XI. THE DIAGNOSIS OF NATURAL RAT PLAGUE.

I. Introduction.

In the following paper an attempt will be made to give a description of certain characteristic features which we have observed on post-mortem examination of a large number of plague rats in the course of the daily routine rat investigation in Bombay. An account of the diagnosis of rat plague may appear at first sight to cover well-known ground and to be consequently unnecessary. Having regard however to its importance the subject seems to have received remarkably little attention, judging from the scattered references to it in the literature.

At least three reasons appealed to us as rendering it imperative to make a thorough investigation of the post-mortem features of plague rats—firstly to estimate the value of naked-eye examination *per se* as an aid in the diagnosis of rats suspected of being plague-infected—secondly to determine to what extent macroscopical examination alone might be

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relied upon for purposes of diagnosis in the daily routine examination of rats sent to the laboratory—and lastly to elicit whether examination of a large number of plague-infected rats might reveal evidence of the mode of infection in these animals in nature—a question of crucial importance from the point of view of the epidemiologist.

The records on which the following account is based have been grouped into two series, which will be referred to hereinafter as Series I and Series II respectively. Series I comprises 200 plague rats (100 *M. rattus* and 100 *M. decumanus*[1]) from those examined during the off season, *i.e.* from July to December 1905, when sporadic cases only were occurring in rats and in men. All these rats were in a fresh condition, *i.e.* they showed no obvious signs of putrefaction. Series II consists of 4000 rats from those obtained during the early period of the epizootic, *i.e.* from the beginning of January to the middle of February 1906.

II. Preliminary Details.

The following preliminary details may be given of the rats in the two series.

TABLE I. *Series I.*

	100 *rattus*	100 *decumanus*	Percentage of total
Young males[2]	9	7	8
Adult males	51	54	52·5
Young females	8	9	8·5
Adult females	32	30	31
Pregnant	4	2	3
Rats brought alive for examination	5	6	5·5

TABLE II. *Series II.*

Analysis of 31,174 rats examined from 1-I-06 to 17-II-06 inclusive		Analysis of records of 4000 plague-infected rats during this period	
21867 were *M. decumanus*	70 %	Alive when brought for examination	0·82 %
9307 were *M. rattus*	30	Dead ,, ,, ,,	99·17
5951 were sent to laboratory alive	19	*M. rattus*	15·4
25223 were brought dead to laboratory	81	*M. decumanus*	84·6
16002 were males	51	Males	58·92
15172 were females	49	Females	41·07
4675 were plague-infected	15		

[1] This group includes both *Mus decumanus* and *Nesokia bengalensis*. The occurrence of this latter species was not at the time recognised: subsequent experience shows that about 1 % of the "decumanus" are in reality *Nesokia*.

[2] Every rat brought to the laboratory for examination is weighed. We have arbitrarily fixed the weight of a young "*rattus*" as being under 70 grammes and that of a young "*decumanus*" as being under 100 grammes. Animals with weights above these limits were regarded as adults.

III. Characteristic Appearances in Plague-infected Rats recognisable by Naked-eye Examination.

(1) *The presence of rigor mortis, subcutaneous congestion, subcutaneous haemorrhages, and subcutaneous oedema*[1].

Rigor mortis is fairly often present in plague rats, and is somewhat characteristic, the limbs projecting stiffly in a distinctive manner from the body. It may persist even when putrefaction has begun, in the internal organs. It was noted in 26·5 % of the rats in Series I.

Subcutaneous congestion is not infrequently a well-marked feature. It may be general but in some cases is limited to the neighbourhood of the bubo. In Series I it was present in 30·5 % of the total while in Series II a note was made of its presence in 69 %, it was well-marked in 7 % and was absent in 23 % of the rats[2]. A peculiar purplish-red appearance of the muscles exposed by reflecting the skin of the thorax and abdomen is obviously due to the presence of congested vessels and, combined with the reddish-pink colour of the subcutaneous tissue, presents an appearance which arouses a strong suspicion of plague at the commencement of the examination.

Emaciation was very rarely observed by us and is certainly not typical of plague. It may be said indeed that when a rat shows emaciation, and has lesions such as abscesses or septic lung conditions, the chances are greatly against it being plague.

Subcutaneous haemorrhages were noted in 40·5 % of the rats in Series I. In 18·5 % the haemorrhages were situated in the submaxillary region, and were associated with the occurrence of a bubo in this region, while in 8 % subcutaneous haemorrhages were noted in the submaxillary region although the bubo was in another situation or occasionally absent altogether. The general statement may be made that when present these haemorrhages are most frequently to be found in the submaxillary region. This depends doubtless upon the fact that haemorrhages are seen generally in the neighbourhood of buboes and that, as will appear later, buboes in rats are most often found in the neck. The next

[1] Unless special mention is made to the contrary all the observations in this paper refer to naturally infected rats.

[2] Subcutaneous congestion may manifest itself in a reddish hue of the skin before the rat is dissected. This is especially visible on the plantar surface of the hind feet which have a pink appearance. The sign is not however absolutely constant nor reliable, since it may not be observed in rats which are plague-infected and may occur in conditions other than plague.

common situation for these haemorrhages is the region of the flank. In young and medium sized rats especially they may be very widespread. We have never observed them in any rat which was not plague-infected. It is a matter of interest that they rarely occur in guinea-pigs infected either experimentally or naturally.

A general *oedema* of the subcutaneous tissue is a feature rarely met with in plague rats. When oedema is present it is usually limited to the region of the bubo. Thus in Series I cervical oedema was present in 10% of the cases. This contrasts with what is found in experimentally infected guinea-pigs in which general subcutaneous oedema is a very characteristic feature.

(2) *Changes in the lymphatic glands—buboes.*

If a dissection is made of a healthy rat the only glands which are large enough to be easily seen are those forming the crescent embracing the salivary glands in the submaxillary region, and the elongated retroperitoneal glands on each side of the middle line in the lower part of the abdomen. For the sake of brevity we will refer to the latter as "pelvic" glands.

In a septicaemic plague rat the glands in any region of the body may be enlarged and congested. Even when a primary bubo is present, secondarily enlarged glands may be found in a different situation. Thus the inguinal glands are not infrequently slightly swollen and congested, and may be surrounded by a characteristic radiating appearance due to an injection of the blood-vessels leading to and from the glands. Enlarged glands of this nature must be sharply distinguished from primary buboes. In the following description the use of the word bubo is restricted to mean a primary bubo and not these secondary glands.

The Austrian Plague Commission in their valuable account of the pathology of the lymphatic system in human plague make a distinction between primary buboes of the second order, *i.e.* glands in the neighbourhood of the primary bubo which have been directly infected from it, and secondary buboes which derive their infection from the blood when a septicaemia supervenes. In rats one occasionally finds both the inguinal and pelvic glands converted into primary buboes, the latter having obviously been infected by way of the lymphatics from the inguinal buboes. Such a lesion conforms to the description of a primary bubo of the second order.

DESCRIPTION OF PLATE VII.

Fig. I. Healthy rat to be contrasted with plague-infected rat.

Fig. II. Plague-infected rat. A composite picture illustrating some of the common naked-eye pathological changes found in various organs and tissues in a plague-infected rat. (All the changes illustrated are rarely met with in a single specimen.)

Note (*a*) Marked subcutaneous congestion causing a peculiar pink appearance of the tissues which contrasts with the condition found in a healthy rat.

(*b*) Submaxillary bubo; the gland has been dissected out and defined for the purpose of illustration.

(*c*) Subcutaneous punctate haemorrhages most frequently found in the neck.

(*d*) Marked congestion and haemorrhages in the thoracic cavity especially in the lungs.

(*e*) Advanced stage of "mottled" and "granular" liver.

(*f*) Enlarged and congested spleen.

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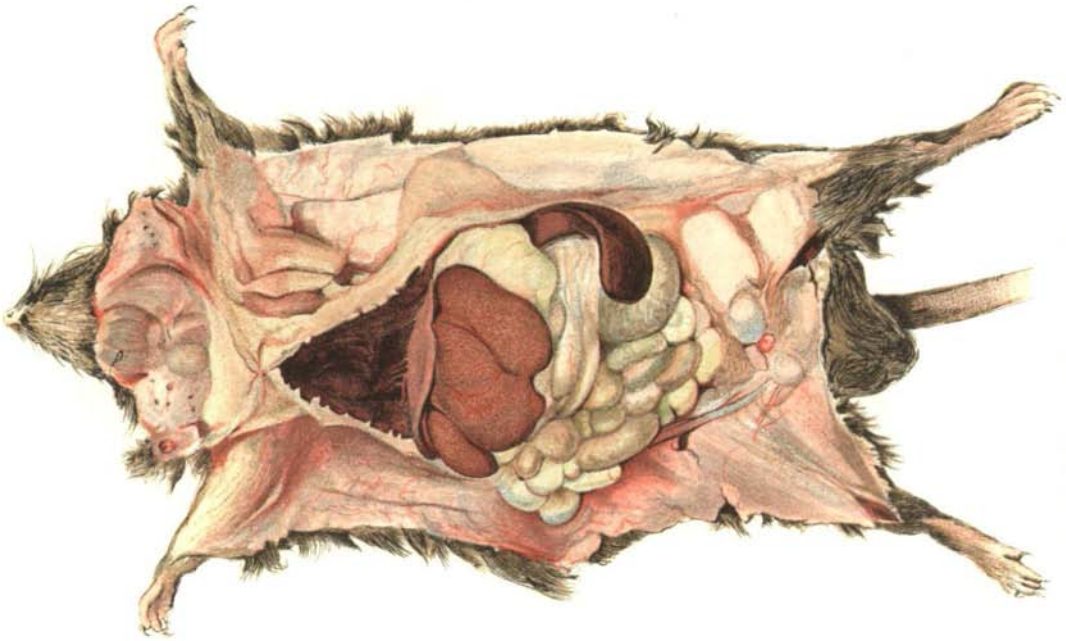
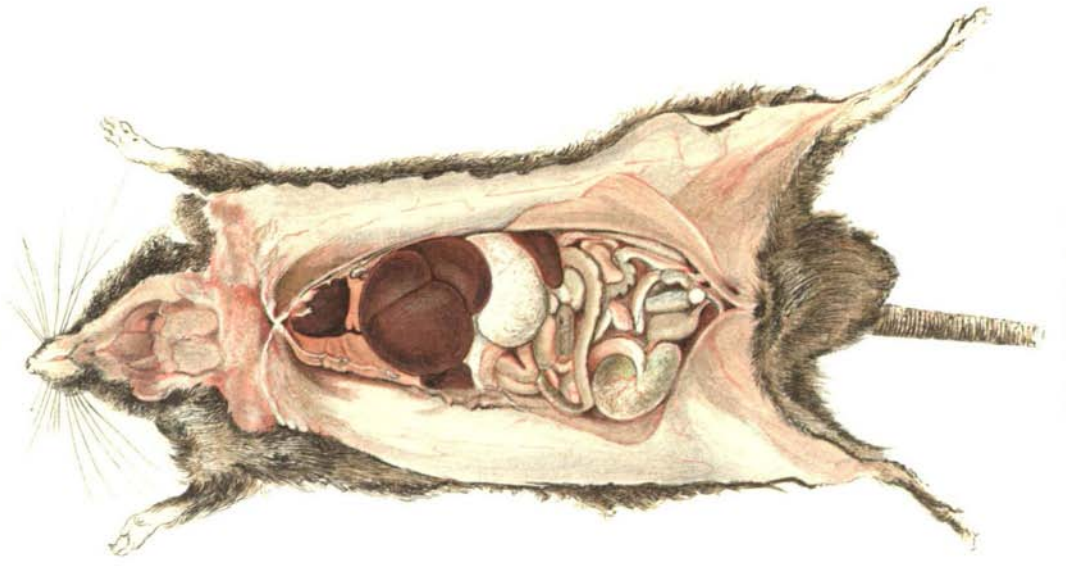


PLAGUE INFECTED RAT.



HEALTHY RAT.

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Edwin Wilson, Cambridge.

Occasionally the primary bubo is seen in the first stage of enlargement and congestion, showing haemorrhagic points when cut across. It may be distinguished from a secondary gland by the surrounding infiltration with perhaps haemorrhages in the subcutaneous tissue overlying it. Infiltration in the neighbourhood of the bubo extending into the subcutaneous tissue is indeed a highly characteristic feature of a bubo in any stage of its development. A localised subcutaneous oedema is sometimes observed. The presence of subcutaneous haemorrhages in the proximity of the bubo may often be noticed and these are frequently associated with marked congestion of the surrounding tissues.

A bubo feels hard when cut across though it has not the tough consistence of a normal gland. The contents of the latter are not easily squeezed out by pressure, whereas in a bubo the substance of the gland is readily broken down by slight pressure with the forceps.

TABLE III.

Showing the occurrence and distribution of buboes in 4000 *rats in Series II.*

Buboes in single situation only	=	2923	=	73·05 %
Multiple buboes	=	467	=	11·67
Bubo absent	=	610	=	15·25
Of the buboes in single situation.				
Neck	=	2194	=	75
Axilla	=	440	=	15·1
Groin	=	178	=	6·1
Pelvis	=	111	=	3·8

TABLE IV.

Showing order of frequency of various combinations of buboes in 467 *rats with multiple buboes in Series II.*

Groin + axilla	in	32·3 %	(151)
Neck + axilla	,,	28·2	(132)
Neck + groin	,,	12·6	(59)
Neck + axilla + groin	,,	7·4	(35)
Groin + pelvis	,,	7·1	(33)
Groin + pelvis + axilla	,,	4·9	(23)
Neck + pelvis	,,	2·9	(14)
Axilla + pelvis	,,	1·29	(6)
Neck + groin + pelvis	,,	1·07	(5)
Neck + axilla + groin + pelvis	,,	1·07	(5)
Neck + axilla + pelvis	,,	0·85	(4)

Note:—Of the rats with multiple buboes 54·5 % had a bubo in the neck.

The typical appearance of a bubo on section is that of necrosis affecting first the medullary portion of the gland, and gradually spreading outwards so that ultimately the glands may be converted into a mass of necrotic tissue enclosed within the capsule. The central portion has consequently a gray appearance, or in a somewhat later stage contains a yellowish cheesy material. Rarely, in a still more advanced stage, the centre has broken down into a rather dry—still more rarely a liquid—purulent material. Buboes with greenish liquid pus are not typical of plague, and those examined specially have not proved to be plague.

At times one finds but little surrounding congestion of the tissues and the bubo itself may have a yellowish-white colour. Such a tissue offers a greater resistance than a normal gland when cut across. Microscopical examination of a bubo of this character reveals the presence of swarms of plague bacilli. Occasionally when a suspicious gland is cut across a creamy fluid exudes which on microscopical examination is found to consist of degenerated leucocytes, cellular débris, and masses of plague bacilli.

There is generally little difficulty in recognising a bubo simply on account of its relatively large size and from the fact that it causes a prominent swelling, *e.g.* in the submaxillary region where several buboes may be fused into a large mass. In many instances however the existence of a bubo in the neck may easily be overlooked for the reason that there is not much apparent swelling even when the neck glands are exposed. The glands in this region should always be arranged in their natural relations, and cognizance taken of the slightest asymmetry. Any suspicious gland should then be dissected out and cut across in several directions. The cut surface may show appearances suggestive of necrotic change and if so a smear should be prepared for microscopical examination. Indeed we made it a practice to cut into the neck glands of every rat examined. A bubo in the neck is sometimes readily found by probing with forceps in the region of the glands. Here it may be detected as a hard nodule like a pea.

Enlarged and congested glands in the groin and axilla ought to be incised and examined in the same way—a yellowish centre, if only the size of a pinhead, speaking strongly in favour of such a gland being a primary bubo.

Axillary buboes may readily be passed over when small, and especially if they are flattened and lie parallel to the inner surface of the arm under the insertion of the pectoral muscle into the humerus.

A routine practice should therefore be adopted of cutting through these muscles in the axilla.

It may be mentioned here that a common and marked feature of a bubo when examined microscopically is the presence of more or less numerous involution forms. Although secondarily enlarged glands may contain numerous bacilli these typically have the normal bipolar appearance.

(3) *Characteristic appearances in the abdominal organs.*

The Liver. The liver may show all degrees of "fatty"[1] change. In the early stages the lobules are clearly demarcated and this, combined with the yellowish appearance of the parts affected contrasting with the reddish colour of the congested areas, constitutes a characteristic picture which—perhaps inadequately—we have been accustomed to describe by the term "mottling." In some instances an extreme degree of "fatty" change is seen. In such a case the liver has a pink tinge, its surface presenting a uniformly smooth appearance and showing no sign of any division into lobules. The whole organ gives the impression of being modelled in wax with the upper surface peculiarly dome-like and the edges sharply defined. It has lost the normal tough resilient consistence, so that it pits on pressure and somewhat easily cracks on bilateral pressure especially when putrefaction has begun. This condition has not been seen in fresh rats other than those which were plague-infected, but in putrid rats an appearance very similar to it, or even indistinguishable from it, is rarely encountered when plague can with certainty be excluded.

Another condition frequently met with in the liver, and one of the greatest importance in diagnosis, is the occurrence of small necrotic foci scattered over its surface and throughout its substance. Tidswell makes mention of "small white points" in a few plague rats examined by him. Skschivan also from his description of several plague rats evidently recognised a similar appearance. We have been in the habit of referring to such a condition as "granular" liver. The gray or whitish granules are most easily observed on the surface; they are typically of the size of a pin's point and give the surface of the organ a stippled appearance as if dusted over with gray pepper. They are invariably discrete and in this respect contrast with the "mottled"

[1] The term fatty is used in reference to the naked-eye appearance only which strongly suggests an excess of fat. Microscopically however the appearance is found to be due to necrosis of the liver tissue.

liver in which there is no well-defined margin to any of the affected areas. They may be so small that only the closest scrutiny of an experienced observer will detect them. When larger the granules are of a yellow colour and vary somewhat in size. When well marked and closely set together they are always uniformly scattered throughout the liver substance, but if faintly marked and very few they may be confined to one lobe or to the edges only of the lobes. In some instances the necrosis assumes the appearance of a delicate gray network enclosing in its meshes the lobules which appear reddish from the presence of congested vessels. In a typical specimen the granules are not raised above the surface of the liver. Very exceptionally this does happen as in the case of an adult *M. rattus* where the liver is described as being "full of small yellowish white necrotic nodules about the size of a mustard seed, those on the surface being raised above the liver tissue that remained." On section the organ was found to be almost entirely replaced by the necrotic deposits, hardly any liver tissue being left. In this case a few typical bacilli only were seen microscopically but a pure culture of *B. pestis*, which gave good stalactites, was obtained from the heart-blood.

This granular condition of the liver is fairly often met with in experimentally infected rats which die about 48 hours after inoculation. The longer the interval between inoculation and death the better marked is the granulation. In a few instances rats killed on the 18th day have shown coarse granulation of the liver with very few plague bacilli present in the smears. With regard to the frequency of its occurrence it may be mentioned that it was noted in 58 % of the rats in Series II. It is occasionally found in a liver which shows "fatty" changes. Even in putrid rats the granules may be recognised as gray points standing out on a black background.

Other pathological conditions met with in the liver in plague rats may be said to be neither constant nor characteristic. Haemorrhages under Glisson's capsule are seen relatively seldom. Enlargement and congestion of the liver, which some writers seem to consider noteworthy signs, are in our view of very little value.

The Spleen. The spleen of a plague rat is typically of firm consistence with a moulded appearance, so that it lies over the stomach in its natural relation to that organ instead of collapsing like a soft normal spleen. Its firm consistence probably accounts for the fact that a good smear is obtained from it on a slide with greater ease than from a normal spleen. Granules or nodules may be very well marked (*e.g.* the

size of a millet seed) and may be confluent. Sometimes a relatively large wedge-shaped portion of the spleen is converted into a cheesy mass in which plague bacilli can be found. A false appearance of granulation is often seen in normal spleens and is doubtless due to the Malpighian bodies showing through the semi-transparent capsule. Apart from this we have never seen a nodular condition except in a plague rat. Analysis of the records of 200 plague rats examined during December 1905 showed that 111 of the livers were granular (55·5 %), while the spleen was granular in 9, *i.e.* 4·5 %. In very rare instances the spleen contains granules although none are to be found in the liver.

Apparent enlargement and congestion of the spleen are of little importance for purposes of diagnosis. The spleen, especially in *M. decumanus*, is often much enlarged although the other organs are apparently normal; such a spleen is usually soft and flabby.

The Kidneys. The kidneys and the suprarenal capsules are often congested. Minute subcapsular haemorrhages are fairly often present viz. in 8·5 % of Series I. The kidneys frequently show "fatty" changes, sometimes appearing quite yellow. A granular condition of the organ is an extreme rarity although occasionally it has been noted.

The Stomach and *the Intestines* usually show no characteristic change. The latter may be acutely congested but subserous haemorrhages are rarely present, contrasting in this respect with plague guinea-pigs in which they are a common and striking feature. Haemorrhages are somewhat rarely seen under the peritoneal coat of the stomach.

Abundant peritoneal effusion is a rare occurrence though slight effusion may be seen, the serous surface having a moist look.

(4) *Characteristic appearances in the organs in the thorax.*

Pleurae and *Lungs.* Haemorrhages occur fairly often in the lungs and pulmonary pleurae but we have never seen them in the parietal layer of the pleurae.

The presence of *pleural effusion* is a very characteristic feature and one which we consider of great value in diagnosis. The effusion is typically quite clear and may be so abundant that when the sternum and portions of the ribs are reflected the heart and lungs appear to be floating in a bath of straw-coloured fluid which overflows, forming a pool in the axilla. It may sometimes be blood-stained. In Series I it occurred in 73·5 % of the rats while in 9 % it was abundant. In

TABLE V.

Serial No.	Date of examination	Species, sex, weight	Naked-eye appearances of lungs	Microscopical examination of lungs	Other post-mortem appearances	Microscopical examination of other organs	Confirmatory tests
1	9/4/06	Male *M. decumanus* 225 gms.	Left lung and right lower lobe consolidated; pleural effusion	Very numerous *B. pestis*	Right submaxillary bubo containing yellowish soft pus, no subcutaneous congestion; suspicious faint granulation in liver	Heart-blood—clumps of plague-like bacilli Spleen—0 Bubo—0	Subculture from bubo gave good stalactites
2	11/4/06	Female *M. decumanus* 350 gms.	Intensely congested with right lower lobe pneumonic; marked pleural effusion	Clumps of plague-like bacilli	Rigor mortis; no primary bubo; marked subcutaneous congestion; liver and spleen typically granular; haemorrhages around calices of kidneys; subcutaneous haemorrhages	Heart-blood—0 Spleen—0 Liver—0	Pure culture of *B. pestis* from lungs. Cultures from liver and spleen sterile. Culture from heart-blood contaminated
3	12/4/06	Male *M. decumanus* 300 gms.	Congested with right lower lobe consolidated; pleural effusion; haemorrhages in lungs	Swarms of plague-like bacilli	Rigor mortis; no primary bubo; slight subcutaneous congestion; liver mottled; spleen congested	Heart-blood—0 Spleen—0	Culture from lungs contaminated but showing plague-like growth, subculture pure plague. Spleen culture sterile; liver and heart-blood cultures contaminated
4	14/4/06	Male *M. rattus* 100 gms.	Deeply congested; lower lobe of right and left lungs consolidated; haemorrhages in lungs; pleural effusion	Swarms of plague-like bacilli	Rigor mortis slight; no primary bubo; subcutaneous congestion; liver mottled; subcutaneous haemorrhages	Heart-blood—a few suspicious bacilli amongst many putrefactive Spleen—0	Cultures of heart-blood, spleen, lungs all contaminated
5	16/4/06	Female *M. decumanus* 410 gms.	Deeply congested; left lung consolidated and showing gray hepatisation; haemorrhages in lungs; marked pleural effusion	Swarms of plague-like bacilli	Rigor mortis; left cervical bubo; very slight subcutaneous congestion; liver mottled	Heart-blood—a few doubtful *B. pestis* Spleen—0 Bubo—a fair number of *B. pestis*	Thick growth of plague in culture from lungs. Liver culture sterile
6	16/4/06	Male *M. decumanus* 300 gms.	Deeply congested; right lower lobe consolidated; haemorrhages in lungs; pleural effusion	Swarms of plague-like bacilli	No primary bubo; anterior mediastinal gland enlarged, congested; marked subcutaneous congestion with haemorrhages; liver mottled	Heart-blood—0 Spleen—0 Mediastinal glands—a few *B. pestis*	Thick growth of plague in culture from lung. Liver culture sterile
7	17/4/06	Female *M. decumanus* 280 gms.	Deeply congested; right lower lobe consolidated and patches of consolidation in left lung; pleural effusion	Swarms of plague-like bacilli in consolidated areas	No primary bubo; very marked subcutaneous congestion; liver granular	Heart-blood—0 Liver—0 Spleen—0	

Series II its presence was observed in 72 %, it was noted as being abundant in 6·9 % and it was absent in 28 % of the rats.

The Lungs vary considerably in appearance and as a rule present nothing characteristic. They may exhibit a patchy congestion but in some cases they appear quite pale. Compared with guinea-pigs granules in the lungs of rats rarely occur—only 2·5 % of the rats showing them in Series I.

An interesting feature somewhat rarely met with in plague rats is a pneumonia which is decidedly lobar in character. The details of seven cases have been collected in Table V. It will be observed that the lower lobe of the right lung was consolidated in six out of the seven rats, and that a double pneumonia was present in three. It will further be noted that microscopical examination of the lungs revealed very numerous plague-like bacilli (which were verified by culture in some of the cases) although relatively few or no bacilli were seen in the other organs. In two a submaxillary bubo was present, but the condition of the others leaves little doubt that they are instances of a typical primary pneumonia. We have observed pneumonic lungs in all stages including typical red and gray hepatisation and even apparent resolution. Portions of consolidated lungs sank when placed in water. 1000 plague rats not included in Series II were examined for the special purpose of noting the frequency of this condition with the result that six cases were found, viz. those numbered 2 to 7 in the table.

The Heart. The pericardium fairly often contains a clear fluid and epicardial haemorrhages occasionally are seen. The vessels on the surface of the heart frequently have an injected appearance. The walls are relaxed with the right cavities usually engorged with blood and the left empty.

IV. The Value of Certain Characteristic Post-mortem Features in the Diagnosis of Plague Rats, including those which have undergone Putrefaction.

A recapitulation may be conveniently given under this head of what we consider the most important post-mortem features for purposes of diagnosis.

The presence of a typical bubo is the most important sign of plague in rats.

The next important sign is the condition we have described as "granular" liver. In our experience this condition is not met with in rats other than those that are plague-infected. The spleen is a much less important organ for diagnostic purposes than the liver—in this respect, indeed, the latter takes the place of the spleen in guinea-pigs.

Haemorrhages, both subcutaneous and in the organs, are very suggestive features. They occurred somewhere or other in no less than 54% of the rats in Series I. We have already noted that so far as our experience goes subcutaneous haemorrhages constitute a most important sign of plague in rats.

Again an abundant clear pleural effusion of itself goes a long way towards establishing a diagnosis of plague.

In putrid rats, at least three of these signs may persist and when recognised are of the greatest assistance, viz. a bubo, granular liver and pleural effusion.

Table VI has been constructed in order to show the frequency of occurrence of most of the characteristic naked-eye features of the rats included in Series I.

TABLE VI.

Showing frequency of occurrence of certain characteristic post-mortem features in the rats included in Series I.

Post-mortem appearance or lesion	100 *M. rattus*	100 *M. decumanus*	Percentage of total
Rigor mortis	27	26	26·5
Subcutaneous congestion (including submaxillary)	22	39	30·5
Subcutaneous haemorrhages ...	44	37	40·5
Submaxillary haemorrhages with bubo ...	17	20	18·5
,, ,, (bubo absent or in another situation) ...	7	9	8
Cervical oedema	9	11	10
Fatty liver	59	50	54·5
Granules in the lungs	1	4	2·5
,, ,, kidneys	0	1	0·5
Pleural effusion	73	56	64·5
Abundant effusion (included in above) ...	11	7	9
Haemorrhages in lungs and pleurae ...	16	32	24
,, ,, kidneys and suprarenals	5	12	8·5
,, ,, epicardium ...	2	5	3·5
,, ,, stomach	4	0	2
,, ,, intestine	0	1	0·5

V. The Occurrence of Plague-like Diseases among Rats.

During sixteen months' continuous rat examination in Bombay, involving the scrutiny of 150,000 animals of which 19,000 were infected with plague, no disease of the rat has been met with which caused any material difficulty in diagnosis[1].

VI. Remarks on the Results of Microscopical Examination.

The importance of the results which have been obtained by us from an analysis of this method of examination relates chiefly to the question of diagnosis.

For staining carbol-thionin blue was used invariably in the routine examinations. This has a certain value as a differential stain in that plague bacilli appear more faintly coloured than adventitious organisms. It brings out to advantage the typical bipolar appearance of *B. pestis.* Very rarely the bacilli in the organs assume the form of a small coccobacillus closely resembling the organism of fowl cholera and causing some doubt as to their real nature.

With regard to the presence of involution forms 56·6 % of the buboes in Series I showed them, while in the same number of spleen preparations examined they were found in only 12 %. In the spleen they occur perhaps most frequently in association with putrefactive organisms. They have never been observed in the heart-blood[2].

Sometimes in rats which give evidence of a relatively chronic form of plague, with well-marked granules in the liver, the bacilli are not uniformly distributed over the preparation, but are present in the form of characteristic clumps. Clumps of bacilli were seen in 9·5 % of the spleen smears in Series I. They rarely occur in the heart-blood, having been seen once only in this series. When in clumps the bipolar appearance is much less often observed than when the organisms are uniformly distributed in the smear, the contents of the bacilli usually appearing very finely and uniformly granular.

[1] It is perhaps worth recording that the leprosy-like disease of rats due to acid fast bacilli described by Stefansky (*Centralblatt für Bakt.*, xxxiii. 1903, p. 481) and Dean (This *Journal*, vol. v. p. 99) has been met with in *M. decumanus* in Bombay and in *M. rattus* in the Punjab.

[2] They have been observed in the heart-blood of plague guinea-pigs, and very rarely (namely on two occasions) in the heart-blood of experimentally infected rats.

The general value of the method of microscopical examination is sufficiently indicated by the fact that in 75 % of the total rats in Series I numerous plague bacilli were seen either in the heart-blood, spleen, or bubo of each rat, or, if not very numerous in the bubo, involution forms were present.

As to the comparative value of the three tissues usually examined, there can be no doubt that the bubo gives a better chance of finding plague bacilli than the spleen, and the spleen than the heart-blood. Thus, out of 150 rats with buboes in Series I, numerous *B. pestis* were noted in 104 preparations of the buboes, 70 preparations of spleens and only 27 preparations of the heart-blood. Results tending in the same direction will be found in the appended statement of an analysis of the microscopical examination of 1000 rats in Series II (Tables VII, VIII,

TABLE VII.

Analysis of results of microscopical examination of 1000 *rats with buboes in Series II.*

	No *B. pestis* seen	Few *B. pestis* seen	Numerous *B. pestis* seen
Heart-blood	13·5 %	53·4 %	33·1 %
Spleen	9·9	17·3	72·8
Bubo	0·9	7·4	91·7

No *B. pestis* seen in smears of bubo, spleen and heart-blood 0·4 %

B. pestis seen in smears of bubo only ... ... 6·6

TABLE VIII.

Analysis of 37 *putrid rats out of the* 1000 *rats with buboes in Series II.*

	No *B. pestis* seen	Few *B. pestis* seen	Numerous *B. pestis* seen
Heart-blood	24·3 %	37·8 %	37·9 %
Spleen	29·7	13·5	56·8
Bubo	5·4	13·5	81·1

B. pestis not seen in any of the three smears 5·4 %

TABLE IX.

Analysis of results of microscopical examination of 100 M. rattus *and* 100 M. decumanus *in Series I.*

	M. rattus			*M. decumanus*		
	None	Few	Numerous	None	Few	Numerous
Heart-blood	19 %	48 %	29 %	29 %	59 %	7 %
Spleen	8	26	64	14	51	31
Bubo	1·3	24	72	8	20	69

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TABLE X.

Comparison of results of microscopical examination of Series I (combined percentages of 100 M. rattus *and* 100 M. decumanus) *and* 1000 *rats with buboes in Series II.*

		Series I	Series II
Heart-blood	= 0 or doubtful	28·5 %	13·5 %
Heart-blood	= few	53·5	53·4
Heart-blood	= numerous	18	33·1
Spleen	= 0 or doubtful	14	9·9
Spleen	= few	38·5	17·3
Spleen	= numerous	47·5	72·8
Bubo	= 0 or doubtful	7·4	0·9
Bubo	= few	22	7·4
Bubo	= numerous	70·6	91·7

Note:—Under the term doubtful are included those cases in which microscopical examination did not reveal the presence of plague bacilli with reasonable certainty.

and X). Even in a very putrid rat the bubo may show many plague bacilli, frequently with involution forms in addition, but with relatively much fewer putrefactive organisms than in the smears of the spleen or of the heart-blood. In a suspicious bubo showing no plague bacilli the presence of degenerated leucocytes and cellular débris serves materially to strengthen the suspicion of plague.

VII. On the Relative Value of the Methods of Naked-eye and Microscopical Examination in the Diagnosis of Rats suspected of being plague-infected.

Any value which our epidemiological inquiries into plague in Bombay may possess necessarily hinges to no small extent upon the accuracy of the daily returns of the plague-infected rats. For this reason it became a matter of importance to acquire as thorough a knowledge as possible of the appearances presented by the plague rats, and of the reliable methods for the diagnosis of rats suspected of being plague-infected. It is obvious, moreover, that especially in the plague season, when as many as 200 rats were returned daily as plague-infected, some system of examination had to be adopted which should give the best results without needless expenditure of time and labour. A description of the methods carried out by us in the daily rat investigation, in so far as they are concerned with the recognition of plague rats, may not be considered out of place here.

At the beginning of the investigation in July 1905, and for several months thereafter, a smear of the spleen of every rat brought to the laboratory was examined microscopically, the post-mortem appearances of the rats being also noted. In addition to this, the spleen or other organ of every rat which was suspected of being plague-infected either by macroscopical or by microscopical examination, and even of those which were regarded with certainty as plague rats, was inoculated cutaneously into a guinea-pig. After a complete and careful examination had been made of 50 plague rats verified in this manner, the procedure was so far modified that only those rats which offered difficulty in diagnosis were submitted to the animal test. The routine microscopical examination of the spleen smears was carried out during the off season, but later, having regard to our increasing experience in diagnosis by macroscopical examination alone, it was decided to forego the former method. The system adopted at this stage may be briefly described.

The rats were cut open by three soldiers attached to the Plague Research Laboratory. These men proved to be very intelligent assistants—their knowledge of the appearances of plague rats as the result of an extensive experience being remarkably accurate. Rats recognised as plague by them were sent to a room in another part of the building, where a detailed examination was carried out by members of the staff. Moreover, two or three members of the staff examined carefully every rat passed over by the soldiers, and any rat with suspicious features was sent for further examination to the room referred to. Thus, a detailed examination was made of every plague rat and every rat suspected of being plague—a record being made of the post-mortem appearances and of the results of microscopical examination of the heart-blood, spleen, and bubo if present. The returns of plague rats were compiled after a consideration of the results of the complete examination. In cases where the diagnosis remained uncertain the organs were inoculated cutaneously into a guinea-pig and, if thought advisable, cultures were made. Nearly 5000 plague rats were examined in this way during the early period of the epizootic. It then became apparent that such a detailed examination might be curtailed without substantially affecting the accuracy of the returns. The method was accordingly limited to a careful checking of every rat by two or three trustworthy and highly trained observers. Only in the case of suspicious rats were the organs examined microscopically.

In order to discover whether any serious error had crept in from the

omission of the microscopical examination of every rat brought to the laboratory, and whether the naked-eye method of examination alone might be considered reliable, it was arranged to carry out a test extending over a fortnight with this object in view. The plan pursued was as follows:—

The soldiers put on one side the rats they believed to be plague-infected, and if they regarded any as suspicious a note to this effect was made on the card affixed to the rat. At the same time the members of the staff whose duty it was under ordinary circumstances to observe the post-mortem appearances recorded their opinion of every rat examined by them. To three experienced microscopists the task was allotted of examining preparations from the spleen of all the rats; they likewise recorded their opinion of every slide examined. Finally, a member of the Commission compared the results arrived at by the two methods. It ought to be explained that in order to avoid any bias which might arise from their inclusion amongst the spleen smears, preparations from buboes were not given in the first instance to the microscopists. An opportunity was, however, afforded of examining smears of buboes or of the heart-blood if the original spleen smear proved negative on repeated examination.

An endeavour was made by the individual superintending the test to be impartial in his appraisement of the results, though it must be admitted that no criterion is available on which to base a final decision in every instance since even the cutaneous test may fail in certain cases. It may be added that when any of the men engaged in carrying out the test disagreed in his opinion with the others he was permitted to have the suspected material inoculated into a guinea-pig. This was done indeed in every case in which there was a wide divergence of opinion as regards the diagnosis. A detailed analysis of the results of the last six days of the test has been arranged in the following Tables (XI—XIV).

A review of this analysis leaves no room for doubt that for purposes of diagnosis naked-eye examination by a competent observer is more satisfactory than microscopical examination alone. It is somewhat remarkable that in a single instance only was a plague rat diagnosed by microscopical examination which the observers of the post-mortem appearances failed to recognise, *i.e.* 0·7 % of the total number of plague rats. On the other hand six rats with plague bacilli in the spleen smear were overlooked by the microscopists, and in seven rats no plague bacilli were found microscopically in any of the organs, *i.e.* 13

rats were missed out of a total of 131, viz. nearly 10 %. Naturally in isolated cases both methods must be employed, but the results clearly show that the omission of the routine microscopical examination of every rat in an investigation conducted on a large scale does not necessarily impair the accuracy of the work, while the saving of labour is of course very great.

It will readily appear from Tables XIII and XIV that the chief difficulty which is encountered in diagnosis by either of the methods

TABLE XI.

Showing analysis of results of experiment to compare macro- and microscopical methods of diagnosis.

Date	Total No. of rats examined	Total No. returned as plague	Plague rats missed by soldiers	Plague rats missed by expert observers of P.M. appearances	*B. pestis* present in spleen smear but overlooked by micro-scopists	*B. pestis* not seen in spleen smear but seen in smear of other organ or in bubo	*B. pestis* not seen in any organ microscopically
21/5/06	205	25	—	—	2	2	1
22/5/06	165	20	—	—	—	—	1
23/5/06	195	17	—	—	—	1	2
24/5/06	171	23	—	—	2	—	1
25/5/06	223	21	—	—	1	2	2
26/5/06	214	25	1	1[1]	1	3	—
Total	1173	131	1	1	6	8	7

[1] It may be explained that this rat was very putrid with an inguinal bubo which had already been opened by one of the soldiers. The appearance of the cut surface of such a bubo rapidly alters by exposure to air, and as this was the only suspicious feature present the failure to recognise the rat as being plague-infected is excusable. Moreover it had already been noted as "suspicious" by the soldier who dissected it.

TABLE XII.

Giving an analysis of the diagnosis of the rats returned as plague which were diagnosed in the first instance either as "suspicious" or "plague" both by the Post Mortem observers and by the microscopists.

Date	Total No.	Diagnosis of "plague" by post-mortem examination	Diagnosis of "suspicious" by post-mortem examination	Diagnosis of "plague" by microscopists	Diagnosis of "suspicious" or "very suspicious" by microscopists
21/5/06	20	20	—	17	3
22/5/06	19	18	1	15	4
23/5/06	14	13	1	13	1
24/5/06	21	17	4	15	6
25/5/06	16	16	—	13	3
26/5/06	20	19	1	16	4
Total	110	103	7	89	21

TABLE XIII.

Giving details of the rats which were proved to be plague by cutaneous inoculation into guinea-pig.

Serial No.	Date	Diagnosis of observers of post-mortem	Diagnosis of microscopists	Post-mortem features of rats
1	14/5/06	Suspicious	*B. pestis* not recognised	Semi-putrid; no primary bubo; subcutaneous congestion; spleen congested and firm; pleural effusion
2	14/5/06	Suspicious	*B. pestis* not recognised	Semi-putrid; no primary bubo; subcutaneous congestion; liver pinkish; lungs deeply congested; pleural effusion
3	14/5/06	Suspicious	*B. pestis* not recognised	Semi-putrid; no primary bubo; slight subcutaneous congestion; liver pink; lungs deeply congested with slight effusion
4	15/5/06	Suspicious	Plague	Putrid; liver pale; guinea-pig died in four days
5	16/5/06	Plague	*B. pestis* not recognised	Putrid; right cervical bubo; coarsely granular liver and spleen; haemorrhages in lungs with slight pleural effusion
6	16/5/06	Very suspicious	*B. pestis* not recognised	Putrid; right cervical bubo?; spleen firm and moulded; lungs deeply congested with pleural effusion
7	19/5/06	Suspicious	Plague	No primary bubo; enlarged and congested secondary glands; subcutaneous oedema and congestion; organs congested; abundant pleural effusion
8	19/5/06	Suspicious	*B. pestis* not recognised	Putrid; no primary bubo; very slight subcutaneous congestion; liver pinkish and firm; lungs congested with pleural effusion; subcutaneous haemorrhages
9	21/5/06	Plague	*B. pestis* not recognised	Putrid; no primary bubo; lungs congested with pleural effusion
10	23/5/06	Suspicious	*B. pestis* not recognised	Semi-putrid; right axillary bubo?; slight subcutaneous congestion; liver pinkish; lungs congested; pleural effusion

Summary of diagnosis in above Table.

Diagnosis of "suspicious" by p.m.	=7	*B. pestis* not recognised by microscopists	8
Diagnosis of "very suspicious" by p.m.	=1	Diagnosis of "plague"	2
Diagnosis of "plague" by p.m.	=2		

TABLE XIV.

Showing analysis of diagnosis of 21 *rats which did not prove to be plague-infected when inoculated cutaneously into guinea-pig.*

	Diagnosis by P.M. examination	Diagnosis by microscope
Not plague	9	7
Suspicious	5	7
Very suspicious	5	3
Plague	2	4

Note:—19 out of the 21 rats were more or less putrid.

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arises from putrefactive changes masking the characteristic appearances in the organs. In this connection it may be convenient to give details of an experiment devised in order to test the ability of those in charge of the diagnosis of the rats in the routine examination to recognise putrid plague rats. A number of Bombay rats were inoculated under the skin of the back with a virulent culture of the plague bacillus. Twelve of these rats which died at about the same time were laid aside exposed to the air in a go-down along with a number of healthy rats freshly killed by chloroform. In all some 50 rats were thus exposed. The rats were labelled in such a way that they gave no indication to those examining them as to whether or not they had been inoculated. After being in the go-down for 48 hours, they were dissected and an independent opinion was given of the diagnosis from post-mortem and from microscopical appearances. As will be seen from Table XV extreme putrefactive changes had set in in most of the rats. The results of the experiment are briefly as follows:—of the 12 plague rats seven were diagnosed as plague by the observers of the post-mortem appearances, in addition two were noted as "suspicious," while the remaining three had become so putrid that it was impossible to put forward any diagnosis. The signs which, although faintly marked (as is generally the case in artificially inoculated rats), gave a clue to the diagnosis of "plague" or "suspicious" were—an appearance of general congestion, some moisture in the pleural cavities, oedema in the axilla in a few (the bubo had already disappeared), and a somewhat firm consistence of the spleen and liver in others.

It would appear from this test, also, that no serious error can be ascribed to the naked-eye method of diagnosis in the direction of failing to recognise rats which show advanced putrefactive changes. As a matter of fact the number of rats brought to the laboratory for examination which showed such extreme putrefaction as those in the experiment just described is so small that it does not deserve to be taken into account.

Material from all these rats was inoculated cutaneously into guinea-pigs, but by an oversight the diagnosis attached to each rat and the results of the cutaneous tests were not correlated. Still, the fact that seven out of the 12 rats gave plague to guinea-pigs serves to strengthen the impression that even in a severe test of this description a reliable method for diagnosis is to be found in the cutaneous method of inoculation.

TABLE XV.

Giving details of plague rats in the test for diagnosis of putrid rats.

Test No. of rat	Extent of putrefaction	Microscopical examination of rat	Cutaneous test in guinea-pigs
2	Putrid and full of maggots	Smears of H.-B. and spleen putrefactive organisms, none plague-like	Spleen and H.-B. inoculated. Guinea-pig died in 5 days of typical plague
8	Very putrid; organs almost entirely eaten away by maggots except those in thorax	H.-B. and spleen—all putrefactive organisms, none plague-like	Heart inoculated. Guinea-pig chloroformed on 5th day: plague
9	Fairly fresh; liver pink and firm; subcutaneous congestion; lungs congested with pleural effusion	H.-B.—many putrefactive; a few plague-like	Spleen inoculated. Guinea-pig chloroformed on 3rd day: plague
13	Similar to No. 8	H.-B.—some like typical plague; involution forms seen, many putrefactive	Heart inoculated. Guinea-pig chloroformed on 6th day: plague
30	Putrid	H.-B.—plague-like and many putrefactive bacilli	Spleen and H.-B. Guinea-pig chloroformed on 6th day: plague
34	Very putrid	H.-B. and spleen—many putrefactive, none like plague	Spleen and H.-B. Guinea-pig chloroformed on 6th day: plague
39	Putrid	Similar to rat 34	Spleen. Guinea-pig chloroformed on 6th day: plague
3	Very putrid and full of maggots	H.-B.—a few like plague, many putrefactive. Spleen—putrefactive only	Guinea-pig failed to take plague
16	Very putrid and destroyed by maggots except hind limbs, muscles of which were soft and pulpy	H.-B. Spleen—putrefactive only—none like plague	Guinea-pig failed to take plague
24	Putrid and full of maggots	Similar to 16	Guinea-pig failed to take plague
41	Putrid	Similar to 16	Guinea-pig failed to take plague
49	Putrid	H.-B.—a few involution + putrefactive bacilli. Spleen—putrefactive only	Guinea-pig failed to take plague

Summary of results obtained by different methods of diagnosis in this test.

1. By p. m. examination 7 were considered to be plague; 2 as suspicious.
2. By cutaneous tests 7 were proved to be plague.
3. By microscopical examination 5 were considered as suspicious.

VIII. On Diagnosis by the Cutaneous Method of Inoculation with Remarks on the Virulence of the Bacillus of Natural Rat Plague.

The following account of a somewhat extensive experience in the diagnosis by means of the cutaneous method in guinea-pigs of plague-infected rats or of rats suspected of being plague-infected is based principally upon an analysis of 330 consecutive examinations carried out according to this method, 150 of which proved to be plague. The rats formed part of the daily collections made in Bombay, and were sent to the laboratory for examination by the Commission during the period between July and December 1905. Plague in rats sent to the laboratory alive was a rare occurrence during this period, so that as a matter of fact in nearly every case the rats were dead when brought to the laboratory and showed varying degrees of freshness or in some cases of putrefaction. It must be remembered that the temperature in Bombay favours rapid putrefaction, thus adding greatly to the difficulties of diagnosis, especially by cultural methods. For the sake of simplicity a division has been made between those which might be considered "fresh," *i.e.* without obvious signs of putrefaction, and those which were decidedly putrid; thus the material inoculated was derived from 123 fresh rats and from 27 putrid rats.

It may be added that the majority of the rats belonged to the species *M. decumanus*.

Before commenting generally on the results, the technique adopted by us may be briefly described, together with the effects which follow inoculation of plague material into the skin of a guinea-pig.

The method was as follows. An area of skin about one inch square of the guinea-pig's abdomen is shaved with a sharp razor, no water nor soap being used. It is important to avoid the use of soap in shaving the skin, as there is good reason to believe that the chances of the guinea-pig dying acutely are thereby greatly diminished. The epidermis is partly removed in shaving, so that a raw, slightly bleeding surface is exposed. Pieces of the organ or organs selected for the test are then removed with sterile scissors and forceps, and rubbed, with some vigour, by means of the forceps, over the shaved area. This procedure is adhered to however putrid the material may be.

In the employment of the cutaneous method as a confirmatory test for rats diagnosed as plague or for rats suspected of being plague-

TABLE XVI.

Serial No.	Dilution	Method	Weight	Local reaction	Result
1	0·000001	Cutaneous	Increased steadily in weight	None	Recovered
2	0·000001	Subcutaneous	Gained weight till 6th day, then gradually lost weight till original weight on 12th day, thereafter gained weight	Small reaction noted on 8th day	Killed by $CHCl_3$ on 23rd day; P. M. very chronic plague
3	0·00001	Cutaneous	Gained weight	None	Recovered
4	0·00001	Subcutaneous	Gained weight till 5th day, then gradually lost weight till death	Slight reaction on 5th day	Died on 13th day; P.M. chronic plague
5	0·0001	Cutaneous	Gained weight till 5th day then lost weight	—	Died on 6th day
6	0·0001	Subcutaneous	Gained weight till 6th day. Final weight same as original	Reaction on 4th day	Died on 13th day
7	0·001	Cutaneous	Gained weight till 6th day, then lost weight. Final weight = original weight—50 grams	—	Died on 18th day
8	0·001	Subcutaneous	Gained weight till 6th day. Final weight = original weight—10 grams	Reaction on 3rd day	Died on 18th day
9	0·01	Cutaneous	Lost weight on 3rd day. Final weight = original weight—30 grams	—	Died on 9th day
10	0·01	Subcutaneous	Lost weight on 3rd day. Final weight = original weight—40 grams	Reaction on 2nd day	Died on 7th day
11	0·1	Cutaneous	Lost weight on 3rd day. Gained weight on 14th day	—	Killed by $CHCl_3$ on 23rd day; P.M. chronic plague
12	0·1	Subcutaneous	Lost weight on 2nd day. Final weight = original weight—50 grams	Reaction on 3rd day	Died on 6th day

infected it is important that the bubo, if present, should be rubbed in. It has been shown above that plague bacilli are more often found and, when present, are more numerous in the bubo of a plague-infected rat than in any other tissue.

The factors which influence the result of an inoculation of plague bacilli into an animal are various.

They are partly illustrated in Table XVI, which compares the effects of graduated quantities of virulent plague bacilli inoculated cutaneously and subcutaneously in a parallel series of guinea-pigs. The culture employed in the experiment was a subculture in broth of a strain recently isolated from the blood of a septicaemic patient. The dilutions were made in fresh normal urine, 0·25 c.c. of each dilution being used for each inoculation.

It is obvious first of all that the question of dosage is an important one. This is apparent in the case of guinea-pigs 1 and 3, which remained unaffected by cutaneous inoculation of small doses of virulent *B. pestis,* although subcutaneous injection of the same amount produced chronic plague in guinea-pigs 2 and 4. Again the influence of individual resistance of the animal experimented upon must explain the case of guinea-pig 11, which received the largest dose of the cutaneous series and which, nevertheless, lived for more than three weeks. Another factor not taken into account in this series, since the material used was the same throughout, is that of virulence of the bacilli inoculated. Yet another circumstance which must be reckoned with is the association with plague bacilli of other organisms in the material used for cutaneous inoculation, *e.g.* putrefactive organisms. This point will be adverted to later.

With regard to the subcutaneous series it will be observed that when a small dose is injected, the animal suffers from a chronic form of plague. In such cases it may continue to gain weight during the first five or six days and the local reaction is delayed even for a week. When larger amounts are given loss of weight is noticed on the second or third day, and at the same time a reaction may be felt in the glands near the site of injection. A very similar state of things seems to hold good in animals inoculated cutaneously, as will appear in the examples to be given shortly.

From the point of view of early diagnosis by the cutaneous method the appearance of a reaction at the site of inoculation and the existence of enlarged inguinal glands deserve attention. One or two actual instances will best illustrate this.

I. A guinea-pig was inoculated cutaneously in the usual manner with the spleen of rat 50237 on 23–XII–05. This rat was considered suspicious on detailed examination. No primary bubo was seen; the liver was granular; there was pleural effusion and subcutaneous haemorrhages were noted. Microscopical examination of the heart-blood and spleen showed no *B. pestis*. Twelve hours after inoculation the guinea-pig showed a slight skin reaction consisting of phlyctenules surrounded by a zone of redness. No bubo could then be felt. Thirty-six hours after inoculation the skin reaction was marked and small inguinal buboes could be felt. The guinea-pig died with typical signs of plague on the fifth day; the weight remained the same throughout.

II. A guinea-pig was inoculated cutaneously with the spleen and bubo of rat 49985 on 23–XII–05. The rat was putrid, but a right submaxillary bubo was found. Microscopical examination of the heart-blood and bubo showed no *B. pestis*, but a few suspicious bacilli were seen in a spleen smear. The detailed examination was considered suspicious of plague. The guinea-pig showed a skin reaction 12 hours after inoculation. A small inguinal bubo was felt for the first time on the 5th day—the skin reaction being then well marked. The animal was killed by chloroform on the 10th day, when axillary and inguinal buboes, containing typical plague bacilli were found on post-mortem examination. There was a very gradual increase of weight throughout, so that the final weight was 30 grams in excess of the original weight.

III. A guinea-pig was inoculated in the usual way with the spleen of rat 50750 on 26–XII–06. The rat was missed in the routine microscopical examination of the spleen smears, but was regarded as suspicious on detailed examination. There was no bubo; the liver was granular, and pleural effusion and subcutaneous haemorrhages were observed. Twelve hours after inoculation a skin reaction was noted, the guinea-pig, however, being well and active; 24 hours later, the inguinal glands on the left side were felt to be enlarged. The guinea-pig died in three days of typical plague. The final weight was 10 grams in excess of the original weight.

These cases exemplify a general experience, namely, that the cutaneous reaction is the earliest symptom, usually appearing about 12 hours after inoculation. If the disease is acute the inguinal glands can be felt to be enlarged 36 hours after inoculation, while in chronic cases the glands may be palpable only after the lapse of several days.

Inoculation of fresh material.

Proceeding now to the consideration of the material examined by us in the course of the routine diagnosis, a matter of considerable importance will be found in an analysis of the varying periods before death of the inoculated animals.

Table XVII, in which the facts have been arranged, makes it sufficiently clear that a large percentage of the guinea-pigs died acutely, that is in from two to four days. Thus, no less than 41 % of the cases died on or before the 4th day, and 62 % in five days. The largest percentage of deaths on any one day occurred on the 5th day, viz. 21·1 %.

TABLE XVII.

Death in days	No. of guinea-pigs which died	
Death in 2 days	3	2·4 %
,, 3 ,,	24	19·5
,, 4 ,,	23	18·7
,, 5 ,,	26	21·1
,, 6 ,,	18	14·6
,, 7 ,,	10	8·1
,, 8 ,,	2	1·6
,, 9 ,,	2	1·6
,, 10 ,,	2	1·6
Chloroformed after 10 days	4	3·2
Chloroformed in 10 days or before 10 days	9	7·3

Since the statement has been made (Klein, 1906) that plague in guinea-pigs following cutaneous inoculation almost invariably takes a subacute form (*i.e.* death in more than three days), we feel justified in claiming that the bacilli in our experiments causing acute death must have possessed a high degree of virulence. Nor can the objection be raised that the acuteness of the disease was due to a massive dose of bacilli. At least this cannot have been the case always as will be seen from a review of Table XVIII, in which the data have been classified with reference to three things—1st, the period before death of the guinea-pigs; 2nd, the microscopical examination of the material inoculated; 3rd, the results of the post-mortem examinations of the rats from which the material was derived. Study of this table leads to the conviction that in a large number of cases the material rubbed in contained highly virulent plague bacilli. This conclusion receives support from the fact that many of the organs when examined microscopically showed

either no plague bacilli or suspicious bacilli only, so that the quantity inoculated was presumably a small one, and yet the guinea-pigs died of acute or subacute plague. If a calculation of the results be made on the basis of the standard of virulence adopted by Kolle and Martini (1902) it will be found that at least 62 % of the strains used in the experiments were fully virulent, *i.e.* caused death up to five days.

TABLE XVIII.

Fresh material inoculated (114 *experiments*).

	Microscopical examination of inoculated material					Post-mortem examination of rat		
Death in days	Suspicious bacilli	No *B. pestis* seen	A few seen	Fairly numerous	Numerous	Typical	Fairly typical or "suspicious"	Nothing typical of plague
Death in 2 days	—	—	1	—	2	3	—	—
,, 3 ,,	10	2	7	1	4	11	10	3
,, 4 ,,	9	3	2	4	5	15	4	4
	19	5	10	5	11	29	14	7[1]
Death in 5 days	9	5	2	3	7	14	11	1
,, 6 ,,	3	3	2	5	5	12	6	—
,, 7 ,,	3	1	1	2	3	8	2	—
,, 8 ,,	—	—	—	—	2	2	—	—
,, 9 ,,	—	—	—	—	2	2	—	—
,, 10 ,,	1	—	—	—	1	1	—	1
Chloroformed after 10 days	—	—	—	1	3	4	—	—
	16	9	5	11	23	43	19	2

The only evidence pointing to an occasional diminution of virulence of the bacilli in natural rat plague may be drawn from the case of four guinea-pigs inoculated separately with material containing numerous *B. pestis*. One guinea-pig was killed by chloroform on the 10th day, two on the 11th day, and one on the 14th day after inoculation, all showing signs of chronic plague. The possibility of an unusual resistance to plague in these particular animals cannot, however, be altogether excluded.

An additional strong argument in favour of the view that the type of bacillus associated with natural rat plague is a virulent one lies in the

[1] The number of rats classed under this head would probably have been much reduced if these experiments had been made later in our experience of rat plague: see the results in Tables XI, XII, XIII and XV.

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fact that out of 300 rats examined the inoculation apparently failed in three instances only of rats which were strongly suspected of being plague-infected. In further proof of the virulence of the bacillus of natural rat plague, we may point out that the cultures used to set alight the epizootics carried on by rat fleas in the go-down experiments were derived from naturally infected Bombay rats.

There is some indication that the variation of virulence for the guinea-pigs is an expression of variations of virulence for the corresponding rats. Table XVIII shows that the rapidity of death of the test guinea-pig varies inversely with the suggestiveness of the post-mortem appearances of the rat. Thus 14 % of the inocula which killed guinea-pigs in four days or less were derived from rats which showed nothing typical of plague on section, while but 3 % of those which produced death at a later period were derived from such indeterminate animals. Excluding races of very low virulence, it is probable that the degree of reaction (*i.e.* the degree to which the naked-eye post-mortem abnormalities are developed) varies inversely with the virulence of the infecting strain of bacillus, and that therefore the races most virulent to guinea-pigs were also most virulent to rats.

Before leaving the subject of virulence, attention may be called to the fact that the rats dealt with in Table XVIII were examined in the off-plague season, *i.e.* when only sporadic cases were occurring in rats and in men. The only statement founded on experiment relating to possible fluctuations in virulence of the bacillus of natural rat plague appears to be that made by Skschivan (1903) in a paper describing the epizootic in Odessa in 1901—1902. Our own experience receives confirmation from the observations of this writer, although it must be said that his conclusions are based upon the examination of only 32 rats. Skschivan concludes that the virulence of the bacilli does not become weakened by successive passages through rats, since, for example, one of the last plague rats found contained virulent bacilli.

These results, taken in conjunction, have an obvious bearing upon the question of the transmission of plague from rat to rat in nature, but they retain their significance only on the view that such transmission is of a more or less direct character, *e.g.* by entrance of the bacilli through an abrasion in the skin. Their import is considerably modified if the assumption be made that an alteration of virulence may be effected in an intermediate host, for example, in the stomach of the flea. Not only do the results affect the general problem of the transmission of plague in rats, but they may bear upon a more specific point, namely, the

seasonal periodicity of plague amongst rats. An opportunity will, doubtless, be afforded later of discussing this subject from every possible point of view.

It is evident from the examples given previously that a cutaneous reaction and the presence of inguinal buboes are outstanding features when the inoculation proves successful. Another symptom equally important is the loss of weight which occurs as the result of infection. When death takes place very acutely, the animal may even gain slightly in weight, otherwise there is a varying loss of weight depending upon the acuteness of the disease. The average daily loss of weight is seen in Table XIX to be the greatest in the case of guinea-pigs which die on the 5th day. An early decrease in weight then gives valuable indication that infection by plague bacilli is in progress. A striking contrast is afforded in the case of guinea-pigs in which infection has failed—the animal steadily gains weight from the beginning, and the skin abrasions rapidly heal.

TABLE XIX.

Death in days	No. of guinea-pigs whose weight was observed	Average daily change of weight
2	3	Gained 1·6 grams
3	19	Lost 5·2 ,,
4	16	,, 7·8 ,,
5	23	,, 8·4 ,,
6	18	,, 6·7 ,,
7	10	,, 7·2 ,,
8	2	,, 7·5 ,,
9	2	,, 8·3 ,,
10	2	,, 3·5 ,,

Inoculation of putrid material.

The value of the cutaneous test for plague is especially manifest in those cases where the material inoculated is derived from rats which have undergone putrefaction. The details of those selected for the purposes of this paper have been collected and arranged in Table XX.

We may note that the percentage of acute deaths (22 % by the 2—4 days' standard—40 % by the 2—5 days') is considerably less than in the series of fresh material. It must be admitted, however, that the percentages have been calculated on small numbers. It is possible that the discrepancy is explicable on the view that a small amount of *B. pestis* was inoculated on account of the presence of other organisms,

or it may be that the virulence of the plague bacillus is diminished by association with putrefactive organisms. The latter view is not, however, supported by the circumstances that very putrid material containing very few *B. pestis* killed some of the inoculated animals with the acute disease.

TABLE XX.

Death in days	Number of guinea-pigs which died
Died 2 days	0
,, 3 ,,	2
,, 4 ,,	4
,, 5 ,,	5
,, 6 ,,	3
,, 7 ,,	4
,, 8 ,,	3
,, 9 ,,	1
Killed after 9 days	4
Killed before 9 days	1

Again it has to be remarked that inoculation by this method apparently failed to produce infection in only three cases out of 27 putrid rats presenting appearances strongly suggestive of plague, *i.e.* it apparently fails in 10 % of putrid rats.

Some writers have stated that rats suspected of being plague-infected have come under their observation which were too putrid to permit of bacteriological examination. It would seem, however, from our experience at least, that the cutaneous method gives an excellent chance of diagnosing plague even in rats far advanced in putrefaction. In support of this statement we would refer to the account given above of an experiment in which 12 experimentally infected rats dead of plague were allowed to putrefy. Although in all these rats the putrefactive changes were very marked, yet no fewer than seven out of 12 gave plague to guinea-pigs when inoculated cutaneously.

Infections through the skin by organisms other than plague.

Considering the frequency with which material of all degrees of putrefaction was rubbed into the skin, it is remarkable how seldom infection by other organisms resulted. The only instances we observed are set forth in Table XXI. It is worthy of note that guinea-pigs 1, 2, and 5 continued to gain weight after the inoculation.

TABLE XXI.

Serial No.	P.M. of rat	Microscopical examination of inoculation material	P.M. of guinea-pig	Microscopical examination of guinea-pig	Cultural tests
1	Putrid; no buboes	Many putrefactive organisms, some like *B. pestis*	Killed by $CHCl_3$ 15 days after inoculation. P.M. small left inguinal gland not congested. Spleen not much enlarged, a few gray nodules	Spleen } = 0 Heart-blood }	Culture from bubo gave an organism which corresponded to *B. enteritidis* (Gaertner)
2	Very putrid. Maggoty. Many enlarged glands	Many putrefactive organisms, a few like *B. pestis*	Killed by $CHCl_3$ in 10 days. Abscess, at site of inoculation. Right inguinal bubo. Spleen decidedly enlarged. Some small nodules scattered through lungs	Nothing definite	Pure culture of cocci
3	Putrid. Congested glands in left axilla; enlarged gland in inguinal region	Numerous putrefactive organisms	Killed by $CHCl_3$ in 9 days; circumscribed abscess size of walnut at site of inoculation, no other pathological changes	Pus—a few slender bipolar bacilli. Spleen = 0	Culture on agar of pus gave growth very like plague. A smear showed cocci (?). Culture inoculated subcutaneously. Killed a guinea-pig in 2 days with a septicaemia; spleen not enlarged, but crowded with very small diplo-bacilli
4	Very putrid; small glands in axillary and inguinal region	Many putrefactive, some like *B. pestis*	Killed by $CHCl_3$ in 11 days, open sore on skin with moderate infiltration at site of inoculation. No buboes—no signs of plague. Spleen small		
5	(Live *decumanus*.) Left femoral abscess, organs congested	Doubtful	Died in 6 days. Large purulent local reaction; no buboes—intestines congested; spleen small	Organisms in spleen, not plague bacilli	

On the comparative value of the cutaneous method in the diagnosis of rats suspected of being plague-infected.

It would appear, from the experiment summarised in Table XVI, that when virulent plague bacilli unmixed with other organisms are inoculated into a guinea-pig, the subcutaneous method is more certain in producing infection than the cutaneous when a small dose is given. In actual practice, however, the chief difficulty arises from the admixture of other organisms in the material used for inoculation. At the commencement of our work in the routine examination of rats, we made emulsions of the organs and injected them subcutaneously into guinea-pigs. Of seven guinea-pigs thus inoculated, three died of a septicaemia due to contaminating organisms. This method had therefore to be abandoned.

With regard to the chances of success by the cutaneous method, we have already mentioned incidentally that the inoculation appeared to fail in the case of three rats only (excluding putrid rats) out of a total of 300 examined, in other words, the method failed in only 1 % of the tests. If putrid rats be included, the method failed in 2 % of the total experiments. In putrid rats the method appears to fail in 10 % of the cases. It must be pointed out however, in qualification of this statement, that the strong suspicion of plague in these cases rested solely upon our opinion of the naked-eye and microscopical appearances of the rats, and that this in turn was the outcome of an experience in the diagnosis of plague rats from post-mortem appearances which was, at the period of examination, comparatively small.

In addition to the subcutaneous and cutaneous methods, others have been recommended, *e.g.* the conjunctival, nasal, and the "Hauttasche" methods. At present we cannot speak from personal experience of these procedures, but the ease with which the cutaneous method can be carried out would seem to give it an advantage over the others.

Pseudo-tuberculosis has never been encountered in our stock of guinea-pigs in Bombay. An epizootic of this disease, or even the occurrence of sporadic cases, would seriously complicate the use of the method for diagnostic purposes. In such an event two plans might be adopted. Either white rats might be used instead of guinea-pigs, or the guinea-pigs should be isolated in separate cages and the weight of each should be taken daily for at least a week before being used for experiment. The weighing should, of course, be continued after the test. The only disease resembling plague which has been observed by us in Bombay as a natural infection in guinea-pigs, is one caused apparently by an

organism belonging to the *B. enteritidis* (Gaertner) group. The disease chiefly affects young guinea-pigs in the monsoon months, viz. July to November. The lesions in the organs bear a remarkable resemblance to those found in plague guinea-pigs and, even on microscopical examination, the bacilli appear as bipolar organisms almost indistinguishable from *B. pestis*. Cultural tests, however, render the diagnosis a matter of no difficulty, the causative organism differing in every essential respect from the plague bacillus.

A possible limitation to the employment of the cutaneous test is the occurrence in fresh plague material of avirulent bacilli. Our experience —a not inconsiderable one—leads us to believe that this must be an exceedingly rare event, if, indeed, it ever happens. We have never isolated from an animal a strain of plague bacillus, the first remove of which can be styled "avirulent," that is, one which fails to kill guinea-pigs by injection of massive doses. We have worked with several such strains, but in every instance the culture has been either one isolated from an old broth culture, or one which has been frequently subcultivated outside the body. Originally these cultures were fully virulent.

IX. Concluding Remarks.

Although the data brought forward in this paper do not lend themselves to being condensed into the form of a summary, yet the facts which appear to us to be of principal interest may be briefly recalled.

It has been shown that plague rats, like human cases, may be divided into two classes, according as to whether or not a bubo is present. The bubo, if present, is the most important diagnostic sign of plague.

Of other characteristic appearances, those occurring in the liver of plague-infected rats have been described in detail, since they are of primary importance from the point of view of diagnosis. Haemorrhages in various parts of the body are commonly met with, and an abundant clear pleural effusion constitutes, when present, a noteworthy sign of plague in the rat.

Analyses of the results of microscopical examination of 1200 plague rats are set forth chiefly in the form of tables. It is apparent from these that the bubo gives the best chance of recognising plague bacilli in large numbers. Not only so, but the value of the bubo as an aid in the microscopical diagnosis of plague is increased by the presence in at least 50 % of those examined of the characteristic involution forms.

Reference has been made to the occurrence of plague-like bacilli or of plague-like diseases in rats. We can only reiterate the statement that in Bombay no difficulty of this kind has been experienced.

The relative value for diagnosis of the macroscopical and microscopical methods of diagnosis has been discussed. The results of tests carried out for the purpose of comparison make it manifest that the naked-eye is markedly superior to the microscopical method as an aid in diagnosis, and as the result of our experience we are prepared to make a diagnosis of plague on the strength of the macroscopical appearances alone, even though the other results of cutaneous inoculation and culture are negative and the animal shows marked signs of putrefaction.

The value of the method of cutaneous inoculation of guinea-pigs has been examined: it would appear to fail only in about 2 % of fresh and about 10 % of putrid rats.

The bacilli found in naturally infected rats are fully virulent: 62 % of the inoculated animals die of acute plague in five days or less.

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