XIII. TRANSMISSION OF PLAGUE BY FEEDING RATS WITH INFECTED MATERIAL.

Introduction.

During the last ten years, in which plague has been experimentally investigated, much work has been done with the view of settling the questions (1) as to whether it was possible to produce the disease in animals by feeding, and (2) as to whether this method of transmission played an important part in the epizootic or epidemic spread of the disease.

The Indian Plague Commission (1901) pointed out that while three workers in India, namely Gibson, Hankin and Simond, had failed to infect rats by means of feeding, both the German and Austrian Plague Commissions had been able to give the disease to animals by this method. They concluded that, while it was possible to produce plague by feeding, this method was probably not a common one of infection in nature. The German Plague Commission (1899) found that plague could be conveyed to rats by feeding them on plague cultures or on the bodies of rats dead of plague. They thought that in a state of nature rats generally became infected in this way, and that the infective material might penetrate into the system through the mucous membrane of the throat or nose and not through the mucous membrane of the alimentary canal. The Austrian Plague Commission (1898), experimenting with gray rats, also found that infection could easily be given *per os*. They never saw any post-mortem appearances to justify an inference that the primary infection had been through the mucous membrane of the stomach. If large amounts of material were used, or if the stomach tube was employed, then intestinal plague was produced. They succeeded, however, in producing in various animals a primary neck bubo, pointing to the entrance of the plague bacillus through the mucous membrane of the mouth.

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Kolle (1901) fed 60 white rats partly with bread soaked in cultures and partly with the organs of plague-infected rats. Of 48 successful infections, 40 had primary buboes in the submaxillary region, four showed a plague pneumonia, two had mesenteric buboes, and two showed foci of infection in the small intestine. The intestinal lesions fell principally on Peyer's patches, which were enlarged, haemorrhagic and frequently showed a necrotic centre. In consequence of these observations Kolle formed the opinion that plague spread naturally among rats by their gnawing the corpses of their dead plague-infected comrades.

Kister and Schumacher (1905) did a large number of feeding experiments. They found that only 50 % of rats fed developed the disease: that if spicules of bone were present in the feeding material the bubo was cervical, but that if soft food was given, intestinal plague resulted. The infected rats died as a rule on the 3rd to 5th day although some lived as long as the 8th, 9th, or 11th day. They attempted to do a series of passages by this method of infection, but failed after the third passage. They thought such a failure might explain the diminution of the natural epizootic. These workers further showed that the natural resistance of rats seemed to be heightened by several feedings with plague material, as a considerable number of animals which had been fed with large amounts over a long period of time were resistant to large subcutaneous injections of virulent culture.

Berestneff (1906) fed white rats on the livers and spleens of septicaemic plague-infected guinea-pigs. There were 12 successful infections, nine rats dying between the 3rd and 6th days. One had a bubo in the neck, another in the inguinal region, while the remaining ten showed the intestinal form of plague. In all these cases mesenteric buboes were present, while they all showed multiple lesions of the lymphatic apparatus of the small intestine. Peyer's patches were swollen and showed as prominent swellings from outside: there were punctiform haemorrhages on the serous surfaces, while the mucous surfaces were inflamed and haemorrhagic. In the caecum of one animal which died on the 7th day there were ulcers with infiltrated and haemorrhagic edges, the mesenteric glands being enlarged and haemorrhagic.

Klein (1906), who had previously failed (with rare exceptions) to infect animals by feeding with fresh plague material, has recently succeeded in giving the disease to rats, mice and guinea-pigs by feeding them with dried gelatine cultures and with dried organs of

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plague-infected animals, either alone or mixed with rice and wheat. In every instance there were present enlargement and congestion of the mesenteric glands, with definite pathological changes in the intestines, the lesions being especially marked in and around Peyer's patches. The infected animals died between the 4th and 6th day.

Methods of observation.

In our present observations we have fed a large number of wild rats with plague-infected material. With the exception of the Punjab experiments, no distinction was made between the species of rat used, but, as a matter of fact, the great majority were *Mus rattus*. In the first and much the larger series the rats were procured from the Health Department of the Municipality of Bombay. They had been caught in traps placed in houses and gullies in the city. Before being used for our experiments they were kept isolated for some time in order to make sure that they were not already plague-infected. With a view of testing the relative susceptibility of wild Bombay rats to plague-infection by feeding in comparison with other wild rats, we did a second small series of experiments, using for this purpose rats which were caught on board ships in the harbour of Bombay and which had presumably not been subjected to an epizootic of plague.

The material used for feeding in these two series was the same. The internal organs of a rat or a guinea-pig, dead of acute plague, were minced up into more or less of a pulp. This pulp was then mixed thoroughly with the food, which consisted entirely of parched Indian corn, known in India as "laya." About 1/6 or 1/10 part of the internal organs of one animal, in which were included lungs, heart, liver, spleen, kidneys and sometimes intestines, was used for each feeding. Care was taken that no spicules of bone were present. Each rat only received one meal of this plague-infected material, so that the period of death after infection could be accurately determined.

Cultures were taken and animal experiments made from all those rats which showed any post-mortem appearances, however slight, pointing to their being plague-infected. It was possible, in this way, accurately to separate those rats which died from plague from those which died from some other cause. All rats which still survived at the end of three weeks were killed and examined post-mortem. This examination revealed the fact that a few animals, which to all appearances were well and healthy, were really plague-infected. A reference will be made to this group later on.

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A third series of experiments was made in the Punjab. As *Mus decumanus* is not found in the villages of this Province, *Mus rattus* was used throughout. The material employed was the livers and spleens of rats and guinea-pigs which had been artificially infected with plague or the same organs of plague-infected rats found dead in the villages. Only one feeding was given, the amount of material being as a rule half a liver and spleen to each animal, but in the case when the infected rat weighed under 100 grammes the whole liver and spleen were used. The amount of material given to these rats was, therefore, considerably greater than in the case of the Bombay rats.

The material used for feeding in all three series of observations was soft material mixed with the ordinary food of the rats. In order to get over a possible objection that feeding with such material might cause different post-mortem lesions or a different distribution of the primary bubo to feeding with material containing bone, such as would occur in nature, a fourth series of experiments was carried out. In this series wild rats caught in the city of Bombay (no distinction being made between *M. rattus* and *M. decumanus*) were kept without food for 24 hours. They were then supplied with the carcases of fresh plague-infected rats which had been found dead in the city, one carcase being given to every two rats. A partial post-mortem examination of each carcase, sufficient for the diagnosis of plague, had been made beforehand. In this way the conditions obtaining in nature were imitated as far as was possible.

Results.

In Table I are summarised the general results of the experiments.

TABLE I.

	Series	No. of rats observed throughout	Number which died of plague on each day								No. found plague infected when killed on 21st day	Percentage of rats which became plague infected
			2nd	3rd	4th	5th	6th	7th	8th-21st	Total		
I.	Bombay rats	415	1	25	26	14	9	4	4	83	6	21·4
II.	Ship rats	41	1	4	6	3	1	1	1	17	1	43·9
III.	Punjab rats	28	3	11	4	1	0	0	0	19	0	67·9
IV.	Bombay rats fed on whole carcases	108								36	5	38·0
	Total	592								155		26·2

From these figures it appears that the ship rats are twice as susceptible as the Bombay rats, both series receiving the same quantity

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of infected food. It is interesting to note that the same difference in susceptibility between these two classes of rat was found in a series of experiments in which infected fleas were the agents by which plague was transmitted. Of the Punjab rats three times as many were infected as of the Bombay rats, and all died on or before the fifth day. The larger percentage of positive results and the more acute form of the disease are probably largely accounted for by the larger amount of infected material given to the rats. The same consideration may well account for great increase of infections in Series IV compared with Series I.

Analysis of the post-mortem examinations of the rats which died of plague.

We have already described in detail the post-mortem appearances which are found in rats which have died from plague in nature. With the exception of two very important points, namely, the distribution of the primary bubo and the condition of the intestinal tract, the pathological lesions seen in our present series were similar to those found in rats naturally infected. Thus subcutaneous congestion was commonly observed, but only in a very few instances (2 %) were subcutaneous haemorrhages present. This relative absence of such haemorrhages is a point worthy of note, as in rats naturally infected they were present in 40 % examined. The liver showed all the changes which we have already described under the terms "fatty," "mottled," and "granular." The spleen was sometimes enlarged and granular, but its appearance was by no means constant. The kidneys and suprarenal capsules were often found congested. In the thorax the lungs sometimes showed a patchy congestion and even punctiform haemorrhages, but there is no record of a lobar pneumonia having been detected. Pleural effusion of the same clear type as previously described was a very characteristic feature. As regards the microscopical examination of the tissues, plague bacilli were always found in the buboes in very large numbers; in the great majority of cases they were also present in the spleen, while the blood showed organisms in 88 % of the cases. The microscopical examination of the tissues, in fact, yielded results exactly the same as those obtained in the naturally infected rats which we have already described.

The above description refers equally well to the 36 animals which died of plague as a result of feeding on the whole carcases of plague infected rats.

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Distribution of the primary bubo.

The first great difference between the post-mortem lesions of the rats of these series and those of naturally infected rats is to be found in the distribution of the primary bubo. We have already defined what we mean by a primary bubo and the various appearances which it may present. The same description applies to the bubo found in our present experiments. When, however, we come to analyse the distribution of the bubo, it is seen (*vide* Table II) that in rats infected by feeding by far the commonest situation is the mesentery. In the series fed on soft material, of 109 animals which had buboes, the mesenteric glands were involved in 79 instances (= 72 %), and the cervical glands in 39 (36 %). In the series of rats (Table III) infected by feeding on bony material, namely, the carcases of plague-infected rats, we find that mesenteric buboes occurred in 76 % of 33 animals which had buboes, and cervical buboes in 31 %. This is in marked contrast to the results obtained in the examination of naturally infected plague rats in Bombay. In a series of 4000 rats (see above, p. 329) no mesenteric buboes were found, nor were any seen in a further series of 1000 which were especially examined with this point in view: on the other hand, 72 % of the animals with buboes had cervical buboes, which occurred only in 32 % of those infected by feeding.

TABLE II.

Animals fed on soft material (viscera).

Total number of rats dead of plague	No bubo	Single bubo		Multiple buboes	
119	10 (8·4 %)	98 (82·4 %)		11 (9·2 %)	
		Mesenteric	Submaxillary	Submaxillary and Mesenteric	Axillary and Inguinal
		69 (70·4 %)	29 (29·6 %)	10 (91 %)	1 (9 %)

72·5 % of animals with buboes had a primary mesenteric bubo.

TABLE III.

Animals fed on whole carcases (including bones).

Total number of rats dead of plague	No bubo	Single bubo		Multiple buboes
36	3 (8·3 %)	30 (83·4 %)		3 (8·3 %)
		Mesenteric	Submaxillary	Mesenteric + Submaxillary
		22 (73·3 %)	8 (26·6 %)	3 (100 %)

75·8 % of animals with buboes had a primary mesenteric bubo.

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Condition of intestinal tract.

We have now to record the second striking difference in the post-mortem lesions between naturally infected rats and those infected by feeding. In the case of naturally infected rats we have already noted (p. 333) that the stomach and intestines show no characteristic change. An analysis of the records of all the rats infected by feeding shows that haemorrhages in the stomach wall were present in 3 % of the cases, and that the intestines were markedly congested in about 27 %. It was also noted that in about a third of the cases (31 %) Peyer's patches were markedly enlarged, showing prominently on the serous surface, and congested and haemorrhagic on the mucous surface, which was very often ulcerated. Smears of these ulcers examined microscopically showed abundant plague-like bacilli.

Rats found plague-infected on being killed on the 21st day.

Eleven rats come under this category. They appeared quite well and healthy, but on being killed and examined post-mortem showed undoubted signs of being plague-infected. In five instances there was a submaxillary bubo, in three cases the bubo was in the mesentery, while in one instance a bubo was present in the axilla. The liver was slightly granular in many of the cases. While a microscopical examination of spleen and blood smears failed to reveal the presence of the *B. pestis* in any instance, the bubo almost always yielded a specimen showing a few bacilli. From two of the rats the plague bacillus was isolated by culture or animal test. It would appear, then, that these animals had all suffered from a slight attack of plague, and that they were all tending towards a complete recovery.

In no instance did we come across any pathological condition similar to that which we have described elsewhere as "chronic plague" (vol. VI. p. 530, and below, under Paper XIX, p. 457). It will be seen that with the exception of the two cases in which the organism was isolated, the diagnosis of plague rested on the naked-eye appearances, which in the hands of an expert we have shown elsewhere to be quite reliable.

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Bombay rats fed on urine from human plague cases.

We have seen above that it is quite possible to infect Bombay rats with plague by feeding them with grossly infected material. We have now to record a series of experiments which were made with the object of testing a theory which is held by not a few plague workers in India, namely, that rats become infected in nature through eating the excreta of plague patients. We fed a large number of rats on the urine of plague cases. The rats used were the wild rats of Bombay, no distinction being made between *M. rattus* and *M. decumanus*.

Each rat received one feeding, from 2 to 3 c.c. of urine being mixed with the food, namely "laya" or parched Indian corn. The urine was collected from 35 cases of acute plague, only four of which recovered (88·5 % mortality). At the same time as the rats were fed a rough estimation of the number of organisms present in the blood of the patients was made, 0·1 c.c. of blood being spread over an agar slope and the resulting colonies counted. Of the 35 cases, 19 had plague bacilli in the blood, the growth obtained on agar varying from a few colonies to a thick layer. The infectivity of the urine was tested by injecting varying quantities subcutaneously into guinea-pigs. It was found that seven of the urines were infective in quantities below 1 c.c., one sample giving plague to a guinea-pig in so small an amount as 0·0001 c.c.

In all 194 rats were fed with this material with the result that not one of them developed plague.

Summary.

1. It is possible to infect wild rats of Bombay with plague by feeding them with the viscera of dead plague rats, 21·4 % being susceptible to this method of infection. Bombay rats show a greater immunity to infection by feeding than rats of the same species, which have not been subjected to a plague epizootic.

A series of experiments were also done with *Mus rattus* caught in the Punjab. Of these rats 67·8 % were susceptible. In this series a considerably larger dose of infected material was given.

We have infected a large number (38 %) of wild Bombay rats by feeding them on the whole carcases of their plague-infected comrades. No difference as regards the post-mortem appearances or the distribution of the primary bubo was found between rats infected in this way and rats infected by feeding on soft viscera.

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2. The general pathological lesions found in all rats infected by feeding are, in the main, the same as those found in rats naturally infected. There are, however, two striking differences :—

(*a*) The distribution of the primary bubo is different. The common site in naturally infected plague rats is in the neck, no mesenteric bubo having been seen out of 5000 post-mortems. In the case of fed rats the common site is the mesentery.

(*b*) In the case of naturally infected rats the stomach and intestines show no marked pathological change. In the case of fed rats well marked pathological lesions are found in the intestines.

3. It would appear that in nature intestinal infection rarely or never takes place, and that in consequence rats do not become infected by eating the carcases of their comrades.

4. A large series of rats were fed on the urine of plague cases. None of these contracted the disease.

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