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REPORT TO THE LOCAL GOVERNMENT BOARD ON AN ENQUIRY INTO RAT PLAGUE IN EAST ANGLIA DURING THE PERIOD JULY—OCTOBER, 1911[1].

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(With Map.)

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Introduction.

This enquiry was conducted in accordance with the instructions of the Board, who directed that attention should first be confined to the area where plague-infected rats had previously been found and that, if such rats were again discovered, an endeavour should then be made to determine the limits of the infection. The area shown by previous investigations to have been infected comprised the rural districts of Samford and Woodbridge, the urban districts of Woodbridge and of Felixstowe and Walton, and the borough of Ipswich. The present enquiry commenced on July 1st, and was continued until October 31st, 1911.

Organisation.

Police Assistance in Rat Collection.

The Board obtained, through the Home Office, the assistance of Captain Mayne, Chief Constable of East Suffolk, throughout the enquiry. Captain Mayne issued instructions to the following effect:

(1) The police officers throughout East Suffolk were to make confidential enquiries and then to report answers to the following questions: (*a*) Are rats known to be dying in any part of the officer's district? (*b*) If so, in what parishes and on whose premises? (*c*) What is the supposed cause of death? (*d*) Has any sort of rat poison or virus been recently laid down? (*e*) Any other information?

(2) The officers in charge of every police station in that part of East Suffolk selected by the Board for the collection of rats were requested (*a*) to ascertain, by local enquiry, in what parts of their districts rats were likely to be found and to inform the Board's rat-catchers accordingly, (*b*) to receive at the police station all rats brought in by these rat-catchers, (*c*) to urge all residents in their districts to

catch rats and bring them in to the police station, and to pay, on behalf of the Board, 2*d.* per rat, (*d*) to disinfect all rats brought in, (*e*) to see that a disc bearing a number was attached to each rat, (*f*) to fill in for each rat a card of identification, bearing specified particulars, and (*g*) to forward the rats and the cards to the Municipal Laboratory at Ipswich.

When it was found necessary to extend the area of enquiry into portions of West Suffolk and the north of Essex, similar assistance was obtained, through the Home Office, from the Chief Constables of West Suffolk, Essex, and Colchester.

Arrangements by Mr Huddart.

Mr Huddart, Assistant Inspector to the Board, (1) made all arrangements with the Chief Constables and their superintendents for the work of the police, (2) engaged rat-catchers and assigned them work week by week, (3) obtained and distributed all material (disinfectants, cards, boxes, rat-traps, etc.) required by the police and the rat-catchers, (4) visited all the police stations and explained to the constables what was required, (5) made periodical tours of inspection throughout the enquiry for the purposes of supervising the rat-catchers and keeping in touch with the work of the police, and (6) took charge of the clerical work in connection with the enquiry.

The Ipswich Laboratory.

On the recommendation of Dr Pringle, Medical Officer of Health for Ipswich, the Council of that Borough placed their Municipal Laboratory gratuitously at the disposal of the Board throughout the enquiry. All rats were received and dissected in this laboratory by Drs G. H. Macalister and R. St John Brooks, who were engaged by the Board from July 1st to October 31st for the performance of this work. The routine clerical work was conducted in a room adjacent to but separate from the laboratory by Mr Leach, a temporary clerk, who worked under the direction of Mr Huddart.

The Board's Pathological Laboratory.

In the case of any rats which on post-mortem examination did not appear to Drs Macalister and Brooks to be free from suspicion of plague, the liver, spleen, and any other suspected material from each of these animals were sent to the Board's laboratory for diagnosis. They were

there examined microscopically, culturally, and by animal inoculation. Dr Klein acted as consultant in cases where it was thought that the diagnosis needed his confirmation.

Routine.

When a rat was caught by one of the Board's rat-catchers he attached to it with string a perforated circular metal disc bearing a stamped number. This number and the place where the rat was caught he recorded in a note-book. It was to his interest to keep these records accurately, as his weekly claims for payment were based on them. He caught his rats with the aid of ferrets, dogs, and traps, but was not allowed to use poison. In addition to the rats so obtained he added to his collection any dead rats he could find; these latter were labelled and recorded in the same way as the caught rats, and, in addition, a piece of coloured tape was tied to each as a distinctive mark.

He brought in his day's bag, in which he was not permitted to include any rats less than half-grown, to the nearest police station. There the constable disinfected them in 2 per cent. lysol and entered on a separate card for each rat the following particulars: (1) the number on the metal disc (termed "local number"); (2) the date; (3) name of catcher; (4) parish where caught or found; (5) exact place where caught or found; (6) whether caught or found dead; (7) whether the occupier had previously laid down virus; (8) if so, when and what kind.

When rats were brought in by voluntary workers, the constable affixed to each a disc bearing a "local number," tied coloured tape to any found dead, and then dealt with them in the same way as with those brought in by the professionals.

The constable packed the rats in tin boxes, each of which was enclosed within an outer wooden box, and forwarded them, generally by carrier, to the Ipswich laboratory. The cards bearing details of each rat were sent to the laboratory by post.

At Ipswich the cards were received in the clerk's room, the rat-boxes in the laboratory.

When the examination of each rat was completed, the circular disc bearing the local number was removed by cutting the string attaching it to the rat, and a square disc bearing a serial number was attached by wire to the circular disc. The serial numbers ran in sequence from 1 upwards, and registered the total number of rats received in the laboratory. The pairs of circular and square discs were collected in two lots,

one bearing the numbers of the rats found free from plague, the other bearing the numbers of those reserved for further examination. After being sterilised, these batches of discs were sent up to the clerk's room. No articles of any sort which might have been in contact with infective material were sent up to the clerk's room without being sterilised.

On receiving the pairs of discs, the clerk identified each card by its local number and copied on to it the corresponding serial number. Each rat was then entered up, according to its serial number, as "normal" (*i.e.* free from plague) or "reserved" (*i.e.* specimens sent to the Board's laboratory for examination). At the end of each day's work the clerk drew up a detailed statement of all the rats examined during the day, giving the names of the parishes from which the rats were received, the numbers of rats received from each, the serial numbers of all "normal" and all "reserved" rats and of all rats found dead, and, in the case of each "reserved" rat and of every rat found dead, the exact address where it was obtained. One copy of this information was posted each evening to the Board's laboratory and another to Mr Huddart.

In the case of each "reserved" rat Drs Macalister and Brooks sent to the Board's laboratory the liver, spleen and any other material considered suspicious, together with a full account of the post-mortem appearances of the rat, including microscopic evidence obtained from smear preparations. This material and information, and also the clerk's daily record, were received the next morning at the Board's laboratory. The specimens were there investigated microscopically, culturally, and by animal inoculation. When a case of plague was fully established, the Board were at once notified of the fact and of the exact place whence the rat was obtained. At the same time Mr Huddart was asked to stop further supplies from the parish whence the rat was obtained, to notify the occupier of the premises where the rat was found of the presence of rat-plague, to warn him of the danger, and to advise him to take energetic measures to destroy any rats on his premises.

BACTERIOLOGICAL DIAGNOSIS IN THE BOARD'S LABORATORY.

Methods.

The routine method of staining film preparations from tissues, fluids, or cultures was as follows: After drying in the air and then fixing for two minutes in absolute alcohol, the smears were stained, as

recommended by Dr Klein, with Czinzinski's mixture of methylene blue and eosin. The formula for this mixture is:

Methylene blue (conc. aqueous solution) ..	50 c.c.
Eosin (soluble in alcohol)	·5 gm.
Alcohol (absolute)	70 c.c.
Water (distilled)	130 c.c.

The stain was applied for at least five minutes, and at the beginning and the end of this period was warmed over the flame until steam rose. The specimens were then washed in water, dried, and mounted in Canada balsam.

For the isolation of cultures slanted agar tubes and agar plates were employed, the medium consisting of ordinary nutrient agar made with beef broth, reaction + 10 to phenolphthalein. The surface of the agar was fairly dry. In the earlier part of the investigation some use was made of MacConkey's neutral-red, bile salt medium, with the addition of mannite or lactose. It was found, however, by comparative experiments with plague-infected tissue, that growth on a bile salt medium was less certain and slower than on agar, and that the bile salt had some inhibitory effect, fewer plague colonies being produced than on ordinary nutrient agar plates inoculated with the same dose of the same material. Again, the fact that intestinal bacteria, the growth of which was not inhibited by bile salt, were commonly present in such contaminated and decomposed tissues as had to be dealt with rendered bile salt preparations of no great advantage as selective media for isolating plague bacilli from a mixture of organisms.

For culture work the spleen was usually taken. Cultures were also made from other material which, after microscopic examination, seemed suitable. After the surface of the spleen, or other organ, had been well seared, a portion of the interior was cut out with a sterile knife and placed on the first plate or culture tube. After this material had been rubbed over the surface two or three more plates or tubes were inoculated from it in series. The temperature of incubation was 30° C.

In addition to the cultural investigation, the specimens in nearly every case were tested by inoculation upon white rats or guinea-pigs or both. Animals were also inoculated, when thought necessary, with cultures or with such material from the first experimental animals as required further investigation. The inoculation was usually made subcutaneously, but in some cases cutaneously.

In the case of the specimens sent from Ipswich for diagnosis the

spleen and liver were usually selected for inoculation. After the surfaces of these organs had been thoroughly seared, portions of the interior were transferred to a small test tube, well rubbed up with a glass rod and then emulsified with normal saline. Other material, such as lymphatic glands, pleural effusion or blood, were also used when thought requisite.

No case was diagnosed as positive until a typical plague culture and typical plague infection of an inoculated animal had been obtained. With the exception of 12 cases where animal inoculation was not considered necessary, no case was settled as negative until it was proved that the material was incapable of producing plague in an experimental animal.

Enquiry was also made into the action of the plague bacilli, after isolation in pure culture, upon various carbohydrates and allied substances and into their virulence, in measured dosage of culture, for experimental animals. Broth cultures showing typical stalactites were also obtained with several of the viruses.

Results.

Out of the 15,332 rats which were dissected at the Ipswich laboratory by Drs Macalister and Brooks, specimens from 151 were sent to the Board's laboratory for diagnosis. They were there examined by the methods described above.

Thirty-five of the rats were found to be plague infected, the remaining 116 being proved negative as regards plague.

ANALYSIS OF RESULTS IN POSITIVE CASES.

Preliminary Data.

Out of the 35 rats proved to be plague infected, 21 had been caught and killed, the remaining 14 had been found dead.

At the time of the post-mortem examination the condition, as regards preservation, of 30 of the above rats was described by Drs Macalister and Brooks as either " good " or " fair " ; the remaining five were either " bad " or " putrid."

Accompanying the selected material submitted to the Board's laboratory for diagnosis, full details were sent of the post-mortem and microscopic appearances which Drs Macalister and Brooks found.

These data may be classified as follows :

Group I.—In this group, to which 28 of the positive cases belong, the appearances were either typical of plague or very strongly suggestive

of it. The predominant features were marked subcutaneous congestion and general distribution in the tissues of bacilli, usually very numerous, which were morphologically identical with *B. pestis*. A good example is Rat 2876, which was described in the following detail: *Macroscopically:* The right inguinal gland was the size of a pea, with a necrotic centre and thick walls; the pelvic glands were enlarged; subcutaneous congestion was intense in the region of the right inguinal gland and was very well marked all over the trunk; no haemorrhages were seen; there was some clear pleural effusion; the liver was large, firm, generally pale, mottled, and showed numerous punctate necrotic foci; the spleen was large and firm, with well-marked granulation. *Microscopically:* A few bacilli, with some involution forms, were found in the necrotic centre and in the walls of the right inguinal gland; some bipolar bacilli were seen in the right pelvic gland; no organisms were found in the pleural effusion; scanty bipolar bacilli were found in the liver and spleen; a few plague-like bacilli were found in the heart's blood.

This case proved to be, in most respects, typical of the group. But in the remaining 27, though glandular enlargement and congestion were usually found, necrosis of a lymph gland only occurred once, and plague-like bacilli were numerous in the organs in 22 cases. Other differences which occurred in some of these 27 cases were minor and were not sufficiently marked to justify classification in a separate group.

Twelve of these 28 cases were rats which had been found dead; three of the rats were found at the post-mortem examination to be "bad" or "putrid."

Group II.—To this group seven positive cases belong. In general, the macroscopic and microscopic data cannot be regarded as affording more than slight suspicion of plague. Some bipolar bacilli were found in each case, but subcutaneous congestion was only marked in one case and was absent altogether in three. Rat 1030 belongs to this group. As this was the first case in the present enquiry which turned out to be positive, the full description of its post-mortem and microscopic appearance is of particular interest. *Macroscopically:* there was some enlargement of the submaxillary glands; there was neither subcutaneous congestion, haemorrhage, nor pleural effusion; the liver showed punctate, white necrotic foci; in the spleen there were remains of a white infarct. *Microscopically:* numerous bipolar bacilli were found in the liver and heart's blood. This is the only case, among the seven, in which bipolar bacilli were recorded as numerous; in one case, in which the tissues were decomposed, "mixed organisms" including bipolar bacilli were

found; in the remaining five cases bipolar bacilli were stated to be scanty.

Two of these seven cases were rats which had been found dead; two of the rats were found on post-mortem examination to be "bad" or "putrid."

Results of Culture Work.

Isolation of the plague bacillus by culture.

In 29 out of the 35 positive cases *Bacillus pestis* was obtained by direct culture of the original material, and in many cases without admixture of any contaminating organisms. Cultures were obtained from the spleen in 26 of these cases; in the remaining 3 they were obtained from the liver; additional cultures were obtained in 8 cases from livers and in 2 cases from lymphatic glands.

In the remaining 6 out of the 35 cases direct cultures from the original material failed. In three instances they were overgrown; in two other cases, where bile salt media alone were used, no plague colonies appeared; in the sixth case no culture was attempted, as the original material was very putrid.

In each of the above six cases cultures of *B. pestis* were obtained from the animals inoculated with the original material.

Characteristics of the plague cultures.

The following were the main characteristics of the cultures, which were obtained by the methods described above (p. 289).

On examination the next day, within 24 hours after inoculation on agar, plague colonies were usually visible with the aid of a hand lens as minute transparent points; when the material inoculated contained numerous plague bacilli, a thin growth over the surface could be seen by the naked eye. The colonies, transparent at first, soon became denser and then exhibited a delicate but characteristic ground-glass appearance; as the colonies grew older they became completely opaque. The typical plague colony, two to three days old, was greyish-white, with a smooth, shining surface; the centre of the colony was slightly raised, the margin thin, grey, translucent and irregular; in this margin minute colonies sometimes developed. The adhesive nature of the culture was an important characteristic, to which special attention has been called by Dr Klein. This peculiarity, which developed early when incubation took place at 30° C., was readily demonstrated by touching

the growth with the platinum needle ; on gently raising the needle, the culture adhered to it and was drawn out in the form of a long thread.

In subculture on agar slants, and also in primary culture from tissues rich in plague bacilli without admixture of other organisms, the surface became covered with a continuous greyish-white layer, exhibiting the characteristic stickiness when touched with the platinum needle. Not uncommonly discrete, dense colonies of plague bacilli grew up above the rest of the surface of a pure culture.

As the cultural results obtained on agar were unequivocal and distinctive and were corroborated in every instance by the production of typical plague in an experimental animal, no further culture work was considered necessary for the purpose of diagnosis.

Incidentally, a few experiments were made on the production of stalactites in broth covered with a thin layer of butter fat and kept at room temperature. Out of the six strains, selected at random, which were tested five produced typical stalactites, the sixth failed to do so.

Fermentation tests.

For the purpose of a comparative study of plague bacilli with other organisms which are often differentiated from each other by means of fermentation tests, each of the 35 plague viruses was submitted to a series of these tests.

To ordinary peptone water, made with tap water and coloured with 10 per cent. litmus solution, ·5 per cent. of one of the following chemically pure carbohydrates or other compounds was added : glucose, fructose, galactose, maltose, lactose, saccharose, raffinose, iso-dulcite, glycerin, mannite, dulcite, adonite, inulin, and salicin. Each of these media was put up in Durham's fermentation tubes. Litmus milk and malachite green peptone water were also used as test media.

With all 35 viruses acid, but no gas, was produced in—glucose, fructose, galactose, maltose, mannite, adonite, and salicin. The reactions with the last two were slow, salicin taking about 6 days and adonite about 12 days.

With all 35 viruses no change of reaction was produced in—lactose, saccharose, raffinose, dulcite, glycerin, inulin, and litmus milk. The period of observation, in each case, was 28 days. During this period no appreciable change was noted in the malachite green medium, but after about 5 weeks' incubation many strains slightly decolourised this medium.

With 4 of the viruses acid was formed, in 20 days, with iso-dulcite; with the other 31 viruses during this period no change was produced with the compound, but in the case of two of these 31 acid was formed after a further incubation of 20 days.

For the purpose of control, all the above tests were performed also with two previously authenticated strains of plague bacilli; for one of these we are indebted to Dr Klein, for the other to Dr Rowland, of the Lister Institute. These two strains produced no change in iso-dulcite; in all the other tests they corresponded exactly to the 35 strains mentioned above.

Results of Animal Experiments.

Inoculation of rats with plague-infected tissues.

With the exception of a few cases where the cutaneous method of inoculation was adopted, the material was inoculated subcutaneously in the right groin.

The day after inoculation the rats usually looked ill, with staring coat, and local tenderness and swelling could be felt on palpation. They rapidly became worse, dyspnoea being marked, and died in from 2 to 8 days; death usually occurred between the second and the fourth day. In every case subcutaneous inoculation produced a fatal result, a tumour being formed at the site of inoculation.

The local lesion was invariably found to be necrotic and oedematous. It was surrounded by an area of congestion which was often intense. There were general redness and vascular engorgement throughout the ventral subcutaneous tissues. These features were constant and particularly conspicuous.

The inguinal and iliac glands were usually enlarged and often congested and necrosed, the extent of the lesions being proportionate to the duration of the animal's life. The axillary and submaxillary glands were sometimes enlarged and congested.

In several cases the spleen did not appear abnormal in size, colour, or consistency. In others it was enlarged, firm, and dry on section. In one rat, which lived for 8 days, the spleen was peppered with grey foci.

In animals which died in from 2 to 3 days the liver generally showed nothing more than congestion. In later cases it showed either small, irregular patches of necrosis or minute irregular grey foci.

Tabular Statement of Results.

Details of Cultural Results and Animal Inoculations in the Diagnosis of the 35 Positive Cases.

* The Roman numerals refer to the grouping on pp. 291 and 292.

Number and Condition of Rats		Results of cultures from original material, as regards growth of plague bacilli	Results of animal experiments			
Number	*Condition		Material inoculated	Animals	Duration of life	Post-mortem-results
1030	II	Discrete colonies from liver on mannite bile salt	Liver	Rat 30	Died, 3 days	Acute plague. Bacilli numerous in liver, spleen, and blood.
			Spleen	G.P. 507	Died, 5 days	Acute plague. Bacilli abundant in spleen.
1264	II (*putrid*)	Cultures, on bile salt media, failed	Liver	Rat 31	Died, 4 days	Acute plague. Bacilli numerous in spleen.
			Liver	Rat 32	Died, 6 days	Acute plague. Bacilli numerous in liver, spleen, and blood. Cultures from Rats 31 and 32 typical of plague.
1691	I (*found dead*)	Cultures, on bile salt media, failed	Liver	Rat 36	Died, 3 days	Acute plague. Bacilli numerous in spleen and liver.
			Liver	Rat 37	Died, 3 days	Acute plague. Bacilli (not numerous) in spleen and liver.
			Spleen	G.P. 514	Died, 8 days	Typical plague. Bacilli fairly numerous in spleen. Cultures from Rat 37 typical of plague.
1694	I (*found dead*)	Discrete colonies from liver on lactose bile salt	Liver	Rat 38	Died, 3 days	Acute plague. Bacilli fairly numerous in liver and a few in spleen.
			Liver	Rat 39	Died, 3 days	Acute plague. Bacilli numerous in liver and spleen.
			Spleen	G.P. 515	Killed, 16 days	Healthy. Cultures from Rat 38 typical of plague.
1769	I	Discrete colonies from liver and spleen on mannite bile salt	Liver	Rat 40	Died, 2 days	Necrotic local lesion; much oedema, spreading over thoracic wall; nothing definite in organs. Two bipolar bacilli seen in spleen, none in liver.
			Pleural fluid	Rat 41	Died, 6 days	Typical plague. A few bacilli in spleen, fairly numerous in liver.
			Spleen	G.P. 540	Died, 6 days	Typical plague. Bacilli numerous in spleen. Cultures from Rat 40 typical of plague.
2064	II	Pure culture from spleen on agar slants	Liver	Rat 51	Died, 4 days	Acute plague. Bacilli rather scanty in spleen.
			Liver	G.P. 561	Died, 4 days	Acute plague. Bacilli abundant in spleen.

2156	II (*found dead*)	Pure cultures from spleen and liver on agar slants	Liver	Rat 52	Died, 5 days	Acute plague. A few bacilli in spleen.
			Liver	Rat 53	Died, 3 days	Acute plague. Bacilli rather scanty in spleen.
			Liver	G.P. 566	Died, 6 days	Acute plague. Bacilli abundant in spleen.
2522	II (*putrid; found dead*)	No culture attempted ...	Spleen and Liver	Rat 72 (*cutaneous*)	Killed, 13 days	Healthy.
			Spleen and Liver	Rat 73 (*cutaneous*)	Died, 6 days	Acute plague. Bacilli scanty in spleen, many in axillary gland. Cultures from Rat 73 typical of plague.
2876	I (*found dead*)	Discrete colonies from inguinal bubo on mannite bile salt slants. Abundant colonies on agar slants from spleen	Spleen	Rat 94	Died, 3 days	Acute plague. Bacilli moderately numerous in spleen.
			Liver	G.P. 652	Died, 3 days	Acute plague. Bacilli abundant in spleen.
3346	I (*found dead*)	Overgrown (liver and spleen)...	Spleen and Liver	Rat 116	Died, 3 days	Acute plague. Bacilli moderately numerous in spleen.
			Spleen and Liver	Rat 117	Died, 4 days	Soft caseous nodule at site of inoculation, without congestion; spleen mottled with greyish necrotic patches; two similar patches in liver. A few bipolar bacilli in spleen. Cultures from liver and spleen of Rat 116 typical of plague. Cultures from spleen of Rat 117 overgrown with coliform growth.
3430	I (*found dead*)	Pure cultures from spleen on agar slants; cultures almost pure from liver on same medium	Spleen and Liver	Rat 118	Died, 2 days	Acute plague. Bacilli moderately numerous in spleen.
			Spleen and Liver	Rat 119	Died, 3 days	Acute plague. No bacilli seen in spleen, fairly numerous in iliac gland. Pure cultures from spleen and liver.
4849	I	Numerous colonies in pure culture from spleen and almost pure from liver—on agar slants	Spleen and Liver	Rat 143	Died, 5 days	Acute plague. Bacilli moderately numerous in spleen and axillary gland.
				G.P. 706	Died, 10 days	Plague lesions well marked. Bacilli in spleen.
5381	I	Pure culture from spleen on agar slants	Spleen and Liver	Rat 144	Died, 8 days	Purulent sinus at site of inoculation, slight injection of subcutaneous vessels; right inguinal gland enlarged; liver and spleen peppered with irregular grey foci. No bipolar bacilli seen in spleen; two seen in liver. Coliform colonies from spleen but no plague colonies.
			Spleen and Liver	G.P. 743	Died, 6 days	Acute plague. Bacilli abundant in spleen.

Tabular Statement of Results (*continued*).

Number and Condition of Rats		Results of cultures from original material, as regards growth of plague bacilli	Results of animal experiments			
Number	*Condition		Material inoculated	Animals	Duration of life	Post-mortem results
5382	I (*found dead*)	Discrete colonies from liver on one agar slant. Three other tubes overgrown	Liver	G.P. 744	Died, 4 days	Acute plague. Bacilli abundant in spleen. Pure culture isolated from spleen.
7643	I	Abundant growth on agar slants from spleen and liver, almost pure	Spleen and Liver	Rat 157	Died, 2 days	Acute plague. Bacilli moderately numerous in spleen, from which a pure culture was obtained.
8086	I (*found dead*)	Discrete colonies on agar slants from spleen	Spleen and Liver	Rat 160	Died, 3 days	Acute plague. Bacilli rare in spleen, moderately numerous in iliac gland.
10169	I	Pure culture on agar slants from spleen	Spleen and Liver	Rat 169	Died, 2 days	Acute plague. Bacilli moderately numerous in spleen, abundant in iliac gland.
10840	I (*found dead*)	Pure culture on agar slants from spleen	Spleen and Liver	Rat 175	Died, 2 days	Necrosis at site of inoculation, with oedema but no congestion; slight enlargement of iliac gland; spleen firm; liver lobules outlined. Numerous bacilli in iliac gland.
10848	I	Discrete colonies on agar slants from spleen and liver	Spleen and Liver	Rat 176	Died, 4 days	Acute plague. Bacilli scanty in spleen, moderately numerous in iliac gland. Pure culture obtained from spleen.
11407	II (*bad*)	Discrete colonies on agar slants from spleen; contaminating organisms numerous	Spleen and Liver	Rat 180	Died, 4 days	Oedematous necrotic tissue at seat of inoculation, without congestion; iliac gland enlarged; spleen firm; liver peppered with irregular grey foci. One doubtful bacillus seen in spleen. Cultures from liver and spleen overgrown. G.P. 876, inoculated from the liver, died in 5 days from acute plague.
11724	I (*found dead*)	Pure cultures on agar slants from spleen	Spleen and Liver	Rat 184	Died, 4 days	Acute plague. No bacilli seen in spleen, but pure culture was obtained from it.
12012	I	Abundant, pure culture on agar slants from spleen	Spleen and Liver	Rat 189	Died, 3 days	Acute plague. Abundant bacilli in iliac gland.
12013	I	Abundant, pure culture on agar slants from spleen	Spleen and Liver	Rat 190	Died, 3 days	Acute plague. Abundant bacilli in iliac gland.
12245	I	Overgrown	Spleen and Liver	Rat 192	Died, 3 days	Very marked local congestion, necrosis, and oedema; organs apparently normal. Bacilli scanty in spleen, abundant in local lesion. Culture isolated from spleen. Rat 197, fed with organs of Rat 192, died in 5 days from plague, with submaxillary bubo and abundant bacilli in spleen.

13663	I (*found dead*)	Pure culture on agar slants from spleen	Spleen and Liver	Rat 199	Died, 3 days	Acute plague. Bacilli abundant in iliac gland and numerous in spleen.
13712	I	Pure culture on agar slants from spleen	Spleen and Liver	Rat 200	Died, 2 days	Highly congested, necrotic local lesion; slight general subcutaneous congestion; iliac gland slightly enlarged; spleen small and rather firm. Bacilli abundant in iliac gland, a few in spleen.
13713	I	Confluent pure culture on agar slants from spleen	Spleen and Liver	Rat 201	Died, 3 days	Acute plague. Bacilli moderately numerous in spleen and abundant in iliac gland. Culture obtained from spleen.
13817	I	Confluent pure culture on agar slants from spleen	Spleen	Rat 202	Died, 2 days	Acute plague. Bacilli abundant in iliac gland and spleen.
14499	I	On agar slants pure confluent growth from lymphatic gland,	Spleen and Liver	Rat 209	Died, 3 days	Acute plague. Bacilli moderately numerous in spleen, abundant in iliac gland.
		discrete colonies from spleen and liver	Gland	G.P. 897	Died, 6 days	Acute plague. Bacilli abundant in spleen.
14816	II	Discrete colonies from spleen on agar slants	Spleen	Rat 220	Died, 4 days	Slightly congested necrotic local lesion; nothing else abnormal. No bacilli seen in smear from spleen. Pure culture of plague from spleen.
14854	I	Pure cultures from liver and spleen on agar slants	Spleen	Rat 221	Died, 3 days	Acute plague. Bacilli moderately numerous in spleen.
14855	I	Discrete colonies from spleen on agar slants	Spleen	G.P. 902	Died, 5 days	Acute plague. Bacilli abundant in spleen.
14911	I (*found dead*)	Pure cultures on agar slants from liver and spleen	Spleen	G.P. 909	Died, 4 days	Acute plague. Bacilli abundant in spleen.
15079	I	Discrete colonies from spleen on agar slants	Spleen and Liver	Rat 230	Died, 4 days	Purulent nodule at site of inoculation; general subcutaneous congestion; necrotic foci in iliac gland. Two bacilli found in smear from iliac gland.
15219	I	Discrete colonies from spleen on agar slants	Spleen	Rat 229	Died, 2 days	Acute plague. Bacilli moderately numerous in inguinal gland.

The lungs were frequently oedematous and congested, but no pleural effusion was found.

Plague bacilli were almost invariably numerous in the local lesion and the nearest glands, being usually irregular in size and shape and often showing involution forms. In the spleen they were often scanty, especially when the animal died early with marked local reaction. In some cases the bacilli were abundantly disseminated throughout the body and in the blood.

Inoculation of guinea-pigs with plague-infected tissues.

The material was inoculated subcutaneously in the right groin, in the same manner as with the rats.

The day after inoculation it was usually found that the animals were quiet and that local tenderness and some swelling could be detected on palpation. Day by day the animals rapidly became more ill and lost flesh, whilst the local swelling increased in size. At death there was usually a firm, prominent swelling and the right hind leg was drawn up. Death occurred between the third and the tenth day; in the majority of cases it was between the fifth and the sixth day.

At the site of inoculation there was found in the subcutaneous tissue an infiltrating tumour which, on section, was seen to be centrally necrotic and to be surrounded by an area of intense congestion and oedema. The size of the tumour varied, being usually larger in the animals which lived longer; the largest was the size of a pigeon's egg and was found in a guinea-pig which had lived for ten days after inoculation.

Subcutaneous congestion and oedema extended for a considerable distance beyond the local lesion, with engorgement of the subcutaneous capillaries and veins; but the oedema was never found to spread completely over the ventral subcutaneous tissues.

The inguinal and iliac lymphatic glands were enlarged and congested, particularly in the cortex; their interior usually showed extensive necrosis.

The spleen was usually enlarged, congested, and either mottled with pale, irregular necrotic areas or studded with discrete, soft, grey nodules ranging in size up to 1 mm. in diameter. In cases of very acute infection ending fatally in about three days the spleen remained small and did not exhibit macroscopic lesions.

The liver, as a rule, was enlarged; sometimes it showed nodules similar to those found in the spleen; in other cases it was peppered with

irregular, grey foci. In cases which terminated fatally in three days no lesions were visible.

The lungs were usually congested and oedematous, sometimes with patches of consolidation. They were frequently studded with soft, greyish-white nodules with a congested periphery. The largest of these nodules were found in the less rapidly fatal cases; in one animal, which had lived for ten days, they attained the diameter of 5–6 mm. Pleural effusion was found sometimes.

In almost every instance the tissues of the animals contained enormous numbers of plague bacilli; in smears from the spleen or the lung nodules this feature was particularly noticeable.

Inoculation of rats and guinea-pigs with plague cultures.

In rats inoculated with culture, the oedema, congestion, and necrosis of the local lesion, the general subcutaneous congestion, and the enlargement, congestion, and necrosis of the inguinal and iliac glands were all features which corresponded closely with the results obtained by the inoculations of infected tissues. As compared with tissue emulsions, pure cultures produced less macroscopic change in the spleen and liver; the spleen, though sometimes enlarged and often firm and dark on section, did not show foci of necrosis; the liver was generally normal in appearance, though occasionally showing a few grey foci. The lungs were generally oedematous and sometimes congested; in a small proportion of the cases (less than a quarter) there was a little pleural exudate.

In guinea-pigs culture inoculations produced very much the same effects as emulsions of tissues.

The Virulence of the Plague Strains.

In the earlier part of the enquiry the cultures isolated from each case of rat plague were tested for virulence and were compared in this respect with two previously authenticated strains of plague bacilli received respectively from Dr Klein and from Dr Rowland at the Elstree Laboratory. It was found that pure cultures of all the strains were of high, and of about equally high, virulence for rats and guinea-pigs. It was not considered necessary to continue these culture tests systematically throughout the enquiry because in no instance was a virus obtained which, when inoculated in the form of a tissue emulsion, afforded any indication of being lower in virulence than the viruses already tested by inoculation both of cultures and of tissue emulsions.

The estimation of dosage was made by weighing on a chemical balance a platinum loop charged with culture and then subtracting the weight, previously ascertained, of the platinum loop. The weighed culture was finely emulsified with the requisite amount of normal saline solution to afford a convenient volumetric measure of dosage.

Most of the inoculations were made subcutaneously, but virulence for rats was also tested by the cutaneous method, one or two loopfuls of dilute emulsion of culture being smeared over the shaved and lightly scarified skin at the root of the tail.

The results of these virulence tests are tabulated below.

Virulence of the Plague Cultures for Rats inoculated subcutaneously.

Number or name of virus	Dose inoculated, in mgms.	Number of rat inoculated	Duration of life, in days
1264	·1	46	3
1030	·1	48	5
1694	·01	42	3
1691	·01	44	3
1769	·01	57	5
2064	·01	58	4
"Klein"	·01	60	3
"Elstree"	·001	62	4
2156	·001	131	3
3346	·001	133	3
3430	·001	135	4
4849	·0001	150	[remained healthy]
4849	·00001	151	5

Virulence of the Plague Cultures for Guinea-pigs inoculated subcutaneously.

Number or name of virus	Dose inoculated, in mgms.	Number of guinea-pig inoculated	Duration of life, in days
1030	1	559	4
1030	·1	560	5
1694	·1	554	5
1691	·1	556	6
1264	·1	557	6
1769	·1	591	3
2064	·1	593	5
"Klein"	·1	595	5
1691	·01	555	5
1264	·01	558	4
1769	·01	592	5
2064	·01	594	3
"Klein"	·01	596	7
"Elstree"	·01	597	4
"Elstree"	·001	598	5
2156	·001	689	13
3346	·001	690	8
3430	·001	692	8
4849	·0001	760	[killed, 18 days; local disease only]
4849	·00001	761	8

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Virulence of the Plague Cultures for Rats inoculated cutaneously.

Number or name of virus	Number of loopfuls of dilute culture emulsion used	Number of rat	Duration of life, in days
1694	1	43	6
1691	1	45	[killed, 15 days; healthy]
1264	2	47	4
1030	?	50	5
1769	1	56	4
2064	1	59	3
"Klein"	1	61	4
"Elstree"	1	63	4
2156	1	132	5
3346	1	134	3
3430	1	136	5
5381	2	152	4
11407	?	174	[killed, 24 days; healthy]
14816	?	231	3

ANALYSIS OF RESULTS IN NEGATIVE CASES.

Preliminary Data.

Out of the 116 cases which were under more or less definite suspicion at the post-mortem examination but proved negative when further investigation was made at the Board's laboratory, 105 had been caught and killed; the remaining 11 had been found dead.

At the time of the post-mortem examination the condition, as regards preservation, of 93 of the above rats was described by Drs Macalister and Brooks as either "good" or "fair"; the remaining 23 were either "bad" or "putrid."

The post-mortem and microscopic details furnished from the Ipswich laboratory may be classified as follows:

Group I.—Five cases fall within this group and resemble the positive cases found in Group I (p. 291) in so far as their descriptions are strongly suggestive of plague. Subcutaneous congestion was present in all and was very marked in one. In four cases bipolar bacilli were numerous and generally distributed; in the fifth they were generally distributed in small numbers. With the exception of one liver, necrotic or granular changes are described in the livers and spleens of all the cases. Pleural effusion was found in every case and glandular enlargement in all but one. One rat (4843) was in bad condition and one (10,510) was found dead.

Tabular Statement.

Details of the 33 Negative Cases where Lesions were produced in Experimental Animals.

* The Roman numerals refer to the grouping on pp. 303, 307.

† "*B. Gärtner*," throughout this table, means a bacillus belonging to the Gärtner group.

Number and Condition of Rats		Results of cultures from original material	Results of animal experiments	
Number	*Condition		Animals	Results
544	III	Discrete colonies from spleen and blood	G.P. 501	Died, 6 days; only slight local reaction, necrotic foci in liver, spleen apparently normal. *B. coli* isolated from spleen.
711	II	Discrete colonies from spleen and liver. *B. Gärtner* isolated from six	G.P. 504	Died, 2 days; septic cellulitis. †*B. Gärtner* isolated from blood and spleen.
			Rat 27	Killed, 5 days; necrosis at site of inoculation, spleen peppered with minute, irregular, grey foci. *B. Gärtner* isolated.
1444	III	Discrete colonies from spleen, liver, and blood	G.P. 513	Died, 1 day; acute infection. *B. coli* isolated from inguinal gland.
			Rat 33	Killed, 7 days; like Rat 27. *B. Gärtner* isolated from spleen.
2353	II	Discrete colonies from pleural fluid. A coccus isolated	G.P. 611	Died, 3 days; necrotic local lesion and subcutaneous congestion. *B. coli* isolated.
			Rat 66 (*cutaneous*)	Killed, 6 days; healthy.
			Rat 67	Killed, 6 days; healthy.
2891	II (*putrid*)	Overgrown	G.P. 656	Died, 1 day; septic cellulitis.
			G.P. 657	Died, 3 days; septic cellulitis. Culture of colon type isolated from spleen.
3023	II	Discrete colonies from spleen. Adhesive culture isolated which fermented saccharose		Rat 113 was inoculated cutaneously with culture isolated from original material.
				Died, 7 days; grey foci in liver. *B. Gärtner* isolated from liver.
			G.P. 658	Died, 1 day; intense oedema and subcutaneous congestion. Culture isolated was inoculated into 2 guinea-pigs which died in 1 and 3 days from acute disease unlike plague, and into 2 rats which remained healthy.
			G.P. 659	Died, 2 days; intense oedema and subcutaneous congestion.
				From each G.P. a culture similar to the one from original material

3030	II	Discrete colonies from spleen. A coccus isolated	Rat 106	Killed, 8 days: no local lesion, grey foci in liver and spleen. *B. Gärtner* isolated.
3051	II	Discrete colonies from spleen. A coccus isolated	Rat 108	Killed, 8 days; purulent nodule at site of inoculation, grey foci in liver and spleen. *B. Gärtner* isolated.
3052	II	Discrete colonies from spleen and liver. A coccus isolated	Rat 109	Killed, 8 days; grey foci in liver and spleen. *B. Gärtner* isolated.
3053	II	Discrete colonies. A coccus isolated	Rat 110	Ditto ditto ditto.
3056	III (*putrid*)	Discrete colonies from liver. A coccus isolated	Rat 111	Ditto ditto ditto.
3138	II	Discrete colonies from spleen and liver	Rat 112	Died, 9 days; no lesions beyond slight local congestion at site of inoculation; colon-like bacillus isolated from liver.
3495	III	Discrete colonies from liver ...	Rat 126	Died, 2 days; slight local congestion only. Some bipolar bacilli in spleen, from which colon-like culture was obtained.
3615	III	Discrete colonies from gland at root of penis, yielding *B. coli*	Rat 127	Died, 3 days; no local reaction, grey foci in spleen. *B. Gärtner* isolated.
3649	II	Discrete colonies from gland at root of penis. A coccus isolated	Rat 128	Killed, 13 days; local abscess, spleen speckled with grey foci. *B. Gärtner* isolated.
3762	III	Overgrown	G.P. 687	Died, 2 days; septic cellulitis; microscopically, no plague-like bacilli.
4187	III	Overgrown	G.P. 697	Killed, 13 days; normal except 2 yellow foci in spleen. Motile bacilli isolated from spleen.
4242	II	Discrete colonies from liver ...	G.P. 696	Died, 3 days; acute cellulitis; no organisms found in spleen.
			G.P. 695	Killed, 13 days; healthy.
4659	II (*putrid*)	Discrete colonies from spleen ...	Rat 141	Died, 2 days; subcutaneous oedema, necrosis and congestion. Bipolar bacilli found in spleen and iliac gland. Saccharose fermenter, isolated from spleen, inoculated in doses of 1 mg. into Rat 149 and G.P. 759; both animals killed, 18 days,—healthy.
6427	II (*putrid*)	Discrete colonies from spleen ...	G.P. 770	Died, 8 days: small abscess only.
8284	II	Discrete colonies from spleen ...	Rat 163	Killed, 12 days; no local lesion; grey foci in liver and spleen; no bipolar bacilli in spleen.

Number and Condition of Rats		Results of cultures from original material	Results of animal experiments	
Number	*Condition		Animals	Results
8439	II (*found dead*)	Discrete colonies from spleen ...	Rat 162	Died, 2 days; slight necrosis at site of inoculation, many bipolar bacilli; a few in spleen. Colon-like colonies were isolated from local lesion and spleen of Rat 162. Rat 166, inoculated from local lesion of Rat 162, was killed in 9 days and found healthy.
10150	I (*found dead*)	Overgrown	Rat 168	Died, 4 days; local necrosis and oedema, a few grey foci in liver; coliform organism isolated.
10549	II	Discrete colonies from liver	Rat 173	Killed, 7 days; no local lesion; grey foci in liver and spleen; no bipolar bacilli in spleen.
10678	III	Discrete colonies from spleen and liver	Rat 174	Died, 7 days; slight necrosis, but no congestion at site of inoculation; grey foci in liver and spleen. *B. Gärtner* isolated.
12194	II (*bad*)	Overgrown	Rat 191	Died, 6 days; local abscess, few grey foci in liver. *B. Gärtner* isolated.
12520	III	Discrete colonies from spleen ...	Rat 193	Killed, 8 days; slight local reaction; grey foci in liver and spleen. *B. Gärtner* isolated.
12919	III (*bad*)	Discrete colonies from spleen ...	Rat 195	Killed, 7 days; slight local reaction; grey foci in liver and spleen. *B. Gärtner* isolated.
14638	III (*bad*)	Discrete colonies from spleen ...	Rat 214	Killed, 8 days; no local reaction; grey foci in spleen and liver.
14790	II	Discrete colonies from spleen, yielding *B. Gärtner*	Rat 217	Died, 6 days; local necrotic nodule, grey foci in liver and spleen; colon-like culture from spleen.
14792	II	Overgrown	Rat 218	Died, 7 days; necrosis at site of inoculation, congestion without oedema; culture from spleen unlike plague.
14793	II	Overgrown	Rat 219	Died, 5 days; small necrotic nodule at site of inoculation, no subcutaneous congestion, spleen firm, irregular grey foci in liver. Culture from liver coliform.
15064	III	Discrete colonies from spleen ...	Rat 224	Killed, 7 days; no local lesion, grey foci in liver and spleen.

Group II.—Sixty-one of the negative cases fall into this group. They resemble the positive cases in Group II (p. 292) in that the macroscopic and microscopic data afford slight, but no more than slight, suspicion of plague. The following are the main general facts concerning these 61 cases:

Macroscopically:—Subcutaneous congestion was present in 39 cases but absent in 22; pleural effusion was present in 42 but absent in 19; mottling or necrotic foci were present in 30 livers but absent in 31; granulation or necrotic foci were present in 26 spleens but absent in 35; enlargement of lymphatic glands was seen in the majority of cases.

Microscopically:—In each case bipolar bacilli were found in some part of the body, though usually they were not numerous; in 54 cases the liver or spleen, or both organs, were examined, and in 53 of these cases bipolar bacilli were found in one or both of these organs.

In eight cases there were found—subcutaneous congestion, bipolar bacilli in the liver and spleen, pleural effusion, and the above-mentioned changes in the liver and spleen.

Eight of the 61 rats had been found dead; 13 were "bad" or "putrid" when dissected.

Group III.—This group comprises 50 cases, each presenting some element of doubt at the time of the post-mortem examination, though it was obvious that further investigation was needed before plague infection could be proved or even definitely suspected.

The following are the main features of these cases:

Macroscopically:—Subcutaneous congestion was present in 18 but absent in 32; pleural effusion was present in 21 but absent in 29; liver changes were present in 25 but absent in 25; spleen abnormalities were present in 21 but absent in 29; enlargement of lymphatic glands was noted in all but 12.

Microscopically:—Bipolar bacilli were only found once in the liver or spleen; they were present in other regions of the body in 33 cases; in 16 cases no bipolar bacilli were found in any part of the body.

Two of the above 50 cases had been found dead; 9 were "bad" or "putrid" when dissected.

Results of Cultures and Animal Experiments.

Cultures were usually made from the spleen; under special circumstances the liver, a lymphatic gland, pleural exudate, or other material was also used.

Cultures were attempted from the original material of all except four of the cases which turned out to be negative, these four being omitted because the material was too putrid to give any prospect of success. In 20 cases the agar media inoculated remained sterile; in 16 the primary cultures were overgrown, so that it was impossible to decide whether or no they contained plague bacilli; and in 76 discrete colonies were obtained, which rendered it possible to decide, either directly or after subculture of any doubtful colonies, that no growth of plague bacilli had been obtained.

The animals inoculated with original material remained healthy in 71 out of the 116 negative cases, and after being kept alive for a sufficient period (from one to two weeks) were killed, and proved by post-mortem examination to be free from disease.

In 12 cases it was not thought necessary to inoculate animals with original material, and in these the microscopic and cultural evidence appeared conclusive. In 2 of these cases the culture media inoculated remained sterile; in 8 discrete colonies, unlike plague, were obtained; and in 1, though the agar cultures were overgrown, cultures on bile salt media yielded discrete colonies but failed to develop any resembling plague.

In the twelfth case, Rat 818, a culture from the submaxillary gland, though more luxuriant than plague, was somewhat sticky in character; it was therefore tested on a guinea-pig and a rat, but failed to produce any disease. It was also observed that the culture fermented raffinose and saccharose.

In 33 cases the original material inoculated produced, in one or more of the animals, lesions, sometimes fatal, which were distinguished from plague either directly by macroscopic and microscopic examination or after further investigation, including the raising of cultures and, where necessary, animal inoculation.

Bacteria of some Special Interest.

Bacilli belonging to the Gärtner group.

An organism belonging to this group was isolated sixteen times, thrice from the original material and in thirteen instances from rats inoculated with original material. A feature of these cases was the occurrence in the tissues of bipolar staining bacilli closely resembling the plague bacillus.

The organisms gave the following reactions. Acid and gas were produced in glucose, galactose, fructose, mannite, and dulcite. In maltose acid was produced, sometimes with and sometimes without gas. In glycerin and iso-dulcite acid was produced but no gas. No change of reaction occurred in lactose, saccharose, raffinose, inulin, or salicin. Litmus milk became acid at first, but afterwards changed to alkaline. The above test media were kept under observation in every instance for at least 18 days.

Several other cases, in addition to the above 16, produced in experimental rats lesions typical of Gärtner infection, but it was not thought necessary to investigate these culturally.

A cocco-bacillus from a rabbit.

In addition to the rats' tissues, specimens from one rabbit were received for examination as being under suspicion of plague.

The animal had been found dead. The post-mortem report stated that there was subcutaneous congestion, a small and granular spleen, a congested liver with necrotic patches, and copious pleural effusion; numerous bipolar bacilli were seen in the liver and the heart's blood, and a few in the spleen.

From the tissues forwarded for examination, the liver and spleen yielded on agar slants pure cultures of a cocco-bacillus; the colonies presented a resemblance to those of plague in their general appearance, but were more translucent; the cultures grew more readily than those of plague and were not sticky.

Rat 212 was killed 13 days after inoculation with an emulsion of the liver of the rabbit. There was an ulcer at the site of inoculation, but no glandular enlargement; with the exception of a few areas of congestion in the lungs, the organs were normal.

G.P. 900 died one day after inoculation with an emulsion of the rabbit's spleen. The local lesion was deeply congested and oedematous, and there was oedema of the adjacent subcutaneous tissue. There was slight excess of peritoneal fluid, but all the internal organs appeared normal. Swarms of very small bipolar bacilli were found in the local lesion, and a few short, thick bacilli in the spleen and blood. A culture from the local lesion proved identical with those obtained from the tissues of the original rabbit.

Rabbit 13 died 3 days after cutaneous inoculation in the ear with a minute dose of culture from the original rabbit's spleen. Post-

mortem, there were some thickening and bluish redness of the ear; the spleen and liver were both covered with a thin fibrinous layer which was easily removed; irregular, greyish foci were then found in the substance of each organ; the lungs were congested and oedematous. In the spleen small bacilli were abundant; in the blood small bacilli with well-marked bipolar staining were numerous.

The cultural characteristics of the organism were as follows: it was non-motile, Gram-negative, and formed a deposit in peptone water without producing turbidity. It formed acid, but no gas, in media containing respectively—glucose, galactose, fructose, saccharose, mannite, and glycerin. It produced no change of reaction in media containing—maltose, lactose, raffinose, iso-dulcite, dulcite, adonite, salicin, or inulin; no change was produced in litmus milk or malachite green.

From the above cultural and animal tests, the organism was evidently the bacillus of rabbit septicaemia.

Colonies bearing some resemblance to plague.

In 19 of the cases which proved to be negative, the primary cultures contained colonies which, during the first two days, bore some resemblance to plague colonies. They became visible within the first twenty-four hours after inoculation as small, almost transparent points. Subsequently their development was slow; they remained small and transparent, but gradually became slightly raised in the centre. Subcultures grew in the form of discrete colonies, and never produced more than a very thin and transparent layer. After the first two days, therefore, there was no difficulty in distinguishing these growths from plague. As a matter of subsidiary interest, however, they received further investigation.

In every case examined the colonies consisted of a Gram-positive coccus with a tendency to assume slightly elongated forms which might perhaps be called very short bacilli.

The organism, when tested after being in culture for a considerable period, was not pathogenic for mice, no more than a small, purulent focus being formed at the site of inoculation. One strain inoculated subcutaneously into a rat and a guinea-pig failed to produce any disease.

In seven cases the coccus produced acid and clot in litmus milk, and acid, without gas, in glucose, galactose, fructose, maltose, saccharose, lactose, mannite, dextrin, and salicin. No change of reaction was obtained in raffinose, dulcite, or inulin. In five other cases the organism was a somewhat less active fermenter, saccharose being unattacked in

one case, mannite and salicin in a second, mannite and lactose in a third, and mannite, lactose and salicin in a fourth. In the fifth case the coccus had no action on litmus milk, and failed to attack lactose, saccharose, mannite and salicin.

It is doubtful whether these cocci were responsible for any pathogenic changes in the rats from which they had been obtained, and it is noteworthy, in this connection, that all the experimental rats inoculated with the original material remained healthy. They were all from cases showing marked pleural effusion, but there is no evidence that the cocci were responsible for this condition.

EVIDENCE AS TO DISTRIBUTION AND APPARENT LIMITATION OF INFECTION.

The Area from which Rats were collected.

The area investigated is shown on the accompanying map, prepared from data furnished by Mr Huddart.

At the beginning of the enquiry attention was confined to the borough of Ipswich and its environment, the rural districts of Samford and Woodbridge, and the urban districts of Woodbridge and of Felixstowe and Walton, this being the area where plague rats had previously been found.

Six rats obtained during the latter half of July from the parishes of Sutton, Bawdsey, and Trimley St Mary, in Woodbridge rural district, and from the parish of Bentley, in Samford rural district, proved to be plague infected.

As soon as these cases were fully established, steps were taken to extend the area of enquiry, whilst continuing the search for infected localities in the original area. The enlarged area comprised a larger field in East Suffolk, a small portion of West Suffolk, and a strip of Essex adjacent to the south of Suffolk. Various reasons determined the actual demarcation of the area. To the north it included certain parishes from which reports had been received in 1910 of rats being found dead in unusual numbers; to the north-west it included the whole of Bosmere and Claydon rural district, which had not been searched during the Board's enquiry in the beginning of the year 1911; to the west it extended far enough to include a parish in which a plague-infected hare had been found in 1910; and to the south it took in a fairly wide strip of land on the right bank of the Stour, including a parish where a plague-infected hare had been found in 1910.

Comparison with the Area previously investigated.

The present investigation has been especially directed to the whole of the area which was not examined during the rat enquiry conducted for the Board during the period January 16th to February 14th, 1911[1].

The area referred to consists of the rural districts of Cosford, Samford, Bosmere and Claydon, and Woodbridge, the urban districts of Hadleigh, Woodbridge, and Felixstowe and Walton, and the borough of Ipswich.

In addition to comprising this area, the present enquiry has extended a short distance to the north and to the south. (*See* Map.)

The result of the former enquiry was that no plague-infected rats were found in a wide peripheral zone surrounding the area omitted from that investigation. In the present enquiry plague-infected rats have been found only in this last-mentioned area, and only in a limited portion thereof, which corresponds closely to, but is slightly more limited than, the area proved to be infected with rat-plague before the former enquiry was instituted.

The Supply of Rats.

During July and August, the first two months of the present enquiry, rats were, as was anticipated, particularly difficult to obtain. They were dispersed in the open country amongst the standing crops and densely overgrown ditches and hedgerows ; and during harvest farmers and their assistants were too busy to spare time for ratting.

There was, however, an important reason for commencing the present enquiry at the beginning of July. Rat-plague usually tends to expand from sporadic outbreaks into epizootic proportions at the beginning of autumn ; therefore it was particularly desirable to locate early cases, in order that preventive measures might be directed to the foci where these were found, before the time arrived when rapid spread of the infection was likely to occur.

This anticipation was justified by results. The first rat (No. 1030) which proved to be plague-infected was caught on July 17th, and by the end of that month infected rats had been secured from five different foci. The Board at once advised the local authorities concerned, and the Board's investigators, as soon as a case was diagnosed, visited the farm or other premises where the case occurred, warned the occupier of the danger, and advised thorough and persistent rat destruction.

[1] Reports to Local Government Board on Public Health and Medical Subjects. New Series, No. 52.

This procedure was maintained throughout the enquiry, whenever fresh cases of rat-plague were detected.

The abnormally hot and dry weather experienced throughout July and August 1911 made rat-catching particularly difficult; at the same time it had the advantage of reducing to some extent the normal proliferation amongst rats, owing to their difficulties in obtaining the moisture requisite for their own subsistence and for suckling their young. Another reason for their scarcity was that a large number of farmers throughout the area investigated had been systematically killing rats throughout the year.

In September and October the area of enquiry had been extended, the harvest was over, the rats were beginning to come back to food supplies near farm buildings, and the farmers and their men had more time to interest themselves in ratting. For these reasons there was then no difficulty about obtaining a daily supply of as many rats as could be dealt with in the Ipswich laboratory; in fact, prompt measures had to be taken to reduce the inflow, which towards the end of September became excessive.

For many reasons the numbers of rats taken from each parish (*see* Map) varied considerably. Parishes differ greatly in size and in the number of farms which they contain; the rat population is very irregularly distributed; and in some districts the farmers are much more enterprising in rat destruction than in others. Special attention was paid to parishes which were under suspicion last year owing either to the discovery of rat-plague or to local reports of excessive mortality amongst rodents. In some of these parishes no plague could be discovered, although every effort was made to obtain for examination as many rats as could be found.

As soon as a positive case was diagnosed, further supplies were stopped from the parish whence the rat had been obtained, as it was the object of the enquiry to determine the limits of the infection rather than to ascertain how many cases of infection could be discovered in particular areas; and, owing to the migratory habits of rats, the discovery of one infected pocket was considered sufficient to stigmatise its environment, within a radius of several miles, as possibly plague-infected.

Although special search for dead rats was invited and the same price was paid for these as for rats which had been caught, out of the total 15,332 rats only 69 dead rats were brought in. During the September and October of the previous year (1910) dead rats were reported in large

numbers, particularly within or near to localities where the existence of rat-plague had been proved. All the known pockets where rat-plague had previously been found were re-examined several times during the present enquiry, but in only a few cases were fresh cases of the disease discovered.

ACKNOWLEDGMENTS.

Dr Klein, F.R.S., has given us valuable assistance in the diagnosis of rat-plague, and, before the commencement of the enquiry, Dr C. J. Martin, F.R.S., and Dr Rowland kindly allowed us to make post-mortem examinations of a large number of rats which had died from experimental inoculation with plague bacilli.

The Lister Institute kindly seconded Dr G. H. Macalister, who had already gained extensive experience in the post-mortem examination of rats, to act as one of our assistants throughout the enquiry. He and his colleague, Dr Brooks, though engaged upon work which was extremely unpleasant, tiring, and of long duration, always exhibited remarkable skill in the selection of the material which they submitted to us for diagnosis.

Dr Pringle, Medical Officer of Health for Ipswich, was most helpful in facilitating arrangements at the Ipswich laboratory, the use of which for the purpose of the enquiry must, we fear, have caused him personal inconvenience in the prosecution of his own bacteriological work. Dr Heath, then Medical Officer of Health for East Suffolk, gave us invaluable advice and assistance throughout the enquiry by placing at our disposal his experience of rat-plague in the district which we were investigating. Dr Hollis, Medical Officer of Health for Woodbridge rural district, and Dr Redpath, Medical Officer of Health for Woodbridge urban district, also took great interest in the enquiry and assisted us in the examination of areas likely to prove plague infected.

The Chief Constables of East Suffolk, West Suffolk, Essex, and Colchester gave us every possible facility for the collection of rats through the aid of the police; and the highly efficient organisation of their forces is responsible in large measure for the maintenance of a satisfactory supply of rats throughout the enquiry. The individual constables undertook the extra work which devolved on them with willingness and enthusiasm, and spared neither time nor trouble in obtaining supplies of rats from the farms on their beats. Special thanks are due to Captain Mayne, Chief Constable of East Suffolk, and his

assistant, Superintendent Staunton, for their keen personal interest in the enquiry and their skill in organisation.

Throughout the four months of the enquiry Mr Gordon Merriman worked daily with the rat-catchers for the purpose of collecting and investigating the fleas from the freshly killed animals. His practical advice, based on the local knowledge thus gained and on his personal experience of ratting, added much to the efficiency of our control over the daily work of the rat-catchers.

SUMMARY.

During the period July 1st—October 31st, 1911, 15,332 rats were examined for plague infection. These rats were obtained (*see* Map facing p. 311) partly from the area which in the previous year had been found to be infected with rat-plague and partly from the districts immediately adjacent to this area.

As a result of the present enquiry 27 farms or other premises were found to harbour plague-infected rats.

These 27 places are all within the area previously pronounced to be infected. No plague-infected rats have been discovered outside this area.

Experience of rat collection during the present enquiry showed that the rat destruction which had been maintained by local enterprise since the end of 1910 had in many localities appreciably diminished the rat population.