; most Malpighian corpuscles shrunk and small, showing necrosis in one or the other part. *B. pestis* here and there only in small groups in necrotic parts.

(3) *Liver.*—Numerous large blood-vessels distended and filled with coagulated blood; within the liver lobules and more or less continued from the interlobular vessels were large irregular spaces filled with coagulated blood; liver cells showed granular degeneration; *B. pestis* difficult to find, if any.

(4) *Lung.*—General congestion, hæmorrhage in some parts; infundibula and small bronchi filled with leucocytes and fibrin. *B. pestis* difficult to find, if any.

(5) *Kidney.*—Large blood-vessels distended and filled with blood; glomeruli of Malpighian corpuscles swollen, capillaries containing moderate amount of blood. *B. pestis* difficult to find, if any. The convoluted tubes appeared in most parts filled with hyaline casts, the lining epithelium fairly well preserved.

RAT 1L.—(1) *Bubo.*—Lymph vessels and lymph spaces around the glands injected and distended with *B. pestis*; the blood-vessels of the gland itself greatly distended by blood.

(2) *Spleen.*—All blood-vessels and blood spaces distended by blood. *B. pestis* only in small masses and not numerous.

(3) *Liver.*—Capillaries of the lobules much distended by blood; liver cells contained fat globules, and many of them showed granular disintegration. Some capillaries—but not in many places—contained plague bacilli.

(4) *Lung.*—Great congestion in all blood-vessels; hæmorrhage in some alveoli. No *B. pestis* discernible.

(5) *Kidneys.* — Great congestion of cortical vessels, in some places hæmorrhage; epithelium of convoluted tubes disintegrating. *B. pestis* to be found only in large vessels amongst the blood corpuscles.

Rat 1o.—(1) *Bubo.*—Almost complete necrosis due to infarct. Masses of *B. pestis* in the cortical lymph sinuses and adjoining cortical necrotic parts.

(2) *Spleen.*—Infarct; blood-vessels full of coagulated blood; Malpighian corpuscles shrunk and partially necrosed. *B. pestis* few, chiefly in necrotic parts of Malpighian corpuscles.

(3) *Liver.*—Infarct; bacilli very sparse, if any.

(4) *Lung.*—General congestion, some infundibula filled with fibrin and leucocytes. *B. pestis* very sparse, if any.

(5) *Kidney.*—Slight congestion, only larger vessels distended by blood. *B. pestis* very sparse, if any.

Rats 1Q and 1R differed in no way from the previous rats, except that the inguinal lymph glands showed extreme filling of the cortical lymph sinuses with masses of *B. pestis*; so also the lymph vessels around the glands were partially or wholly filled with *B. pestis.*

IV.—*Mice inoculated cutaneously with B. pestis of Attenuated Type.*

There was no difference, in the condition of the organs and in the wide and copious distribution in them of *B. pestis*, between mice of this series and the mice described in reference to the virulent series. The extreme prevalence of *B. pestis* in connected masses in the lymph spaces and lymph sinuses of the bubo, in the spleen pulp, in the vessels of the liver and lung, is a remarkable feature; just as conspicuous as that described of the mice in connection with the virulent type of *B. pestis.*

From this it appears that as regards the mouse both types of plague act in the same manner; that for both types of plague bacilli the tissues of the mouse offer a most excellent nidus for growth and multiplication. But it is different with the rat. In this animal a distinction between the two types can be made in view of the result of the changes produced in the spleen and by the distribution of the *B. pestis* in this organ, in the lung and liver, and in the blood in general.

In the attenuated type the spleen of the rat is in a large per-

centage of cases only slightly enlarged or not appreciably so, as will readily be understood from the description of the microscopic examination of the organ: not only was there found stasis and coagulation of the blood in most vessels, but also a distinct shrinkage of the Malpighian corpuscles due to necrosis. The number of bacilli in the spleen was small and not to be compared with what is the general rule in the case of the virulent type of plague. As regards the liver, lung, and kidneys, the absence of any appreciable number of *B. pestis* appears a noteworthy feature.

Since the inguinal bubo (*i.e.* the proximal local effect) contains just as vast an amount of *B. pestis* as in the case of the virulent type, the conclusion is justified that in the attenuated type the condition of the organs (congestion, hæmorrhage) is mainly toxic; that is to say, is due to absorption into the circulation of the toxin *formed in the bubo* by the *B. pestis*. Further, it appears that in its attenuated type *B. pestis* does not find in the viscera suitable conditions for its existence and multiplication as does the *B. pestis* of the virulent type, and that owing to *B. pestis* of the virulent type thriving and multiplying well in the viscera the amount of toxin circulating in the system in animals inoculated with it must be greater than in the case of the attenuated type. This would not only account for the more rapid course and more rapidly fatal issue of the disease in the former case, but would seem to indicate that in the attenuated type the delayed death permits sustained action of the toxin, enabling it to produce the conspicuous necrotic changes in the bubo, in the spleen, and in the liver upon which stress has been laid.

One further important consideration deserves notice—namely, that owing to the generally copious presence of *B. pestis* in the vessels of the viscera (lung, intestine, kidneys) of animals inoculated with the virulent type, the excretions of these rats would be liable to be charged with the contagium and to be to a corresponding extent dangerous; whereas with the attenuated type the excretions of the rats, as judged by the above description of the microscopic character of their viscera, would seem to be but sparsely charged with plague contagium, and with contagium not necessarily as dangerous to the human subject.

Plague artificially induced in the Guinea-pig and some other Animals.—We have on several occasions in the preceding pages described the results of the infection

of guinea-pigs with plague materials or with culture of *B. pestis*, and now we wish to give a general summary of the observations concerning these rodents.

As has been repeatedly mentioned, the guinea-pig injected subcutaneously in the groin with a small dose of virulent plague material or of virulent culture develops rapidly (within twenty-four hours) a soft swelling, which within forty-eight hours reaches a considerable size—filbert to pigeon's egg; the animal is now distinctly quiet, sits in the corner of its cage with curved back—"is lumpy,"—and does not feed. It is found dead in or before seventy-two hours. On post-mortem examination the inguinal glands of the injected side are enlarged, firm, and on incision show hæmorrhages; the subcutaneous tissue around the gland is much œdematous and contains many petechiæ and effused blood; this hæmorrhagic condition of the peri-lymphatic tissue, being sometimes very extensive, may reach to the middle line of the abdomen and even beyond, and may in some cases extend upwards and over the chest. In the hæmorrhagic œdematous tissue the *B. pestis* largely abound, as is shown by film specimens and by culture. The inguinal lymph gland itself is packed with *B. pestis*, which on staining are distinctly bipolar. On opening the abdomen the large, dark, firm spleen and liver attract attention; film specimens of a cut surface of these organs and culture yield enormous masses of *B. pestis*, bipolar in staining. In some cases the intestines (small and also large) show numerous punctiform hæmorrhages in their serous coat; the small intestine is relaxed and contains sanguineous mucus, the mesenteric glands in these instances being swollen and showing hæmorrhage. In these positive

instances, that is positive as regards the intestines, the sanguineous mucus and the juice of the mesenteric glands always yield *B. pestis*, and injection of animals with the intestinal mucus yields positive result—acute plague. But in other instances the intestines show nothing marked beyond general injection. In some instances the urine of the bladder is tinged with blood, and then the urine injected into rodents causes plague.

The liver, the suprarenals, the kidneys, and the lungs show congestion. The heart's blood and the blood of all viscera contain *B. pestis*, and, as culture proves, in considerable numbers, although film specimens may, owing to the scattered distribution of the comparatively few *B. pestis*, not suggest it.

If the infecting material is, however, of subnormal virulence, the subcutaneous injection does not cause the above acute form of plague, but leads to the subacute form, death occurring after four or five days or later. On post-mortem examination it is found that the bubo shows necrotic foci in the lymph gland, the spleen is more or less enlarged, granular, and mottled with numerous necrotic nodules. The liver in the early fatal cases may contain only very few punctiform grey dots of necrotic change; in late fatal cases it is generally crowded with them. The lungs in early fatal cases show punctiform or patchy hæmorrhages; in the late fatal cases one or both lungs show necrotic consolidations, some punctiform, others patchy and extensive, involving occasionally a whole lobe. Film specimens of these patches show continuous and dense masses of *B. pestis*, with well-marked bipolar arrangement on staining.

Inoculated cutaneously—care being taken that the

inoculation does not extend into the subcutaneous tissue —the guinea-pig, as mentioned already, develops the subacute form of plague. Only in very rare instances, *i.e.* in cases of exceptionally virulent material, have I seen acute plague in the guinea-pig after cutaneous inoculation, that is plague showing the symptoms of the acute form, death ensuing in or about seventy-two hours. Amongst the many dozens of guinea-pigs, which I have had occasion to experiment on during the last half-dozen years, I have only once dealt with material of such virulence that the two guinea-pigs inoculated cutaneously with it developed the acute and not the subacute form. I have had, however, on several occasions, guinea-pigs which died in four days, and which showed something of a transition between acute and subacute plague—that is, it showed the appearances of the bubo and of the spleen which to the unaided eye were those of acute plague as described above, but on more minute examination in the inguinal lymph gland of the bubo there was nevertheless found one or the other necrotic focus, and the spleen also contained a few minute white punctiform nodules. The subcutaneous injection of the guinea-pig is in my experience an excellent test for deciding whether a given material is of normal or subnormal virulence, and whether the *B. pestis* of it corresponds to type 1 (man) or type 2 (rat); for if the material injected in small dose causes the acute form of plague it may be accepted to be of the normal, if it causes the subacute form it may be considered of the subnormal virulence. But the cutaneous inoculation of the guinea-pig is not of this diagnostic value, since, except in the few rare cases mentioned, the guinea-pig responds to this form of inoculation with the subacute form of

plague only. Although the more or less pronounced and more or less extensive character of the necrotic change in the bubo, spleen, liver, and lung is no absolutely reliable guide, since slight variations being noticed occasionally in different guinea-pigs inoculated with the same material, as a rule the more extensive and more pronounced the necrotic changes are, and the longer death is delayed, the less virulent may be considered the material used for the inoculation.

Observations which I have made for the Local Government Board during 1905-1906, which await publication, were directed to ascertain the varying susceptibility of various races of rats, such as may play a part in carrying plague on board ship and of disseminating it on shore. These experiments, not having been published yet in full by the Board, can only be indicated here in some of their general results; and in connection with it, it is in place to state that Captain Liston has already drawn attention to the varying susceptibility to plague of different races of rats in India.

He found that while the Indian domestic rat is very susceptible to plague and dangerous as regards the transmission of plague to man, the wild rat is less so, and appears therefore to play a subordinate part in such transmission.

From a considerable number of experiments which I have made, it appears that, of all rat races that were tested, the tame or white rat (white, white and black) is the most susceptible to plague, more so than other races living in a wild state; not only is this race more highly susceptible, but it yields *B. pestis*, originally derived from a virulent source, of a highly

virulent character when tested on other rats or on the guinea-pig (see above). Next in susceptibility is the brown ship rat or brown dock rat (brown on dorsum and flanks, grey on belly and chest); this rat, which reaches a good size and is fairly wild, is by the expert rat-catcher supposed to be present in ships coming from South America and the Cape. Next in susceptibility to the dock rat is the black rat; this is a smaller and more timid animal than the brown rat, and is of a more plum-coloured appearance; it is caught on ships coming from India. The next rat race which I received is a brown rat, big and wild; it differs from the former brown rat by being cream-coloured on abdomen and chest; it is caught on ships coming from Norway. This rat is distinctly less susceptible to plague than the former species. Least susceptible is our common sewer rat. This last race has the great disadvantage of all the others, in respect of experimental work, that it is difficult to keep it in captivity, 25 to 30 per cent being liable to succumb spontaneously when kept in cages, and that it is very wild and difficult to handle.

All these rat races are susceptible to infection with plague, but in different degrees. While the white tame rat takes plague in every way and even when inoculated with attenuated *B. pestis*, the Norwegian rat and the sewer rat take it only if the *B. pestis* is of the virulent type; using for cutaneous inoculation the attenuated type of *B. pestis* (type 2 or rat type) failures are more common than successes; while in the case of the brown ship rat and the black ship rat, inoculated with attenuated type of *B. pestis*, successes are more common than failures, though failures do occur. But both the brown ship rat and the

black ship rat take plague readily if inoculated with virulent type of *B. pestis*, and in this respect they differ from the Norwegian and the sewer rat, since even with virulent *B. pestis* failures in them are not exceptional.

Another important fact observed is this: whereas the white tame rat, and to a certain extent also the brown ship rat, breed true the virulent *B. pestis*, showing no diminution in the virulence of the *B. pestis* after one or two passages, it is otherwise with the other races of rats. *B. pestis*, at starting of the virulent type, when passed through the black ship rat for one or two generations is of a distinctly attenuated type; that is to say, the less susceptible black rat and the less susceptible Norwegian rat infected with type 1 (human) yield a *B. pestis* of a distinctly less virulent character, *i.e.* type 2 (rat type). This is to a certain extent also true of the brown ship rat, but to a less extent; it certainly and distinctly holds good for the sewer rat.

This attenuation which *B. pestis*, originally of the human or virulent type, undergoes on its passage through the black ship rat, and may undergo in the brown ship rat, may account for the fact of the notoriously less virulent form of plague in Occidental countries, and the no less notorious fact, as pointed out on a former page (by Dr. Thomson), of the numerous cases where plague in rats on board ship failed to be transmitted to human beings.

Calmette's statement, that *B. pestis* bred in one species of rodents and virulent for that species need not be virulent for another species, may therein find its explanation. As far as my own experiments are concerned, I have always found that *B. pestis* of virulent race, when bred in the white rat, loses none of its virulence for

the guinea-pig. But it is different with the *B. pestis* (virulent type) when bred in the black ship rat, for here as a rule a *B. pestis* will be the result, which, tested on the guinea-pig, behaves like an attenuated *B. pestis*, inasmuch as even when injected subcutaneously it does not cause acute but only subacute plague.

The same explanation may also be applied to Hankin's statement, to the effect that *B. pestis* passed through a series of rats loses its virulence. As mentioned just now, this is certainly not correct if expressed in this general way, for it depends through which race of rats it is passed. It is, however, incorrect when applied to the guinea-pig, for in this animal there occurs no attenuation, provided the race of *B. pestis* at starting is the right one and the infection is made by subcutaneous injection.

The above explanation would at the same time furnish us with the possibility of understanding the reason why transmission of plague introduced by ship rats to those on shore takes, according to all accounts (see also Dr. Ashburton Thomson's *Reports on Plague in Sydney*), a considerable time for its eventual transmission to human beings on shore. It is probable that the type of *B. pestis* bred by those rats is the attenuated type 2, and therefore it requires a particularly inflammable material, *i.e.* highly susceptible human individuals, for starting human plague; but once having had access to a human being it again may readily revert to its former virulent type, and having access to other human beings (pneumonic forms, septicæmic forms of plague) becomes rapidly disseminated and takes effect also among less susceptible human beings.

Plague artificially induced in the Monkey.—When the monkey (macacus) is injected subcutaneously in the

thigh with a few minims of emulsion of recent agar culture of virulent *B. pestis*, the disease does not declare itself before two or three days, by which time the animal appears quiet and refuses food. The next day the animal is manifestly ill, is drowsy, and sits with curved back in a corner of its cage. It is found dead the next morning.

On post-mortem examination the following appearances are found:—The inguinal lymph glands are swollen and inflamed, the surrounding tissue is œdematous. The juice of the lymph gland contains crowds of *B. pestis*. The spleen is enlarged; film specimens show abundance of *B. pestis*. The most strikingly affected organ is the liver, it being considerably enlarged and its vessels engorged with blood. An impression of the cut end of an incision into the liver substance shows all the blood-vessels engorged with blood, comparatively few bacilli in the vessels, but the spaces in which the cylinders of liver cells are contained—the lymph spaces —contain great abundance of *B. pestis*. The lungs show great congestion; the blood contains numerous *B. pestis*, but less so than the liver substance. Stained film specimens of all the above organs show the *B. pestis* markedly cylindrical and bipolarly stained. Cultures of the juice of the inflamed lymph glands, of the spleen, liver, and heart's blood yield pure growth of *B. pestis*.

Inoculation of rats and guinea-pigs with the juice of the swollen lymph glands proves that the *B. pestis* is highly virulent for these rodents.

CHAPTER VII

MODES OF INFECTION OF ANIMALS WITH PLAGUE[1]

THE manner in which plague is or can be transmitted, whether to man or to the rat, has long been the subject of much discussion, observation, and experiment; so that our knowledge in these matters is by no means restricted in character. Nevertheless, some observers seem to regard the chief method of transmission of plague as likely to be parallel with that observed in regard of diseases like malaria and yellow fever.

Malaria is accepted as solely transmitted by the bite of an Anopheles mosquito, and the like is probably true of yellow fever (Stegomyia). But in malaria the Anopheles, having sucked, along with blood from a human being, the malarial parasite in a certain phase of this parasite's life, becomes the host of such parasite in further phases of its development; until, that is, the parasite is ripe again for infection of the human subject by means of the bite of the mosquito which has harboured it. There is no other way of transmission of malaria at present proved.

As an agent in the transmission of plague, the flea has

[1] Report of the Medical Officer of the Local Government Board, 1904-1905.

been indicted by Ogata, Simmonds, Tideswell, Ashburton Thomson, and others; while Hankin has gone a step further, considering the flea a real intermediary host of plague in much the same fashion as the mosquito is host to malaria.

As to this latter hypothesis, that of an intermediary insect host of plague, a number of considerations deserve notice which militate greatly against its acceptance even provisionally. But with reference to the flea as a mere agent in spread of plague, Captain Liston in a paper read before the Bombay Natural History Society, as reported in the *Lancet* of February 25, 1905, is inclined to attribute to the flea, proper to a particular species of rat, an important rôle not only in the transmission of plague from rats to other animals, but also from the rat to the human subject. Captain Liston's suggestion that plague is not readily transmitted from the wild rat to man is quite in accord with my observation as to the lesser susceptibility of the sewer rat to plague. It harmonises also with the results of my own observations (as described in my reports to the Medical Officer of the Local Government Board for 1902-1903 and 1903-1904) as to the lesser virulence of *B. pestis*, type 2, or the "rat type," and the probably greater virulence of *B. pestis* of the domestic rat, which, living in India more in relation with human dwellings, is therefore more likely than the wild rat to be subject to the extra virulent or "human type" of *B. pestis*. As to Captain Liston's view that plague is commonly transmitted from the domestic rat to man by means of the flea proper to that species of rat, more evidence is wanting. Transmission of plague in this way, though theoretically quite possible, does not seem

to me to have a basis of direct experiment as regards the species of rat and flea in question.

Plague has been experimentally proved (see previous chapters) to be transmissible in a number of ways, which under natural conditions must needs frequently find their counterpart. Thus, no one doubts that just as in the laboratory cutaneous inoculation of the *B. pestis* is an infallible means of producing plague in the rat, so also under natural conditions an abrasion of the skin of this animal may serve to give entrance to *B. pestis*. Similarly, numerous cases of plague in human beings who, having some broken skin on the hand, have handled plague materials and developed axillary plague buboes on the injured side, as also others who in a similar fashion have contracted femoral plague bubo of one side owing to a cutaneous injury on the foot of that side, are in no want of explanation. Again, rats in their wild state are rarely without some objective sign of their fighting habits—wounds on tail or snout, for instance. Application of *B. pestis*, present in infected food, filth, or other substances, to such wounds on tail or snout would, no doubt, often result in plague of the animal thus, as it were, inoculated. Further, no one doubts that the expectoration of a case of pneumonic plague is charged with *B. pestis*, or that such expectoration is highly infectious if it finds entrance into a healthy person, as, for instance, along with microscopic droplets of mucus coughed out into the atmosphere by the affected patient. In a word, no one doubts that so long as the *B. pestis* is present and virulent in quality, its direct access to the tissues of a susceptible human being or of a rat could, and often would, cause the disease. Obviously, therefore, it is

unnecessary for the successful transmission of plague to man or to rat that *B. pestis* should have previously been taken up by, not to mention stored within, an intermediary host like the flea.

The essential point about the transmission of the disease is that the *B. pestis per se* of a plague case (man or rat) should obtain entrance into a new individual. *B. pestis* of the laboratory has no particular phases in which only it is specially active; in no sense, therefore, is it parallel with the malaria parasite in this respect. Active *B. pestis* has practically but a single phase, namely, that of a bacillus multiplying by fission. This bacillus, whether taken from an active animal source or from an actively growing culture, is always, so long as its normal virulence has not deteriorated, effective on inoculation.

As was pointed out in a previous chapter as regards a large percentage of rats dying quickly of virulent plague, the blood of the circulation just before death contains the *B. pestis* sometimes in great numbers, as is shown both by microscopic examination and by culture experiment. It is therefore quite possible that fleas fed on the blood of an animal dying in this state might contain the *B. pestis*. It was also pointed out that in the virulent or "human" type of plague in the rat the bacilli are more copiously present in the blood of this animal than in the less virulent or "rat" type. Wherefore fleas fed on the blood of a rat affected with the first type of *B. pestis* would be more likely to contain *B. pestis* than if fed on the second or "rat" type, and the more competent, therefore, by hypothesis, to cutaneously inoculate other rats in proceeding at once to bite them.

Subject to these reservations, the possibility of trans-

mission of plague from rat to rat by means of fleas cannot be on theoretical grounds denied. But the question to which answer is wanted is the extent to which this method of transmission is common, as compared with the more obvious methods previously mentioned in reference to this animal, viz. by cutaneous abrasions or wounds coming in contact with plague-infected matter, by ingestion, or by way of the respiratory tract. In order to assign to these different modes of transmission their proper rôle it is necessary to bear well in mind—(1) the distribution of *B. pestis* in an infected animal (rat), and (2) the experimental evidence at present to hand in regard to production of plague in the rat by one or another method.

In a rat acutely affected with the virulent or human type of plague *B. pestis* is distributed throughout the body. If an animal has been cutaneously inoculated the nearest lymph glands are crowded with *B. pestis*; the blood of the general circulation contains the bacillus in great number; the spleen is crowded, sometimes literally packed with them; and in the blood-vessels of the liver, the lungs, and the kidneys *B. pestis* is abundant.

In more than fifty per cent of the animals the *small intestine* is found relaxed and much congested; and its cavity contains blood-tinged mucus, which under the microscope shows along with blood corpuscles numerous bipolar-stained bacilli. Guinea-pigs injected subcutaneously with this mucus, and rats inoculated with it cutaneously, develop in the great majority of instances definite plague. Whenever, in fact, a rat dies of plague and shows the above condition of the small intestine, viz. congestion of the wall and blood within the cavity, the probabilities are

that this sanguineous mucus contains also the *B. pestis* derived from ruptured vessels; and certainly there is no difficulty in producing plague in rats or guinea-pigs injected subcutaneously or inoculated cutaneously with such sanguineous mucus. As regards rats, therefore, suffering acute (septicæmic) plague, and the subjects consequently of hæmorrhage into the intestines, it is reasonable to anticipate that their bowel discharges will contain *B. pestis*; that this is so is indeed clearly indicated by experimental evidence.[1]

The *kidney* is another organ which in acute virulent plague of the rat shows not only great congestion (the vessels of all parts being distended by and filled with blood), but exhibits also actual capillary hæmorrhage, particularly amongst the convoluted tubes of the cortex and in the Malpighian tufts. On careful examination of microscopic sections of the plague-kidney of the rat *B. pestis* are found not only in the distended capillaries of the glomeruli and between the uriniferous tubules, but I have seen them and have described them as occurring in the cavity of the Malpighian capsule and in uriniferous tubules themselves. It follows, therefore, that occurrence of *B. pestis* in the urine of such animals is to be confidently expected; indeed, in several animals (rats and guinea-pigs) dead of (inoculated) acute virulent plague the urine of the bladder was found distinctly tinged with blood; and such urine on injection into other rats and guinea-pigs produced typical plague.

Two instances will be sufficient to further prove this:—

[1] This I maintain notwithstanding that Mr. Hankin in an article in the *Journal of Hygiene*, vol. v. No. 1, appears to discard this mode of transmission, viz. by the bowel discharges of a plague rat. (But as to this see positive experiments which follow.)

(*a*) A rat was inoculated on April 26 with virulent agar culture of *B. pestis*. The animal was dead on 29th of the month with acute typical plague. The bladder was found distended by blood-tinged urine. With this urine one rat and one guinea-pig were injected subcutaneously; both animals died of plague with copious presence of *B. pestis* in the bubo and spleen. (*b*) A guinea-pig was injected subcutaneously with recent agar culture of *B. pestis* on February 18. It was dead on the 22nd of the month with typical plague. The bladder was distended with urine. A guinea-pig was injected subcutaneously with this urine on the same day. The animal was found dead with typical subacute plague on March 1, the bubo, spleen, liver, and lungs containing in the necrotic nodules crowds of *B. pestis*. Blood must therefore have found its way into the cavity of the bladder in these cases and with it presumably *B. pestis*. Hence the urine of a rat affected with the acute virulent (septicæmic) type of plague has to be regarded as very possibly infective.

The *lung* of the plague rat is always more or less congested, occasionally showing minute capillary hæmorrhages. Sections show in some lobules effusion of blood, and in films thereof *B. pestis* is detected. But I have not been able to satisfy myself that the mucus of the mouth, pharynx, or larynx of the rat dead of acute plague commonly contains *B. pestis*; several experiments made in this direction have not been productive of positive results, the mucus having been taken carefully without injuring the blood-vessels of the mucous membranes.

The lung appearances are, however, altogether different in rats which do not succumb with acute plague, but

which pass on into the subacute or even chronic stage; rats, that is, which, though affected with plague, do not succumb within five or six days. I have pointed out that under these conditions the lungs are profoundly and intensively affected; that they show extensive consolidation, the bronchi being filled with inflammatory products containing crowds of plague bacilli;[1] and I have described experiments showing that the oral, pharyngeal, and laryngeal purulent exudation of a rat so affected produces on cutaneous inoculation typical acute plague.[2] From this it was inferred by me that a rat of this kind would by its bite of another rat be capable of communicating plague (by cutaneous inoculation) to such other rat. A chronically affected rat would, I noted, have many chances of doing this, seeing that such rat is generally drowsy and sulky, and that when disturbed by other animals it generally bites them, a method of positive inoculation actually observed under experimental conditions.

[It may be added that the German Plague Commission have produced plague in rats by simply placing plague material into their conjunctival sacs; and further, it has been observed that rubbing in plague material (taken from a plague animal or from culture) into the skin of the nostrils or mucous membrane of the nasal cavity of guinea-pigs was sufficient, in a majority of instances, to produce plague.]

Ways and opportunities of experimentally infecting rats with plague with materials derived from plague rats are in fact so many and various that it is probable that

[1] See Plate II. in Report of the Medical Officer of the Local Government Board for 1902-1903.

[2] *Loc. cit.*, see Fig. 9, Plate III. p. 690, Report of the Medical Officer for 1902-1903.

abundant opportunity of rat infecting rat can occur also under natural conditions; so that the transmission of plague by fleas from the affected to the healthy rat would at best but represent but one among many ways, one moreover that would require more conditions for fulfilment than many others. The chances, for instance, that the bowel discharges, the kidney secretions, or the secretions of the diseased lung of a plague rat would find contact with an abrasion of the skin of the body, or even with the skin and mucous membrane of nose and mouth of healthy rats, are obviously greater than that a flea from an infected rat retaining *B. pestis* after biting the plague rat would settle on a fresh rat and communicate the disease to it by its bite. I do not mean to doubt this latter possibility—in fact, as I have already said, I consider this theoretically quite possible. All I would urge is: that the chances that it actually occurs, and, as some would have it, frequently occurs, are far fewer than those other chances mentioned above, viz. those that might almost be called ever present during prevalence of plague among rats. Dr. Tideswell's positive experiment appears to me not cogent as to the transmission of plague from rat to rat by the bite of the flea, and, so far, numerous observations as to the presence of the true *B. pestis* in fleas taken from rats affected with plague (including the later experiments by Simmonds) have yielded negative results (see the Report of the Bombay Plague Laboratory for 1902).

Hankin (*Journal of Hygiene*, vol. v. No. 1) describes a single observation as to a flea which contained in its stomach *B. pestis*, as shown by microscopic examination and by culture. This, however, proves no more than that

a flea taken from a plague rat may contain the *B. pestis*; it is far from justifying the proposition that such a condition indicates one of the general modes of the transmission of plague, and affords no warrant at all for supposing that the flea represents a real intermediary host in which the *B. pestis* multiplies and acquires virulence. Nevertheless, Hankin attempts to explain in the latter way certain epidemics of plague in man which have appeared in particular localities some considerable time after the introduction of a case of plague into them.

So far we have left out of consideration one possible channel of transmission of plague which must be, and has always been, in the mind of every one acquainted with plague as it occurs in nature. This is transmission of infection by means of food, *i.e.* infection by way of the alimentary canal.

First of all, what are the epidemiological facts observed hitherto? It is an occurrence of no uncommon kind for rats to die of plague in places where fodder and food-stuffs are accessible to them, *e.g.* fodder, grain, rice, and flour stores, larders on ships, etc., and in circumstances consistent with such rats having contracted the disease by eating food-stuffs previously specifically contaminated by plague rats. For instance, a plague rat living in stores would most probably be capable of polluting these food-stuffs by its intestinal discharges, by its urine, possibly also after death by dispersal of plague bacilli previously stored in its tissues.

In the second place, there is no theoretical reason why both in the rat, as also in the human subject, the mucous membrane of the digestive tract, including that of the mouth and fauces, should not be as amenable (perhaps

more so) to the entrance of the *B. pestis* as is the corium of the skin. In the mucous membrane there is no protective dry cuticle to be overcome as in the skin; seemingly, therefore, *B. pestis* has only to be lodged in the soft surface epithelium — which, under the many mechanical operations involved in the process of mastication, deglutition, and digestion, might occur—in order to become actually "inoculated." The introduction with positive result by the German Commission of *B. pestis* into the conjunctival sac of rats would be an illustration in point; and here the mechanical action of the eyelids would be capable of ensuring such "inoculation."

It is matter of history, however, that numerous experiments made in the laboratory in feeding rats and other susceptible animals with plague cultures, and with fresh organs of animals dead of plague, have commonly failed. Leaving out those positive instances[1] in which cervical buboes and plague occurred in a rodent (guinea-pig, rat, mouse) after gnawing the bones as well as the viscera of a plague animal—positive instances which might have been really due to inoculation of an abrasion caused by sharp points of bone splinters,—negative results have hitherto

[1] In the large number of experiments of feeding made by the German Plague Commission, there were only very few cases indeed in which a direct infection *via* the intestine could, from the anatomical lesions found on post-mortem, be considered to have actually occurred—leaving out those instances in which infection clearly is referable to having taken place in the fauces; and in the positive instances (second type of the Commissioners) the intestinal infection appears to have occurred in rats only after feeding them with rats dead of plague.

The Austrian Plague Commission, in their feeding experiments, seem to have had positive cases of septicæmic plague in guinea-pigs and rats, but of a character which clearly denoted infection to have taken place from the mouth or fauces; but they appear to have had some few positive intestinal infections (Peyer's patches hæmorrhagically infiltrated or necrotic, and the same condition of the mesenteric glands) in guinea-pigs only, after feeding them on animals dead of plague. But most of their feeding experiments (on rats and mice) were negative *quâ* intestinal infection; positive infection clearly occurred from the mouth or fauces.

been in the main recorded. I know from a personal communication made to me by Professor Haffkine, that at the Bombay Plague Laboratory attempts at producing plague in rats by feeding have been uniformly unsuccessful.

I myself have to record a considerable number of failures of the same kind. Guinea-pigs, rats, and mice were repeatedly fed by me with milk culture and with broth culture of *B. pestis*, as also with fresh organs (bubo, spleen, liver, lung) of animals dead of typical plague; but without result — the animals remained unaffected. After a fortnight or so they were subjected to inoculation with active *B. pestis* in an abrasion of the skin, in a puncture of the oral mucous membrane, or by rubbing the material well into puncture of the skin of the nostrils; and in all cases acute plague was the result. Only once out of twenty experiments on sewer rats have I had positive result by feeding. This rat had been fed (at the same time as others) with plague organs of a rat dead of inoculated plague; and it too died of plague. The intestine showed general congestion, and the mesenteric glands were enlarged and much congested. But I did not consider this a proof that the animal had become infected by way of the intestine, because such a condition occurs also not unfrequently after subcutaneous or cutaneous inoculation; and, further, because in the above rat the lungs and bronchial glands were in the same condition as the intestine and mesenteric glands. Be this, however, as it may, it occurred to me that the probable reason why simple feeding with plague organs or with active culture of *B. pestis* is rarely followed by infection is that the *B. pestis* is commonly destroyed by the secretion of the

stomach during digestion. I suspected that if *B. pestis* could reach the small intestine unscathed—*i.e.* in a living state—its power of multiplication would not only not be interfered with, but that the alkaline condition there might help it. A good parallel illustration is to hand in the case of the bacillus of fowl cholera, viz. a microbe which manifests its activities in the small intestine of that bird, this being the organ principally affected by the disease. It is extremely difficult to produce in the laboratory a positive result by feeding fowls or rabbits—both highly susceptible to inoculation—with culture of the bacillus of fowl cholera. Nevertheless, it is notorious that when once the disease is introduced into a poultry farm it rapidly spreads, and that it can do so obviously only by means of matter escaping from the alimentary canal of the fowls; the droppings of a diseased fowl teeming with the specific microbe infecting the soil, and consequently the food distributed on such soil. There must, therefore, exist here conditions which essentially differ from those in the laboratory experiment; and it is reasonable to suppose that the contagium mixed with the soil and food is better secured against the action of the gastric juice than when mixed as a broth culture with food. It has hence occurred to me that it might be possible to obtain greater success in plague-feeding experiments by first protecting the contagium (*B. pestis*) by drying it along with the food, or by administering it with the food in such a state that some of it at any rate might pass unscathed through the stomach.

Experiment 1.—As already mentioned, I have made a number of experiments in feeding guinea-pigs, rats, and mice with virulent cultures of *B. pestis* (emulsion of

recent gelatine and agar cultures, of broth cultures, of milk cultures), as also with the various fresh plague organs (lymph glands, spleen, liver, and lung[1]), containing abundance of *B. pestis*; all of which experiments were without positive results. Of these experiments it is sufficient to mention the following:—

Two rats and two mice were fed with milk cultures (incubated five days at 37° C.), which when tested by subculture were crowded with living *B. pestis*. For feeding purposes the milk culture was mixed with bread. After the lapse of a week the same four animals and one additional rat were fed with similar milk culture and bread. All the animals, however, remained unaffected. A fortnight later they were cutaneously inoculated with a trace of a similar milk culture, and promptly succumbed with plague between the third and fourth days.

In the same way two guinea-pigs and two rats were fed with the organs (minced and mixed with green food and bread) of one of the above rats. No result followed. After ten days these animals were inoculated (the guinea-pigs subcutaneously, the rats cutaneously) with agar culture derived from the spleen of one of the rats of the above experiment. They all died of plague.

In all experiments referred to here the rats used were, unless otherwise stated, tame or white rats. As I have explained in my reports to the M.O. of the L.G.B. for 1902-1903 and 1903-1904, tame white rats are highly susceptible to plague infection in all the various ways. Not so sewer rats. These rodents, although susceptible to plague infection, are so to a lesser degree than tame rats,

[1] These were derived from guinea-pigs and rats that had died with acute virulent plague. Such organs inoculated in minute doses into rodents produced acute and typical plague.

and I have therefore limited myself to only few experiments with them. It would seem that tame rats (white or white and black) are in respect of susceptibility more nearly related to *Mus rattus* (the Oriental rat) than to *Mus decumanus* (the brown sewer rat).

Experiment 2.—Three rats were fed with a mixture of bread and sloped surface gelatine and agar cultures of *B. pestis*. The gelatine cultures had been kept growing at 20° C. for some six, those on agar for some three weeks. At the margin of the slopes of these cultures both the gelatine and agar were dried up, but the free sloped surface was covered with typical colonies of *B. pestis*; colonies which were angular, granular, opaque, raised, and dry-looking. After melting the gelatine by immersing the tubes in warm water—in the case of the agar the glass tubes had to be broken—the contents of the tubes were mixed with bread. The above animals were fed with this mixture on May 2. In the morning on May 5 two of the rats, No. 1 and No. 2, were found very ill, in fact dying; one died at about 10 A.M., the other at noon. The third rat, No. 3, looked quiet, its coat was rough, and seemingly it did not feed. This rat did not improve for the next few days, but on May 8 was noticed to be again lively and to feed normally. It was killed on May 10. The post-mortem examination of this animal did not reveal any pathological changes, and cultures made from the spleen and heart's blood proved sterile.

But the post-mortem examination of rats No. 1 and No. 2 showed the following appearances:—

Rat No. 1.—No swollen subcutaneous lymph glands. The omentum much congested with numerous petechiæ; in the lower ileum a patch of hæmorrhage around a

swollen and partially necrotic Peyer's patch, the latter itself much injected, but well marked off from the hæmorrhagic tissue around it; the mesenteric glands swollen, deep purple in colour, hæmorrhagic. Both testes showed the parenchymatous and superficial lymphatics filled with blood, and the lymphatics of the spermatic cord and pelvic lymph gland were in like condition.[1] The spleen was much enlarged, dark; both lungs congested; liver, kidneys, and suprarenals in same condition.

Film specimens were examined and cultures made—(*a*) of the mesenteric glands, (*b*) of the spleen, (*c*) of the heart's blood. In all these instances the film specimens showed crowds of *B. pestis*, and the cultures were crowded with colonies of *B. pestis* in pure state.

The hæmorrhagic patch of the ileum, and the Peyer's gland, the kidney, liver, spleen, lung, mesenteric gland, and testes were placed in Müller's fluid, then in spirit. After completion of the hardening process these organs were used for preparing sections.

Rat No. 2.—The post-mortem examination showed almost exactly the same appearances as those of Rat 1, except that the testes appeared free from any change. The lower ileum showed a hæmorrhagic patch in which there was a swollen, prominent, congested, and partly necrotic Peyer's gland. The spleen, kidneys, mesenteric glands, and lungs were similar to those of Rat 1.

Film specimens and cultures of the hæmorrhagic mesenteric glands, of the spleen, and of the heart's blood showed that these organs contained crowds of *B. pestis*. The hæmorrhagic patch and swollen Peyer's gland and

[1] I have seen this condition of the testes occasionally also in guinea-pigs which have succumbed with acute plague after subcutaneous injection.

the mesenteric glands were preserved in Müller's fluid with a view to afterwards making sections.

On examination, the stained and mounted sections of the intestinal hæmorrhagic patches and Peyer's glands, as also sections of the mesenteric glands, showed in both cases (Rat 1 and Rat 2) the same condition. It will suffice therefore to describe the appearances in one animal only.

Rat 1—the appearances are shown in Figs. 75, 76, and 77. Fig. 75 shows a transverse section of the swollen Peyer's gland with margin of surrounding tissue, under low magnification ($\times 25$). As is well seen, the Peyer's gland projects considerably on the serous (peritoneal) surface, and opposite this projection—*i.e.* towards the cavity of the intestine—is a mass of débris extending over the surface of the mucous membrane beyond. Under a high power masses of bipolar-stained *B. pestis* are recognisable in this débris as small and large clumps; they appear as dark stained patches. In some places, *e.g.* in the neighbourhood of line 1, a large mass of *B. pestis* may be seen embedded in finely granular material, which is in fact a remnant of one of the original colonies in the agar with which the animal had been fed. The superficial part of the Peyer's gland—below the central mass 1—is necrotic tissue with a large amount of diffused blood. At 5, the mucous membrane (villi) is devoid of its surface epithelium; its mucosa shows numerous vessels distended by blood, and in its tissue is effused blood; so also around the crypts there is a good deal of effused blood. Masses of *B. pestis* are seen not only everywhere between the tissue elements of the villi, but also in the effused blood. At 2 and 3 in the space between swollen lymph follicles,

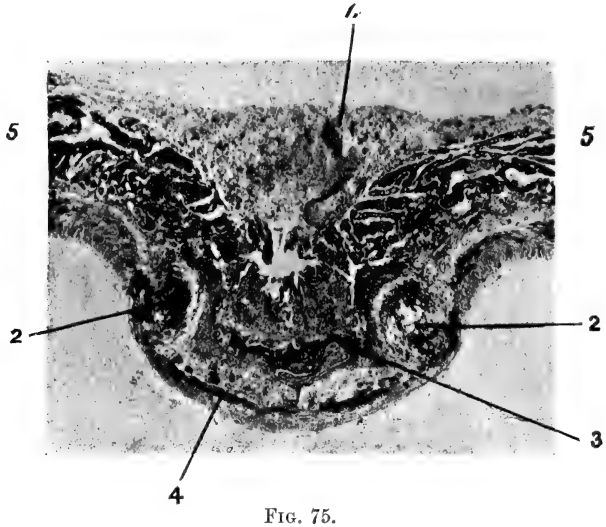

FIG. 75.

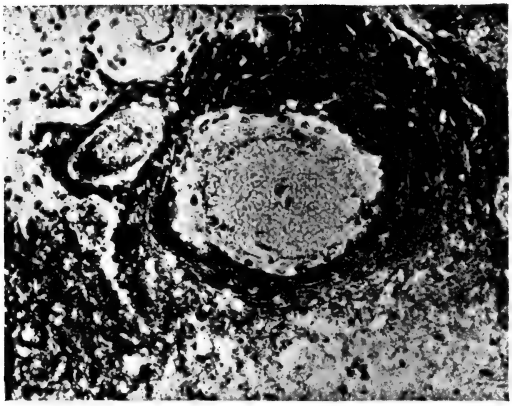

FIG. 76.

FIG. 75.

Reproduced from a section through the affected Peyer's patch of the ileum of a rat dead of plague after feeding; seen under a low magnifying power.

At 1. Piece of "food" (crowded with *B. pestis*) wedged in and fastened at the pit of the mucous membrane corresponding to the internal portion of the Peyer's gland.

At 2. A swollen and partially necrotic lymph follicle.

At 3. Lymph spaces surrounding a large blood-vessel and filled with continuous masses of *B. pestis*.

At 4. Lymph vessels filled with *B. pestis* between the layers of the outer muscular coat.

At 5. Necrotic mucous membrane filled with extravasated blood and masses of *B. pestis*. × 25.

FIG. 76.

Large blood-vessel of the submucosa (3 of Fig. 75) containing numerous *B. pestis* amongst the blood corpuscles, surrounded by lymph space filled with *B. pestis*. × 300.

FIG. 77.

Part of Fig. 76, more highly magnified.

A, Blood corpuscles within the blood-vessel.

B, *B. pestis* in surrounding lymph spaces. × 1000.

FIG. 78.

Transverse section through mesenteric gland of a rat dead of acute plague after feeding.

A, An afferent lymph vessel filled with *B. pestis*.

B, Cortical lymph sinuses filled with *B. pestis*.

All the light areas of the figure are the parts containing extravasated blood, the dark parts are masses of *B. pestis*. In the actual preparation, which was stained with methylene blue and eosin, the contrast between the masses of *B. pestis* (blue) and the extravasated blood (pink) was particularly striking. × 20.

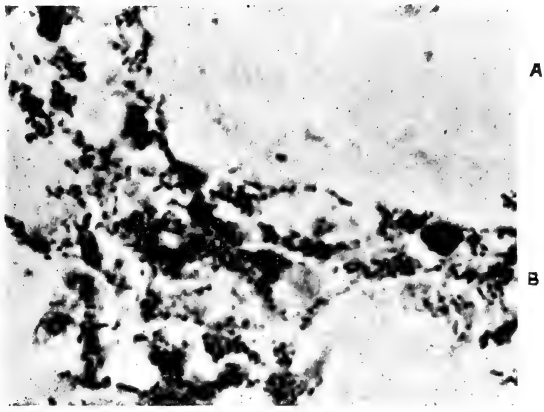

Fig. 77.

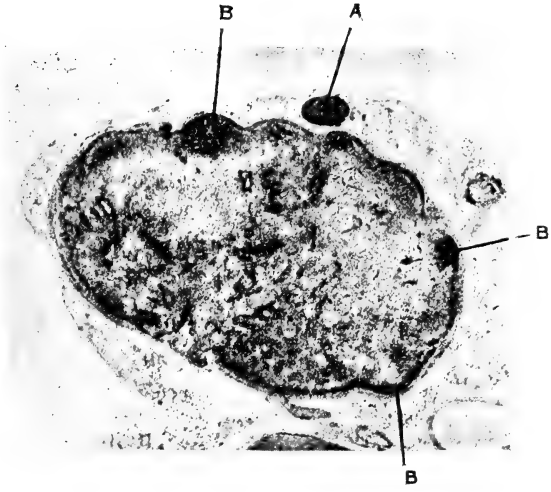

Fig. 78.

in the submucous tissue, is a large blood-vessel distended by blood, and surrounding it is a lymph space densely filled with a continuous mass of *B. pestis*. Similarly at 4, lymph vessels situated between the inner circular and outer longitudinal layer of the muscular coat are filled with continuous masses of *B. pestis*. In the figure these lymph spaces at 3 and 4 appear dark; but in the actual specimen stained with methyl-blue and eosin these parts are deeply blue, while the blood of the blood-vessels is pink. Under a sufficiently high magnification the fact that the masses are composed of *B. pestis* is easily recognised.

In this animal, as also in others yet to be described—rats, guinea-pigs, and mice—sections through the hæmorrhagic patches of the mucous membrane over and around the affected Peyer's glands show some villi which present a remarkable appearance. Continuous streaks and masses of *B. pestis* (Figs. 80, 81, 87, and 89) not only cover the surface of the villi denuded of their epithelium, but extend between the tissue elements in the same reticular fashion as chyle does during absorption; moreover, in some instances the central chyle vessel appears distended and filled with the *B. pestis*. In particular villi the tissue is completely deranged by effused blood in which appear numerous *B. pestis*. Continuous masses of *B. pestis* are found also around the fundus of the Lieberkühn crypts, surrounding these like lymph spaces, as also within the cavities of the crypts.

In Fig. 76 the blood-vessels mentioned at 3 (Fig. 75) are shown somewhat more magnified (× 300). They are filled with blood in which abundance of small and large masses of *B. pestis* can be seen (dark in the figure); the

blood-vessels are surrounded (perivascular lymph spaces) by the continuous masses of *B. pestis* mentioned above. This is specially shown in Fig. 77 (× 1000) from a point where the *B. pestis* are less densely packed, and therefore recognisable as such.

Fig. 78 represents a section through the hæmorrhagic mesenteric gland, photographed under a low magnification (× 20). The gland is seen surrounded by fat tissue. Near the middle of the upper part, close to the capsule, is an afferent lymphatic (dark) densely packed with *B. pestis*. At the right upper angle, within the fat tissue, is a blood-vessel containing masses of *B. pestis* (dark). In several places on the inside of the capsule, in situations corresponding to cortical lymph sinuses, are continuous masses (dark) of *B. pestis*; so also in the medullary part. A good deal of the cortical part is more or less necrotic, and contains effused blood (light). In the actual specimens, stained double with methyl-blue and eosin, the contrast between the masses of *B. pestis* (blue) and those of blood corpuscles (pink) is very striking.

Sections through the spleen, kidney, liver, and lung show not only that the blood-vessels up to their finest branches are distended with blood, and contain large numbers of *B. pestis*, but that in many places there is blood effused outside the vessels between the elements of the parenchyma, in which blood a great many *B. pestis* in small and large masses are seen. The spleen pulp is in many places literally packed with continuous masses of *B. pestis* in streaks and patches. Sections through the testis show the intertubular lymph spaces filled with blood, and amongst this continuous streaks and irregular

clumps of *B. pestis* (see Fig. 79). In the liver the capillary blood-vessels within the acini appear in some places almost as if injected with *B. pestis*, while the liver cells appear shrunk or full of fat globules. Sections through the lungs show, besides *B. pestis* present in some parts in the distended veins and capillaries of the minute bronchi and alveolar walls, that the alveoli contain blood and homogeneous exudation. Extravasation of blood *en masse* occurs in many parts of the peribronchial and interlobular connective tissue. Similar extravasations of blood *en masse*, containing numerous *B. pestis*, are met with in many places in the connective tissue between the cortex and medulla of the kidney. And small branches of veins in the cortex of the kidney, as also the capillaries of the glomeruli, contain numbers of *B. pestis*, forming in many capillaries continuous blocks.

[As mentioned above, the naked-eye appearances and cultural results of rat 2 were the same as those of rat 1, and it is not necessary to describe again the appearances of the sections made through the hæmorrhagic swollen Peyer's patch, the mesenteric glands, the spleen, the liver, and the kidney. They showed the same copious distribution of *B. pestis* in the blood-vessels, in the lymphatics, and in the parenchyma.]

From these observations it is seen that out of three rats fed, two succumbed to plague; that they showed not only definite changes of the intestine, but in unmistakable manner the exact spot or portal of the infection. Further, it is seen that not only was there abundance of *B. pestis* in the interior cavity of the affected intestine, but also that the lymphatics of the affected part of the intestine (villi, mucosa, submucosa, and muscular coat),

the mesenteric glands, and the vascular system in general, had become literally crowded with the *B. pestis.* This is a result more intensive in respect of the distribution of the *B. pestis* in the affected animal than that which occurred after subcutaneous or cutaneous injection.

Experiment 3.—Four rats, Nos. 4, 5, 6, and 7 (kept in couples in cages), were fed on May 10 with bread mixed with gelatine cultures of *B. pestis.* The cultures were six weeks old and contained abundance of typical colonies; but the gelatine around them was almost dry, and the colonies themselves were dry-looking and shrivelled.

Rat No. 4 was found dead on May 13; rats Nos. 5 and 6 on May 14. Rat No. 7 appeared quiet at this date, but by May 17 had become quite normal, and it remained so.

The post-mortem examination of rat No. 4 showed the following appearances:—Both mammary glands much congested and showing streaky hæmorrhages; sections through the hardened tissue showed a great deal of effused blood in the alveolar tissue, with numerous *B. pestis.* The omentum was congested, and there were numerous petechiæ in the ileum; a distinct patch of hæmorrhage was seen around a swollen Peyer's gland; the mesenteric glands were swollen and hæmorrhagic; the spleen was typically enlarged, dark, and firm; the other viscera were congested. Cultures and film specimens of the mesenteric glands, of the interior of the intestine at the seat of hæmorrhage, of the spleen, and of the heart's blood showed copious presence of *B. pestis.*

Rat No. 5.—The whole of the lower ileum was relaxed and filled with a sanguineous mucus; several Peyer's

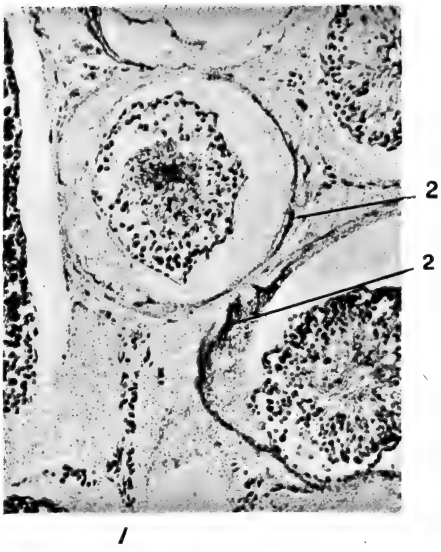

FIG. 79.

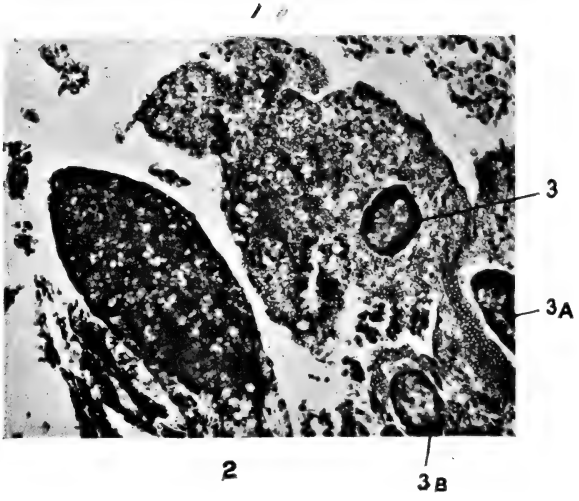

FIG. 80.

FIG. 79.

From a section through the testis of rat mentioned in Fig. 75.

1. A small arteriole showing a rupture (at its upper end). The tissue around it and the lymph spaces between the seminal tubules, seen here in cross section, are filled with the extravasated blood.

2. Masses of *B. pestis* in this extravasated blood. × 120.

FIG. 80.

Reproduced from a section through the ileum, at the site of hæmorrhage, of a mouse dead of acute plague after feeding.

1. A villus containing a large amount of extravasated blood; its central lymph vessel, 3, seen in section, is filled with *B. pestis*; the same appears at 3*A* and 3*B*.

2. A villus denuded of its epithelium and permeated in reticular form by masses of *B. pestis*; the same as during absorption with chyle. × 165.

FIG. 81.

Part of a similar villus to that in Fig. 80, more highly magnified.

At 1. Surface of villus covered with continuous masses of *B. pestis*, which extend (2) in reticular formation between the tissue elements of the villus; exactly the same appearances as are shown by a villus in an active state of an ordinary absorption with chyle, with the difference that in this particular case the chyle globules are replaced by *B. pestis*. × 1000.

FIG. 82.

Transverse section through the affected part of the ileum of a rat dead after feeding with plague material, *i.e.* with plague spleen dried in wheat.

In the interior of the gut is a particle of material fixed a little above the section to the inflamed and necrotic Peyer's gland. This particle consists of a central mass of spleen tissue full of *B. pestis*, surrounded by semi-digested wheat.

The villi contain abundance of *B. pestis* similar to what was shown in Figs. 80 and 81. × 25.

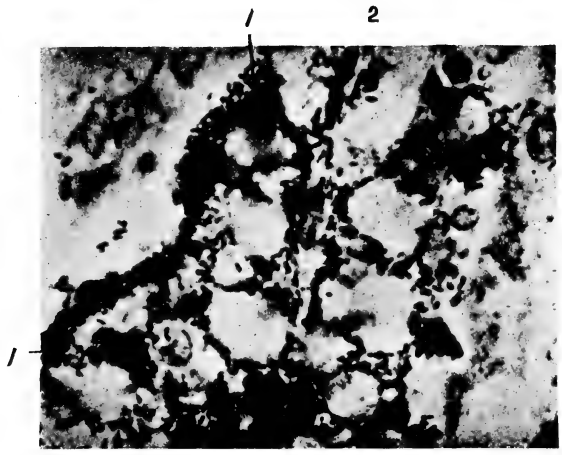

FIG. 81.

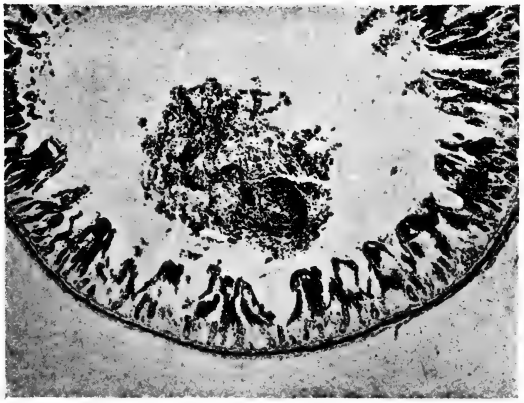

FIG. 82.

glands were swollen and showed punctiform hæmorrhages; the mesenteric glands were swollen and showed hæmorrhages; the spleen and kidney were large and dark; the testes showed several hæmorrhagic patches, and their lymph vessels were injected with blood.

Cultures, film specimens, and sections of the hardened organs showed the same copious presence of *B. pestis* as mentioned in regard of the former animals. Sections through the various organs showed the same appearances of distended vessels and hæmorrhage, along with copious presence of *B. pestis*, as was described in the case of rat No. 1.

Rat No. 6.—Several of the Peyer's glands of the ileum were enlarged and showed hæmorrhages; mesenteric glands slightly enlarged, congested; both testes showed lymph spaces and lymph vessels injected with blood; the spleen was large and dark. Cultures and film specimens of the Peyer's glands, of the mesenteric glands, of the spleen, and of heart's blood proved to be crowded with *B. pestis*. Sections through the various organs showed appearances as in the former case.

Here, then, there was positive result in three out of four cases; definite pathological changes of the intestine with intensive and extensive distribution of *B. pestis*.

Experiment 4.—Two guinea-pigs, Nos. 8 and 9, were fed at the same time (May 10) and with the same stock of gelatine cultures as were the rats of the previous experiment; with this difference only, that for the guinea-pigs the gelatine cultures were mixed with soft green food—cabbage leaves finely cut up. In all these feeding experiments it was of course essential that no pointed particles should be in the food, in order to obviate

the possibility of inoculation of the fed animal by pricks of its skin or mucous membrane. As a matter of fact, in no case was any such accident noticed—the animals either succumbed with definite lesions of the ileum, denoting the place of entrance of infection, or they remained alive.

Guinea-pig No. 8 was found dead on May 16.

The post-mortem examination showed a considerable portion of the lower ileum swollen, deep purple in colour, and hæmorrhagic; several Peyer's glands swollen and necrotic; the interior of this part of the ileum, as also of the adjoining portions, appeared filled with sanguineous matter, which in film specimens yielded *B. pestis* in pure culture. The mesenteric glands were swollen and hæmorrhagic, showing also several whitish-grey necrotic foci. The liver and spleen were crowded with grey punctiform nodules.

Cultures were made of the intestinal contents, of the heart's blood, and of the spleen. As a result, in all cases pure cultures of *B. pestis* were obtained, except as regards cultures of the intestinal contents, which contained a small number of *B. coli* in addition.

Two mice were inoculated cutaneously with a trace of the sanguineous intestinal contents of guinea-pig No. 8. One of these mice was found dead on the third day, the other on the fourth day. Both showed all the appearances of typical acute plague; the spleen and heart's blood were literally packed with *B. pestis*.

Sections were made through the hardened intestine, the mesenteric glands, the spleen, and the liver of the above guinea-pig, No. 8, and the results were practically the same as were described in regard of previous rats. In the swollen Peyer's patches the blood-vessels were dis-

tended, with extravasation of blood *en masse*, and with *B. pestis* everywhere; the mucosa over and around the lymph follicles was filled with extravasated blood. Both the tissue of the mucosa and that of the lymph follicles were necrotic; the lymph vessels and sinuses in the mucosa and submucosa were distended and filled with continuous masses of *B. pestis*. Necrotic patches were found in the mesenteric glands, in the liver and in the spleen associated with masses of *B. pestis*.

Guinea-pig No. 9, which had been kept in the same cage as guinea-pig No. 8, remained alive. On May 30 it was injected subcutaneously with a trace of gelatine culture of the heart's blood of its dead companion, guinea-pig No. 8. It died on the seventh day with all appearances of subacute plague: necrotic bubo; liver and spleen full of minute necrotic points; lungs congested with necrotic patches.

Film specimens and cultures were made of the bubo, the spleen, and the lung, and these showed copious presence of *B. pestis*.

This experiment is instructive in that it bears out a fact on which in former reports I have repeatedly insisted, viz. that of two animals kept together in one cage, one may succumb to plague—its intestine, kidney, spleen, and blood in general literally swarming with *B. pestis*,—whereas its companion may remain, and this notwithstanding blood-sucking insects with which guinea-pigs are well provided, quite unaffected. If after twenty days the animal which has thus escaped illness be inoculated with *B. pestis* derived from its dead companion, it promptly succumbs to plague.

Experiment 5.—Two mice, Nos. 1 and 2, were fed on

May 4 with bread mixed with minced fresh spleen of a rat which had died of typical plague after cutaneous inoculation. The spleen in question was typical of plague: large, dark, and crowded with *B. pestis.* These mice remained unaffected. On May 16 they were inoculated cutaneously with a trace of a gelatine culture derived from the rat-spleen with which they had been fed. Both were dead of typical plague on the third and fourth days respectively.

Experiment 6.—Two mice, Nos. 3 and 4, were fed on May 20 with gelatine cultures of *B. pestis.* These gelatine cultures were sixteen days old, and showed on their sloped surface crowds of typical colonies, somewhat dry at the margin.

Both mice were found dead in the morning of May 24. One of them must have been dead for some hours as its abdomen was in an advanced state of putrefaction. But the other, which was in a good state of preservation, showed the following interesting appearances on post-mortem examination:—In the ileum a number of hæmorrhagic patches surrounding injected Peyer's glands; mesenteric glands swollen and hæmorrhagic; liver pale; spleen large, dark, and firm; kidneys much injected. Film specimens and cultures showed that the heart's blood and spleen were packed with *B. pestis.*

Sections through the hæmorrhagic parts of the intestine showed appearances similar to those already described in regard of rats fed in like manner. Not only was the actual infecting particle (full of *B. pestis*) found fixed to the intestinal mucosa, that is within the lumen, but the surface of the mucosa (villi) was denuded of its epithelium and covered with a continuous layer of

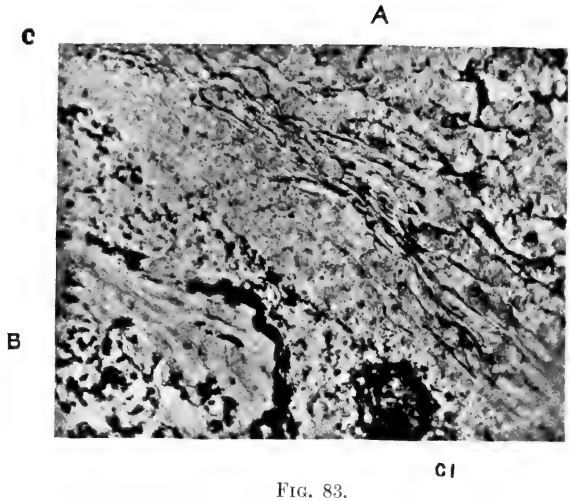

FIG. 83.

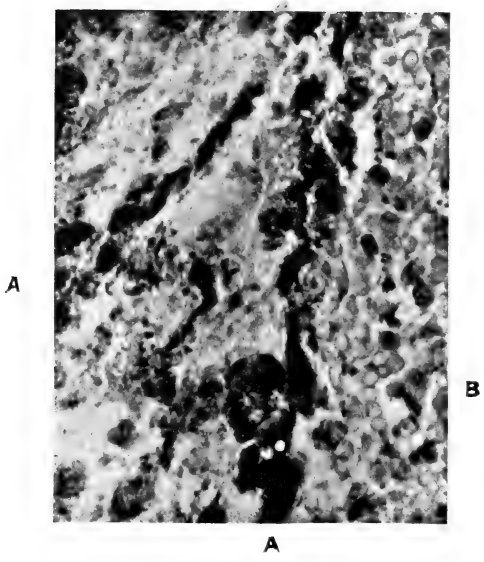

FIG. 84.

FIG. 83.

A portion of the previous particle (Fig. 82) more highly magnified.

A, Gluten bodies and separating membranes.

B, The same, but permeated by masses of *B. pestis.*

*C*1-*C*, Bit of plague spleen with numerous *B. pestis.* × 300.

FIG. 84.

Part of previous figure more highly magnified.

A-*A*, Semi-digested wheat permeated by masses of *B. pestis.*

B, Piece of plague spleen (blood corpuscles are well shown) with numerous *B. pestis* forming aggregations at the margin of spleen and wheat. This tends to indicate that the wheat does not offer any antagonism or inhibition to the multiplication of *B. pestis* brought in contact with it. × 700.

FIG. 85.

The same particle of plague spleen in wheat as shown in Fig. 82. The middle portion corresponds to the particle of plague spleen, surrounded by remains of wheat. × 85.

FIG. 86.

Section through mesenteric gland of a sewer rat dead after feeding with semi-dried plague organs.

The light parts are filled with extravasated blood, the dark parts contain masses of *B. pestis* filling the cortical and medullary sinuses.

1. Two efferent lymph vessels in section filled with *B. pestis*; there is een at the hilum (2) an efferent lymph vessel filled with *B. pestis*, just merging from the gland.

1*A*. An afferent lymph vessel filled with *B. pestis*. × 45.

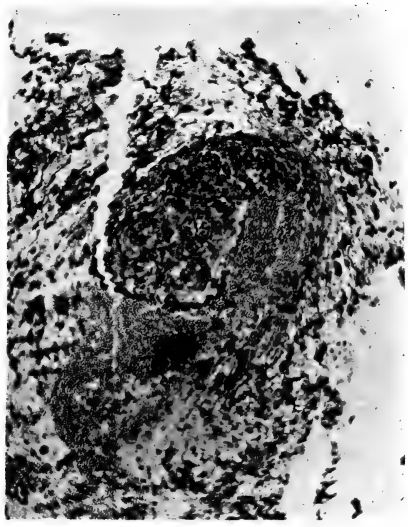

FIG. 85.

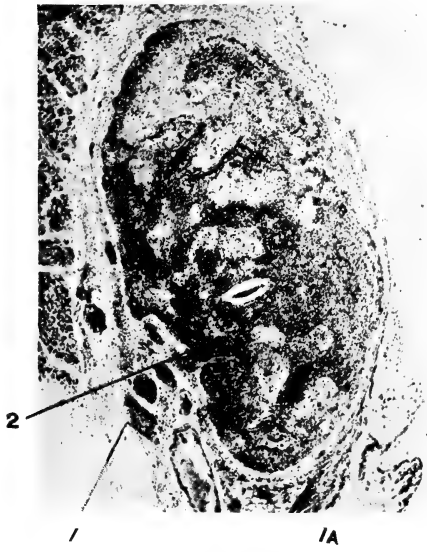

FIG. 86.

B. pestis. These bacilli could be traced in almost continuous masses (see Figs. 80 and 81) into the villi, into the lymph spaces of the mucosa around the Lieberkühn crypts, and into the lymph vessels of the submucosa around and between the lymph follicles. The mesenteric glands were literally crammed with *B. pestis* forming denser masses corresponding to the subcapsular and medullary lymph sinuses.

Experiment 7.—Having thus satisfied myself that rats, mice, and guinea-pigs can be infected with plague by feeding them on old and drying gelatine and agar cultures, and that the intestines (ileum and Peyer's glands) of the experimental animals demonstrate in unmistakable manner the exact place where infection occurred, I directed my attention to similar feeding experiments with grain, *i.e.* with wheat and rice, which, having been mixed with minced plague organs of guinea-pigs or rats, or with gelatine cultures, had been allowed to dry. It is obvious that these further experiments more nearly represent conditions to which rats may become subjected as it were naturally.

The bubo, spleen, liver, and lung of a guinea-pig that had died of plague after subcutaneous injection (guinea-pig No. 9 of experiment 4) were, on June 6, finely minced, and then well mixed in separate plate dishes, partly with wheat, partly with rice. The two lots were placed over sulphuric acid to dry. On June 7, *i.e.* after twenty-four hours, the materials were found well dried, and were now used for feeding.

One dish (wheat and organs) was supplied to two rats in one cage, the other dish (rice and organs) to two other rats in a separate cage. Thus :—

Rats Nos. 10 and 11 were fed on June 7 with plague organs dried with rice. These two animals were distinctly affected on June 10, and they were found dead on the morning of June 11. On post-mortem examination the lower ileum in both animals was found intensely inflamed and showed hæmorrhages; the mesenteric glands were swollen and hæmorrhagic; spleen typical, large, dark, firm, and crowded with *B. pestis*.

Rats Nos. 12 and 13 were fed on June 7 with plague organs dried with wheat. One was found dead in the morning of June 10, the other remained unaffected. The post-mortem examination of the dead rat showed in the ileum five different spots where hæmorrhage had taken place over and in the neighbourhood of Peyer's glands; within the cavity of the ileum was a good deal of blood, and mingled with it crowds of typical *B. pestis*; in the mesentery around the mesenteric glands was a big hæmorrhagic patch, while the mesenteric glands themselves were swollen and exhibited petechiæ. The spleen was large, dark, firm, and crowded with *B. pestis*; both kidneys, which were twice the normal size, were deeply congested in all parts; both lungs were congested with numerous hæmorrhagic spots. Film specimens and cultures of the intestinal sanguineous contents of the spleen and of the heart's blood yielded *B. pestis*. Sections were made of the hardened intestine through the hæmorrhagic spots, and Figs. 82, 83, 84, and 85 show the appearances observed.

Fig. 82 is from a transverse section through a hæmorrhagic part of the intestine close to a swollen Peyer's gland ($\times 25$). In the lumen of the intestine is seen a mass which is only the outlier of a larger mass attached to the inner portion of the Peyer's patch. Part of this

central mass is shown more highly magnified (× 300) in Fig. 83. Here may be recognised between two masses of wheat A and B—or what is left of wheat undigested—a streak of tissue CC_1, which, examined under a higher power, × 1000 (as in Fig. 84), shows itself to be a mass of tissue, most probably a piece of spleen; and, further, there is seen in this tissue, as also in the part of wheat next to it, at B, continuous masses and clumps of *B. pestis* which appear dark in the figure. Likewise there is seen in Fig. 83 at C_1 a dense mass of *B. pestis* surrounded by what under high power is recognisable as a necrotic portion of tissue, probably spleen. At A in Fig. 83 there are gluten globules and septa between them. Here, then, is demonstration of a striking fact. In the lumen of the intestine, exactly at a hæmorrhagic spot and next an inflamed Peyer's gland, is a piece of the infected food—*i.e.* plague tissue wedged in between two portions of wheat —which would seem to have become attached to a Peyer's patch during the life of the animal. In this food particle there are recognisable more or less undigested parts of wheat (gluten portions) and part of the original plague organ that was dried with it. Further, it would appear there had been active multiplication of plague bacilli in the adjoining loosened wheat particle.[1]

Fig. 84 shows under a high power these streaks and masses of *B. pestis* between the layers of the remains of the wheat A A, B being the original plague material dried in the wheat. It remains to be noted that many villi of Fig. 82 showed, when examined with a high power, crowds of *B. pestis* on the surface (denuded of epithelium)

[1] This observation does not lend support to Hankin's contention that the wheat offers antagonism and inhibition to the life and multiplication of *B. pestis* brought in contact with it.

and in the tissue of the villi. Sections through the affected swollen Peyer's glands showed appearances like those previously described (Fig. 75). Particularly some villi showed hæmorrhages with numerous *B. pestis*, and others were crammed with *B. pestis* in the manner represented in Fig. 80.

Sections through the hardened kidney showed appearances already described, but more pronounced, in respect of numerous hæmorrhages in the cortex and at the boundary layer. Almost all glomeruli showed some capillaries degenerated, others blocked with *B. pestis*; in many of the Malpighian corpuscles the cavity of the capsules contained blood and *B. pestis*. A like appearance was observed in many of the convoluted tubules.

It should be mentioned here that while in the actual specimens double stained with methylene-blue and eosin the distribution of the *B. pestis* in the tissues is at once strikingly apparent owing to the contrast between the bacilli stained blue and the blood corpuscles red, in the figures submitted this contrast is lost; owing to the low magnifying power and to dense packing of the *B. pestis* these collections are only indicated as dark masses.

In addition to the above experiments, I have obtained a number of other positive results from feeding rats with wheat and rice mixed with finely minced plague organs and dried over sulphuric acid for two or even three days. It is, however, unnecessary to describe all of them in detail; they fully confirm the above observations.

Experiment 8.—Parts of the organs (spleen and liver) of plague rat No. 12 were finely cut up and mixed in separate dishes, with wheat and rice, and placed on June 11 over sulphuric acid. After forty-eight hours (June 13)

the materials were found quite dry and extremely hard and brittle. They were now given as food to two white mice, Nos. 5 and 6 (rice plague organs), and to three wild mice, Nos. 7, 8, and 9 (wheat plague organs). The animals ate all the food within twenty-four hours. One of the white mice was found dead on June 16. It had extensive skin disease and a big worm in the liver, but its intestine and spleen were not affected. The other white mouse and the three wild mice remained alive and unaffected. From this it would seem that the material had been dried too much, and that probably all plague bacilli in them had been killed.

Experiment 9.—This experiment was made in repetition of experiment 3. Wheat and rice were mixed with old gelatine cultures of *B. pestis* (derived from blood and spleen of rats and mice dead of plague after feeding), and the mixed materials dried over sulphuric acid for twenty-four hours. The materials were then, June 21, given to rats, two for each lot. One of the rats, No. 14, fed with wheat gelatine culture was found dead in the morning of June 25; the companion, No. 15, pregnant, was very ill and was killed. On post-mortem examination this animal (15) contained seven dead fœtuses, nearly full time; there was a great deal of peritonitis; the spleen was small and had no plague bacilli; there was no appearance of plague.

But the post-mortem examination of rat No. 17 showed lesions in the ileum and Peyer's glands, mesenteric glands, and spleen, identical with those already described. It is not necessary, therefore, to further refer to them beyond saying that film specimens and cultures of the hæmorrhagic mesenteric glands, of the spleen, and of the

heart's blood showed abundance of *B. pestis*. The rice-fed rats both remained alive, and showed no alteration in their condition.

Experiment 10.—A guinea-pig which had been inoculated with plague having died, its organs—bubo, spleen (full of *B. pestis*)—were finely minced, and then mixed well with wheat and flour, and placed over sulphuric acid. After twenty-four hours the material, which was now perfectly dry and hard, was given (June 28) as food to three white and to three wild mice.

On July 1 one of these wild mice was found dead. The ileum was relaxed and full of sanguineous mucus; petechiæ in the mucous membrane; mesenteric glands much injected and swollen; spleen literally packed with *B. pestis*. The kidneys, lungs, and the liver were hardened, and sections were made. In all these organs great congestion of the blood-vessels was found, and in the large and small vessels numerous *B. pestis*; in the kidney some of the capillaries of the Malpighian tufts were quite blocked with them.

The other two wild mice remained alive.

Of the three white mice one was found dead on July 11, *i.e.* after thirteen days, and post-mortem examination showed the following conditions:—Ileum much congested; at one spot, around a swollen more or less necrotic Peyer's gland, the mucous membrane was hæmorrhagic; the mesenteric glands swollen and hæmorrhagic; the spleen much enlarged, dark, and firm.

Sections through the swollen Peyer's gland and surrounding membrane showed almost complete necrosis, the tissues being permeated with effused blood, and the lymphatic vessels blocked with continuous masses of *B.*

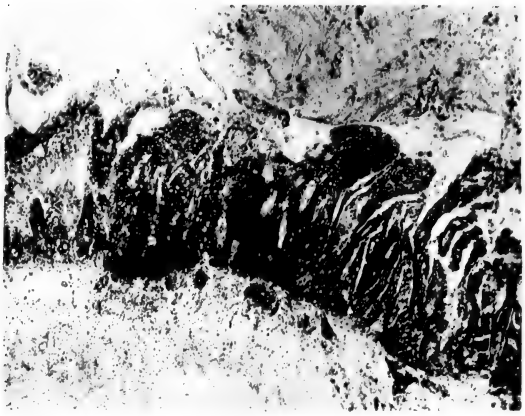

FIG. 87.

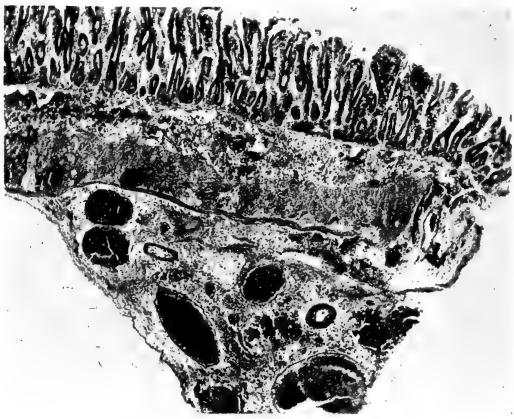

FIG. 88.

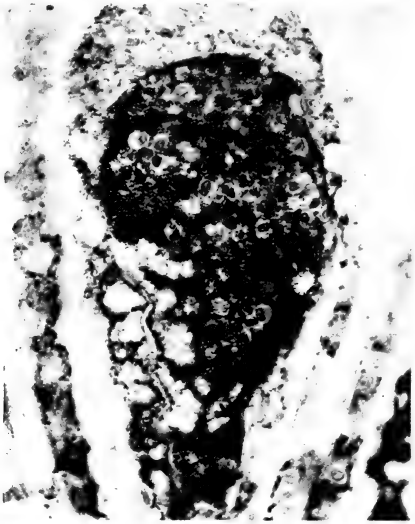

FIG. 89.

FIG. 87.

Section through the hæmorrhagic patch of the ileum of a guinea-pig dead of subacute plague. The figure shows the mucosa and part of the submucosa; on the top of the mucosa is a mass of blood-clot pervaded by *B. pestis* (dark). The villi and the rest of the mucosa are crowded with *B. pestis* (dark). × 65.

FIG. 88.

Section through the same portion of the ileum at its mesenteric attachment, shown in the lower part of the figure. In this attachment are seen (about the middle) two blood-vessels in section filled with blood; in the upper left and lower right of the mesentery are chyle vessels filled with *B. pestis*. × 30.

FIG. 89.

A villus of Fig. 87, showing copious absorption of *B. pestis* (dark). The epithelium of the surface of the villus is detached both on right and left. × 300.

pestis. The mesenteric glands were necrotic, the lymphatics here being filled and distended with masses of *B. pestis.*

The two other white mice remained alive.

In a number of other experiments old gelatine cultures of plague bacillus or actual particles of plague organs were mixed with wheat, rice, and flour, and were then left over sulphuric acid for more than three days. As a result the materials became thoroughly dry; too dry, in fact, as negative result of feeding rats and mice with them showed —the animals remaining unaffected. More than forty-eight hours drying over sulphuric acid appears to preclude expectation of *B. pestis* remaining present in the material in a living state.

From these experiments it appears :—

(1) That rats, mice, and guinea-pigs are capable of infection with plague by feeding them with old[1] and drying gelatine cultures alone; with old gelatine cultures mixed with wheat or rice and well dried previously; and with pieces of plague organs mixed with wheat or rice or flour and dried previously.

(2) That of animals thus fed a considerable percentage become infected; that the infection takes place in the ileum at or about the Peyer's glands; and that the infected animals die between the fourth and sixth days.

(3) That hæmorrhage with great multiplication of plague bacilli occurs in the ileum at the point of infection, and this not only in the cavity of the intestine and on the free surface of the mucous membrane, but also within the villi themselves, in the absorbents of the villi and of all

[1] By old I do not mean gelatine cultures which are practically dead and have lost all virulence, but gelatine cultures from two weeks to two months old and which yield active and living subcultures.

parts of the intestinal wall; and further, in the mesenteric glands, whence the vascular system of the other viscera become crowded with the bacilli.

(4) That the positive results obtained by feeding with semi-dried or more completely dried gelatinous plague materials—*e.g.* gelatine cultures and plague organs dried by themselves or mixed with food-stuffs (wheat, rice, flour)—are in marked contrast to the negative results of feeding with fresh plague materials—*e.g.* fresh milk cultures, broth cultures, watery emulsions of cultures of *B. pestis* and fresh plague organs.

(5) That it is, therefore, justifiable to assume that the positive results in the former cases are due to the gastric juice failing to reach the central portions of the infective food particles, the plague bacilli in these central portions being left undisturbed and unaffected owing to the protection afforded them by the outer dried shell of material.

In a word, I am inferring that on their passage through the stomach the infective materials would retain some of the plague bacilli protected and living; that on reaching the ileum the infective particles would, by the absorptive tendency of the Peyer's glands, become fixed, as it were, on these glands, and that the living plague bacilli within the material thus affixed to the mucous membrane would at once commence to multiply *in situ*, causing thereby the changes which were actually found, viz. hæmorrhage and necrosis and charging of the absorbents with *B. pestis*, with the result of general infection of the rest of the body.[1]

[1] As direct evidence bearing on this point, the following observation deserves to be mentioned:—

A guinea-pig was inoculated cutaneously, on April 11, with a particle of the spleen of a rat dead of acute plague. This guinea-pig was found dead on April 17, showing the following post-mortem appearances: large necrotic bubo with hæmorrhage around it, while the spleen, liver, and lung contained numerous

Similarly I am inferring that the negative results of feeding with fresh materials are due to all the ingested plague bacilli becoming readily exposed to the action of the gastric juice, so that no living plague bacilli are capable of reaching the ileum, and that the animal therefore escapes.

It must be obvious from the above description that the discharges of the intestine and the secretion of the kidney in animals (rats, mice, or guinea-pigs) affected with plague by feeding, in all probability contain abundance of plague bacilli. It has been seen that the materials within the cavity of the ileum of such animals swarm with *B.*

necrotic nodules crowded with the *B. pestis*; that is to say, the animal presented the appearance of subacute plague which follows the cutaneous inoculation. But in addition a portion of the lower ileum of this guinea-pig showed great hæmorrhage with a blood-clot in its cavity firmly adhering to the mucous membrane, while the mesenteric glands were enlarged and showed hæmorrhages; there were hæmorrhages also — besides those mentioned — in the subcutaneous tissue surrounding the inguinal bubo, also in the testis and omentum. Sections were made of the hæmorrhagic portion of the ileum and of the mesenteric glands. These showed: (*a*) in the ileum and firmly adhering to the mucosa (villi) a mass of blood, in which were large numbers of nests of *B. pestis*; the tissue of the villi was full of *B. pestis*, forming reticulated masses, as described and figured of former cases, in which infection had started from the intestine; the central chyle vessel, as also the lymph vessels of the mucosa, were literally crowded and injected with *B. pestis*, as were also the lymph vessels of the submucosa and of the outer muscular coat; in the mesenteric margin of this part of the intestine the efferent chyle vessels were distended and filled with blood and with masses of *B. pestis*; the large blood-vessels of the same parts were also filled with blood, amongst which some *B. pestis* could be recognised. (*b*) The mesenteric glands showed the same appearances as were described on a former page, viz. the afferent and efferent lymph vessels were distended and filled with *B. pestis*, so also were the cortical and medullary lymph sinuses; a great deal of hæmorrhage was noticeable in the cortical lymph follicles.

The appearances above described appear to me to admit of one interpretation only, viz. this: in the ileum hæmorrhage had taken place, witness the presence of a blood-clot in the cavity; the *B. pestis* in this clot being in a suitable place—the ileum—had multiplied and had become readily absorbed by the villi in which indeed they abounded; the same process had followed in all the absorbents of the intestinal wall and mesenteric glands. This was evidently made possible by the duration of the disease (six days). So that a direct lodgment of *B. pestis* in the intestinal cavity having occurred while the animal continued to live, time was given for a *secondary infection* of this part of the ileum of the same character as that observed in the other cases in which the *B. pestis* had reached the ileum by way of the food.

pestis, and that these materials on inoculation promptly produce plague. Likewise it appears that plague bacilli are abundant in the Malpighian corpuscles (glomeruli and capsules) and in the uriniferous tubules.[1] These facts in no way support a contention such as that put forward by Hankin in the *Journal of Hygiene*, to the effect that the intestinal or renal discharges of the plague rat are not to be regarded as materials capable of causing infection. On the contrary, the observations I have recorded go to justify a contention that, as regards rats which have contracted plague by feeding, the dejecta in question, teeming as they needs must with *B. pestis*, are to be regarded as in all probability infective. Indeed, in view of the ready transmission of plague to the rat by means of feeding with infected grain, rice, flour, and the like, the foregoing experiments afford strong suggestion that the excreta of a plague rat becoming mixed and dried along with food-stuffs may be the starting of extensive plague infection of the rats feeding on these substances contaminated by stale plague dejecta.

In connection with the above propositions, it has to be borne in mind that the anatomical lesions in the ileum which I have described cannot be of a single day's duration; that the necrotic changes in the mucous membrane and in Peyer's glands caused by the multiplication of *B. pestis* within the cavity of the ileum must have occupied time in development, and that accordingly there

[1] As a matter of fact I have made a number of experiments (which need not be described in detail) in which the sanguineous mucus of the inflamed intestine of animals (rat and guinea-pig) dead of plague after inoculation was used for cutaneous as also subcutaneous injection, and by which fatal plague of the animals so inoculated (guinea-pigs and rats) was the result. I have also made inoculation experiments (subcutaneous) of guinea-pigs, employing the urine drawn from the bladder of guinea-pigs or of rats which had succumbed to (hæmorrhagic) plague, and the result was positive in the majority of instances.

must have arisen ample opportunity for *B. pestis* to pass into and out from the large intestine with the alvine evacuations of the infected animal.

It is not possible to deny that similar conditions might occur in respect of human beings; that food-stuffs having become polluted with, say, excreta of plague rats preserved in a semi-dried or dried condition, might cause plague in persons ingesting them, the point of infection in such case being the intestine itself.

Simpson's contention (Report on Plague in Hong-Kong) that the septicæmic form of plague in man (in which the whole blood system and all organs contain abundance of *B. pestis*) may be caused by infection of man's intestine by means of food, was based on experiments in which wild rats were fed with fresh plague organs; these rats, or some of them, having died, plague was considered to have been induced in them by way of the intestine. This inference seems to me, however, not justified, or at least not proven, and for two reasons:—

(1) The fact that the rats in question died, as is said by Simpson, from the septicæmic form of plague does not afford presumption that they were infected by ingestion; the septicæmic form of plague being that under which both rats and mice and also guinea-pigs commonly die after inoculation.

(2) No indication is given by Simpson of any such conspicuous and definite local infection (hæmorrhage) in the ileum and in Peyer's glands as that described by me as resulting in those of my feeding experiments which proved positive.

The general congestion of the small intestine mentioned by him (in addition to sanguineous mucus in the intestinal

cavity) is not, be it observed, an uncommon feature in the rat and mouse dead of plague contracted otherwise than by feeding; it is not uncommon too in rodents which have died of septicæmic diseases other than plague. In the guinea-pig, dead of plague after subcutaneous injection, I have not unfrequently found the whole of the intestine showing punctiform hæmorrhages. Also I have observed hæmorrhage in the testis, whence the effused blood could be traced by the absorbents of the spermatic cord into the pelvic lymph gland.

On the other hand, it is possible, in view of the experiments I have been describing, that Dr. Simpson's view may prove on the whole to be correct, and this mainly for the reason that in the septicæmic form of plague of the human subject the blood and the organs appear crowded with *B. pestis*, as also in the animals of my feeding experiments. Before, however, definitely pronouncing on this point, it would be necessary to make careful post-mortem examination of septicæmic plague in man for the purpose of ascertaining whether local infection (ileum and Peyer's gland) and changes of the mesenteric glands similar to the conditions described here in regard of experimental animals, has actually occurred.

Among the many interesting facts concerning the spread of plague in India ascertained by the Indian Plague Commission are those pointing to persistence for some considerable time of the contagium of plague in rooms and streets from which all plague cases had been removed; rooms or streets, for instance, are cited in which plague broke out afresh on the return to them of the inhabitants. Hankin, however, it appears (see also Report of Indian Plague Commission) had made experiments on the vitality

of the *B. pestis* in soil, and had found this to be of very short duration. These experiments of Hankin have been relied on by commentators as justifying the view that the observed persistence of infectivity in rooms and streets after removal therefrom of infected persons might have been due to retention of the contagium by rats or by fleas; in a word, to the contagium having been kept alive as it were by these means.

Before, however, accepting such interpretation of the facts it seemed to me necessary to ascertain whether the vitality of *B. pestis* in soil is really of short duration; whether the result might not have been different if, instead of distributing *B. pestis* in the soil, as practised by Hankin, in the form of a broth culture, it had been applied under conditions more nearly approaching those in which it would naturally find access to soil, *e.g.* in blood, in discharges of a suppurating bubo, in mucous expectorations, in the mucoid discharges from the bowels of a plague rat, in the juices of plague organs, or in similar viscid or slimy materials.

Accordingly I have made a series of experiments in this direction, and have thereby ascertained that *B. pestis* planted in or mixed with soil in the above manner has considerable power of persistence. Also I have made experiments with wheat and rice on the same lines; that is, I have sought to ascertain for what length of time the bacilli in plague material (cultures or organs) mixed with wheat or with rice can retain their vitality.

Gelatine cultures, about six weeks old, were melted in warm water and poured over fine earth (gravel) contained in a glass dish (July 2), and over fine sand (July 1) contained in a separate glass dish. The earth and the sand were

in each instance thoroughly mixed with the gelatine and then placed over sulphuric acid, of course in a hermetically closed space. The earth as also the sand were (after being kept not more than twenty-four hours over sulphuric acid) found perfectly dry and hard. After having been thus dried during one, two, three, or more days, a small amount (about $\frac{1}{2}$ to 1 gramme) of the infected earth or sand was in each instance taken out, placed in a sterile watch-glass and distributed (rubbed down) in a few cubic centimetres of warm sterile water so as to form a turbid emulsion. Of these turbid emulsions small quantities in each instance (about $\frac{1}{10}$, $\frac{1}{5}$, $\frac{1}{2}$ cc.) were injected subcutaneously into guinea-pigs. This method of procedure was chosen in preference to mere cutaneous inoculation of rats, for the reason that the object was to ascertain not whether living plague bacilli were present in a small droplet (the quantity that could be introduced by cutaneous inoculation) but whether *any* living plague bacilli were left in fair quantities of the materials.

Experiment 1.—Guinea-pig No. 1 injected with "sand-gelatine," after twenty-four hours' drying, on July 2. Guinea-pig No. 2 injected with "earth-gelatine," after forty-eight hours' drying, on July 4.

Guinea-pig No. 1 developed a big bubo and died on July 7 with typical subacute plague. Post-mortem examination showed a necrotic bubo with crowds of *B. pestis*; spleen and liver dotted all through with white nodules full of *B. pestis*.[1]

[1] It may not be unnecessary to state that in all experiments mentioned here, inclusive of all animals without exception dead or killed after plague infection, stained film specimens of all affected organs, and culture on gelatine from the bubo (when present), from the intestine (when affected), from the spleen and from the heart's blood, were made invariably and as a matter of ordinary routine.

Guinea-pig No. 2 developed a bubo slowly; it was found dead on July 11. Post-mortem examination showed typical subacute plague.

Experiment 2.—Guinea-pig No. 3 was injected (on July 4) with "sand-gelatine," dried for three days. Guinea-pig No. 4 was injected on July 5 with "earth-gelatine," dried for three days.

Guinea-pig No. 3 developed bubo slowly; this became a big abscess by July 13. The same condition was observed with guinea-pig No. 4. But both animals appeared otherwise lively. Both were killed, and on post-mortem examination the pus of the buboes was found to contain, amongst cocci, crowds of *B. pestis.* The viscera appeared unaffected.

Experiment 3.—Both the dishes containing "sand-gelatine" and "earth-gelatine" were removed from over the sulphuric acid after lapse of three days and were then kept under simple glass cover (raised about half an inch at one side) at the temperature of the laboratory.

Six days after withdrawal from the influence of the sulphuric acid, the "sand-gelatine" was used for subcutaneous injection (on July 9) of guinea-pig No. 5; and five days after withdrawal the "earth-gelatine" was used in similar manner for injection (on July 9) of guinea-pig No. 6.

Guinea-pig No. 5 was on July 15 without tumour and quite lively; and it remained so. Guinea-pig No. 6 died on this day (July 15). On post-mortem examination there was evidence of typical subacute plague: big necrotic bubo with crowds of plague bacilli, liver and spleen permeated with minute white nodules containing numerous *B. pestis.*

Experiment 4. — After a further week's (July 16) withdrawal from sulphuric acid, the samples having been kept meanwhile at the room temperature and in the air of the laboratory, the same "sand-gelatine" and "earth-gelatine" were used for injection of guinea-pigs Nos. 7 and 8 respectively. The "earth-gelatine" appeared now as dry and hard as brick. The "sand-gelatine" guinea-pig, No. 7, remained without swelling and quite well; whereas the "earth-gelatine" guinea-pig, No. 8, developed a small firm nodule at the seat of inoculation. This, however, almost entirely disappeared in the course of a fortnight, the animal remaining lively and well.

From these experiments it appears that gelatine cultures mixed with sand and earth can be dried over sulphuric acid for three days, until, for instance, the material becomes distinctly hard and dry, without the plague microbes losing their vitality; and, further, it appears that the "earth-gelatine" kept for additional five days, till it had become as hard as brick, still retained living and active *B. pestis.*

Experiment 5.—In this experiment gelatine cultures about six weeks old were (June 30) melted in warm water and poured over wheat and rice in separate glass dishes. After being well mixed in each instance the materials were placed over sulphuric acid, of course in a hermetically closed space.

On July 1, *i.e.* after twenty-four hours, the materials being now quite dry, a little of each sample was placed in warm water and a guinea-pig was injected subcutaneously with the emulsion thus obtained; viz. guinea-pig No. 9, with "rice-gelatine," and guinea-pig No. 10 with "wheat-gelatine." Both animals developed big tumours in the

groin. Guinea-pig No. 10, which appeared otherwise lively on July 5, was killed on that date, and on post-mortem examination showed the following conditions :—

Extensive œdema over abdomen and chest; about the place of inoculation a big cavity filled with grumous fluid. Spleen not enlarged, no bacilli in it. In the inguinal fluid were crowds of diplococci and streptococci; as also numerous bipolar bacilli like *B. pestis*. With a dilution of the inguinal fluid an agar plate was made, and this brought forth crowds of colonies just like those of *B. pestis*. A guinea-pig, No. 11, was injected with one of these plague-like colonies. This animal was found dead on the fifth day, and gave evidence of typical plague with the typical distribution of *B. pestis* in the bubo and spleen.

Guinea-pig No. 9, which had been injected with the "rice-gelatine," developed a big abscess in the groin and thigh; on the eighth day it was quiet and off its feed, and the bubo having opened spontaneously was discharging pus. This animal, which was better and fairly lively again on the thirteenth day, was now killed, and showed the following post-mortem appearances :—In the inguinal region and about the thigh was a big cavity containing grumous creamy pus; spleen and liver and also lungs showed numerous minute white nodules. In the pus of the inguinal abscess were numerous clumps of *B. pestis*.

In the next series a number of animals were inoculated with earth and with sand which, after addition of small particles of plague organs, had been dried for various periods at the ordinary temperature of the laboratory.

Experiment 6. — Of a guinea-pig dead on July 7

with typical subacute plague, bits of the necrotic bubo, and pieces of the spleen and liver (full of minute nodules), were finely minced and mixed separately in glass dishes with earth (fine gravel) and fine sand. These materials were then so placed as to obtain fairly free access of air,[1] and were left to dry spontaneously.

On July 14, *i.e.* after one week, a small amount in each case of the dry material was emulsified in warm water, and of each emulsion about $\frac{1}{4}$ to $\frac{1}{2}$ cc. was subcutaneously injected into a guinea-pig. These guinea-pigs will be designated :—

No. 12.—"Sand-plague" guinea-pig.

No. 13.—"Earth-plague" guinea-pig.

Guinea-pig No. 12 was found dead on July 18, *i.e.* after four days; it had, however, been dead for some time, July 17 being a Sunday. The post-mortem examination showed typical plague (early stage of subacute), viz. necrotic bubo, and few minute nodules in spleen and liver. Bubo and spleen were crowded with *B. pestis*, as shown by film specimens and by cultures.

Guinea-pig No. 13 was dying on July 21. On post-mortem examination there was found a big necrotic bubo crowded with *B. pestis*; the spleen, liver, and the lungs were crowded with necrotic nodules and patches; film specimens and cultures yielded pure cultures of *B. pestis*.

Experiment 7.—The materials of experiment 6 were used for inoculation of guinea-pigs after a further week of drying, viz. on July 21. By this date the materials appeared perfectly dry and hard as bricks.

[1] The dishes were kept under a bell glass, raised all round about $\frac{1}{2}$ to 1 inch and placed by the open window.

Guinea-pig No. 14 was injected with emulsion of the "sand-plague" organs.

Guinea-pig No. 15 was injected with emulsion of the "earth-plague" organs.

Guinea-pig No. 14 remained without swelling, and was altogether unaffected; it was killed on August 2, and gave totally negative result as regards plague. But guinea-pig No. 15 was distinctly ill on July 26 and had bubo. It was killed on the same day, and showed the following appearances: necrotic bubo; spleen, liver, and lungs crowded with necrotic nodules and patches. Film specimens and cultures of the bubo, spleen, and lung yielded crowds of *B. pestis.*

Fig. 24 shows, in a section under a low power, a necrotic nodule of the spleen containing aggregations (dark) of *B. pestis*; these aggregations being in reality networks of blood spaces of the pulp tissue.

Fig. 21 shows, in a section (× 100) through the lung, one of the nodules (this being in reality an infundibulum with its alveoli) all blocked with masses (dark) of *B. pestis.*

From this experiment it appears, therefore, that while "sand-plague" material dried for fourteen days at the ordinary temperature was barren of infective power, "earth-plague" material still retained its efficacy. The former was therefore discarded, but the latter was retained for further experiment.

Experiment 8.—With emulsion of the same "earth-plague" material now dried for three weeks, guinea-pig No. 16 was injected on July 28. This animal appeared unaffected on August 3, *i.e.* after six days. It was killed and found in all respects normal.

Thus, earth subjected to admixture with the juice and particles of plague organs, and allowed to dry, retained its infective power for a fortnight, but after lapse of three weeks proved barren of infectivity. It is to be noted that these experiments were carried out during the hottest part of the year, when the drying was fairly rapid and thorough.

Experiment 9.—The blood, bubo, spleen, and liver of a rat dead of acute (inoculated) plague were, on July 8, finely minced and mixed with sand and earth; these materials being then put away to dry spontaneously, a fair amount of ventilation being in each instance allowed. In this condition the materials were kept for eight days (July 16); they were then placed over sulphuric acid for three days (July 19), by which time they were quite dry and as hard as bricks.

On this date, *i.e.* July 19, emulsions were made and used for the subcutaneous injection of guinea-pigs.

Guinea-pig No. 17 was injected with "sand-plague" organs.

Guinea-pig No. 18 was injected with "earth-plague" organs.

Guinea-pig No. 17 developed a small abscess on the abdomen, but otherwise remained lively. Guinea-pig No. 18 showed a gradually increasing soft tumour (abscess) in the groin, which by the end of the week (July 26) had reached the size of a pigeon's egg; the animal, however, appeared otherwise lively. Both were killed on July 27, but with negative result *qua B. pestis*. Guinea-pigs injected with the pus of guinea-pig No. 17, as also with the pus of guinea-pig No. 18, remained unaffected.

Experiment 10.—Rice and wheat were in each instance mixed, on July 11, with finely minced bits of spleen and lung of a guinea-pig dead of typical subacute plague, and placed to dry spontaneously in the same manner and under the same arrangement as in the previous experiment.

On July 21, that is after ten days, the materials were found quite dry, and were used for making emulsions in warm water for injection subcutaneously of two guinea-pigs :—

Guinea-pig No. 19.—" Rice-plague " organs.

Guinea-pig No. 20.—" Wheat-plague " organs.

Both animals remained without tumour and lively. They were killed after one week and were found quite normal.

Experiment 11.—A guinea-pig died on September 27 from typical subacute plague. Its organs (bubo, spleen, liver, and lungs) were finely minced, mixed with sand and with earth respectively, and placed to dry spontaneously in the laboratory under the same conditions as in previous experiments. By the end of a week (October 4) the materials seemed fairly dry, and were used to inject guinea-pigs :—

Guinea-pig No. 21.—" Sand-plague " organs.

Guinea-pig No. 22.—" Earth-plague " organs.

Guinea-pig No. 22 was found dead on October 7 with very intensive acute plague ; guinea-pig No. 21 was found dead on October 10 with typical subacute plague.

Experiment 12.—The same " sand " and " earth " plague materials were used for injection of guinea-pigs on October 11, that is after a fortnight's drying :—

Guinea-pig No. 23.—" Sand-plague " organs.

Guinea-pig No. 24.—"Earth-plague" organs.

Both animals were found dead on October 17 with typical subacute plague.

Experiment 13.—The materials of September 27 were used for experiment on guinea-pigs on October 20, *i.e.* after twenty-three days' drying :—

Guinea-pig No. 25.—"Sand-plague" organs.

Guinea-pig No. 26.—"Earth-plague" organs.

Both animals remained alive and unaffected.

Experiment 14.—Gelatine cultures of *B. pestis*, directly derived from rats and mice dead of plague after feeding and about fourteen weeks old, were on October 8 melted in warm water, mixed with sand and with earth, and left spontaneously to dry in the laboratory in the manner described in previous experiments. Nine days later (October 17) these materials were used for injection of guinea-pigs :—

Guinea-pig No. 27.—"Sand-gelatine" plague.

Guinea-pig No. 28.—"Earth-gelatine" plague.

Guinea-pig No. 28 was found dead on October 24 with typical subacute plague.

Guinea-pig No. 27 was found dead on October 26 with typical subacute plague.

Experiment 15.—The above materials were used again for injection of guinea-pigs on October 26, *i.e.* after eighteen days' drying :—

Guinea-pig No. 29.—"Sand-gelatine" plague.

Guinea-pig No. 30.—"Earth-gelatine" plague.

Guinea-pig No. 29 died November 1 with typical subacute plague.

Guinea-pig No. 30 was found dead on November 2 with typical subacute plague.

Experiment 16.—These materials were again used on

November 7, *i.e.* after thirty days' drying, for injection of guinea-pigs :—

Guinea-pig No. 31.—"Sand-gelatine" plague.

Guinea-pig No. 32.—"Earth-gelatine" plague.

Guinea-pig No. 31 was found dead on November 16 with typical subacute plague.

Guinea-pig No. 32 was found dead on November 14 with typical subacute plague.

Experiment 17.—The materials were again used for injection of guinea-pigs on November 22, *i.e.* after six weeks and three days' drying :—

Guinea-pig No. 33.—"Sand-gelatine" plague.

Guinea-pig No. 34.—"Earth-gelatine" plague.

Guinea-pig No. 34 was found dead on November 26 with typical subacute plague.

Guinea-pig No. 33 remained unaffected. It was tested after a fortnight by the subcutaneous injection with plague culture and was found fully susceptible.

Experiment 18.—The same "earth-gelatine" plague was again used on November 29, *i.e.* after seven weeks and three days' drying, one guinea-pig, No. 25, being injected with it. The animal was found dead with typical subacute plague.

Experiment 19.—The last experiment with this same material, viz. "earth-gelatine" plague, was made on December 14, that is after nine weeks and four days' drying, one guinea-pig, No. 36, being injected subcutaneously with turbid emulsion. The animal remained unaffected. This animal a fortnight later was injected with *B. pestis* of culture and promptly succumbed to plague.

From this series it appears that the vitality of *B. pestis* in earth and in sand to which the micro-organism

had been added in the form of gelatine culture remained, during the autumn months, for a long period unimpaired. Indeed, *B. pestis* was present in a living state in the earth even after seven and a half weeks; in the sand for six weeks and three days. These are periods of considerable duration, much longer than were found in the previous experiments (experiment 7) in which the infective materials were exposed to drying during the summer months. In these later experiments of early winter the infective materials were old gelatine cultures, not plague organs, and they were left to dry spontaneously under conditions such as might occur naturally. The fact that plague contagium is, under gelatinous or viscid conditions and during the cooler months of the year, capable of retaining its vitality in sand, and particularly in earth, for a considerable time, is a circumstance having no small importance, seeing that under natural conditions during plague seasons plague expectoration and intestinal mucus charged with masses of the *B. pestis* cast upon the earth may very possibly in like manner long retain dangerous infective property.

As a last series I have to mention some experiments in which plague organs were, by themselves, without admixture with other material, exposed to drying, and were then used for feeding rats, both tame and wild. The organs in question—bubo, spleen, lung, and liver, containing abundance of *B. pestis*—were obtained from animals (guinea-pig and rat) dead of typical acute plague. These organs, after having been cut into fair-sized pieces, were put to dry over sulphuric acid.

Experiment 20.—The organs of a guinea-pig dead of

subacute plague which had been cut up in small bits and kept over sulphuric acid for seven days were by this time perfectly dry and hard. Two rats were fed with this material. Both animals remained unaffected; so they were at the end of a week injected cutaneously with agar culture (twenty-four hours old) of *B. pestis* derived from a recent case of plague in man (case from s.s. *Weybridge*) which had been landed at Denton in December 1904. Both were dead of acute plague in three days.

Experiment 21.—The organs (bubo, spleen, liver, and lungs) of two rats, dead of plague after having been inoculated cutaneously with the blood of a guinea-pig dead of acute plague, were cut up into small bits and dried over sulphuric acid for eight days. With this material, by this time quite dry and hard, two wild rats were fed. Both animals remained unaffected.

Experiment 22.—The organs of a guinea-pig dead on February 22 of acute plague were cut up into bits and dried over sulphuric acid for forty-eight hours. By this time the outside of the material was well dried, though the inside remained still moist. With this material two tame rats in one cage, and two wild or sewer rats in a second cage, were fed on February 24.

One of the sewer[1] rats was found dead on February 28, the other remaining, by then and afterward, seemingly unaffected. Both tame rats at this date seemed ill, and showed rough coats. The post-mortem examination of the dead sewer rat showed the following conditions:—The greater part of the lower ileum congested,

[1] The wild rats used in these experiments were all of the common brown sewer rat type.

relaxed, and filled with blood and mucus; at one point of the congested ileum a Peyer's gland appeared swollen, much projecting, and necrotic, the membrane forming around it a well-marked ring of hæmorrhage; the mesenteric glands were enlarged and hæmorrhagic (Fig. 86); the spleen large, dark, and firm; the lungs of both sides were much congested and showed hæmorrhages. The sanguineous mucus of the ileum, the mesenteric glands, the spleen, and the lungs were crowded with *B. pestis* (as was proved by film specimens and cultures); the blood also contained abundance of *B. pestis*.

In this case, therefore, there was distinct and direct evidence that plague had been contracted by the feeding; for here, as in former positive cases, the point of infection was anatomically easily located in the ileum at a Peyer's gland. Sections made through these parts fully confirmed this inference.

A small particle of the sanguineous mucus from the ileum of the dead sewer rat was injected subcutaneously into a guinea-pig, and the rest of the mucus was mixed with bran and oats, dried over sulphuric acid, and then given as food to two wild rats. As a result, the two rats thus fed remained unaffected, whereas the inoculated guinea-pig sickened and became affected with typical subacute plague, the subcutaneous injection causing a huge hæmorrhagic and necrotic bubo in which was abundance of *B. pestis*.

It is of interest to notice in regard of this experiment 22, that a plague rat had been in the same cage with another rat which remained unaffected, though fed with plague material like its fellow—as indeed had happened in previous experiments, Series I. and II. In these condi-

tions, therefore, there was abundant opportunity for transmission of plague by fleas or other blood-sucking insects, such as lice, from the affected animal to its companion living with it in the same small cage; moreover, the animal that sickened with and died of acute plague about the fourth or fifth day had plenty of *B. pestis* in its blood. It is not to be supposed that common sewer rats such as were used in these experiments were without fleas or lice, nor can it be supposed that the fleas of the rat that died did not leave this rat for the living rat, a practice of fleas noticed and recorded by several observers. On making the post-mortem examination no fleas but only lice could be found on this dead rat,[1] though during life the animal was frequently observed to scratch itself in the manner customary with rats. Notwithstanding, therefore, all these circumstances specially favourable for the transmission of plague, the companion rat remained unaffected. Here was a rat the blood of which was teeming with *B. pestis*; further, close at hand was a companion rat ready for any supposed fleas or lice to migrate to, and yet this companion rat remained unaffected by plague. Nevertheless this companion rat, when later on (after lapse of a week) it was cutaneously inoculated into the skin of the root of the tail with a trace of gelatine culture directly made of the blood of its former dead companion, died on the third to fourth day with acute plague (just like other rats after cutaneous inoculation), showing hæmorrhagic swollen glands in the groin with dense crowds of *B. pestis*, the spleen large, dark, and firm,

[1] It should be here added that I have not been able as yet to find "fleas" on sewer rats examined after death—caused spontaneously by plague or after killing them by chloroform; lice alone, but these in abundance, have been hitherto found on their dead bodies.

crowded with *B. pestis*; that is to say, with the appearances described in detail on a former page.

This failure of transmission of plague from a plague rat to a healthy rat living amicably in the same or in an adjoining open wire cage is not an isolated instance.[1] In my reports to the Medical Officer of the Local Government Board for 1902-1903 and 1903-1904 I have mentioned a good many such instances; and in the present chapter I have recorded several in addition where only one of two animals (rats, mice) contracted and died of plague, be it by inoculation or by feeding, the companion remaining unaffected, though highly susceptible, as proved afterwards by inoculation with active plague material.

While, then, the transmission of plague from animal to animal is experimentally established both as regards cutaneous inoculation and feeding with semi-dry infective material, there is a distinct failure of evidence that transmission of the disease is effected by fleas or lice from an infected animal to a healthy one. It is not, therefore, in my view, justifiable to regard this mode of transmission, if, indeed, it happens at all under natural conditions, as anything but exceptional, at any rate as far as the sewer rat and the tame white rat are concerned. Theoretically, such a transmission is possible and easily imaginable, as I have discussed on a former page; it is possible, I mean, that a flea or louse which has just sucked from a rat blood well charged with *B. pestis* may, by biting a neighbouring rat, directly inocu-

[1] Hitherto I have searched in vain during autumn, winter, and spring for fleas on sewer rats and on the tame or white rats. I have been able to discover only lice. I would further point out that, as mentioned on a former page, the above experiment is not altogether satisfactory, since the sewer rat is not an animal with high susceptibility for plague. It is probable that in these respects an important distinction may have to be drawn between this species and the Oriental rat or *Mus rattus*.

late this latter. But what I wish to insist on is, that such an occurrence is not likely under natural conditions to be anything but exceptional; there is no direct evidence that this has happened, and in cases where it might have been expected to happen—*e.g.* in many experiments recorded by me—it certainly did not do so.

As already mentioned, Hankin (*l.c.*) does not favour direct transmission of plague by fleas. He regards the flea as a true host, a living body in which the *B. pestis* multiplies and in which it acquires virulence, believing, it would seem, that not until these further phases have been accomplished is the flea capable of transmitting plague to a new individual (rat or man). From what I have already said in this chapter it is, however, clear that definite support of proof for this view of Hankin's has yet to be furnished.

CHAPTER VIII

AGGLUTINATION OF *BACILLUS PESTIS*

The Physiological Action of Solid Sterilised Masses of Plague Bacilli.—All[1] observers who, as regards plague, have worked with the bacillary growth *en masse* have found that the injection into the animal body of the sterilised material in sufficient amounts has a protective action. Haffkine himself (*cf.* the evidence before the Plague Commission) laid stress on this. It is, in his view, the amount of the bacillary sediment in the prophylactic fluid which determines the dose, the amount of precipitate and the size of the dose standing in inverse proportion. Calmette (Harben Lectures, London, 1900) also relies almost entirely on the bacillary bodies (sterilised) as being the essence of the prophylactic material. The same applies to the German Plague Commission's recommendation.

In my Plague Report for 1896 (Report of the Medical Officer for 1896-1897) I had already described (p. 297) a number of experiments in which the bodies of plague bacilli, scraped from the agar surface and sterilised by heat, were injected in repeated doses subcutaneously and intraperitoneally into guinea-pigs and rabbits. I there

[1] Report of the Medical Officer of the Loc. Gov. Board for 1901-1902, p. 360 and *passim*.

showed that sterilised cultures (solid growth on gelatine and agar broth cultures) repeatedly injected, in large doses, subcutaneously or intraperitoneally, into guinea-pigs, do not confer absolute protection on the guinea-pigs against further infection any more than does repeated injection of sub-fatal doses of living plague bacilli. In fact, I showed in an unmistakable manner that the guinea-pig is an animal which it is extremely difficult to immunise against plague. This fact was subsequently verified in a series of experiments which Professor Haffkine and I together carried out in my laboratory with his plague prophylactic. We found that even injection of enormous doses of Haffkine's plague prophylactic, such as yielded positive results in protection of rats, did not confer absolute protection on the guinea-pigs against subsequent infection.

In the same report I have also described experiments which conclusively show that, as was to be anticipated from the above negative results, the blood of guinea-pigs which had recovered from induced plague (produced by injection of sub-fatal doses of living plague bacilli) is devoid of immunising or germicidal substances in appreciable amounts. I have in further experiments sought to ascertain whether the blood of guinea-pigs previously prepared, either by repeated injection of sub-fatal doses of living cultures or by repeated injection of large doses of sterile cultures, possesses any agglutinating action on emulsion of plague bacilli; and it is these experiments which I propose here to describe.

It is now well established that by repeated injections of an animal with a particular microbe the blood and tissues of this prepared animal undergo certain changes

the result of which is the development in them of various new substances. Amongst these, two at any rate have been studied carefully: (*a*) agglutinins; and (*b*) lysins, or germicidal or immunising substances. I do not discuss here—the matter being outside this treatise—the different theories that have been put forward as to the probable nature of these substances, viz. whether they are ferments (Roux, Emmerich) or are some highly and complexly constituted organic bodies other than ferments (R. Pfeiffer, Ehrlich, and others). I am content to give consideration to their mode of action as observed in actual experiment.

The Agglutinins.—Bordet and Gruber were the first to show that the blood serum of an animal subjected to repeated injections with the typhoid or cholera microbe sooner or later (usually in about a fortnight) acquires a new property: that when a small quantity of the serum is added to an emulsion of the typhoid or the cholera microbe respectively the microbes soon lose their motility, are attracted together, and become at the same time "agglutinated" or "clumped," so as to form smaller or larger masses. These, on account of their weight, gradually sink to the bottom of the tube in which the emulsion is contained, and the previously turbid fluid thus becomes clear. This process of "clumping" or "agglutinating" has been also studied in other cases besides those of the typhoid bacillus or the cholera vibrio, and it has been shown that the phenomenon is of a fairly general nature; that the blood of an animal which has been repeatedly injected with a given microbe—pathogenic or non-pathogenic—acquires the power to "agglutinate" an emulsion of the particular microbe with which it has been, so to speak, "prepared." It had been further shown that the degree

to which the agglutinating power of the blood can be raised differs, *cæteris paribus*, in the different animals for the different microbes; that it differs also in regard to diverse methods of administration, and again as to the time at which the agglutinating power makes its appearance. A few instances may be mentioned in illustration. After intraperitoneal injection into guinea-pigs of sub-fatal doses of living culture of cholera vibrio, the blood serum of the animal shows some weeks later (two to three) distinct agglutinating power with an emulsion of cholera vibrios in the proportion of 1 : 20 or even 1 : 40. By repeated (three) intraperitoneal injection of culture of living cholera vibrios this agglutinating power of the blood serum may be raised to 1 : 100 or even 200. I have, like other persons, obtained a high degree of agglutinating action by injecting subcutaneously first a large dose of sterilised and then, from week to week, gradually increasing doses of living cholera culture. A fortnight after the last (fifth) injection the blood serum of the guinea-pig possessed so strong an agglutinating power that one part of the serum agglutinated completely, and within a few minutes 200 parts of bouillon emulsion of living (recent) agar culture, or, better still, a twenty-four hours old peptone salt solution culture of the vibrio.

As regards the typhoid bacillus, a high agglutinating action of the blood serum of guinea-pigs can be produced in just the same way. After a fifth injection the blood serum agglutinates completely in the proportion of 1 in 400, within a few minutes, the bouillon emulsion of the typhoid bacillus being made from a forty-eight to seventy-two hours old gelatine culture.

There is, however, a difference between the two

microbes, the vibrio of cholera and *B. typhosus*, as regards the above reaction. The agglutinating action of the blood serum of a cholera-prepared animal does not ensue immediately, say within a few days, but takes some time, at least a fortnight, to develop; whereas in the case of a typhoid-prepared guinea-pig the agglutinating action can be shown to have set in within a few days of the injection, though of course it increases somewhat as time passes. This fact was first noted in the case of typhoid fever in man by Widal. He found that the blood serum possesses early in the acute stage of the illness an agglutinating action, and that the fact therefore is of great value for diagnosis. As regards cholera in man, on the other hand, it has been shown that the agglutinating action of the blood cannot be demonstrated till about two or three weeks after the disease has passed off.

I now proceed to consider how and to what extent the blood of animals previously "prepared" with plague culture acquires agglutinating action.

Experiments in Agglutination with Culture of *B. pestis*.

Various observers—Paltauf (*Wiener Kl. Wochenschrift*, 1897, N. 22); German Plague Commission (*Arbeiten aus dem Kaiserl. Gesundh.* Band xvi.); Russian Plague Commission (*Annales de l'Institut Pasteur*, 1897, N. 7); Vagedes (*Arb. des K. Gesundh.* Band xvii.)—have described positive results on plague culture with the blood of persons that have passed through and become convalescent for some weeks from an attack of bubonic plague. Similar positive results have been noted with the blood of

animals that had previously been prepared and protected by injection of non-fatal doses of plague culture or by injection of Haffkine's prophylactic. Thus Leumann, in various reports from the Bombay Plague Laboratory, mentions such positive agglutination results; Zabolotny [1] likewise describes such positive results; Dr. Markl [2] and others have stated the same. But there is no unanimity of these observers, either as to the degree of dilution or as to the time in which the addition of the blood serum produced the agglutination; there is in most of their descriptions merely the statement that positive results were obtained. Further, there is no detailed account of the manner in which the test was applied. As I have pointed out in the *Lancet* (February 16, 1901), it is extremely difficult to obtain an emulsion of plague culture suitable for the agglutination test, owing to the fact that the *B. pestis* has in all media a tendency to grow in coherent masses, the individual bacilli becoming naturally agglutinated by an interstitial (intercellular) sticky substance. As is well known, and as I have pointed out in my report for 1896, the *B. pestis* forms in broth cultures granules and flocculi of agglutinated masses, which, even on shaking, do not readily or to any large extent break up into their constituent elements. Wherefore a broth culture cannot be used for the test since the plague bacilli are already showing agglutinated masses. The same is the case with agar cultures. As is known, and as has been repeatedly pointed out (*l.c.* 1896), the *B. pestis* grows on the surface of agar as a characteristic filmy translucent sticky layer; so that when, with a platinum needle, a particle is

[1] *Archives des Sciences Biologiques de St. Petersbourg*, vol. viii. N. 1.
[2] *Centralbl. für Bakteriologie*, etc., vol. xxix. No. 21, p. 810.

attempted to be removed, the growth is drawn out along with it as a slimy thread. When an attempt is made to emulsify such growth the utmost that can be achieved by shaking it up in broth or salt solution is a breaking up into larger or smaller flocculi, on account of the presence of the gelatinous interstitial substance by which the bacterial cells are agglutinated. To use, therefore, for the agglutination test an emulsion in which from the outset there are present small and large masses, would not only be useless, but might be altogether misleading. While these difficulties are found with broth cultures and with cultures on agar and on serum, they apply in much less degree to cultures of *B. pestis* on the surface of gelatine; for on this medium the *B. pestis* forms a growth which is fairly dry and not of a viscid nature, although here also the bacilli are intimately aggregated. A fairly uniform emulsion can, however, usually be obtained from a surface gelatine culture by shaking up a particle of the growth in bouillon or in salt solution. By shaking up gelatine growth in distilled water an excellent emulsion can also be rapidly established. Similarly, by shaking up a recent agar culture in salt solution an emulsion is made which, filtered through filter-paper, forms a workable fluid.

First as to the bouillon and salt emulsion of gelatine culture of the bacillus.

After a considerable amount of experimentation with gelatine cultures, recent and old, I have found that gelatine cultures of a recent or fairly recent date, *e.g.* established from two to ten or twelve days, are most suitable. A particle of the growth is removed with a platinum needle or platinum loop and distributed by agitation in, say, salt solution so as to render the fluid

slightly turbid. As in other similar experiments on agglutination, too great turbidity, *i.e.* too thick an emulsion, is to be avoided. While in most instances an emulsion is obtainable in this manner, in others it is not possible to get rid of small aggregations of the bacilli. No amount of shaking can dissociate these, and such an emulsion is for obvious reasons not of sufficient reliability for application of the agglutination test. When such is the case I adopt the following plan for making a workable emulsion: I make (*a*) a thick emulsion and filter this through a double filter-paper, by which means all the larger aggregations are kept back and only the isolated or fairly isolated or very minute groups are let through; or (*b*) I take a particle of growth from the gelatine culture tube and rub it over the slanting surface of a fresh gelatine tube, after which a few cc. of salt solution are poured over this new surface and the tube slightly shaken till the fluid has worked off the matter from the surface.

I have made also an extensive series of observations with regard to the mere *sedimentation* of emulsions (broth, salt solution, water) of *B. pestis* in tubes, in capillary pipettes, etc. As a result I have found that any conclusion as to positive sedimentation and agglutination resulting from addition to them of blood has no value whatever, and for the simple reason that some (in fact many) emulsions of plague bacilli sediment in such tubes and capillary pipettes spontaneously without the addition of anything. Similarly, they sediment after the addition of various indifferent fluids, *e.g.* normal blood serum, aqueous humour, strong salt solution, peptone solution. All these cause the bacilli to settle down to the bottom of the fluid, which itself becomes quite limpid. I would go so far as

to warn all observers from relying on the agglutination test with emulsions of non-motile bacteria in which is involved similar liability to mere sedimentation. At any rate, my experiments on agglutination with emulsion of *B. pestis* by sedimentation have given very unsatisfactory results, and I have therefore abandoned them and have relied solely on *the agglutination test under the microscope.* In using this it is necessary to subject a large drop of the mixture of emulsion and blood serum to microscopic examination, so as to give the suspended bacilli a fair chance of coming together. If the test be made by covering a small drop of the mixture deposited on a glass slide with a covering glass, and thereby exposing the bacilli only in a very thin layer, there would be no chance given to the non-motile (plague or other) bacilli, if any were present, to respond to the agglutination force.

A further point in connection with these experiments which needs to be insisted on is that the agglutination test is valueless with bouillon emulsions. If a good and workable bouillon emulsion of *B. pestis* is prepared from a gelatine culture in the manner already described — an emulsion, for instance, in which the great majority of the bacilli are well isolated, and in which perhaps only here and there small groups of two or three bacilli occur—if, I say, such bouillon emulsion is then watched under the microscope it will be found that agglutination occurs in a comparatively rapid way. Clumps of fair size are formed within ten to fifteen minutes or even less, so much so that in some experiments with no addition of any material whatever " complete " agglutination in fairly large masses and disappearance of all single bacilli as such occurs in fifteen to thirty minutes. Bouillon emulsions of plague

bacilli for the object of making the agglutination test are therefore altogether useless. More than that: I have shown (*The Lancet*, February 16, 1901) that the addition of sterile bouillon to a good and otherwise permanent salt emulsion of *B. pestis*, in the proportion of 1 to 20 or even 1 to 40, causes within fifteen to thirty minutes distinct agglutination. I am disposed to think that the unreliable and unsatisfactory agglutination results obtained by some observers with the *B. pestis* have been due to their using bouillon emulsions, such, for instance, as are very useful in the case of *B. typhosus*, *Vibrio choleræ*, and other microbes.

Another kind of emulsion to be avoided in testing for agglutination with the plague bacillus is the watery emulsion. The first experiments that I made with a watery emulsion of gelatine plague culture were remarkable and deserve to be described in detail. Removing a particle of the growth from a week's old gelatine culture (slanting surface) and placing it in sterile distilled water, it was noticed that even a comparatively slight agitation produced an excellent emulsion; very soon neither with the unaided eye nor with the microscope could any aggregated mass be recognised, the bacilli formed indeed a uniform excellent emulsion. The blood serum of an animal which I had previously (and also afterwards) ascertained to possess distinct agglutinating power in dilution of 1 in 20, was added in the same proportion (viz. 1 in 20) to the above watery emulsion of plague bacilli. The result was quite unexpected, inasmuch as there occurred complete and striking agglutination within five minutes. It was, however, noticed that on adding the blood as such (1 part) to an equal amount (20 parts) of water

there occurred the well-known discoloration of the fluid (washing out of the hæmoglobin of the blood corpuscles), in consequence of which the blood became laky and only the discoloured stroma of the blood discs was left. The agglutinated masses of the plague bacilli in the mixture were seen to be especially associated with the discoloured stromata of the blood discs. A comparative experiment made with blood of a normal rabbit (as also with blood of normal guinea-pig, normal rat, and normal man) produced exactly the same result; and I must accordingly attribute to the hæmolysis occurring when a small quantity of blood is placed in a large volume of water this phenomenon of agglutination of the bacilli in watery emulsion of plague culture.

For making, therefore, a trustworthy test experiment as to agglutination of the bacilli of plague by a given sample of blood, neither bouillon emulsions nor watery emulsion of plague culture must be used. The test should always be made with a salt solution emulsion prepared as described above. A good salt emulsion in a control microscopic specimen sealed up shows, even after twenty-four hours, the bacilli in an isolated condition.

Next as to the blood to be tested. Comparative observations which I have made in this respect show that care is required to add to the emulsion the defibrinated blood or blood serum, not blood in which complete separation of the fibrin has not yet taken place; because in the process of coagulation apparent agglutination of the bacilli through entanglement of them in fibrin threads might easily be mistaken for real agglutination.

Another point that requires elucidation is this: Granted that a proper salt emulsion of plague bacilli is

being used, and granted also that the sample of blood to be tested is used after the complete separation of the fibrin, the question arises, In what dilution and for what length of time should the test be carried out? As is well known in the case of an animal well "prepared" with typhoid culture, or with cholera culture, agglutination is positive even when the animal's blood serum is used in very high dilutions—*e.g.* 1 in 200, 1 in 500, and even 1 in 1000—agglutination (under the microscope) occurring in a decided fashion within the hour.

In the observations of some authors (Bordet) the addition of normal serum, the placing of the mixture in the hot incubator, and various other factors are introduced which have an accelerating influence on the agglutination. These and similar observations are no doubt, both from a theoretical and practical point of view, of value. But in the case of the agglutination of plague culture I have limited myself preferably to making the test in as simple and uniform a manner as possible, so as to avoid the introduction of quasi-extraneous, for the most part unknown, new factors. The explanation of the process and nature of the phenomenon of agglutination is in itself complex and difficult in its most simple form, and it is made considerably more complex, without adding to the better understanding of it, by introducing a number of unknown additional factors. I have convinced myself early in my experiments that the blood serum of rats previously protected against plague, as also the blood serum of guinea-pigs previously prepared by repeated injection of sub-fatal doses of plague culture, has unmistakably the power to agglutinate the plague bacilli in a salt emulsion of gelatine plague culture when used in

dilution of 1 in 20, this agglutination taking place within the half-hour.

As will be presently described, the agglutination test has been made in a considerable number of cases of protected animals, and as a result it has been found that a dilution of 1 in 20, left at rest for half to one hour, is a fair standard and index for deciding one way or another. In the first place, I found that if, in a given instance, the agglutination test was positive with a dilution of 1 in 20 within the half-hour, it was positive also at 1 in 30 within the hour, but doubtful with 1 in 40 within the hour; and, on the other hand, that if the agglutination test was negative at 1 in 20 within the half-hour, it was equally negative at 1 in 10, or less, dilution within the half-hour.[1] In all the statements to be made here the test was declared positive if in dilution of 1 in 20 distinct clumping was observed within the hour in a preparation made in the manner of the hanging drop.

I.—Experiments of Agglutination with Blood of Guinea-pigs

1. *Guinea-pigs* 1 *and* 2.—(α) These two guinea-pigs were injected, February 18, with a salt emulsion of the laboratory *B. pestis*,[2] scraped from the slanting surface of an agar culture. The emulsion was thick and strongly turbid, and had been sterilised at 70° C. for fifteen minutes. Cultures made after the sterilisation did not yield any

[1] Dr. Markl's observations, *Centralbl. f. Bakteriologie*, etc., p. 810, vol. xxix., N. 21, lose a great deal of their value because he uses dilutions as low as 1 : 2, 1 : 5, and speaks of twenty-four hours' duration.

[2] In all experiments described here and subsequently the strain of plague bacilli was one derived from a case of plague pneumonia that had occurred in 1896 in the London Docks (L.P. I.).

colonies of *B. pestis*. Each animal received subcutaneously the whole growth covering the surface of one agar tube (6 centimetres by 2 centimetres).

(*b*) The same two guinea-pigs were reinjected subcutaneously on February 28 (ten days later) with exactly the same kind and same amount of sterilised culture.

On March 11 (*i.e.* eleven days after the second injection) blood of both guinea-pigs was withdrawn, allowed to clot, and test was made with the serum on salt emulsion of the laboratory *B. pestis* from gelatine culture, the dilution being 1 in 20.

[It is to be understood that in all experiments (without exception) of agglutination of emulsion of plague bacilli a control specimen was made of the emulsion alone without the blood, so as to make sure that in the particular emulsion agglutination did not occur spontaneously. Such has taken place in few instances for reasons unknown and undiscovered. In such an instance the experiment with the blood was rejected and repeated on a subsequent day.]

In ten to twenty minutes there was an indication of the formation of small clumps. In one hour the number and size of the clumps had increased; but altogether the agglutination was slight and not very pronounced.

(*c*) The same two guinea-pigs were therefore reinoculated on March 11 with the same amounts and the same kind of sterilised plague culture as in the previous instances.

On March 27, that is sixteen days later, the blood serum of these two guinea-pigs was again tested on salt emulsion of *B. pestis* (from gelatine culture), dilution 1 in 20. In thirty minutes the agglutination was distinct, some largish

clumps having formed, and in one hour it was unmistakable, and practically most bacilli had aggregated into loose masses.

It follows from these experiments that after three subcutaneous injections of guinea-pigs with large masses of solid growth of sterilised plague bacilli the blood of these animals acquired unmistakably the power to agglutinate plague bacilli.

The blood of one of these guinea-pigs was used for an agglutination experiment on two different strains of plague bacilli, the one derived from a plague rat which died in a dock warehouse in Cardiff, the other from a plague rat which died in Cape Town. In both these instances the test (dilution 1 in 20 for half an hour) proved as positive as with the strain of laboratory plague bacilli.

(*d*) The same two guinea-pigs were again injected on March 27 with about a platinum loopful of living plague bacilli from an agar surface culture about seven weeks old; that is to say, they were injected with a comparatively small and presumably non-fatal dose of an attenuated culture of living plague bacilli. This was done because former experience (see my Report, 1896-1897, p. 287) has shown that it is extremely difficult to render guinea-pigs immune against largish doses of living plague bacilli. As a matter of fact, both the above guinea-pigs developed buboes in the course of the next few days, and one animal (guinea-pig No. 2) was found dead on the tenth day after the last injection. The other animal recovered completely. Nineteen days after the last injection the blood serum of this animal was tested on salt emulsion of *B. pestis* (dilution 1 : 20), two different strains being used—(*a*) the laboratory strain; (*b*) a strain

derived from a case of bubonic plague that had occurred in a sailor at Llandaff. The blood serum of the above guinea-pig caused decided agglutination of both strains in ten minutes, the control specimen of the salt emulsion showing no alteration.

The single injection, therefore, of living culture had decidedly enhanced the agglutinating power of the blood serum of this animal.

(*e*) The further history of this animal is as follows :— On April 22 it was reinoculated subcutaneously with one loopful of living bacilli taken from a recent gelatine culture; it was further injected on May 2 and May 28 and June 17, each time with two to three loops of living gelatine culture. On July 9 its blood serum was tested (dilution 1 : 20) and found to produce complete agglutination in about five minutes, certainly within ten minutes. On same day (July 9) it was again injected with two to three loops of living culture. Its blood serum was tested (dilution 1 : 20) on July 16, and gave complete agglutination of plague emulsion in ten minutes.

On July 18 the guinea-pig was further injected with two to three loops of living culture. On July 29 its blood serum was tested (dilution 1 : 20); result, no agglutination in fifteen minutes, but distinct in forty minutes. It was again injected on July 31. On August 9 its blood serum produced no agglutination in thirty minutes. The animal was reinjected on August 26. Its blood serum was tested on September 10; no agglutination in thirty minutes. Reinjected on September 16. Blood serum showed no agglutination on October 1, but gave distinct and complete agglutination within ten minutes on October 15 and on October 17.

It will be seen from this experiment that the agglutinating power of the blood serum of this animal (No. 1) showed a gradual increase in degree as time went on, corresponding to the increasing number of injections, but that this proceeded only to a certain point. After a while further injection not only did not enhance this power, but failed to maintain it; though still later the power became, after additional injection, again restored. These results, *qua* plague, are quite in harmony with those already obtained in regard of parallel experiments as to agglutinins (with typhoid bacillus, cholera vibrio, and bacillus coli), and as regards also antitoxins with the microbes of diphtheria and tetanus. As in former experiments (*l.c.* 1896-1897) with plague, so now, even a sixth injection was followed by the appearance of a bubo, though the animal remained otherwise lively and fed well.

This guinea-pig No. 1 will be again referred to at a later stage of this report.

2. *Guinea-pigs Nos.* 3 *and* 4.—These two animals were subjected to repeated injections with at first sterile, later with living culture, of *B. pestis*; but the experiment differs from the previous experiment in that all injections were made intraperitoneally. Procedure was as follows:—

[TABLE

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	May 6 .	Intraperitoneal .	The whole growth scraped from the surface of gelatine culture and sterilised for 15 minutes at 70°C.
2nd	„ 22 .	„ .	„ „ „
3rd	June 5 .	„ .	„ „ „
4th	„ 14 .	„ .	Small dose of living culture (gelatine).
5th	July 9 .	„ .	„ „ „
6th	„ 18 .	„ .	„ „ „
7th	„ 31 .	„ .	„ „ „
8th	August 27 .	„ .	„ „ „
9th	September 16	„ .	„ „ „

The blood serum was tested (dilution 1 : 20) on the following dates, and with the following results in one hour :—

No.	Date.	Result.
1st	June 4 . .	Negative.
2nd	„ 13 . .	„
3rd	„ 26 . .	Positive (?).
4th	July 9 . .	Rapid agglutination within 10 minutes.
5th	„ 29 . .	Distinct clumping in 10 minutes.
6th	August 9 . .	Complete agglutination in 10 minutes.
7th	September 12 .	Positive in 5 minutes.
8th	October 1 . .	Indication of agglutination in 10 minutes, but not better in 30 minutes nor in 1 hour.

It is seen from this series that the triple intraperitoneal injection of sterile culture was less effective as to the production of agglutinins than had been subcutaneous injection of like material; that the subsequent two intraperitoneal injections of small doses of living culture brought about distinct presence in the blood of agglutinins; and that while further similar injections continued to enhance the agglutinating power of the blood, the ninth injection was followed by a distinct decrease of this power.

3. *Guinea-pigs Nos.* 5 *and* 6.—These two animals (half-grown) were repeatedly injected subcutaneously with *Haffkine prophylactic.*

The animals were injected on the following dates, and with the following amounts :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	June 27 .	Subcutaneous .	10 cc. of Haffkine's prophylactic.
2nd	July 3 .	„ .	5 „ „ „
3rd	„ 18 .	„ .	10 „ „ „
4th	August 1 .	„ .	10 „ „ „
5th	„ 29 .	„ .	10 „ „ „
6th	September 17	„ .	10 „ „ „
7th	October 21 .	„ .	10 „ „ „

The blood serum was tested (dilution 1 : 20) on the following dates, and with the following results :—

No.	Date.	Result.
1st	July 8 . .	Agglutination doubtful in 1 hour.
2nd	„ 15 . .	Negative in 1 hour.
3rd	„ 30 . .	„ „
4th	August 28 . .	„ „
5th	September 13 . .	„ „
6th	November 19 .	„ „

The subcutaneous administration, many times repeated, of Haffkine's prophylactic had therefore no result in producing agglutinin such as was demonstrable after repeated injection of small doses of living culture.

4. *Guinea-pigs Nos.* 7 *and* 8.—These, which were half-grown, were injected intraperitoneally on the following dates, and with the following results :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	June 17 .	Intraperitoneal .	5 cc. of Haffkine's prophylactic.
2nd	„ 27 .	„ .	5 „ „ „
3rd	July 11 .	„ .	5 „ „ „
4th	„ 18 .	„ .	5 „ „ „
5th	August 1 .	„ .	5 „ „ „
6th	„ 29 .	„ .	6 „ „ „
7th	September 17	„ .	10 „ „ „
8th	October 21 .	„ .	10 „ „ „

The blood serum was tested (dilution 1 : 20) on the following dates, with the following results :—

No.	Date.	Result.
1st	June 27 . .	Indication of agglutination in 15 minutes.
2nd	July 8 . . .	Complete clumping in 10 minutes.
3rd	„ 30 . .	Slight clumping in 20 minutes.
4th	August 8 . .	Negative in 1 hour.
5th	September 9 . .	„ „
6th	November 19 .	„ „

These experiments are in accord with those in which the blood after repeated injections acquired a gradually increasing agglutinating power, and later on, notwithstanding continued injection, again lost it. They, moreover, tend to show that the intraperitoneal injection of small doses of Haffkine's prophylactic into guinea-pigs is more conducive to the formation of agglutinin than the subcutaneous administration of doses twice as large.

II.—Experiments of Agglutination with Blood of Rabbits

In this series rabbits were substituted for guinea-pigs, and, as will be shown, the rabbit proved very much more satisfactory.

5. One half-grown rabbit, No. 1, was injected *intravenously* (ear vein) repeatedly with at first sterile and then living plague culture :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	May 6 .	Intravenous.	Salt emulsion of plague growth scraped from the surface of a gelatine culture and sterilised for 15 minutes at 70° C. The amount used for each animal was about $\frac{3}{4}$ of a culture (6 centimetres by 2 centimetres).
2nd	„ 28 .	„ .	Same amount and same kind of material.
3rd	June 13 .	„ .	„ „ „ „
4th	July 9 .	„ .	Small amount of emulsion of living culture.
5th	„ 18 .	„ .	„ „ „ „

This rabbit was found dead on July 20. On post-mortem examination the spleen was seen to be permeated by small grey nodules, which on microscopic and cultural examination proved to be of the nature of pseudo-tubercle. No plague bacilli were demonstrable either in the blood or the organs.

The blood of the rabbit was, however, tested for its agglutination (dilution 1 : 20) on various occasions, with the following interesting results :—

No.	Date.	Result.
1st	May 24 . . .	Negative.
2nd	June 5 . . .	Distinct agglutination in 30 minutes.
3rd	July 7 . . .	Complete agglutination in 10 minutes.
4th	„ 16 . . .	„ „ within 5 minutes.

From this it appears that after the first intravenous injection of sterile culture no agglutinin had yet been formed, but that after the second such injection there was, eight

days later, distinct agglutinin present; further, that this became enhanced by a similar third injection, and was still more distinct after a fourth injection with living culture.

6. One half-grown rabbit, No. 2, was injected into the ear vein in precisely the same manner and at the same time as the rabbit of the previous experiment. A fortnight after the third injection with sterile culture (first injection May 6, second injection May 28, third injection June 13), *i.e.* June 27, the animal's blood serum was tested (dilution 1 : 20) and was found to produce complete agglutination in ten minutes; the same result as was observed in the previous experiment. The animal, unfortunately, was found dead on July 8. The post-mortem examination showed extensive cysticercus disease of the omentum.

7. A half-grown rabbit, No. 3, was injected *subcutaneously* with Haffkine's prophylactic at the following times :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	June 17 . .	Subcutaneous	10 cc. of Haffkine's prophylactic.
2nd	July 3 . .	,,	10 ,, ,, ,,
3rd	,, 18 . .	,,	10 ,, ,, ,,
4th	August 1 .	,,	10 ,, ,, ,,
5th	,, 29 .	,,	10 ,, ,, ,,
6th	September 17 .	,,	10 ,, ,, ,,
7th	October 21 .	,,	20 ,, ,, ,,
8th	November 21 .	,,	20 ,, ,, ,,

The results of the testing of this rabbit's blood serum on emulsion of *B. pestis* (dilution 1:20) were the following:—

No.	Date.	Result.
1st	June 27 . .	Negative in 1 hour.
2nd	July 2 . . .	„ „
3rd	„ 8 . . .	„ „
4th	„ 31 . . .	„ „
5th	August 26 . .	Positive in 10 minutes.
6th	September 13 . .	Negative in 1 hour.
7th	October 2 . .	„ „
8th	„ 15 . .	„ „
9th	January 16 . .	Positive and distinct in 15 minutes.

This experiment in its early stages contrasts markedly with similar stages of experiment 5; for it shows that the *subcutaneous* injection of Haffkine's prophylactic, even after repeated (three) administration of considerable amounts (10 cc. each time), did not succeed in producing agglutinin in the animal's blood.

8. *Rabbits Nos.* 5 *and* 6.—In this experiment the administration of Haffkine's prophylactic was effected in two rabbits by *intravenous* followed by *subcutaneous* injection. As regards rabbit No. 5:—

[TABLE

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	July 1 . .	Intravenous .	5 cc. of Haffkine's prophylactic.
2nd	„ 11 . .	„	5 „ „ „
3rd	„ 18 . .	Subcutaneous	10 „ „ „
4th	August 1. .	„	10 „ „ „
5th	„ 29 .	„	10 „ „ „
6th	September 17 .	„	10 „ „ „
7th	October 21 .	„	20 „ „ „

The animal died on November 4. On post-mortem examination the liver was found atrophied, fatty; the stomach was much distended, showing few hæmorrhagic patches in the serous covering; in the abdominal cavity were numerous cysticerci.

The blood serum of this animal (rabbit No. 5) had been tested for agglutination on the following dates, and with the following results :—

No.	Date.	Result.
1st	July 8 . . .	Negative.
2nd	„ 16 . . .	Complete agglutination in 15 minutes.
3rd	„ 31 . . .	Negative in 1 hour.
4th	August 26 . .	Positive in 10 minutes.
5th	September 9 . .	Negative in 1 hour.

As to rabbit No. 6 :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	July 1 . .	Intravenous .	5 cc. of Haffkine's prophylactic.
2nd	„ 11 . .	„	5 „ „ „
3rd	„ 18 . .	Subcutaneous	10 „ „ „
4th	August 1. .	„	10 „ „ „
5th	„ 29 .	„	10 „ „ „
6th	September 17 .	„	10 „ „ „
7th	October 21 .	„	18 „ „ „
8th	November 21 .	„	20 „ „ „

The blood serum of this animal was tested (dilution 1 : 20) :—

No.	Date.	Result.
1st	July 16 . . .	Positive in 20 minutes.
2nd	„ 31 . . .	Negative in 1 hour.
3rd	November 14 . .	Indication in 15 minutes.
4th	January 1 . .	Distinct agglutination 10-15 minutes.
5th	„ 16 . .	„ „ „

This experiment is confirmatory of that with the previous animal, No. 5. A twofold intravenous injection of Haffkine's prophylactic brought about production of agglutinins. But at this stage a single subcutaneous injection of 10 cc. not only did not enhance the aggluti-

nating power, but, on the contrary, seemed to destroy it, though it reappeared after several further subcutaneous injections. Another important fact is brought to light in this as in experiment No. 7, viz. the distinct agglutination possessed by the blood serum nearly two months after the last injection.

I shall have to reconsider later on other points concerning these animals, but at present I proceed with the examination by further experiments of the agglutination phenomenon.

III.—Experiments in Agglutination after Injection of Filtrate of Haffkine's Prophylactic

9. In this experiment two half-grown guinea-pigs were injected subcutaneously with the clear *filtrate* of Haffkine's prophylactic. This was obtained by simply opening some of the sealed tubes in which the prophylactic had been preserved, decanting the clear fluid and passing it through a Pasteur-Chamberland filter. The filtrate was of course perfectly limpid.

Guinea-pigs Nos. 9 and 10 (half-grown) were injected *subcutaneously*, each receiving 10 cc. of the above filtrate :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	September 10 .	Subcutaneous .	10 cc. of the filtrate.
2nd	„ 17 .	„ .	„ „
3rd	October 21 .	„ .	„ „

One of these guinea-pigs died December 23. The animal was extremely emaciated, but no cause for its death could be found.

The blood serum was tested (dilution 1 : 20) :—

No.	Date.	Result.
1st	September 29 . .	Negative.
2nd	October 12 . . .	,,
3rd	November 21 . . .	,,

10. The half-grown guinea-pigs Nos. 11 and 12 were injected *intraperitoneally*, each receiving 10 cc. of the filtrate :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	September 10 .	Intraperitoneal	10 cc. of the filtrate.
2nd	,, 17 .	,,	,, ,,
3rd	October 21 .	,,	,, ,,

The tests with the blood serum were made as above, but with completely negative result.

11. Two half-grown rabbits, Nos. 7 and 8, were *subcutaneously* injected with the same filtrate, each animal receiving 20 cc. :—

[TABLE

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	September 10 .	Subcutaneous .	20 cc. of the filtrate.
2nd	„ 17 .	„ .	„ „
3rd	October 21 .	„ .	„ „
4th	November 21 .	„ .	„ „
5th	January 2 .	„ .	„ „

The blood serum of both animals was tested (dilutions 1 : 20 and 1 : 10 in each instance) :—

No.	Date.	Result.
1st	October 2 . . .	Negative.
2nd	November 20 . . .	„
3rd	January 1 . . .	„

From this it appears that neither in the rabbit nor in the guinea-pig was there any agglutinin formed after repeated injection of the *filtrate alone* of Haffkine's prophylactic. It was altogether unexpected that half-grown rabbits should, after receiving a total of 100 cc. of this filtrate, yield blood serum which showed no sign of agglutinin even in dilution of 1 in 10.

IV.—Experiments with Blood Serum of Rats

I have already mentioned that rats which had passed through the disease (rats which had first been prepared by

Haffkine's prophylactic, 10 cc. subcutaneously injected) and were then injected with more than an ordinary fatal dose of living culture of the plague bacillus, developed a distinct bubo which suppurated. This after some days healed completely, and the animals' blood tested along with salt emulsion of gelatine culture showed in a conspicuous degree agglutination (dilutions 1 : 20 and 1 : 40) in ten minutes. I have had occasion to repeat this experiment on other rats which had passed through a non-fatal attack of plague, and have been able fully to confirm it.

12. In this experiment three rats were injected subcutaneously with Haffkine's prophylactic, each animal receiving 10 cc. :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	November 21 .	Subcutaneous .	10 cc. of Haffkine's prophylactic.
2nd	December 31 .	„ .	„ „ „

As was ascertained from the experiments carried out in conjunction with Prof. Haffkine in 1899, a single injection of 10 cc. of this prophylactic into rats suffices to give them protection against an otherwise fatal dose of living plague. In the present instance no further injection beyond the above 20 cc. was employed. The blood serum of one of the rats (after the animal had been killed) was tested (dilution 1 : 20) on January 30, and it showed no distinct agglutination within one hour.

13. Four rats were injected on December 31 subcutaneously with Haffkine's prophylactic, each animal

receiving 8 or 10 cc.; that is to say, two full-grown animals received each 10 cc., other two half-grown 8 cc. in each instance.

On February 5 one of the small animals was killed and its blood serum tested (dilutions 1 : 20 and 1 : 10), with negative result in one hour.

It follows from these experiments that injection into rats of Haffkine's prophylactic in amount more than sufficient to protect them against a fatal dose does not cause the production in their blood of any agglutinins.

14. In this experiment three rats were subcutaneously injected with the *filtrate* of Haffkine's prophylactic, each animal receiving 10 cc. on November 21 and other 10 cc. on December 31.

Their blood serum was tested on February 2 (dilution 1 : 20 and 1 : 10), with completely negative result. This result was to be expected, considering that the injection of the prophylactic as such did not in previous experiments produce agglutinins.

The results, then, of the numerous experiments here described can be thus summarised :—

1. The blood of rodents (guinea-pigs, rabbits, rats) which have been repeatedly injected with large masses of the *sterilised* bodies of *B. pestis* possesses the power of agglutinating a duly prepared emulsion of the *B. pestis.* Especially in rabbits is this manifest.

2. The same agglutinating action is observed with respect to the blood of rodents which having been injected with sub-fatal doses of *living* plague bacilli had as a consequence been affected with the disease and recovered from it.

3. The increase in agglutinating action of the blood of

these "prepared" animals is not in a direct ratio to the amount of material injected, or to the number of the different injections. It appears to go on increasing up to a certain degree, and then to decrease or be lost entirely.

4. The repeated administration of Haffkine's prophylactic into guinea-pigs, when injected *subcutaneously*, produced no agglutinin in the blood; whereas the repeated *intraperitoneal* injection of the prophylactic into guinea-pigs appears to have produced agglutinin which, however, was soon lost, even during continuance of "treatment."

5. In the rabbit, on the other hand, the repeated injection (intravenous, less when subcutaneous) of Haffkine's prophylactic did produce agglutinins at one or another stage, although on the whole such production was uncertain.

6. In the rat the repeated injection subcutaneously of Haffkine's prophylactic failed to produce agglutinin.

7. The repeated injection (in whatever way) of the *filtrate* of Haffkine's prophylactic into rodents (guinea-pigs, rabbits, rats) failed to produce agglutinins.

Besides the experiments on agglutinins above summarised, investigation has been made of the power of the blood serum of guinea-pigs and rabbits, and in a few instances also of rats, previously immunised in various ways, in inhibiting the action of the living *B. pestis*, when such serum along with a lethal dose of plague was injected into a susceptible or unprepared animal. In other words, account having been given of the agglutination or test *in vitro*, what follows will deal with Pfeiffer's test or the test *in corpore*.

V.—Experiments in Testing Blood of Prepared Animals for the Presence of Germicidal Substance.

(*a*) *Guinea-pigs.*—With reference to the guinea-pig of experiment 1, guinea-pig No. 1, referred to at page 212, it will be convenient here to restate, in tabular form, the several occasions on which this animal had been injected, and the material used for injection in each instance :—

No.	Date of Injection.	Method of Injection.	Material Injected.
1st	February 18 .	Subcutaneous .	Sterile agar culture.
2nd	„ 28 .	„ .	„ „
3rd	March 11 .	„ .	„ „
4th	„ 27 .	„ .	Living agar culture.
5th	April 22 .	„ .	„ „
6th	May 2 . .	„ .	„ „
7th	„ 28 . .	„ .	„ „
8th	June 17 . .	„ .	„ „
9th	July 9 . .	„ .	Living gelatine culture.
10th	„ 18 . .	„ .	„ „
11th	„ 31 . .	„ .	„ „
12th	August 26 .	„ .	„ „
13th	September 16 .	„ .	„ „

Test *in corpore* of the blood of this animal was performed as follows :—Blood serum of the animal was mixed with living plague emulsion, and the mixture injected subcutaneously into the groin of a guinea-pig, a like amount of living plague emulsion being at the same time injected into a control guinea-pig. The two animals were of about the same body-weight. Thus :—

(1) July 16.—Guinea-pig No. 11 was injected with 100 cubic millimetres, 1000 cubic millimetres being equal to 1 cubic centimetre, of plague emulsion, plus 25-30

cubic millimetres of blood serum. At the same time 100 cubic millimetres of plague emulsion were injected into control guinea-pig No. 12.

Both guinea-pigs developed big buboes. The control guinea-pig died on the thirteenth day, the other guinea-pig which had received plague culture plus blood serum died on the seventh day, both of plague; that is to say, the control animal died later than the other. The blood serum had therefore had no effect whatever of neutralising the fatal dose of plague culture.

(2) July 29.—Guinea-pig No. 17 was injected with a mixture of 100 cubic millimetres of plague emulsion and 50 cubic millimetres of blood serum. At the same time a control guinea-pig No. 18 was injected with 100 cubic millimetres of plague emulsion alone.

Guinea-pig No. 17 developed no bubo, and remained quite lively and well. Guinea-pig No. 18, on the other hand, showed distinct bubo on the third day; this enlarged till the eighth day, then gradually diminished and disappeared, the animal quite recovering. This experiment is therefore faulty in this, that the control animal did not succumb to plague, the dose injected not proving a fatal dose. But the experiment apparently indicates that some inhibiting effect was produced by mixing the blood of the prepared guinea-pig with a dose of plague culture that served in a control animal to cause a distinct bubo. Referring to the table, it will be seen that at the date on which the blood serum was obtained from the guinea-pig No. 1 this animal had been injected three times with sterile culture, and seven times with small doses of living culture of plague bacillus, and that therefore by this time its blood seemed to contain

some active germicidal substance, although in a small amount.

(3) August 10.—Guinea-pig No. 26 was injected with a mixture of 100 cubic millimetres of emulsion and 100 cubic millimetres of blood serum. At the same time guinea-pig No. 27 was injected with 100 cubic millimetres of emulsion alone.

The control guinea-pig No. 27 died of plague on the sixth day, the other guinea-pig, No. 26, died of plague on the seventh day. This experiment does not denote the presence of germicidal substance in the blood of guinea-pig No. 1, even when serum and plague emulsion have been injected in equal amount.

The guinea-pig No. 1 had been injected on September 16, a thirteenth time, this time with a double dose of living gelatine culture. On October 17 the animal was killed, and it showed nowhere any pathological appearances. As already mentioned, its blood serum at this stage gave on agglutination test (dilution 1 : 20) completely positive result in ten minutes. Its blood serum, as also a salt extract of the spleen (this organ looked quite normal), were now used in the following manner :—

(1) 200 cubic millimetres of plague emulsion were mixed with 200 cubic millimetres of blood serum, and the mixture injected into guinea-pig No. 40.

(2) 200 cubic millimetres of plague emulsion were mixed with 50 cubic millimetres of blood serum, and the mixture injected into guinea-pig No. 39.

(3) 200 cubic millimetres of plague emulsion were mixed with 200 cubic millimetres of thick spleen emulsion, and the mixture injected into guinea-pig No. 41.

(4) 200 cubic millimetres of plague emulsion were injected into control guinea-pig No. 42.

The result was this :—Guinea-pigs 42, 39, and 41 died of plague on the fifth day ; guinea-pig 40 died of plague on the sixth day. All the animals had typical bubo and enlarged spleen crowded with plague bacilli. From this it appears that neither the blood nor the spleen of guinea-pig No. 1 were capable of exerting any appreciable germicidal action. That the guinea-pig No. 1 was distinctly protected by September 16 is proved by the fact that an injection on that day of a considerable dose (certainly more than double the ordinary fatal dose) did not cause any illness whatever in this animal. As a matter of fact, some time previous to the above date the guinea-pig did not react on the injection of an otherwise fatal dose of living plague culture. And yet the last-named experiments prove that this animal possessed neither in its blood nor in its spleen those substances (antitoxins, germicidal substances, etc.) which we associate with protection, *i.e.* substances produced by repeated injections of the microbe, as in diphtheria protection and cholera protection. It is justifiable, therefore, to conclude from the above very striking experiment that, as regards the guinea-pig, the injection (thirteen times) of the plague microbe does not result in the production of demonstrable amounts of anti-bodies—germicidal substances, Pfeiffer's lysins—in the experimental animal.

The second guinea-pig used for experiment was that already referred to as guinea-pig No. 4, p. 216.

This animal had been injected at the following periods :—

No.	Date of Injection.	Method of Injection.	Material (and Amount) Injected.
1st	May 6 . .	Intraperitoneal	Large dose of sterile gelatine culture.
2nd	„ 22 . .	„	„ „ „
3rd	June 5 . .	„	„ „ „
4th	„ 14 . .	„	Small dose of living gelatine culture.
5th	July 9 . .	„	„ „ „
6th	„ 18 . .	„	„ „ „
7th	„ 31 . .	„	„ „ „
8th	August 27 .	„	„ „ „
9th	September 16 .	„	„ „ „

The test *in corpore* was made as follows :—

(1) July 29.—Guinea-pig No. 19 was subcutaneously injected with 100 cubic millimetres of living plague emulsion mixed with 50 cubic millimetres of blood serum. At the same time 100 cubic millimetres of the emulsion was injected into a control animal.

Both these animals remained alive. The experiment is therefore useless, the amount of living culture injected having been insufficient to cause death in the control animal.

(2) August 9.—The experiment was repeated. Guinea-pig No. 28 was subcutaneously injected with 100 cubic millimetres of living plague emulsion mixed with 100 cubic millimetres of blood serum of guinea-pig No. 4. At the same time 100 cubic millimetres of the emulsion were injected into a control animal.

The control guinea-pig died of plague on the sixth

day; the other, No. 28, died of plague on the ninth day.

(3) September 12. — Guinea-pig No. 35 was subcutaneously injected with 100 cubic millimetres of living plague emulsion mixed with 100 cubic millimetres of blood serum. At the same time 100 cubic millimetres of the same emulsion were injected into a control guinea-pig.

The guinea-pig No. 35 died of plague on the fifth day; the control guinea-pig died of plague on the seventh day.

From this series confirmation of the previous result is obtained, viz. that even after several injections of living cultures into the peritoneum of the guinea-pig no appreciable amounts of germicidal substances are present in the blood of the protected animal.

Seeing, then, that no germicidal effect can be produced with the blood serum of guinea-pigs repeatedly injected with living culture, it was not to be expected that a positive effect could be produced with the blood serum of guinea-pigs repeatedly injected with the Haffkine prophylactic. But, nevertheless, experiments as to this were actually made, as follows :—

Guinea-pig No. 5, already mentioned (p. 218) as having been injected subcutaneously with Haffkine's prophylactic on seven separate occasions, furnished blood serum which was tested for the presence of germicidal substance twelve days after the fourth injection, and again fourteen days after the sixth injection. The result in both instances was negative.

Guinea-pig No. 7, referred to at p. 219 as having been injected intraperitoneally on eight separate occasions

with Haffkine's prophylactic, furnished blood serum which was tested for germicidal effects after the fourth and again after the seventh injection. The result was in both instances quite negative.

In the face of these results I have not thought it expedient to test the blood serum of the guinea-pigs which had been injected with the filtrate *alone* of Haffkine's prophylactic.

(*b*) *Rabbits.*—The blood serum of the following three protected rabbits was submitted to the germicidal test, namely:—

Rabbit No. 1, referred to at p. 220 as having been *intravenously* injected at first (three times) with sterile, then (twice) with small doses of living culture; rabbit No. 3, referred to at p. 222 as having been repeatedly injected *subcutaneously* with Haffkine's prophylactic; and rabbit No. 5, referred to at p. 223 as having been repeatedly injected, first *intravenously* and then *subcutaneously*, with Haffkine's prophylactic.

Rabbit No. 1.—Test of this rabbit's serum was made on July 16, that is to say, one week after the fourth intravenous injection.

(1) 100 cubic millimetres of plague emulsion mixed with 50 cubic millimetres of the blood serum were injected into guinea-pig No. 13.

(2) 100 cubic millimetres of plague emulsion mixed with 100 cubic millimetres of the blood serum were injected into guinea-pig No. 14.

The result was negative; both animals died of plague, No. 13 on the sixth day, No. 14 on the seventh day.

Rabbit No. 3.—The blood serum of this rabbit was

tested on July 15, *i.e.* after the second subcutaneous injection with Haffkine's prophylactic.

100 cubic millimetres of plague emulsion mixed with 100 cubic millimetres of its blood serum were injected into guinea-pig No. 9.

This animal, No. 9, died on the fourth day of typical plague.

The test was repeated on July 31, *i.e.* thirteen days after the third injection of 10 cc. of Haffkine's prophylactic.

100 cubic millimetres of plague emulsion mixed with 100 cubic millimetres of the blood serum were injected into guinea-pig No. 23 ; and at the same time 100 cubic millimetres of the emulsion alone were injected into control guinea-pig No. 24.

The control, No. 24, died on the eighth day, the other guinea-pig, No. 23, died on the twelfth day.

The test was repeated on August 26, again with negative result.

Rabbit No. 5.—The blood serum of this rabbit was tested on three different occasions—July 16, July 31, and August 26. But, as was the case with rabbit No. 3, the result proved negative in all three instances.

As regards rabbits Nos. 3 and 5, the test on the last occasion, viz. August 26, was altered in this way. Instead of mixing equal parts of plague emulsion and blood serum, a double volume of blood serum was used, viz. 100 cubic millimetres of plague emulsion were mixed with 200 cubic millimetres of blood serum. The guinea-pigs injected with the mixture died, notwithstanding, of typical plague.

From these experiments it follows that, as with the guinea-pig so with the rabbit, neither repeated intra-

venous or subcutaneous injection, first of sterile and then of living culture, nor repeated subcutaneous or intravenous injection of Haffkine's prophylactic, produces germicidal substances in the blood of this animal in appreciable amount and in a way to render its serum serviceable for neutralising the fatal effect upon the guinea-pig of a dose of living culture of plague bacillus. It will have been noticed from the above control experiments that the dose of plague emulsion, viz. 100 cubic millimetres, generally used, was by no means a large dose, since some of the control guinea-pigs did not die, while in most instances death was delayed. It will be remembered from my Report 1896-1897 that the normal fatal dose for a guinea-pig is the one that kills a half-grown animal between forty-eight and seventy-two hours. In the cases now in question some of the (control) animals die as late as the seventh or eighth day.

(*c*) *Rats.*—One rat, which had been twice *subcutaneously* injected with Haffkine's prophylactic, November 21 and December 31, was killed on January 30.

250 cubic millimetres of plague emulsion mixed with 250 cubic millimetres of blood serum of above rat were injected into one rat, No. 1, and

250 cubic millimetres of emulsion only into a control rat, No. 2.

Both animals died of plague, No. 1 on the fifth, No. 2 on the fourth day.

A further experiment was the following :—

A rat which had been protected by a first injection of 10 cc. of Haffkine's prophylactic was a week later injected with an ordinarily fatal dose of living plague culture.

The animal developed a bubo that suppurated and which in the course of two weeks had completely healed up. This rat was killed about five weeks after the first injection, about two to three weeks after the last, and its blood serum tested *in corpore* both on a guinea-pig and on a rat. In each case 250 cubic millimetres of living plague emulsion were mixed with 250 cubic millimetres of blood serum. Both animals died of plague, as did the control in each instance.

It follows from these experiments that no conspicuous amount of germicidal substance is produced in the blood of the rat when it has been efficiently protected, whether by Haffkine's prophylactic alone or by passing in addition through a mild form of the plague.

Plague therefore differs from some other infective diseases (cholera, diphtheria, etc.) in the circumstance that a previous immunisation of an animal against plague does not necessarily create in the blood of this animal an appreciable amount of germicidal substance which may be in turn used for conferring either passive immunity on a fresh animal or for neutralising a fatal dose of living plague culture. It is necessary, however, to remember that in all these experiments only relatively small amounts of blood serum was used, and it is quite possible that if huge doses of the serum, say several cubic centimetres, had been injected, the result might have been different. But for our purposes, viz. to see whether appreciable amounts of germicidal (specific) substances were present in the blood, large doses of the serum were not to be recommended. Recent research has shown that large doses of blood serum of even normal blood contain substances which act deleteriously on microbes. The above negative

results do not touch the question of specific antitoxins, these being different from lysins or germicidal substances ; it is quite possible that such specific antitoxins may have been present in the blood of the prepared animals, antitoxins, that is, which could be utilised for therapeutic purposes, such as recommended by Yersin, Calmette, and others.

After collecting evidence on the subject, the Indian Plague Commission have expressed the view (Report, vol. v.) that the agglutination test for purposes of diagnosis is not of sufficiently reliable kind, and a similar view has been expressed by Dr. Simpson in his *Treatise on Plague.*

Nevertheless, I am inclined to think, from the large number of observations which I have made, that under certain conditions of experimentation the agglutination test is of value, and a similar view has also been published in Dr. Chalmers' Report on Plague in Glasgow, in 1900.

Recently Captain Holmes of the Indian Veterinary Service has made a number of experiments in my laboratory in the same direction. Guinea-pigs and rats, which had been previously protected by injection of my new organ prophylactic, were tested with virulent culture of *B. pestis.* They had been found immune against plague, and several weeks later their blood was subjected to the agglutination test on culture of *B. pestis.* The proportion of blood serum and emulsion of *B. pestis* was 1 : 1 and 1 : 2. Whereas the blood serum of normal guinea-pigs and normal rats gave invariably a negative result, the blood serum of both the guinea-pigs and the rats which had survived the plague test gave striking and unmistakable positive results. Although the dilution

used was not great, yet a comparison with normal blood proved conclusively that the test with plague blood is distinctly of value.

I have recently had occasion to test the blood serum of two monkeys which had been previously protected by injection of my organ prophylactic (see later), and had then been tested for immunity by subcutaneous injection of virulent *B. pestis.* Fourteen days after the subcutaneous injection of a multiple fatal dose of virulent *B. pestis* into the previously protected monkeys, and which injection caused no abnormal symptom of any kind, the blood of these monkeys was tested on salt emulsion of *B. pestis.* To three drops of the emulsion a small loop of the blood serum of monkey 1 was added, the proportion of serum to emulsion was about 1 : 20. After twenty minutes there were distinct signs of agglutination; after thirty minutes agglutination was striking and complete, all the bacilli of the emulsion having become massed together into smaller and larger clumps. The blood serum of monkey 2 used in the same way acted also distinctly but not quite so markedly as that of monkey 1, since here, even after one hour, agglutination was not complete, although numerous smaller and larger clumps could be met with.

CHAPTER IX

PROTECTIVE INOCULATION AGAINST PLAGUE[1]

SINCE the appearance of epidemic plague at Bombay in 1896 Dr. Haffkine has employed on a considerable scale as a protective inoculation the sterilised broth culture of virulent plague bacilli, which is now known as Haffkine's plague prophylactic fluid. From time to time since 1898 Reports have been published from the Bombay Laboratory and from different localities in India giving an account and statistical tables of the results of the prophylactic injection of the fluid by Haffkine, his assistants, and different medical officers. From these Reports it appears that as to the real prophylactic value of these injections there can be no manner of doubt. The Indian Plague Commission (Report, vol. v.) critically sifted the evidence brought before them by Haffkine and by many other medical officers, and although some of the statistics produced were not admitted by them to be satisfactorily collected and did not conclusively prove in the particular instances the value claimed for the injections, there nevertheless were available statistics from a number of places, such as jails and hospitals, which in the opinion of the Commissioners fully established

[1] Report of the Medical Officer of the Local Government Board for 1901-1902, p. 357 and passim.

the value of a prophylactic injection of the Haffkine fluid.

Simpson (in the *Practitioner* for December 1905) says: "The Indian Plague Commission, while accepting the facts which proved the general protective effect of the prophylactic, were indisposed to follow Haffkine in his conclusion as to protection in the incubation stage. They recorded their opinion that, 'in view of the short incubation period of plague, and in view of the fact that our experience in the case of other diseases, both in animals and man, indicates that protection is not at all rapidly established, it seems to us unlikely that the anti-plague inoculation can exert any favourable influence on persons who are already incubating plague.'[1] Further facts laid before them appear, however, to have somewhat modified their view, for in the conclusion to their Report they state that 'inoculation does not appear to confer any great degree of protection within the first days after the inoculation has been performed.'[2]

"Calmette and the Oporto International Commission went still further, for they demonstrated, by actual experiment on mice, that Haffkine's prophylactic rendered these animals immune only after an elapse of eight to ten days, and that the prophylactic, given simultaneously with a small and feeble dose of the plague virus, increased the virulence of the disease, rendering death certain even in cases in which there might otherwise be a percentage of recoveries.

"Calmette and Salimbeni, in their Report, conclude that 'it is certain that after injection of the prophylactic,

[1] Report of the Indian Plague Commission, vol. v. p. 252.
[2] *Ibid.* p. 262.

and until the immunity it confers is established—that is to say, during eight to ten days after the vaccinal injection (as always exists after active immunisation by living microbes or by their toxins)—the organism is, for the time being, sensitive to an infection even very slight." [1]

Experiments which I have made on rats with my organ prophylactic (see later) show, however, that these animals do not show any increased susceptibility to plague when injected with my organ prophylactic, even when the testing with *B. pestis* is made as early as two to four days after the injection of the prophylactic. Rats tested six days after the injection of the prophylactic were fully protected against an otherwise fatal dose of *B. pestis* inoculated cutaneously.

Haffkine himself admitted that owing to the stress of circumstances (the demand for the fluid in India alone was, owing to the spread of the plague, enormous) a great many points concerning the precise nature and the best method of employment of the fluid remained still unsolved. For instance, the Plague Commissioners justly point out that, from the evidence brought before them, there does not seem to be any advantage in reinjection of the same individual, provided the dose in the first instance has produced the anticipated physiological action. Further, as the Commissioners justly observe, there is as yet a great deal of uncertainty as to the dosage for each injection. Haffkine states, provisionally, that the dose for a human being should be such as to cause, a few hours after subcutaneous injection, a rise of temperature of at least 2-3 degrees Fahrenheit. But the determination of the dose on these lines would on *a priori* grounds

[1] *Annales de l'Institut Pasteur*, No. 12, 1899.

meet with great difficulties, because it can hardly be supposed that all human beings injected with the same amount would react in the same manner. Another method — also provisional — adopted by Haffkine in judging the dose of the prophylactic is the amount of solid matter (bacterial growth) present in the culture. But this method is evidently beset with quite as great difficulties, for, even assuming that simple inspection could approximately determine the relative amount of growth in different brews, there must, owing to the bacterial growth being largely in the form of granules and flocculi, be great uncertainty in any attempt to distribute this solid matter in a uniform manner during the customary decantation of the fluid into the several small bottles or tubes ready for use.

Of not less importance, and of equal uncertainty, is the action of the different constituents of the prophylactic, *e.g.* the bacterial bodies, and the fluid in which they are suspended. The Plague Commissioners, for instance, cannot satisfy themselves as to the presence in the fluid, *per se*, of any toxin or of any bacterial products necessary for the object of prophylaxis.

A further point which unquestionably must be assumed to be of importance is the quality of the strain of the plague bacilli used in establishing a culture. On grounds of analogy—*e.g.* vibrio choleræ, bac. of typhoid —it is to be anticipated that the initial virulence of the microbe determines, *cæteris paribus*, the degree of protective potency of the ensuing culture. And as a last point worthy of consideration, there is the question of the nature of the change in the blood and tissues of a human being or of an animal injected with the prophylactic—

that is to say, the question of the degree of immunity conferred by inoculation against infection, and to what extent are agglutinating, antitoxic, and germicidal substances to be met with in the blood of the injected animal.

All the points above enumerated require to be elucidated before claim can be made to a fair understanding of the action of the prophylactic—before, that is, a rule-of-thumb practice can be superseded by a scientifically proved method of standardising and of using the prophylactic.

I now proceed to describe the experiments and observations which have been undertaken towards the elucidation of some of these matters.

The Preparation of Haffkine Plague Prophylactic.—The fluid which Haffkine (*Proceedings, Royal Society*, June 1900) prepares for distribution and transmission is a broth culture of *B. pestis* incubated for four to six weeks, and then sterilised at 65-70° C. for one hour. It is next decanted into and preserved in special bottles, each containing about 0·5 per cent carbolic acid.[1] The prophylactic employed in the experiments to be recorded was in most respects prepared like Haffkine's broth culture, with addition, that is, of a few drops of sterile clarified butter. It was decanted into test tubes, each capable of containing 32-36 cc., which were then sealed and finally sterilised at 70° C. for one hour. No preservative, however, was added.

Haffkine has pointed out that different brews of the prophylactic started from the same plague culture—

[1] At present (1906) the prophylactic is preserved in Bombay without the addition of preservative.

notwithstanding that the broth itself, the addition of clarified butter, the temperature, and all other conditions are as far as possible the same—show after a like period of incubation different amounts of bacterial growth in the form of floccular and granular sediment. My experience fully bears this out. Thus of a group of flasks (8-12) treated seemingly, and indeed intentionally, in exactly the same manner—*i.e.* same make of peptone broth, same amount of ghee added, same stock culture of plague used for infection, same platinum needle used in this process, same temperature of incubation, same duration of incubation—not all show the same amount of sediment of solid growth. While some flasks of this brew contain a comparatively large amount of the floccular and granular sediment, others show this to a conspicuously less degree. I have found that, *cæteris paribus*, the incubation of the flasks at 25° C. for the last fortnight or three weeks of preparation—the first fortnight having been passed by the flasks in an incubator at 37° C.—yields the greatest amount of sediment, certainly greater than if the flasks are kept for the whole five weeks at 37° C.

Another point in which I fully confirm Haffkine's statement, and one which I think of importance, is this: The presence of a thin layer of droplets of ghee on the surface of the broth is an excellent and sure means of increasing the amount of bacterial growth. This is particularly well shown after the inoculated flasks are transferred to a temperature of 25° C. The ghee drops at this temperature become solid flat platelets; and in connection with them, and extending underneath them rapidly, the growth appears in the form of a whitish scum (with stalactites), which, on shaking, becomes

readily detached and falls to the bottom of the fluid, only to be in the course of a few days replaced by a similar fresh scum. In this way, viz. by keeping the flasks for the last two to three weeks at the temperature of 25° C. and shaking the flasks every three or four days, the greatest amount of sediment (granules and flocculi of growth) will be obtained. It is a fact, although it does not well agree with a general assumption, that not even after four weeks is the growth finished, *i.e.* is the nutritive material in broth exhausted. This may appear strange, since in the case of a rapidly growing microbe like the *B. pestis* one would *a priori* expect that at a temperature of 37° C. the growth would have been completed and the broth "exhausted" in the space of ten days or a fortnight. But this is manifestly not the case; after a fortnight's incubation at 37° C. the flasks, on transference to a temperature of 25° C., exhibit a conspicuous further growth, which continues even to the end of six weeks. In regard of all flasks so treated, subcultures were made on gelatine and agar with a platinum loop, a single loopful being transferred from the flask to the new culture medium. In every instance great numbers of colonies developed, particularly in the gelatine tubes. This proves that the bacilli in the flasks were still living and active; that they had not, as was to be anticipated, died off in the course of a few weeks in large numbers. I think, with Haffkine, that the addition of the clarified butter is the important means by which the bacillary growth is maintained and its amount increased.

In the course of the last two years I have sealed and sterilised nearly 12,000 tubes (each containing

32 to 36 cc.) without a single failure. All of them showed, when kept in an upright position, a perfectly clear fluid with the granular and floccular sediment. Accidental and extraneous contamination did not occur.

Constitutional Changes in Animals injected with Plague Prophylactic

In order to supply means of comparing the effects of injections of the plague prophylactic, I submit here a tabular account of the changes of the temperature and of body weight of rabbits submitted to injection of, at first sterile, afterwards living (solid) culture of *B. pestis.*

Rabbit No. 1

First subcutaneous injection, May 6, with sterile culture :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
Before injection	102·6	1155	May 16 . .	103·4	1160
May 7 . .	105·3	1105	,, 17 . .	102·8	1122
Animal off its feed.			,, 18 . .	102·7	1148
May 8 . .	104·4	1090			
Animal appears all right and feeds again.			,, 20 . .	103	1097
May 9 . .	104	1115	,, 21 . .	103·1	1074
,, 10 . .	102·6	1087	,, 22 . .	102·5	1090
,, 11 . .	104·8	1068	,, 23 . .	103·3	1090
,, 13 . .	103	1065	,, 24 . .	103	1103
,, 14 . .	103·2	1089	,, 25 . .	103·2	1128
,, 15 . .	102·4	1115			

Second intravenous injection with sterilised culture on May 28 :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
Before injection	103·2	1102	June 6 . .	103·6	1257
May 29 . .	103·8	1129	,, 7 . .	103·6	1315
,, 30 . .	104·4	1108	,, 8 . .	103·4	1302
,, 31 . .	103·3	1126	,, 10 . .	103·2	1281
June 1 . .	103·3	1144	,, 11 . .	103·8	1283
,, 3 . .	102·4	1146	,, 12 . .	103	1266
,, 4 . .	103	1180	,, 13 . .	103·3	1313
,, 5 . .	103	1221			

Third injection of the rabbit per ear vein with sterilised culture :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
June 14 . .	103·7	1284	June 22 . .	103·5	1319
,, 15 . .	104·4	1303	,, 24 . .	102	1319
,, 17 . .	102·6	1269	,, 25 . .	102·8	1312
,, 18 . .	102·6	1242	,, 26 . .	103	1335
,, 19 . .	101·6	1277			
,, 20 . .	102·5	1343	July 9 . .	103	1280
,, 21 . .	102·4	1281			

Fourth intravenous injection with small dose of emulsion of living culture :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
July 10 . .	102·6	1310	July 16 . .	106·5	1353
,, 11 . .	105·1	1355	,, 17 . .	104	1278
,, 12 . .	106·1	1352	,, 18 . .	105	1286
,, 13 . .	106·4	1324	,, 19 . .	103·6	1245
,, 15 . .	106·8	1345	,, 20 . .	103·4	1177

Rabbit No. 2

First intravenous injection with sterilised culture on May 6 :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
Before injection	102·5	1105	May 17 . .	102·7	1061
May 7 . .	104	1069	,, 18 . .	102·8	1088
,, 8 . .	104	1015	,, 20 . .	103	1097
,, 9 . .	104	1062	,, 21 . .	102·8	1071
,, 10 . .	103·7	1046	,, 22 . .	102·8	1102
,, 11 . .	105	1039	,, 23 . .	102·7	1113
,, 13 . .	103·7	1031	,, 24 . .	103	1109
,, 14 . .	103	1062	,, 25 . .	102·7	1128
,, 15 . .	103	1095	,, 28 . .	102·8	1093
,, 16 . .	103·4	1124			

Second intravenous injection with sterilised culture :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
May 29 . .	103·8	1098	June 6 . .	103·2	1208
,, 30 . .	103·8	1080	,, 7 . .	104	1266
,, 31 . .	103·1	1102	,, 8 . .	103	1288
June 1 . .	103·4	1121	,, 10 . .	102·6	1250
,, 3 . .	103	1132	,, 11 . .	103·6	1236
,, 4 . .	103·4	1154	,, 12 . .	102·8	1236
,, 5 . .	103	1179	,, 13 . .	103·2	1279

Third intravenous injection with sterilised culture :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
June 14 . .	103·6	1256	June 20 . .	102·4	1322
,, 15 . .	103·4	1287	,, 21 . .	102·4	1283
,, 17 . .	103	1261	,, 22 . .	103·4	1310
,, 18 . .	102·4	1243	,, 24 . .	103·2	1349
,, 19 . .	102·4	1275	,, 26 . .	103	1409

From these records it appears that—

(1) No definite conclusion can be drawn from the alteration of body weight as to whether or not the injection of sterile or even of living culture exerts any influence on the animal's metabolism.

(2) The injection of sterile culture appears to cause a distinct and immediate (in twenty-four hours) rise of the body temperature, which lasts a few days. The second injection of the same culture causes a renewed rise of temperature, but this sets in not twenty-four but forty-eight hours after the injection, and is of shorter duration than that following the first injection. From the experiment on rabbit No. 1 it is further seen that intravenous injection of living

culture following on a third intravenous injection of sterile culture causes, on the second day, a very considerable rise of the body temperature, lasting for more than a week. It is curious, however, to find that the animal did not lose in weight till the temperature again commenced to fall. But, as mentioned above, the body weight is of very uncertain value. It has to be added in this connection that the animals were always kept as to food and all other conditions under precisely the same favourable conditions.

Rabbit No. 3

First subcutaneous injection with 10 cc. Haffkine prophylactic on June 17 :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
Before injection	102·3	1176	June 24 . .	102·6	1161
June 18 . .	105·5	1129	,, 25 . .	102·2	1167
,, 19 . .	105·4	1262	,, 26 . .	102·6	1185
,, 20 . .	103·7	1138	,, 27 . .	102·1	1193
,, 21 . .	101·7	1110	,, 28 . .	102	1186
,, 22 . .	102·5	1150	,, 29 . .	101·4	1138

As has been recorded in connection with various experiments here reported on, this animal was injected and reinjected subcutaneously a second, third, fourth, fifth, and sixth time, on each occasion with 10 cc., and a seventh and eighth time, on each occasion with 20 cc. of Haffkine's prophylactic. But there was no corresponding alteration observed in the body temperature. The body weight, however, was constantly varying upwards and downwards in an erratic fashion. It is, however, seen that the first injection caused a sudden and considerable rise of temperature, lasting for two or three days—that is to say, a change of the same kind as was noted in the case of rabbit No. 1 after the first *intravenous* injection of that animal with sterilised bacteria taken from solid medium. *Subcutaneous* injection, therefore, into a rabbit of under 1200 grammes

weight of 10 cc. of the plague prophylactic caused the same decisive constitutional disturbance, as manifested by a rise of body temperature of over 3° F., as did *intravenous* injection of a mass of bacilli amounting to three-fourths of the growth uniformly covering the slanting surface (6 centimetres by 2 centimetres) of an agar culture. It is important to bear this in mind, since the fact will again be referred to when comparison is made of the relative merits of injection of sterilised (solid) culture and of Haffkine's (broth) prophylactic. In order to show that this constitutional disturbance, *qua* body temperature, is real, I mention here an experiment made on a companion rabbit, No. 4, which animal unfortunately succumbed immediately after the second injection, and on post-mortem examination was found to be affected with extensive psorospermosis.

Rabbit No. 4

First subcutaneous injection of 10 cc. plague prophylactic on June 17 :—

	Temperature, 12 noon.	Body Weight.		Temperature, 12 noon.	Body Weight.
	°F.	Grammes.		°F.	Grammes.
Before injection	102·6	1367	June 24 . .	103·6	1365
June 18 . .	105·5	1315	,, 25 . .	103·2	1360
,, 19 . .	105·7	1333	,, 26 . .	103	1364
,, 20 . .	103·2	1331	,, 27 . .	102·6	1350
,, 21 . .	102·6	1292	,, 28 . .	102·4	1321
,, 22 . .	103	1340	,, 29 . .	102·2	1255

There is here decided rise of temperature (3° F.) the day after the injection, and this was maintained on the day following. Also there is observed a curious initial fall of body weight, with rise again as the temperature decreased after an initial rise.

Rabbits Nos. 5 and 6.

Both these animals were for the first time injected *intravenously* on July 1 with 5 cc. of Haffkine's prophylactic :—

	Temperature, 12 noon.		Body Weight.	
	No. 5.	No. 6.	No. 5.	No. 6.
	°F.	°F.	Grammes.	Grammes.
Before injection . .	102·1	102·4	1395	1605
July 2 . . .	103·9	104·8	1336	1514
,, 3 . . .	103·6	104·2	1325	1543
,, 4 . . .	103·8	103	1321	1526
,, 5 . . .	103·2	103	1320	1590
,, 6 . . .	104	104·6	1363	1565
,, 8 . . .	102·6	102·8	1300	1506
,, 9 . . .	103·1	103	1288	1493
,, 10 . . .	102·6	102·4	1282	1490
,, 11 . . .	102·1	102·4	1275	1489
,, 12 . . .	103	103·1	1270	1497
,, 13 . . .	103	103·4	1233	1486
,, 15 . . .	102·8	102·7	1253	1515
,, 16 . . .	102·5	102·4	1224	1504
,, 17 . . .	102	103·3	1110	1480
,, 18 . . .	102	103·6	1121	1483
,, 19 . . .	103·2	103·8	1163	1432

From this it appears that intravenous injection of the prophylactic was followed by a little less decisive rise of temperature than was subcutaneous injection in the former cases. The body weight fluctuated considerably—fluctuation which was on the whole fairly parallel for the two animals; that is to say, when it rose in one it rose also in the other rabbit, and *vice versa*.

Rabbits Nos. 7 and 8.

These two animals, as mentioned on p. 227, were injected subcutaneously with 20 cc. of the *filtrate* of Haffkine's prophylactic on three separate occasions.

First subcutaneous injection of 20 cc. of the filtrate on September 10 :—

	Temperature, 12 noon.		Body Weight.	
	No. 7.	No. 8.	No. 7.	No. 8.
	° F.	° F.	Grammes.	Grammes.
Before injection . . .	103	103·4	1645	2120
September 11 . . .	104	103·7	1638	2155
,, 12 . . .	103·2	102·4	1651	2169
,, 13 . . .	103	102·6	1688	2196
,, 14 . . .	102·8	102·1	1697	2207
,, 16 . . .	102·4	102·4	1640	2170
,, 17 . . .	102·5	102·2	1604	2110

Second injection with 20 cc. of the filtrate :—

	Temperature, 12 noon.		Body Weight.	
	No. 7.	No. 8.	No. 7.	No. 8.
	° F.	° F.	Grammes.	Grammes.
September 18 . . .	104	103	1670	2169
,, 19 . . .	103·2	102·3	1626	2123
,, 20 . . .	103	102·1	1671	2138
,, 21 . . .	102·6	102·4	1653	2128
,, 23 . . .	102·4	102	1614	2072

It appears from this series that the first injection of 20 cc. of the filtrate did not cause any but an insignificant rise of the body temperature (1° F. in rabbit No. 7, 0·3° F. in rabbit No. 8), a rise so small as to be well within the limits of natural fluctuation. The second injection of 20 cc. of the filtrate appears to have been

a little more active in temporarily raising the body temperature (1·5° F. in rabbit No. 7, 0·8° F. in rabbit No. 8); but here also the rise is not sufficiently striking to warrant assumption of any specific action. It is possible that the effect of injecting subcutaneously 20 cc. of any fluid containing in solution chemical substances of various composition, such as naturally result from the long-continued (four to six weeks) growth of bacteria in broth, would be similar as regards temperature. However this may be, it is certain that the blood of these animals did not contain agglutinating substances even after the fourth injection of 20 cc. of the filtrate.

Observations as to Protection against Plague Infection conferred on Animals by previous Injection of Haffkine Plague Prophylactic.

A.—*Guinea-pigs.*

From the experiments recorded in Chapter VIII. it will have been obvious that repeated injection of the dead (*i.e.* sterilised) bacilli taken from the surface of solid media confers on the guinea-pigs and rabbits a certain degree of immunity. The subsequent injection of living culture of *B. pestis* did not cause a fatal issue. But as in 1896-1897, so also now, the immunity thus conferred on the guinea-pig, viz. by this injection of sterilised bacilli, was not of a high order, since on each subsequent subcutaneous injection of living culture the animals reacted, as shown by the development of a bubo on the inoculated side, which bubo soon, however, suppurated and healed up. I have at present in the laboratory a guinea-pig which exactly a year ago, May 6, was injected the first time with the growth scraped from the whole surface (6 centimetres by 2 centimetres) of a gelatine culture of *B. pestis.* The growth was sterilised as a salt emulsion

and then injected intraperitoneally; and injection in this way was repeated in the same amount on May 22 and on June 5. The animal was then injected intraperitoneally with small doses of *living* culture on eight separate occasions. Finally, on December 28, it was injected subcutaneously with an ordinary lethal dose of living culture. The animal remained alive, but it developed a fair bubo, which suppurated and healed up in about a fortnight.

On May 15, 1901—a year, that is, after the first injection—it was injected, for the thirteenth time, subcutaneously with an ordinary lethal dose of plague material. The animal remained alive and fairly lively, and it fed well, but it again developed a bubo in the inguinal region which extended on to the thigh and became nearly as big as a pigeon's egg. This bubo suppurated by the end of a week, and had not quite healed by the end of a fortnight.

This is by no means an isolated instance as regards the guinea-pig. I have had several such cases in 1896-1897 (see my Report to the Local Government Board), and I have quite a number of similar instances in the present investigation. All show that a high degree of immunity in the guinea-pig does not exist even after a good many previous injections of sterilised and also living plague bacilli. The animals acquire no doubt a certain resistance against fatal infection, but this resistance does not prevent the development of a well-marked bubo with living bacilli in the early stages.

In view of this experience, it was not to be expected that the injection of the Haffkine prophylactic could

produce results more favourable than the repeated injection of living plague bacilli. In 1899-1900, with Professor Haffkine, at the instance of the Local Government Board, I made a number of experiments with the Haffkine plague prophylactic. In these a series of guinea-pigs received by a single subcutaneous injection various amounts of the plague prophylactic—5 cc., 10 cc., 30 cc., 40 cc., and even 60 cc. They were subsequently injected with lethal dose of living plague bacilli, as were, at the same time, a similar series of control guinea-pigs. The result was this:—Of the eight control guinea-pigs all died of plague. Of the eight prepared guinea-pigs seven died of plague; in the eighth animal the bubo suppurated and ultimately healed. During later experiments several guinea-pigs were, as mentioned in a previous chapter, repeatedly (as often as eight times) injected (subcutaneously and intraperitoneally) with Haffkine prophylactic, and afterwards with living culture. They developed typical bubo. I have at present a guinea-pig which had been injected eight times intraperitoneally with Haffkine prophylactic between June 17 and October 21, receiving altogether 51 cc. of the fluid. On December 28 it was injected subcutaneously with small dose of living culture of *B. pestis*. It developed typical bubo. On May 5 of this year it was again injected with an ordinary lethal dose. It remained alive, but by the end of the week it had a bubo of the size of a pigeon's egg.

The same negative results were obtained by injecting guinea-pigs with the clear filtrate of Haffkine's prophylactic; and it is not necessary to detail them beyond noting that neither the subcutaneous nor the intraperi-

toneal injection of 10 cc. in each instance of this clear filtrate on three separate occasions (within six weeks) had any influence in inhibiting the ordinary results of subsequent injection of small and large doses of living *B. pestis.*

B.—*Rabbits.*

The experiments made in conjunction with Professor Haffkine on rabbits, although numerous, were on the whole of an unsatisfactory nature, mainly because of the difficulty and uncertainty in ascertaining what is a normal fatal dose of plague culture for the rabbit. A large series of rabbits were carefully noted as to body temperature and body weight, before and after injection, with varying amounts of the prophylactic (10 cc. to 34 cc.). Control rabbits, equally carefully noted as to body weight, were kept separate, but corresponded as nearly as possible in body weight to the prepared rabbits. In due time each animal in both sets was injected with what ought to have been a fatal dose of living plague culture. The result was disappointing, because an equal proportion of prepared rabbits and control rabbits succumbed to plague.[1] The percentage of deaths in each class of rabbit was, however, less than 50, whereas in the case of both guinea-pigs and rats (used as controls) a fatal issue can always be ensured.

The experiments which I have made in the course of the present year on rabbits with sterilised solid plague culture, with Haffkine prophylactic, and with the filtrate of the prophylactic, were of an equally unsatisfactory nature, and for the same reason, viz. unless extra large

[1] I note with surprise the statement by Professor Balfour Stewart (*Thompson-Yates Laboratory*, vol. ii. p. 19) that he found 2 cc. of the Haffkine prophylactic sufficient to immunise a rabbit of 1400 grammes body weight.

doses of living plague culture be injected (against which, of course, no prophylactic would be efficacious) the control animals did not die.

A series of rabbits were prepared, some by repeated injection of sterilised solid culture, others by repeated injection of Haffkine prophylactic, and still others by repeated injection of the filtrate of Haffkine prophylactic.

At the proper phase of the experiment they, as also corresponding rabbits (*i.e.* corresponding in body weight), were injected with what in a preliminary experiment on a control rabbit acted as a fatal dose, *i.e.* half of a gelatine culture (6 centimetres by 2 centimetres surface) three days old. Unfortunately this crucial experiment failed to elucidate the point and to answer the question, viz. whether or no any, and if so which, of the prepared rabbits were protected. The control rabbits did not any of them die.

I have not therefore repeated the experiments, because I think it proved that, owing to its varying and unstable susceptibility, the rabbit is not a suitable animal for this kind of experiment.

C.—*Rats.*

1. *Action of the complete Haffkine Plague Prophylactic.*—Fortunately in the rat we possess a test animal which, in my experience, is thoroughly reliable. I have in the course of preparing the large amounts of the Haffkine plague prophylactic (about 60,000 doses) invariably used rats for testing this prophylactic, and my test has always been carried out in the following manner:—With each particular brew (comprising 6 to 12 flasks of the same broth, inoculated at the same time under the same con-

ditions, decanted, sealed, and sterilised at the same time) I have generally inoculated four rats, each being made to receive subcutaneously 10 cc. of the finished product. Except in the earliest experiments, white rats, half to full grown, have been employed, as I find these highly susceptible to plague, much easier to handle, and less liable to accidental and spontaneous death than the brown (wild) sewer or house rat.

Eight to ten days (on occasion twelve days) after this injection with the prophylactic the prepared rats, together with two fresh or unprepared rats, are injected with *living* plague culture, in amount sufficient to cause death from typical plague in both controls, while permitting the prepared rats to remain (if protected) alive. As a result of my experience in the above sense of a considerable number of brews of the prophylactic on a considerable number of rats, I am prepared emphatically to maintain that 10 cc. of the Haffkine prophylactic is capable of fully protecting a rat (half-grown to adult) against a subsequent injection of a dose of living plague culture that acts lethally without fail on control rats. And a similar experience was obtained in association with M. Haffkine, when he and I tested on rats the prophylactic brought by him from Bombay, viz. those which received an injection of 10 cc. of the prophylactic withstood subsequent infection with living culture of *B. pestis*.

It is not necessary to detail all the above experiments that were made on the rats with the prophylactic prepared by me here in London. I have already given their general purport, and I therefore proceed to describe the equally satisfactory results which I obtained with the sterilised bacillary bodies from the prophylactic, as also the results

of certain experiments with the clear filtrate after removal of the bacilli.

2. *Action of Sterilised Bacillary Bodies.*—The bodies of the sterilised bacilli were employed in the form in which they occur in the Haffkine prophylactic. The manner of obtaining them was very simple. As was mentioned on a former page of this chapter, the prophylactic was preserved by me in sealed tubes without the addition of any preservative, each tube containing 30, 32, and 36 cc. When left standing upright all the bacillary bodies settle at the bottom of the tube, whereas the fluid above becomes and remains quite limpid. From such a tube—after opening it by breaking the sealed end—the fluid is siphoned off, a process very easily achieved. The remaining sediment is then distributed in salt solution in amount equal to the quantity of broth siphoned off. Of this emulsion 10 cc. are used subcutaneously per rat. As a matter of fact, the 30 to 36 cc. of the fluid in a given tube, salt solution plus bacillary sediment, were used for three rats. The result was complete protection in each instance by this preliminary injection against the subsequent injection of living culture. I have made two series of such experiments, each comprising three prepared rats and one control, and in both series the result was positive, viz. death of the control rat and survival of the prepared animals.

3. *Action of the Filtrate* per se *of Haffkine's Prophylactic.*—From what has just now been stated, viz. that practically the bacillary sediment and the complete Haffkine prophylactic are equally protective, it was to be inferred that the fluid itself—*i.e.* the broth in which the bacilli had been growing, but minus the bacilli—is of no

use in assisting or in sharing in the protective action of the Haffkine prophylactic. As a matter of fact, the Indian Plague Commission deny (vol. v.) any action, toxic or otherwise, of this fluid *per se*, and as far as can be gathered from the questions put by some of the members of the Commission to Professor Haffkine during his examination before the Commission in Bombay, it seems as if Professor Haffkine had been reproached for having used broth culture (of course, sterilised) as plague prophylactic, seeing that his cholera prophylactic was an emulsion made from the growth of the cholera vibrio on solid media. And, further, Calmette, in his Harben Lectures (1900), assumes that all the prophylactic action of a sterilised culture is and must necessarily be lodged in the bacillary bodies themselves. Moreover, Professor Haffkine himself has repeatedly pointed out (see his evidence before the Plague Commission) the importance of copious bacillary growth in his broth culture; as a matter of fact, the prophylactic dose recommended per human individual has in practice been estimated according to the amount of bacillary growth in the flask, the amount of the dose being in inverse ratio to the turbidity, *i.e.* amount of bacillary growth.

While all these are facts of common knowledge, I venture seriously to doubt the assumed inefficacy and superfluity of the fluid part of the prophylactic. And my doubts are based on good experimental grounds as follows:—

(*a*) Four half-grown rats were injected with filtrate of the prophylactic, *i.e.* with the clear fluid siphoned off from a tube (see above) and passed through a Pasteur-Chamberland filter. Each rat received subcutaneously

10 cc. of this filtrate on two separate occasions, November 21 and December 31. On February 6, *i.e.* thirty-seven days later, they were injected with a dose each of living culture which killed a control rat on the fourth day. Of the four prepared rats, two died of plague on the fifth day, the nature of this fatal malady being confirmed on microscopic, macroscopic, and cultural evidence. The two others survived.

(*b*) Four half-grown rats received each 10 cc. of this filtrate on March 11, and again 10 cc. on March 18. On May 5 they were injected with living plague culture at the same time that a control rat received an equal dose of this infection. The result was instructive. The control rat died of typical plague on the third day; one of the prepared rats died of plague on the fourth day; another of the prepared rats died of plague on the sixth day; a third of these prepared rats died of plague on the twelfth day. But the fourth survived.

From these experiments I think it is justifiable to conclude that, although the effect of the filtrate (20 cc. per animal) was small, it was nevertheless of a positive nature, since the prepared animals behaved somewhat differently from the control rats. Some of the former were found distinctly protected, while those not protected died always later than the control rats.

The[1] principle underlying the preparation of plague prophylactics such as have hitherto been employed has its basis in the well-known fact that immunity of an animal to plague may be induced by injecting into it a certain

[1] Preliminary Report to the Local Government Board on a New Plague Prophylactic, December 19, 1905.

dose of culture of dead plague bacilli or their extracts. Thus Haffkine uses a broth culture containing a large amount of bacillary growth, which culture has previously been subjected to heat (70° C.) sufficient to kill the bacilli. As Haffkine has shown, and as is generally the practice wherever this prophylactic is used, the amount of bacillary growth determines in a somewhat rough-and-ready manner the efficacy and therefore the dosage of the prophylactic. With Calmette, as also at the Pasteur Institute, the prophylactic is a sterilised emulsion of the bacillary growth taken from the surface of solid agar cultures. The German Plague Commission also recommended an emulsion of agar culture sterilised at 65° C. On the other hand, Lustig uses in preparation of his prophylactic, or rather his curative serum, the precipitate obtained from an alkaline emulsion of an agar culture of *B. pestis*. Similar processes are employed by others in preparation of their plague prophylactic. According to numerous results hitherto published in India, South Africa, and elsewhere, the first two prophylactics, viz. Haffkine's and prophylactic prepared in the manner adopted by Calmette and the Pasteur Institute, are those which are most reliable. Such disadvantages as are attached to them are the disadvantages generally inherent to all fluids prepared from artificial cultures, viz. the difficulty of preservation, and, above all, the difficulty of securing, when large amounts of material are prepared at one time, uniformity of strength, *i.e.* efficacy for every single dose.

The above disadvantages have led me to investigate the matter in a new direction — to attempt, that is, to obtain a prophylactic free from the above defects. The

results so far obtained by me in a large number of experiments carried out with this view justify, I think, the claim I am making of having succeeded in my object.

The procedure which I adopt in preparing this new prophylactic is based on the following considerations and observations :—

(1) Investigating the vitality of *B. pestis* in certain organs (bubo, spleen, lung) of animals dead of plague—organs which had been subjected to drying on various materials (wood, cloth, linen) at various temperatures over sulphuric acid — I found that, after all *B. pestis* originally contained in such plague organs had been killed in the process of drying, emulsion made of these dried organs, and injected in definite amount into mice and rats, was capable of causing death of these rodents within twenty hours or less — the animals exhibiting phenomena not differing from those observed in acute plague, except, of course, that their tissues did not after death contain any *B. pestis.* Also I found, when employing for injection an amount of emulsion insufficient to cause speedy death, that the animals, though made ill—exhibiting, for instance, local tumour and more or less constitutional disturbance,—commonly recovered; and, further, that these recovered animals when tested later on by injection with virulent *B. pestis* were refractory to plague infection. From this it would seem that the dried plague organs, though not containing any living *B. pestis*, are nevertheless imbued with a powerful plague toxin which in appropriate dosage may serve as a prophylactic.

(2) I have shown in my Reports to the Local Government Board for 1901-1902 and 1902-1903 that guinea-

pigs inoculated *cutaneously* with plague material (by an abrasion or a scratch of the cutis) develop, as a general rule (unless, indeed, the infective material be of extreme virulence), plague in what I have termed the "subacute form"; a form marked by necrotic bubo, necrotic nodules in the spleen and liver, and particularly by necrotic nodules and necrotic patches in the lungs. In these cases death occurs generally in from four to seven or nine days (rarely earlier than four or later than nine days), provided the infecting material be of a moderate degree of virulence, such, for instance, as on *subcutaneous* injection causes death in three to four days, without the above necrotic changes.[1]

Examining sections of the organs containing the necrotic nodules and necrotic patches of guinea-pigs dead of subacute plague, it is seen that while the central parts of the necrotic nodules are crowded with *B. pestis*, the peripheral portions (except their vessels), although quite broken down into dead débris, contain few, if any, bacilli. From this it may be inferred that, as is the case in other bacterial diseases associated with necrotic changes of the tissues, such necrosis is not caused by the mere presence of the bacilli themselves, but by the toxin produced by them. As an illustration may be mentioned the necrotic action of the diphtheria toxin on a mucous membrane.

In view of the above two considerations, I determined to inject into a series of animals the dried organs (containing organ-toxin and dead bacilli) of various rodents dead of plague, with the purpose of ascertaining the ability

[1] As I have on a former page pointed out, there exists in respect of *cutaneous* inoculation a marked difference of reaction between the guinea-pig and the rat; in the latter animal cutaneous inoculation is the most reliable way of causing acute plague with fatal issue in two to three days.

of these materials to protect similar rodents against subsequent infection with virulent *B. pestis.*

A considerable number of experiments and observations were in the first instance made by me in order to ascertain the best mode of preparing and preserving prophylactic material of this nature, as well as to determine which portions of the body of an animal dead of plague are the most efficacious for the purpose. It is not necessary to describe in detail here all the steps adopted; they will be fully dealt with in a forthcoming Report of the Medical Officer of the Local Government Board. Suffice it to say that they comprised experiments with—

(*a*) Dried material of all the organs of mice, of guinea-pigs, and of rats dead of acute virulent plague;

(*b*) Dried material of the bubo and spleen alone of mice, guinea-pigs, and rats dead of acute plague;

(*c*) Dried material of all the organs of guinea-pigs dead of subacute plague, *i.e.* of guinea-pigs in which death occurred after four or five days with necrosis of the bubo, necrotic nodules of the spleen, liver, and lungs; and

(*d*) Dried material of those organs alone which showed necrotic changes—that is, bubo, spleen, lungs, and liver of guinea-pigs dead of subacute plague.

These several materials were dried—

(*e*) At the temperature of the laboratory over sulphuric acid;

(*f*) At the temperature of 20° C. over sulphuric acid;

(*g*) At the temperature of 37° C. over sulphuric acid;

(*h*) At the temperature of 46° to 47° C. over sulphuric acid.

The result of these experiments showed that a variety

of tissues—the bubo, the enlarged spleen, and the affected lung containing abundance of necrotic masses, as also the liver when it contains abundance of necrotic nodules—of guinea-pigs dead of subacute plague (*i.e.* dead after five to nine days), cut out and finely minced aseptically, spread out in thin layers in sterile glass plate dishes and dried over sulphuric acid at 46° to 47° C., yield a material which not only can be very easily and rapidly prepared, but which is of a uniform and reliable efficacy, and in every way, indeed, superior to any of the other prophylactics.

The guinea-pig, as compared with the rat, being a "clean" animal, appears greatly preferable for the above purpose. There is no difficulty in preparing and preserving the above necrotic organs, which yield comparatively the greatest amount of material, in a clean manner, and the exposure to 46° C. prevents growth and multiplication in them of any stray or accidental bacteria. A guinea-pig of about 300 to 400 grammes weight will yield 5 to 7 grammes of dry powder prepared from the bubo, spleen, lungs, and liver; and as the reliably protective dose for an adult rat (see below) is 10 to 15 milligrammes, it follows, therefore, that one large guinea-pig can yield about 400 to 600 doses. Three days' drying in thin layers over sulphuric acid at 46° C. was found more than sufficient to devitalise all *B. pestis* contained in these organs. After three days' drying the dry scales of material are rubbed down to a fine powder in a sterile mortar; this powder is then transferred to a sterile wide-mouthed bottle, plugged with sterile cotton-wool, which is placed for two to three days at 37° C. in order to thoroughly complete the process of drying. At the end

of these three additional days the cotton-wool plug is replaced by a glass stopper, and the prophylactic is ready for use. It can be thus preserved indefinitely in a dry state by a layer of paraffin over the stopper. Such material tested by cultivation is found sterile; it yields no growth of any kind.

In preparing the prophylactic for use the desired amount of powder is weighed out, well rubbed down in a desired amount of sterile warm distilled water, and the turbid emulsion thus obtained is injected subcutaneously. The principal consideration is, of course, the amount of dry powder; the amount of water used per dose is immaterial. I generally use $\frac{1}{2}$ cc. of water per dose, but there is no reason why $\frac{1}{4}$ cc. or 1 cc. should not be used.

As I have indicated, the material contains not only the acutely active toxin, but also the dead bodies of all the *B. pestis* originally present in large numbers in the necrotic organs (bubo, spleen, liver, and lungs), with addition probably of other substances of an undetermined nature and action. That the prophylactic efficacy of the material is not solely due to the bacillary bodies (known to possess both toxic and prophylactic action), retained after drying, can be gathered from the fact that an amount of dead bacilli from culture considerably larger than the quantity contained, say, in 10 milligrammes of dry spleen material, possesses neither the same toxic nor equal immunising efficacy as the latter. For instance, 5 cc. of Haffkine prophylactic strongly turbid with flakes and masses of bacilli does not confer immunity on an adult rat; 10 cc. is the required dose. On the other hand, 10 to 15 milligrammes of the dry

powder above referred to does confer immunity on the adult rat.

I now summarise the results obtained by using the above-described dried material in a large number of experiments, comprising several dozen white mice, five to six dozen guinea-pigs, and considerably over 150 rats, chiefly white :—

(1) The above prophylactic kills a large percentage of mice within twenty to twenty-four hours in doses of 1 to 5 milligrammes.

(2) It kills a percentage (12 to 25) of half-grown white rats in doses of 5 to 8 milligrammes, if the material is derived from acute virulent cases ; but if obtained from the necrotic organs of guinea-pigs dead of subacute plague (death five to nine days) as much as 10 to 12 milligrammes are required for fatal effect. The dead animals show local swelling, with œdema and punctiform hæmorrhages ; the spleen is enlarged, the lungs congested. No *B. pestis* are, however, to be found in their bodies anywhere.

(3) Even as much as 20 milligrammes fails to kill a guinea-pig of 200 to 300 grammes weight.

(4) An adult rat (weighing 120 to 200 grammes) injected with 10 to 15 milligrammes of the prophylactic of medium virulence such as I recommend, or with two doses of 10 milligrammes each at an interval of nine to ten days, is secured complete protection against even the most virulent *B. pestis.* I have a considerable number of rats which, after injection with the prophylactic, were tested by cutaneous inoculation (the most successful mode of infection of the rat) with virulent *B. pestis* at various periods from one to thirteen weeks. These were all found fully protected, whereas the control animals inoculated

cutaneously at the same time and with the same material died from typical acute plague in thirty-six to seventy-two hours.

(5) As is well known, guinea-pigs are less susceptible to plague than white rats, the latter being, of all the rat races which I have experimented with, the most susceptible to plague. In the case of the guinea-pig a dose of 20 milligrammes of the prophylactic now in question twice injected at intervals of ten to fourteen days does not afford protection in more than 50 per cent of the animals; the remainder die on subsequent injection with virulent material of plague, though their death is delayed several days (death in nine to twelve days or later), and they show in the great majority of instances suppurating bubo. The disease induced in these animals differs, however, from the subacute plague in an unprotected (control) guinea-pig as follows:—(*a*) There is but slight enlargement of the spleen, with few necrotic nodules; (*b*) the liver contains either no necrotic nodules, or such nodules only very sparingly; (*c*) there is scarcity of *B. pestis* in the bubo and in the spleen—in unprotected guinea-pigs dead of subacute plague these two tissues being crowded with *B. pestis*; and (*d*) there is much greater amount of necrosis in the lungs, the necrotic parts being packed with *B. pestis.* It seems, therefore, that while in the unprotected guinea-pig the bubo, spleen, and liver are more involved and richer in *B. pestis* than in the protected guinea-pig, the reverse is the case as regards the lungs.

In the experiments which in 1899 I along with Dr. Haffkine made with his prophylactic, and in the experiments which I have repeatedly made since with Haffkine prophylactic as prepared by myself, it was shown that for

an adult rat 10 cc. are required to ensure protection; an amount which represents, according to the statistics in India and elsewhere, at least double that required for protection of an adult human being. I assume, therefore, that 5 to 7 milligrammes of the dry prophylactic might suffice as a dose for the human subject. On this estimate a single large guinea-pig dead of subacute plague would, by means of its necrotic bubo, spleen, liver, and lungs, yield something like 800 to 1000 human doses of the new prophylactic, an amount of protective material equal to 3 to 5 litres of Haffkine's fluid.

When it is borne in mind—(1) that this dried prophylactic does not require more than about ten to twelve days for its preparation—Haffkine's requires four to six weeks; (2) that a large amount can be prepared of uniform strength; (3) that its efficacy is easily standardised by injection into the rat; (4) that, being dry and sterile, it can be preserved without any antiseptic and unaltered for any length of time; and (5) that the protection afforded by its injection into the rat is of considerable duration, certainly many weeks; and last, but not least, that the cost of preparation is incomparably smaller, the superiority of this *organ-prophylactic* to Haffkine's prophylactic must be obvious.

As regards size of dose, it is true that the prophylactic prepared by Calmette and the Paris Pasteur Institute, which is an emulsion of dead bacilli derived from agar surface, is capable of protecting an adult white rat in doses so small as 1 to 2 cc. But here, again, different cultures cannot be relied upon as being of equal potency, and hence no uniformity can be ensured for different sets of cultures, or, for the matter of that, for two separate

cultures from the same source; whereas greater uniformity is to be anticipated from material derived from the same breed of guinea-pigs, all animals being of about the same weight and inoculated with material of like activity.

There remains to be determined the interesting and important question: Whence is derived the especial efficacy of the new prophylactic, which contains, be it remembered, not only the dead bodies of plague bacilli and associated tissue-toxin, but also, in all probability, other tissue constituents? In this connection I have made experiments which prove that the clear filtrate from a watery emulsion of the prophylactic powder possesses undoubted prophylactic efficacy. Thus, injection of an amount of clear filtrate corresponding to, *i.e.* obtained from, 20 to 25 milligrammes of dry powder, confers immunity on the adult rat against a subsequent cutaneous infection with virulent *B. pestis*. This, of course, shows that the new prophylactic does not act solely by means of the bodies of the dead bacilli which it contains.

These experiments also show, as might be expected, that the whole organs prophylactic—*i.e.* the entire powder—is more efficacious than the watery extract, since of a definite sample of the organ prophylactic, of which 15·6 milligrammes of the entire material is sufficient to protect a rat, 20 to 25 milligrammes are required to yield an equally efficacious watery extract. Roughly speaking, the proportion between a given entire prophylactic and its watery extract (filtrate) is as 15 to 25.

A further important point ascertained was this, viz. that the filtrate of a watery emulsion (filtered simply through filter-paper) can be sterilised by heating it to 70° C. for fifteen minutes, and that even twice so heated,

i.e. on successive days, it loses none of its protective efficacy. Tested by culture this so heated filtrate is found reliably sterile. It can in this form easily be preserved in sealed tubes.

A large number of rats were tested with twice-heated filtrate of organ prophylactic, and it was ascertained that, supposing the dose of the mother-substance is 12 to 16 milligrammes, the dose per rat of the filtrate would amount to about one-third more, *i.e.* would correspond to 20 to 25 milligrammes of the entire prophylactic.

CHAPTER X

MODES OF DESTRUCTION OF *BACILLI PESTIS*

1. *By Drying.* 2. *Spontaneous.* 3. *By Chemical Disinfection.*

1. *Drying.*—Already in Chapters VII. and IX. we have fully described experiments by which it was shown that plague materials exposed in the laboratory to various forms of drying on different materials—cloth, linen, wood, grain, in earth, and in sand—lose their infective power, *i.e.* their efficacy *qua B. pestis*; that is to say, the *B. pestis* contained in these materials become sooner or later devitalised; and there is no reason to doubt that such would be the case also under natural conditions. Experiments of drying thin watery or salt emulsions or broth cultures, applied as thin films on cover-glasses—a method practised by some observers in determining the death point of *B. pestis* by drying—are not of much practical value, since this method of drying does not occur in nature. The experiments with plague organs and gelatine cultures which I have described in Chapter VII. were fully in harmony with and in imitation of what we might suppose to occur under natural conditions. The *B. pestis*, being a non-sporing microbe, comports itself like other non-sporing bacteria, inasmuch as when thin films of salt or

watery emulsions of cultures, or prepared direct from broth cultures, are exposed to drying such as can be effected at 45° to 46° C. in less than twenty-four hours, at 37° C. in twenty-four hours, over sulphuric acid at ordinary temperature in forty-eight hours, the microbes of such films become thereby quite devitalised; for if the cover films are, after drying, placed in nutrient broth and incubated, no growth of *B. pestis* takes place. It stands to reason, and direct experiment proves it, that the period of drying of *B. pestis* in a viscid material—*e.g.* blood, spleen tissue, gelatine culture (melted in warm water)—requires to be considerably extended. Take, for instance, the *B. pestis* in blood or spleen tissue exposed to 46° C. even over sulphuric acid. Under these conditions the *B. pestis* cannot with certainty be considered dead after twenty-four hours. I have had cases when even after thirty-six hours not all *B. pestis* were dead, though three days of such drying can be fully relied on.

2. *Spontaneous.*—It has been pointed out in a former chapter that transferring *B. pestis* from one culture to another its virulence gradually diminishes; this is a fact also observed with other microbes. As regards *B. pestis* we have pointed out an important difference, viz. this, that while some strains lose in artificial cultures their virulence slowly, others do so very rapidly, even with complete extinction of virulence. We have shown that in this respect the rat type plague bacillus (type 2), at starting already endowed with lesser virulence than the human type (type 1), is apt to lose its virulence in a relatively very short time, and not only to become less and less virulent, but to become ultimately a quite harmless saprophyte. Might not this find its counterpart under

natural conditions? That is to say, provided a certain race of *B. pestis* is restricted to carrying on its existence in outside nature, and is prevented from finding entrance into an animal body, in which, like other non-sporing microbes, it might be able to again regain or enhance its virulence—in other words, provided the *B. pestis* is doomed to live as a saprophyte, and is prevented from resuming its parasitic existence—might this not have the result that by and by it would altogether cease to be possessed of infective power for the animal body? The experiments which we have mentioned of type 2 certainly seem to warrant such an assumption. Assuming that this is possible in nature, we could understand how *B. pestis* (in a concrete plague case), when introduced into a locality in which it is not endemic, if debarred by preventive sanitary measures from gaining access to a further human being, would, owing to the saprophytic conditions to which under these restrictions it would be doomed to live, soon become deprived of all further infective power. This would particularly apply to the rat type bacillus (type 2), which, rapidly losing its virulence altogether, would equally rapidly cease to be infective. The *B. pestis* is not particularly selective in its nutritive materials; it, like, for instance, the *Proteus vulgaris*, can grow almost in any medium containing albuminous materials; it can grow well in neutral, in alkaline, and even in slightly acid materials—that is to say, it can maintain its existence in most localities and in most ordinary materials of filth in which *Proteus*, *Staphylococci*, *B. coli*, *B. typhosus*, and *Vibrio cholerœ* can exist and multiply, particularly when the medium is in a solid form. There is no reason to suppose that *B.*

pestis introduced into a locality cannot and does not carry on its existence, grow, and multiply in outside nature; but the important point is that it should be prevented from gaining access to an animal body, for if restricted to a saprophytic life it would soon cease to be dangerous.

The gradually diminishing capability of the attenuated or rat type *B. pestis* (type 2)—although capable of continuous transference from rat to rat—to infect human beings, as has been exemplified in several instances (see previous chapters), would ultimately lead to cessation of infection of the human subject.

3. *Chemical Disinfectants.*—In respect of the great and constantly widening subject of the various disinfectant agents commonly employed for destroying bacteria and infective microbes, it has to be remembered that laboratory experiments in which pure cultures of one or the other microbe are exposed for a stated time to the action of a given agent are not exactly analogous to what occurs in practice when infective materials in the form of secretions, excretions, and tissues of an infected animal body are subjected to disinfection. I have shown this by direct experiments both with regard to *Staphylococcus aureus* and coli-typhoid bacilli. I quote in illustration a paper which I published in the *British Medical Journal*, July 2, 1904:—

> The usual method of testing and comparing disinfectants consists in exposing for a given time cultures or emulsions of a particular microbe to dilutions of the disinfectant. This, no doubt, is of value in comparing with one another various disinfectants or their dilutions, and although it affords a sufficiently reliable index of the efficacy and power of a given disinfectant in a definite dilution and for a definite time exposure, it cannot, for reasons to be mentioned

presently, supply an absolute guide for the application of the disinfectant in actual practice. In practical life a disinfectant is applied by a surgeon, by the sanitarian, or by the nurse, not to an artificial culture or to an emulsion of an artificial culture, but to materials, such as excretions, secretions, morbid products, and morbid tissues, derived directly or indirectly from the human or animal bodies. The microbes to be acted upon by the disinfectant are embedded in, surrounded by, and mixed with various materials. This condition cannot obviously be compared with that of a microbe in a broth culture, or with a watery or salt emulsion of the growth taken from an artificial culture. The surgeon who wishes to disinfect a wound and to keep it antiseptic has to apply the disinfectant to tissues and not to a watery emulsion; the nurse who is supposed to disinfect a typhoid stool with a given disinfectant is not acting on a watery or saline emulsion of a culture of the typhoid bacillus, but on materials of a highly complex composition, and altogether of a different nature from the laboratory culture.

An illustration is sufficient to prove the difference between the one kind of disinfectant and the other. *Staphylococcus pyogenes aureus* (of a recent and active culture) is justly considered one of the hardiest non-sporing microbes, at any rate as far as chemical disinfectants are concerned, and for this reason bacteriologists are in the habit of using this microbe in their laboratory tests.

Now, the *Staphylococcus aureus*, taken from a culture (broth or agar) and placed into a watery solution or distributed in a disinfectant, is by simple agitation in most cases readily emulsified; the fluid viewed under the microscope shows the microbes almost uniformly distributed as single cocci or as diplococci or as very small groups. Compare with this a microscopic specimen of the contents of an abscess or of the secretion of a wound or ulcer. Here we find, besides crowds of various tissue elements, leucocytes, débris, tissue fibres, fatty granule-cells, etc., large and small clumps of cocci and diplococci not only in the fluid menstruum, but within the protoplasm of leucocytes, within débris, and within masses of fibres.

The disinfectant which is to act on the microbe so surrounded and lodged has a task to fulfil quite different from what it has when it is added to a broth culture or other uniform emulsion of the microbe. The same, *mutatis mutandis*, applies to diphtherial membrane, to sputum, to the typhoid stool, and other animal excretions. In the following experiments I wish to show by direct observation that in a

number of tests which I have made in this direction, marked differences exist in the action of a certain disinfectant, with which I have been well acquainted for some years, to wit, izal, as applied to microbes, such as *Staphylococcus aureus*, *B. coli*, *B. typhosus*, in different surroundings; in one case the microbes were taken from artificial cultures, and in the other were in their natural habitat. The izal used was the fluid sold as ordinary izal in the ordinary pharmacy.

Method

1. In the case of pus of acute abscess, the pus and blood, immediately after collecting them from the abscess in sterile test tube, were diluted with sterile distilled water, 1 part of pus being added to 49 parts of water. From this dilution a control culture was made on agar, one platinum loopful of the mixture for one agar surface culture. The result of this control culture indicated the amount of staphylococcus present, and allowed the number of colonies in the control tube to be compared with those that appeared in the culture tubes after medication. To the above dilution of the pus was added a definite amount of izal direct as sold, and then well shaken up. The proportion of izal to the volume of diluted pus represented the strength of izal actually used in the experiment.

2. In the case of urine a definite amount of izal was added, well shaken; then, after being kept for the required time, the necessary cultures were made on agar and in broth.

3. In the case of the typhoid stool only typical fluid (pea-soup) stools were used; of these a definite quantity was added to a definite quantity of sterile water. This was well shaken up; to it was added a definite amount of izal as in the other cases.

Also in the last two instances control cultures on agar with one platinum loop were made from the diluted materials previous to the addition of the izal, so as to be able to compare the actual number of microbes of the coli-typhoid type before and after exposure to the disinfectant.

After the addition of izal, cultures were made at the desired periods from the medicated fluids on agar surface and in broth, each culture tube receiving three platinum loops of the medicated material; these tubes were then incubated at 37° C. Inspection and notification were made after the tubes had been incubated for three or four days, for by this time all growth, if any, had become complete.

Experiments

First Series.—Pus of Acute Abscess.

Experiment 1.—Of pus and blood of an acute abscess of the female breast, 1 ccm. was added to 49 ccm. of sterile distilled water (1 in 50).

One platinum loop yielded about 100 colonies of *Staphylococcus aureus.*

Added to diluted pus 0·1 ccm. of izal well shaken up. This made, therefore, 1 izal in 500.

Cultures were made with 3 platinum loops for each tube after 5, 15, and 30 minutes with this result:—

a. After 5 minutes: agar tube has 12 colonies of *Staphylococcus aureus*; broth-tube uniformly turbid.

This broth-tube was subcultured, with the result that it proved a pure culture of *Staphylococcus aureus.*

b. After 15 minutes: on agar 2 colonies of *Staphylococcus aureus*; broth-tube uniformly turbid.

Subculture proved this broth-tube to be a pure culture of *Staphylococcus aureus.*

c. After 30 minutes: on agar 2 colonies of *Staphylococcus aureus*; broth-tube greatly retarded growth, only faintly turbid after 3 days' incubation.

Considering that a single loop of the diluted pus yielded before the addition of the izal about 100 colonies of *Staphylococcus aureus*, whereas 3 loops (same loop as used in control) yielded after 5 minutes' exposure to the izal only 12 colonies, the process of disinfection must be considered already after this short space of time of a fair degree; this is well shown in experiment *c*, after 30 minutes' exposure, when 3 loops yielded only 2 colonies; that is to say, of 300 microbes only 2 had survived. The broth-tube bears witness to this, since the broth showed greatly retarded and scanty growth.

The result of this experiment 1 is, then, that izal 1 in 500 may for practical purposes be considered a fair, though not complete, disinfectant for pus of acute abscess (*Staphylococcus aureus*) after 30 minutes' exposure.

Experiment 2.—The same pus as above was used; 1 ccm. of the

pus was added to 39 ccm. of sterile distilled water (1 in 40), then 0·1 ccm. of the izal was added (1 in 400), well shaken, and cultures were made as above after 5, 15, and 30 minutes on agar and in broth, using 3 platinum loops for each tube.

The result was this:—

a. After 5 minutes' exposure: on agar 15 colonies of *Staphylococcus aureus*; broth uniformly turbid.

Subculture of this proved pure culture of *Staphylococcus aureus.*

b. After 15 minutes: both tubes free of growth.

c. After 30 minutes: both tubes free of growth.

From this it follows that izal 1 in 400 completely disinfects in 15 minutes; in 5 minutes already the number of living microbes being greatly reduced (300 to 15).

Experiment 3.—Pus of an acute submental abscess (mixed with blood) was obtained; 1 ccm. of the pus was diluted with 39 ccm. of sterile distilled water (1 in 40).

With 1 loop of this dilution made control agar culture. In this about 12 dozen colonies came up; these were partly colonies of streptococci, partly colonies of *Staphylococcus aureus* and *albus.*

0·1 ccm. of izal was added to the diluted pus (1 in 400); after well shaking it and keeping it for 5, 15, and 30 minutes, cultures were made on agar and in broth, using for each tube 3 loops. Incubated at 37° C. for 4 days.

The result was this:—

a. After 5 minutes: on agar 15 colonies of mixture of *Staphylococcus aureus* and *albus*; no colonies of streptococci; broth slightly turbid.

Subculture of the broth showed mixture of *Staphylococcus aureus* and *albus*, but the great majority were those of *Staphylococcus aureus*; no colonies of streptococci.

b. After 15 minutes: both tubes were free of growth.

c. After 30 minutes: both tubes were free of growth.

From this experiment it follows that izal 1 in 400 disinfects completely streptococcus in 5 minutes; that it reduces living *Staphylococcus aureus* and *albus* in 5 minutes to a considerable degree (from 432 to 15), and that it completely disinfects them in 15 minutes.

The following table summarises the preceding results as regards the capability or incapability of izal to disinfect the microbes of pus of acute abscess:—

TABLE I

Sample.	Nature of Dilution.	Time of Exposure.		
		5 minutes.	15 minutes.	30 minutes.
Pus No. 1 . .	1 izal in 500	+ reduced	+ reduced	+ greatly reduced
,, ,, 1 . .	1 izal in 400	+ reduced	–	–
,, ,, 2 . .	1 izal in 400	+ reduced	–	–

Positive sign means growth, negative sign means absence of growth, after medication.

Experiment 4.—This experiment is a control to the preceding experiments with *Staphylococcus aureus*, and serves to compare the effect of the disinfectant when used on a culture of the microbe in watery emulsion.

Of a 48 hours' agar active culture of *Staphylococcus aureus* added to sterile distilled water, sufficient to form slight but distinct turbidity.

One loop of emulsion brought forth on agar an almost confluent mass of *Staphylococcus aureus.*

a. To 50 ccm. of the staphylococcus emulsion added 0·1 ccm. izal, 1 in 500.

b. To 60 ccm. of the staphylococcus emulsion added 0·1 ccm. izal, 1 in 600.

c. To 75 ccm. of the staphylococcus emulsion added 0·1 ccm. izal, 1 in 750.

After 5 minutes' exposure, made with 3 loops 1 agar and 1 broth culture, incubation at 37° C., with result as follows :—

a. 1 in 500, 5 minutes' exposure : no growth in either tube.

b. 1 in 600, 5 minutes' exposure : no growth in either tube.

c. 1 in 750, 5 minutes' exposure : agar shows 18 colonies.

Broth uniformly turbid with *Staphylococcus aureus.*

Table II. summarises the preceding results :—

TABLE

TABLE II

Sample.	Nature of Dilution.	Time of Exposure 5 minutes.
Watery emulsion of culture of *Staphylococcus aureus*	1 in 500	–
,, ,, ,,	1 in 600	–
,, ,, ,,	1 in 750	+ greatly reduced

From this it follows that for *Staphylococcus aureus* of culture izal 1 in 600 is a complete disinfectant in 5 minutes. This result is in so far interesting as it shows that *Staphylococcus aureus* of culture is disinfected quicker and with greater dilutions of izal than when it is acted upon in its natural habitat, that is pus. It will be remembered that for pus izal 1 in 400, 15 minutes' exposure, was the limit for completely disinfecting *Staphylococcus aureus*, whereas for disinfection of this microbe in watery emulsion 1 in 600 izal for 5 minutes' exposure was sufficient.

This result emphasises the importance of testing the disinfecting power of any given substance on the pus microbes while in their natural media, for only thus is an absolute index for practical guidance acquired.

Second Series.—Typhoid Stools.

Experiment 5.—Typical (pea-soup) typhoid stool (1) of a case of typhoid fever, 22nd day of illness.

This stool was tested in high dilutions by means of Drigalski Conradi plates, and was found to contain about 14 millions of *B. coli*, about the same number or a little more of streptococci, and 3 millions of the *B. typhosus* per 1 ccm. of original stool.

The disinfection experiment was made in the following manner:—

1 ccm. of the stool was added to 49 ccm. of sterile distilled water (1 in 50); this was well shaken up till a fair and uniform emulsion was obtained. Control agar culture with 1 loop showed innumerable colonies of above microbes. To it was then added 0·1 ccm. of the izal and well shaken up; this made, then, a dilution of 1 izal in 500.

Cultures were made after 15 minutes', after 30 minutes', and

after 1 hour's exposure, 3 loops of the medicated fluid being added to each agar and broth tube.

The result was this :—

a. After 15 minutes : on agar 1 colony of streptococci ; broth slightly turbid.

Subculture of this broth showed colonies of streptococci only.

b. After 30 minutes : on agar no colonies ; broth very slightly turbid.

Subculture of this broth showed colonies of streptococci only.

c. After 1 hour : on agar no growth ; broth very slightly turbid (streptococci).

From this experiment it follows that the microbes of the coli-typhoid type were completely disinfected in 15 minutes by izal 1 in 500 ; but the streptococci, although disinfected for agar in 30 minutes, were not quite disinfected for broth though very largely reduced in number in 1 hour.

Experiment 6.—The same stool (2) as in experiment 5 was treated with izal 1 in 600, after exactly the same manner as described above.

Result :—

a. After 15 minutes : agar free of growth ; broth slightly turbid (*B. coli*).

b. After 30 minutes : both tubes free of growth.

c. After 1 hour : both tubes free of growth.

Experiment 7.—The same stool (3) treated with izal 1 in 750.

Result :—

a. After 15 minutes : agar showed 5 colonies (*B. coli*) ; broth uniformly turbid (*B. coli*).

b. After 30 minutes : agar shows 3 colonies (*B. coli*) ; broth very slightly turbid (*B. coli*).

c. After 1 hour : agar no growth ; broth no growth.

The result of these two experiments, 6 and 7, is then :—

Izal 1 in 600 completely disinfected the microbes of the coli-typhoid group in 30 minutes, but not in 15 minutes. Izal 1 in 750 completely disinfected these microbes in 1 hour, but not in 30 minutes.

Experiment 8.—Typical (pea-soup) typhoid stool (4) of a case, 19th day of illness, was treated in exactly the same manner as the stool in experiment 5—namely, 1 in 500—with this result :—

a. After 15 minutes : no growth on agar or in broth.

b. After 30 minutes : no growth on agar or in broth.

c. After 1 hour : no growth on agar or in broth.

In this case, then, izal 1 in 500 caused in 15 minutes complete

disinfection of the microbes of the coli-typhoid type as well as of the streptococci. (Control experiments made of this stool in high dilution by means of Drigalski and Conradi plates showed: 16 millions *B. coli*, 2 millions *B. typhosus*, and about 10 millions streptococci per 1 ccm.)

Experiment 9.—The same stool (5) as used in experiment 8 was treated with izal 1 in 600.

Result:—

a. After 15 minutes: agar, no growth; broth, no growth.

b. After 30 minutes: agar, no growth; broth, no growth.

c. After 1 hour: agar, no growth; broth, no growth.

From this it follows that izal as 1 in 600 completely disinfected in 15 minutes.

Experiment 10.—The same stool (6) as in experiments 8 and 9, izal 1 in 750.

Result:—

a. After 15 minutes: agar shows 2 colonies (*B. coli*); broth uniformly turbid (*B. coli*).

b. After 30 minutes: agar, no growth; broth uniformly turbid (*B. coli*).

c. After 1 hour: both cultures free of any growth.

From this it follows that izal as 1 in 750 completely disinfected in 1 hour, but not in 30 minutes.

Experiment 11.—Semi-solid stool (7) of a case of typhoid, 10th day of illness. Added 1 gramme to 50 ccm. of sterile distilled water. (From this dilution, 1 in 50, made with one loop an agar culture, with the result that after incubation the agar surface was covered with innumerable colonies, in many cases confluent.) To the above dilution, 1 in 50, added 0·1 ccm. izal, that is 1 in 500. Cultures from the medicated fluid were made after 15 and 30 minutes and after 1 hour, using for each culture tube 3 loops.

Result:—

a. After 15 minutes: agar shows 1 colony of sporing *B. mesentericus*; broth-tube turbid, due to cocci and streptococci (no bacilli).

b. After 30 minutes: both tubes free of growth.

c. After an hour: both tubes free of growth.

In this experiment, then, izal 1 in 500 disinfected the microbes of the coli-typhoid group in 15 minutes, though not those of cocci.

Experiment 12.—The same stool (8) was used as above, izal 1 in 600.

Result :—

a. After 15 minutes: agar free of growth; broth free of growth.

b. After 30 minutes: agar has 1 colony (*B. coli*?); broth free of growth.

c. After 1 hour: both tubes free of growth.

From this it follows that izal 1 in 600 completely disinfected the microbes of the coli-typhoid group in 1 hour; but already in 30 minutes the reduction of all microbes was enormous, since only the agar tubes showed one single colony, but it was not quite clear whether *B. coli* or not. For practical purposes 30 minutes would therefore suffice.

Experiment 13.—The same stool (9) was used as above, izal 1 in 750.

Result :—

a. After 15 minutes: agar shows large number of coli-like colonies; broth clear.

b. After 30 minutes: both tubes without growth.

c. After 1 hour: agar shows 1 colony of sporing *B. mesentericus.*

The following Table III. summarises the results of all experiments made with typhoid stools in respect of the microbes of the coli-typhoid group :—

TABLE III

Sample.	Nature of Dilution.	Time of Exposure.		
		15 minutes.	30 minutes.	1 hour.
Typhoid stool (1)	1 in 500	–	–	–
,, ,, (2)	1 in 600	+reduced	–	–
,, ,, (3)	1 in 750	+reduced	+reduced	–
,, ,, (4)	1 in 500	–	–	–
,, ,, (5)	1 in 600	–	–	–
,, ,, (6)	1 in 750	+reduced	+reduced	–
,, ,, (7)	1 in 500	–	–	–
,, ,, (8)	1 in 600	–	?	–
,, ,, (9)	1 in 750	+	–	–

Third Series.—Urine.

Experiment 14.—Turbid urine of a case of typhoid (end of second week). An agar control culture was made with 1 loop of the urine; it showed after incubation 36 colonies of various kinds of microbes, chiefly belonging to the coli-typhoid group. To 70 ccm. of the urine (practically the whole of the urine obtainable) 0·1 ccm. izal was added; this represents, therefore, 1 in 700.

Cultures were made, using 3 loops for each culture tube, after 5, 15, and 30 minutes, with this result:—

a. After 5 minutes: agar, no growth; broth turbid.

b. After 15 minutes: agar, no growth; broth, no growth.

c. After 30 minutes: agar, no growth; broth, no growth.

From this experiment it appears that 15 minutes was sufficient for izal 1 in 700 to cause complete disinfection of this urine.

Experiment 15.—Normal urine was filtered and then sterilised by heating it for 10 minutes at 70° C. To the sterile clear urine added of a recent culture of *B. typhosus* sufficient to produce slight turbidity. One platinum loop of this was transferred to the surface of gelatine. It brought forth a very large number of colonies of the *B. typhosus.*

a. To 50 ccm. of the typhoid urine was added 0·1 ccm. of izal, this being equal to izal 1 in 500.

b. To 60 ccm. of the typhoid urine was added 0·1 ccm. of izal, this being equal to izal 1 in 600.

c. To 75 ccm. of the typhoid urine was added 0·1 ccm. of izal, this being equal to izal 1 in 750.

After 5 minutes' exposure, cultures were made on agar and broth, each tube receiving 3 platinum loops. Incubation 37° C.

The result was as follows:—

a. 1 in 500, 5 minutes' exposure: no growth in either tube.

b. 1 in 600, 5 minutes' exposure: no growth in either tube.

c. 1 in 750, 5 minutes' exposure: good growth in both tubes, proved to be pure *B. typhosus.*

It follows from this that 1 in 600 for 5 minutes is the limit of izal disinfection for *B. typhosus* distributed in urine.

Experiment 16.—For control and comparison of the above experiments sterile distilled water was substituted for the stools and urine. In other respects all conditions remained the same; namely, of a recent culture of *B. typhosus* (same as used above) sufficient

growth was added to the water so as to produce slight, though distinct, turbidity.

A single platinum loop of the watery emulsion yielded a very large number of colonies of *B. typhosus.*

a. To 50 ccm. of the typhoid water added 0·1 ccm. izal, 1 in 500.

b. To 60 ccm. of the typhoid water added 0·1 ccm. izal, 1 in 600.

c. To 75 ccm. of the typhoid water added 0·1 ccm. izal, 1 in 750.

After 5 minutes' exposure made cultures on agar and in broth with 3 loops. Incubation at 37° C. with the following result:—

a. 1 in 500, 5 minutes' exposure: no growth in either tube.

b. 1 in 600, 5 minutes' exposure: no growth in either tube.

c. 1 in 750, 5 minutes' exposure: no growth on agar; slight turbidity of broth due to *B. typhosus.*

This, then, agrees with experiment 15 as to complete disinfection of *B. typhosus* by izal 1 in 600 in 5 minutes; but as regards izal 1 in 750, the typhoid watery emulsion appears to be more amenable to disinfection than the typhoid urine, for while in the latter good growth appeared in the broth-tube, in the former the broth only showed retarded and diminished growth of the *B. typhosus.* That the slight turbidity of this broth was really due to *B. typhosus* was proved not only by subcultures but by the positive agglutination test, the bacilli becoming completely agglutinated by typhoid serum (of a typhoid protected rabbit) 1 in 200 in 5 minutes.

Comparing these experiments of watery emulsion of *B. typhosus* with those of the typhoid stool the difference becomes obvious; for while izal 1 in 600 completely disinfected the watery emulsion in 5 minutes, and even as 1 in 750 greatly reduced the number of bacilli in 5 minutes (no growth on agar, slight turbidity in broth), in the typhoid stool (2) izal 1 in 600 did not disinfect in 15 minutes, though it succeeded in doing so in 30 minutes; in another case (stool 9) izal 1 in 750 had no effect in 15 minutes, though it caused complete disinfection in 30 minutes.

Coming now to experiments of disinfection of culture of *B. pestis* with cyllin, phenol, and formalin, I quote a paper which I published in *Public Health*, June 1904:—

"The following experiments were undertaken to test and compare the relative actions of 'cyllin,' 'phenol,' and 'formalin' on *B. pestis* of a virulent strain. This

had been originally derived from the bubo of a fatal case of bubonic plague in a man in Cardiff in 1901. The strain had been kept up in the laboratory by continued subcultures till the commencement of this year, when it was used for infecting rats. A trace of an agar culture of recent date (forty-eight hours at 37° C.) inoculated into a superficial incision of the skin of a rat caused typical fatal plague within three days. The culture actually used in the experiments to be described here was derived from the spleen of a rat dead on March 23, 1904, which rat had been cutaneously inoculated on March 20, 1904. In all our tests, of a forty-eight hours old agar culture—copious growth over the whole of the sloped surface (6 centimetres by 2 centimetres)—an emulsion was made with sterile distilled water. The emulsion was a strongly turbid fluid. A single platinum loop of this emulsion spread over a sloped agar surface brought forth innumerable colonies of the *B. pestis*. In all instances to 5 cc. of the disinfectant contained in a sterile test tube, 5 drops (from a definite drop bottle) of the plague emulsion were added and well shaken, and after the required time of exposure cultures were made; in all instances three loops of the medicated fluid being spread out over a sloped surface of gelatine or agar, or added to nutrient broth, as the case might be. The culture tubes were then incubated at 21° C. (gelatine), or at 37° C. (agar or broth), and inspected from time to time until for some days no further change was noticeable. Amongst the several dozen cultures thus made, in no single instance was there any accidental or stray microbe observed; the tubes contained either no growth at all, or they contained colonies of the *B. pestis* in pure culture only, in varying

numbers, as will presently be described. In this connection it will be necessary to bear in mind that three platinum loops of the mixture (5 cc. of the disinfectant and 5 drops of the emulsion of *B. pestis*) yielded in the positive instances innumerable colonies of *B. pestis* in the culture tubes. This disposes at once of an objection that might possibly be raised, viz. that the amount of disinfectant carried over from the mixture to the culture tubes might inhibit the subsequent growth of the microbe, although the latter might still be alive. It also disposes of a further criticism, viz. that the number of microbes carried over by three platinum loops might not be large enough. As just mentioned, in the fully positive instances the whole culture surface was covered with crowds of colonies.

Series I.—March 25, 1904.

A.—Watery solution of pure phenol . . 1 in 200
B.—Watery solution of pure phenol . . 1 in 100
C.—Watery solution of formalin (40 per cent) 1 in 100
D.—Watery solution of formalin (40 per cent) 1 in 50

Time of exposure of *B. pestis*, 5, 10, and 15 minutes.

Cultures after exposure were made on sloped gelatine (21° C.), and inspected on April 5, with the following result :—

1.—Phenol 1 in 200, 5 minutes' exposure.	Innumerable colonies—confluent mass.
10 ,, ,,	,, ,,
15 ,, ,,	,, ,,
2.—Phenol 1 in 100, 5 minutes' exposure.	Innumerable colonies.
10 ,, ,,	About 7 doz. colonies.
15 ,, ,,	6 colonies.

3.—Formalin 1 in 100, 5 minutes' exposure. Innumerable colonies.
10 „ „ „ „
15 „ „ „ „
4.—Formalin 1 in 50, 5 minutes' exposure. Innumerable colonies.
10 „ „ „ „
15 „ „ „ „

Series II.—March 28, 1904.

A.—Watery distribution of cyllin . . 1 in 1500
B.— „ „ „ . . 1 in 1000
C.— „ „ „ . . 1 in 500

Time of exposure, 5, 10, and 15 minutes.

Cultures after exposure were made on sloped gelatine (21° C.) and inspected on April 10, when all tubes were found to be free of any growth.

Series III.—April 8, 1904.

A.—Watery distribution of cyllin . . 1 in 1500
B.— „ „ „ . . 1 in 1000

Time of exposure, 5, 10, and 15 minutes.

Cultures after exposure were made on sloped agar and in broth, and incubated at 37° C., inspection being made on April 16, 1904.

Result: All tubes free of any growth.

Series IV.—April 7, 1904.

A.—Watery solution of phenol . . 1 in 80
B.— „ „ formalin . . 1 in 30
C.—Distribution of cyllin . . . 1 in 2000
D.— „ „ . . . 1 in 1800
E.— „ „ . . . 1 in 1600

Time of exposure, 5, 10, and 15 minutes.

Cultures were made on gelatine (21° C.) and agar (37° C.), and inspected on April 16, with the following result :—

1.—Phenol 1 in 80, 5 minutes' exposure.	On gelatine 3 doz. colonies.
	On agar „ „
10 „ „	In both tubes no growth.
15 „ „	„ „ „
2.—Formalin 1 in 30, 5 minutes' exposure.	Crowds of colonies in both tubes.
10 „ „	Numerous colonies.
15 „ „	About 5 dozen colonies.
3.—Cyllin 1 in 2000, 5, 10, and 15 minutes' exposure.	All tubes free of growth.
1 in 1800 „ „ „ „	
1 in 1600 „ „ „ „	

Series V.—April 11, 1904.

A.—Watery distribution of cyllin . . 1 in 2400

B.— „ „ „ . . 1 in 2200

Time of exposure, 5, 10, and 15 minutes.

Cultures were made on agar (37° C.) and gelatine (21° C.), and inspected on April 18, with this result :—

1.—Cyllin 1 in 2400, 5 minutes' exposure.	Gelatine tube had one colony of *B. pestis*. The agar tube had a small amount of growth in the condensation water; this was allowed to spread over the sloped surface of the agar, with the result that copious growth of *B. pestis* covered the surface next day.
10 „ „	No growth in any tube.
15 „ „	„ „ „

2.—Cyllin 1 in 2200, 5 minutes' exposure. All tubes free of any growth.

10 ,, ,, ,, ,, ,,

15 ,, ,, ,, ,, ,,

From these experiments it follows: (1) that formalin even 1 in 30 failed to disinfect *B. pestis* in fifteen minutes; (2) that phenol 1 in 80 failed to disinfect *B. pestis* in five minutes, but did disinfect in ten minutes; and (3) that cyllin 1 in 2400 failed in five minutes, but succeeded in ten minutes.

From the following tables it will be seen that—

(1) Phenol 1 in 80 is a stronger disinfectant for *B. pestis* than formalin 1 in 30; and

(2) Phenol 1 in 80 stands in about the same position as cyllin 1 in 2400, inasmuch as both failed to completely devitalise the *B. pestis* in five minutes, though they both succeeded in doing so in ten minutes. Expressed in a different form, cyllin possesses for watery emulsion of *B. pestis* a disinfecting power which is more than 27·5 (in fact about 30) times as great as that of absolute phenol.

TEST No. 1 (April 22, 1904).

B. PESTIS, 48 HOURS' AGAR CULTURE AT 37° C. ROOM TEMPERATURE 15°-18° C.

Sample.	Dilution.	Time Culture exposed to Action of Disinfectant. Minutes. 5	10	15	Subculture. Period of Incubation.	Temperature.	Remarks.
Formalin (40%)	1:100	+	+	+	7 days	37° C.	Copious growth.
	1:50	+	+	+	,,	,,	,,
	1:30	+	+	+	,,	,,	Slight reduction in 15 minutes.
Pure Phenol .	1:80	+	−	−	,,	,,	Reduction in 5 minutes.

Carbolic acid coefficient of formalin is under 0·37.

TEST No. 2 (April 22, 1904).

B. PESTIS, 48 HOURS' AGAR CULTURE AT 37° C. ROOM TEMPERATURE 15°-18° C.

Sample.	Dilution.	Time Culture exposed to Action of Disinfectant. Minutes. 5	10	15	Subculture. Period of Incubation.	Temperature.	Remarks.
Cyllin . .	1:2400	+	−	−	7 days	37° C.	Great reduction in 5 minutes.
	1:2200	−	−	−	,,	,,	−
	1:2000	−	−	−	,,	,,	−
	1:1800	−	−	−	,,	,,	−
Pure Phenol .	1:80	+	−	−	,,	,,	Reduction in 5 minutes.

Carbolic acid coefficient of cyllin is 30·0.

I conclude here with the description of the result of experiments on disinfection with izal on culture of *B. pestis* directly derived from a bubo :—

Sample.	Dilution.	Time of Exposure. 5 min.	10 min.	15 min.	Temperature.
Izal ordinary	1 : 2200	+	+	−	37° C.
,, ,,	1 : 2000	+	−	−	,,
,, ,,	1 : 1800	+	−	−	,,
,, ,,	1 : 1600	−	−	−	,,
Phenol .	1 : 100	+	+	−	,,
,, .	1 : 80	+	−	−	,,
,, .	1 : 60	−	−	−	,,

Carbolic coefficient between 22·5 and 26·6.

Taking a certain dilution of carbolic acid as being capable of completely disinfecting in five minutes *B. pestis* of culture, the figures of carbolic coefficient for cyllin and izal work out something like 24 to 30 for cyllin, 22·5 to 26·6 for izal. It will be seen from this that cyllin and izal leave phenol, and still more formalin (when used in fluid form) far behind, and, with the exception of bichloride of mercury, are more powerful disinfectants than certain disinfectants which, when tested under similar conditions on non-sporing microbes, have a coefficient inferior to phenol. Although I have made no special experiments on *B. pestis* with chloros, kerol, or other agents, either belonging to the group of strong oxidisers or to the group of tar products, judged by what these substances are capable of effecting when tested on *B. typhosus* I have no doubt that they will be found powerful disinfectants also for *B. pestis.*

Be it added here that although the figures given here of phenol, formalin, cyllin, and izal apply to experiments on watery emulsions of pure culture of *B. pestis*, it does not follow that they apply in exactly the same degree if disinfection is performed on plague organs direct, such as would have to be dealt with in actual practice. For under the latter conditions the relations of disinfectant to infective material, *i.e.* open and free contact of the disinfecting fluid with the *B. pestis* contained within the viscid and gelatinous albuminous materials—blood, intestinal contents, sputum, viscera,—may be, and probably are, different from what they are when watery emulsions of pure cultures are used. Moreover, the degree of efficacy of one as compared with the other disinfectant may be altered. Not that cyllin and izal, chloros and kerol, and some

others would be found to be less powerful than phenol or formalin, but that their actual phenol coefficient need not be the same as when working with pure culture. This is shown by the experiments of Kenwood and Hewlett (*Public Health*, February 1906, p. 269), by which it was ascertained that, using cyllin and izal for disinfection of *B. typhosus* in fæces or urine, the coefficient is lower than when using pure culture of the *B. typhosus*—as I myself had already previously demonstrated, — and further, that the relative position of these two substances *quâ* phenol coefficient may be equal or even reversed.

THE END

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