PLAGUE IN MANCHURIA.

I. OBSERVATIONS MADE DURING AND AFTER THE SECOND MANCHURIAN PLAGUE EPIDEMIC OF 1920–21.

II. THE RÔLE OF THE TARABAGAN IN THE EPIDEMIOLOGY OF PLAGUE.

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(With Plates VI, VII, VIII.)

(*From the Plague Prevention Service Laboratory, Harbin, China.*)

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PART I.

OBSERVATIONS MADE DURING AND AFTER THE SECOND MANCHURIAN PLAGUE EPIDEMIC OF 1920–21.

I. INTRODUCTION.

THE first Manchurian Epidemic of Pneumonic Plague came upon the world suddenly in the winter of 1910, raged for six months, and was suppressed in April, 1911. On this occasion nearly 60,000 persons died of the disease throughout Manchuria and North China. Apart from some new clinical observations made upon the infection, the urgent work of prevention monopolised the attention of our medical staff and left little or no time for experimental studies. Such specific experiments as were then made upon Pneumonic Plague were mostly carried out in Mukden about the time of the International Plague Conference (March–April, 1911).

In 1912 the Manchurian Plague Prevention Service was established upon a permanent footing by the Chinese Government to cope with any epidemic that might again arise, and since then has successfully undertaken the task of epidemic prevention in these regions; its officers also take part in the duties of public health administration and perform general clinical work in the many hospitals under its care.

Hence the second epidemic of 1920–21 found us ready to deal with the emergency, in which we received the cooperation of the official and merchant classes. In the outbreak, limited practically to North Manchuria and East Siberia, only 8500 lives were lost among a population which had been doubled during the last ten years.

This state of affairs was particularly fortunate, because the time, formerly spent upon education and propaganda purposes among the ignorant masses, was more fruitfully utilised in carrying out the clinical and scientific observations we had in mind for furthering our knowledge of this extremely fatal infection.

Nobody realises better than ourselves the incompleteness and inadequacy of our investigations, as these were rendered difficult by stress of circumstances, which frequently demanded their postponement in favour of more urgent duties connected with the combating of the epidemic. It is hoped, however, that our record may at least contribute to a narrowing-down of the various questions at issue. Should another occasion arise for further researches, we trust that our present observations may prove useful as preliminary data.

II. EXPERIMENTS WITH PLAGUE SPUTUM.

Experiments on a large scale have in the past been conducted by various observers upon the vitality of the *B. pestis* and its resistance to various physical and chemical factors. All this work, however, was necessarily restricted to cultures of the bacillus or to material from plague buboes; rarely were the

experiments performed with sputum of pneumonic plague patients. In view of this scarcity of evidence we undertook some investigations upon plague sputum. These are now reported in the form of tables prefaced by introductory remarks.

A. Exposure of Plague Sputum to Direct Sunlight and to Diffused Daylight (Tables I and II).

During the first Manchurian outbreak Shibayama[1] found plague sputum smeared upon glass to be sterile after 2–5 hours' exposure to direct sunlight, "the time varying according to the thickness of the layer of the sputum." When exposed to diffused light no growth was obtained after 18 to 21 hours, including 12 hours during the night. Toyoda and Yasuda (1912)[2] found sputum smeared on cover glasses to become sterile after exposure to direct sunlight for one hour or to diffused daylight for six hours.

In our experiments we allowed plague patients to expectorate into sterile Petri dishes and exposed the untouched sputum to direct sunlight for half to ten hours in the open air. The results of this investigation are seen in Table I. The temperature, which appeared to have a marked influence in previous experiments with plague cultures[3, 4] seemed as important in our tests. With a temperature above 12° C. it was found by us that after a period longer than two hours the cultures proved either negative or showed only a few plague colonies. There was little difference whether the Petri dishes were open or covered. In every case the condition of the sputum after exposure was noted. The fact that we obtained some growths in almost half of the cases where the sputum appeared quite dry is not remarkable when remembering the great influence of the temperature on all drying experiments with plague cultures or smears.

The number of our experiments is too small to permit definite conclusions, but it seems evident, that an exposure to direct sunlight for nine hours even at a low temperature and in covered Petri dishes killed the plague bacilli, irrespective of whether the sputum looked dry, half dry or unchanged. It must be noted that to complete the full period of exposure the sputum was kept overnight in a cool place. This, however, seems unimportant as all our exposures from six to eight hours were also left overnight, and yet positive results were often recorded.

To supplement the above experiments we kept some dishes with sputa on the window-sills of rooms where no direct sunlight could reach them. The Petri dishes were not entirely closed, two pieces of wire separating the lower from the upper dish. The room as a rule attained an average temperature of 10° C.; in some instances an unheated room was used with a temperature differing

[1] *Mukden Report*, p. 48.
[2] *Zeitschr. f. Hyg. und Infektionskr.* LXXIII.
[3] Simpson (1905), *Treatise on Plague*, p. 91.
[4] Rosenau (1901), *Bull. 4. Hyg. Lab. U.S. Marine Service.*

but little from that of the outside cold air. Again positive cultures were obtained from quite dry sputa. As can be seen from Table II we had positive results in two instances after 48 hours, whereas a dry sputum exposed for 96 hours in an unheated room with an outside mean temperature of + 3° C. proved negative for *B. pestis*[1].

B. Exposure of Plague Sputum to artificial cold surroundings (Table III).

The *Bacillus pestis* shows remarkable resistance to intense cold *in vitro*[2] and in dead bodies[3]. Plague sputum has been proved to possess similar resistant powers. Wu Lien Teh stated that exposure of sputum in agar tubes to intense cold in a shaded place for three days did not affect the vitality of the bacillus. Strong also found that if plague sputum was exposed in open air for one week, freezing each night, the bacilli remained alive and virulent[4].

During the Harbin epidemic we collected the sputa of several plague patients in sterile Petri dishes, enclosed them in tightly fitting tin boxes, and placed these in a biscuit tin in the ice-cellar for periods varying from 26 to 208 days at a temperature not below freezing point. During the warmer period the sputa were kept in a cold-storage room with a constant temperature of − 3° to − 4° C. As can be seen from the table the bacillus survived for 99 days, whereas the last examination, made after 208 days, was negative the sputum being dry.

C. Exposure of Plague Sputum on Wood and Gauze (Table IV).

Besides laboratory tests which showed that *B. pestis* could survive (*a*) on wool, cotton and similar materials for periods up to 76 days[5] and (*b*) on fabrics contaminated by plague rats, some experiments (*c*) akin to ours have been performed with plague sputum. The German Plague Commission found that silk, linen, etc., impregnated with sputum from pneumonic plague patients and kept under ordinary conditions, were sterile after eight days; Shibayama[6] found plague sputum to be sterile when smeared upon cloth and exposed to direct sunlight for six hours daily for five days and when exposed to diffused light in the shade, for 13 days. Toyoda and Yasuda (*loc. cit.*) after smearing sputum upon Soya bean cake and Hemp bags obtained the following results:

	Sunlight	Diffused light
Bean cake . .	Sterile after 6 hours	Sterile after 20 hours
Hemp bag . .	„ „ 14 „	Not sterile after 20 hours

It is to be noted that in the experiments extending over 14 hours the sputum had to be kept for another 16 hours during the night; for the 20 hour test, an extra 34 hours was required.

[1] We regret being unable to furnish accurate readings of humidity on the days of our experiments.

[2] Kasansky (1879), *Centralbl. f. Bakteriol.* xxv.

[3] *Mukden Report*, pp. 87, 444, 446. [4] *Ibid.* p. 89.

[5] Cf. Forster, Löffler and Gladin (1899), *Centralbl. f. Bakteriol.* p. 722.

[6] *Mukden Report*, p. 48.

Experiments with plague sputum applied to inanimate objects were performed on a large scale by Russian observers. Zlatogoroff (1912)[1] reported positive results after drying the sputum on paper and exposing it to light for six days. Askanoff (1912)[2] found sputum kept on cotton in tubes in diffuse daylight at a temperature of 22–23° C. to be virulent after one month in one instance; sputum kept at room temperature on filter paper in test tubes on the window-sill and exposed to sunlight daily for three hours for 20 days was found by him to be still virulent when incubated in bouillon for 10 days.

In our own experiments the patients expectorated on pieces of wood or gauze placed in sterile Petri dishes. These, usually covered with sterile gauze, were kept in the corner of a slightly-heated room. As is seen from Table IV we again obtained positive results from a number of dried sputa. From the Petri dishes not covered by gauze we cultivated *B. pestis* from the sputum on wood after 24 hours, but not after 30 or 48 hours. The specimens on gauze in one instance still gave positive results after 48 hours. Sputa exposed in gauze-covered Petri dishes for 72 hours showed positive results for wood and gauze; but were negative after 96 hours.

D. Experiments upon Plague Sputum and Earth (Tables V–VII).

Many tests of this kind have been made by former observers. Tidswell[3] found *B. pestis* dying out after three weeks when mixed with sterile dust. Yersin (1897)[4] detected bacilli like *B. pestis*, but of lessened virulence, in soil at a depth of 4–5 cm. beneath an infected house. Although many similar tests were made in India, in no instance were plague bacilli isolated by a trustworthy observer, and it is doubtful if Yersin's bacillus was the real *B. pestis* because the cultural characters were not well known at that time[5]. Experiments with artificially infected earth were performed by Gladin[6] and Marsh[7]. Gladin found the bacillus surviving in moist sterile garden earth up to three months, whilst Marsh cultivated plague bacilli from moist sterilised garden earth 13 days after contamination and found them to survive in moist sterile cow-dung for many months. Rosenau (*loc. cit.*) found that the bacillus lived a long time in moist sterile earth, but drying and a temperature above 30° C. quickly killed it. When unsterilised earth was used the plague bacilli were found dead by most observers after a few days; only Gladin (*loc. cit.*) was able to detect them after two months. In Mackie and Winter's experiments at the Plague Research Laboratory, Bombay, with unsterilised cow-dung earth from the floor of an Indian house, *B. pestis* were recovered from one of the samples 96 hours after the contamination, and one out of four animals injected with a suspension of this earth died of plague. In the experiments recorded in

[1] *Second Report on Bacteriology and Epidemiology.* Moscow.
[2] Chmara-Barshefski, *Plague Epidemic in Far East.* Harbin.
[3] Simpson, p. 93.
[4] *Ann. Inst. Pasteur,* XI. 81.
[5] *Journ. of Hygiene,* VI. 1906, 509.
[6] *Centralbl. f. Bakteriol.* XXIV. 588.
[7] *Indian Plague Commission,* 1901, v. 101.

"Reports on Plague Investigations in India" (1916)[1] two different kinds of floors, as encountered in India, were tested. The one floor was of cow-dung, the second of *chunam* (a mixture of sand and lime). The conclusions reached were:

(1) Floors of cow-dung grossly contaminated with *B. pestis* remain infective for 48 hours, the infectivity being tested by rubbing scrapings into susceptible animals.

(2) Floors of *chunam* grossly contaminated with *B. pestis* do not remain infective even for 24 hours, the infectivity being tested by rubbing into susceptible animals.

(3) Floors of cow-dung grossly contaminated with *B. pestis* remained infective for 12 hours but not for 24 hours to susceptible animals which were allowed to run about freely on them.

(4) Floors of *chunam* grossly contaminated with *B. pestis* remained infective for 6 but not for 12 hours, the infectivity being tested by allowing susceptible animals to run about freely on them.

At the Mukden Conference 1911, Strong stated that infection could not be disseminated by dry dust, and that the foregoing Indian experiments should not be considered conclusive for Manchuria because the sputum would dry much more slowly than the moistened floors in India and so remain infectious for a longer period. Moreover, the possibility of sputum becoming frozen in Manchuria had to be taken into consideration[2]. According to Lancelin (1912)[3] "cultures magnifiques" of *B. pestis* were obtained from the earth clinging to the shrouds of exhumed bodies during the first Manchurian outbreak.

In our experiments with plague sputum and earth three different methods were adopted: (1) sputum was *exposed* on fresh earth in the same manner as on wood and gauze, (2) sputum expectorated in sterile Petri dishes was *covered* with fresh or sterile earth, (3) sputum expectorated in such dishes was *mixed* with fresh or sterile earth. We made these experiments with the object of determining (*a*) how far plague sputum actually expectorated on the ground by patients would be a source of infection, and (*b*) how far the bacteria normally contained in the earth influenced the growth of *B. pestis*; the tests with sterilised earth acted as controls. In the first series (Table V) the longest exposure after which *B. pestis* could be recovered was seven hours. In no instance did thoroughly dry sputum yield a positive culture. In our second series (Table VI) we obtained positive cultures after 12 hours' exposure, and in the third (Table VII) after six hours. In both latter instances fresh and sterilised earth furnished the same results. The experiments were purposely discontinued.

E. Influence of Various Disinfectants on Plague Sputum.

Under this heading we are reporting only upon laboratory experiments with different liquid disinfectants and with lime. Gaseous disinfectants will be considered separately.

[1] *Journ. of Hygiene*, VI. 509–518. [2] *Mukden Report*, p. 230.
[3] *Arch. de Méd. et Pharm. Nav.* XCVIII. 353-374.

(1) *Action of Liquid Disinfectants upon Plague Sputum* (Tables VIII and IX).

Many experiments *in vitro* have been made to determine the resistance of the *B. pestis* to solutions of various disinfectants: Kitasato found that the bacillus was killed in two hours by carbolic acid 1 : 200. Ogata attained the same result in 15 minutes, but a 1 : 20 solution killed them almost instantly. Corrosive sublimate 1 : 1000 was effective in five minutes. Klein (1906)[1] found pure phenol 1 : 80 killed in ten minutes, izal 1 : 2000 in ten minutes and 1 : 1600 in five minutes. Shibayama[2] found sublimate 1 : 1000 killed in one minute, carbolic acid 1 : 100 in ten minutes, lysol 1 : 100 in ten minutes. Hankin[3] found permanganate of potash killed in five minutes even in a strength of 1 : 10,000. Simpson[4] states that corrosive sublimate, though powerful in dilutions of 1 : 5000 and 1 : 10,000, "is apt to lose its germicidal effect on discharges, sputum and the like by forming a coating of albuminate of mercury which protects the microorganism from being destroyed." For this reason he recommends, wherever possible, the addition of chloride of sodium and hydrochloric acid to the solution of perchloride of mercury so as to attain a strength of sublimate of 1 : 725 or 1 : 1500. As for permanganate of potash, which has the same disadvantages as sublimate when acting upon organic matter, he recommends a strength of 1 or 2 per cent. which was found to be sufficient for even an excess of organic matter[5]. At the Mukden Conference 1911, carbolic acid (with soap) or alkaline coal-tar products (cresols) were recommended for sputum disinfection; perchloride of mercury was condemned.

In Harbin in 1921 we conducted several series of experiments with carbolic acid, perchloride of mercury, lysol, phenoid, potassium permanganate, peroxide of hydrogen, izal, lysoform, antiformin and alcohol and employed the following technique:

Small cotton swabs mounted on iron wires were placed in test tubes and sterilised by dry heat. 5 c.c. of fresh solutions of the antiseptic in different strengths were placed inside each tube. At the right moment the swab was dipped into the sputum contained in a Petri dish, suspended in the antiseptic solution for the requisite number of minutes and then washed in sterile normal saline solution to remove excess of antiseptic. After this, the swab was introduced into a fresh agar tube and a cultivation made. The same proceeding was repeated with the other solutions, care being taken to allow for as little discrepancy as possible in the quantity of sputum tested. The cultures were examined as a rule after 48 hours in the incubator kept at 30° C.

As can be seen from Tables VIII and IX the following concentrations of disinfectants were necessary to ensure the sterilisation of plague sputum:

		Time required for sterilisation
Carbolic acid . . .	1 : 10	5 minutes
Perchloride of mercury . .	1 : 500	20 ,,
,, ,, . .	1 : 1000	30 ,,
Lysol	1 : 50	20 ,,
Concentrated alcohol . .		4 ,,

[1] *Bacter. and Path. of Oriental Plague.* [2] *Mukden Report*, p. 47.
[3] Simpson, p. 395. [4] *Loc. cit.* p. 394. [5] *Loc. cit.* p. 395.

The following were *not reliable* even after 30 minutes:

Phenol	1 : 50	Lysoform	1 : 50
Potassium permanganate .	1 : 500	Antiformin . . .	1 : 10
Peroxide of hydrogen . .	1 : 3 (30 %)	Alcohol	1 : 2 (50 %)
Izal	1 : 50		

As compared with the results obtained *in vitro*, it can be seen that the popular method of sterilising plague sputum with the usually recommended liquid disinfectants is unsatisfactory. Therefore we used them as little as possible in our disinfection work, relying principally upon methylated spirit for the disinfection of the hands, gloves, etc. We are still using it constantly in connection with our experimental work. Its inflammability should, however, be taken into consideration.

(2) *Action of Slaked Lime and Milk of Lime upon Plague Sputum* (Tables IX and X).

Slaked lime or milk of lime (usually in the strength 1 : 100) has been much recommended[1] in all cases where fumigation cannot be applied for the disinfection of plague-contaminated objects. Jochmann (1914)[2] states that milk of lime sterilised plague-contaminated faeces in 1–2 hours. At the Mukden Conference chloride of lime and slaked lime were mentioned among the useful disinfectants for plague sputum (p. 393).

We performed in 1921 a few experiments with lime powder and milk of lime (1 : 10) in the same manner as in the case of the liquid disinfectants and found them both reliable against plague sputum after 30 minutes; milk of lime even after 20 minutes (Table IX). When plague sputum was covered with lime, no positive growths could be obtained after 30 minutes, only doubtful ones after 15 minutes. Sputum expectorated on a layer of lime in a sterilised Petri dish gave a doubtful culture after two hours, a negative one after $4\frac{1}{2}$ hours. It is perhaps well to remember that in the Indian experiments with grossly plague-contaminated floors referred to above there was a marked difference in favour of the *chunam* floors which consist partly of lime, provided there were no pools of plague bacilli bouillon on them; otherwise the absorptive cow-dung floor was comparatively less infective.

F. Feeding Experiments with Plague Sputum (Table XI).

Feeding experiments, mostly upon rats, with plague-contaminated materials have been made by different observers with partly positive results, but as far as we know, plague sputum was employed for such tests only by Broquet[3] who fed four mice with food mixed with sputum from plague patients and had one positive result.

We fed four animals (two guinea-pigs and two rabbits) with plague sputum mixed with their ordinary food. One guinea-pig and one rabbit died, but neither showed anatomical or bacteriological signs of plague.

[1] Simpson, *loc. cit.* p. 393.
[2] *Lehrb. der Infektions-Krankh.* p. 231.
[3] *Mukden Report*, p. 229.

G. Inoculation of Conjunctiva with Plague.

Human infection through the conjunctiva is extremely rare. A notorious case occurred in 1897 in the Parel Hospital (Bombay), where a nurse was infected by receiving in the eye a particle of sputum coughed up by a pneumonic plague patient. She developed a bubo behind the ear and died. A similar case was reported in Hongkong. No new data in this connection were brought forth at the Mukden Conference. In the 1920–21 outbreak we did not observe any infection traceable to the conjunctiva.

Of two experiments upon susceptible animals, made by rubbing a swab dipped in fresh plague sputum upon one conjunctiva, we succeeded in producing positive results in a rabbit after four days. At autopsy *B. pestis* was found in an abscess in the subconjunctival region.

III. EXPERIMENTS CARRIED OUT IN PLAGUE WARDS.

A. Experiments on Coughing.

During the first Manchurian outbreak Toyoda and Yasuda[1] found that plague bacilli were ejected to a distance of $3\frac{2}{3}$ feet by forcible coughing, whilst Strong and Teague[2], by exposing a plate for 12 minutes, obtained positive results up to a distance of about two metres.

We performed only a few experiments, in which the time of exposure was a short one (just as long as the patient was coughing naturally). The results are recorded in Table XII; we had one doubtful result at a distance of five feet, two doubtful at three feet, and one positive at two feet. We discontinued the experiments because those of Strong and Teague's seemed to be indeed the last word on this subject.

B. Infectivity of Rooms occupied by Plague Patients and their Disinfection (Tables XIII–XV).

The subject of the infectivity of rooms or houses where plague cases had been confined and of their disinfection has attracted much attention from both its theoretical and practical standpoints. Much research in this respect (bacteriological and animal) has been carried out in India. All this work, however, has no direct bearing on the problem of pneumonic plague, because the main carriers of the infection in bubonic plague, namely, the rat and its fleas, play no part in pneumonic plague, a fact emphasised at the Mukden Plague Conference, 1911[3]. At this Conference the most divergent views were held by different observers in regard to the infectivity of houses and their disinfection[4]. The opponents of disinfection asserted that there was not much infectivity left after the patients had been removed from the rooms, so that disinfection seemed merely a waste of labour and materials. They cited instances where

[1] *Mukden Report*, p. 49.
[2] *Philippine Journ. Sci.* Sec. B, VII. 137.
[3] *Loc. cit.* p. 299.
[4] Pp. 249, 298, 230.

people staying in undisinfected houses had not contracted the disease, while others contracted the disease in spite of disinfection. The advocates of disinfection pointed out that in the latter case, perhaps infection had been contracted in some other way and cited cases where infection had taken place in undisinfected houses months after the first case. That danger lurked in "definite foci of infection from the sputum" (Strong) was supported by the investigations of Mischenski[1] who found "sputum retain its infection, even when half dried, for a month." Farrar's statement "that no definite evidence to the effect that houses or their contents remained infective had been put before the Conference" was contradicted by the Chairman, and the Conference eventually recommended disinfection of houses in the following manner:

Houses should be sealed up for a few hours immediately after removal of the patient or the corpse. Search should be made with a good light for visible contamination with blood, sputum, etc. Such contaminated matter must be forthwith disinfected or removed and burnt. The house must then be sprayed and swabbed with a disinfectant solution. Slaked lime may be spread evenly on a mud floor. When a house can be rendered air-tight, fumigation with formalin may be employed. The disinfection of furniture may be included in that of the house, or carried out by fumigation and exposure to sun. The spittoon must be thoroughly disinfected. The *k'ang*-mat and all rubbish should be burnt. House burning should not be carried out where it is found reasonably possible to disinfect.

1. *Infectivity.*

Some tests were made in 1921 with agar plates in sick rooms (Table XIII) by exposing them for ½–1 hour at different heights away from the patients. A further series of experiments consisted in exposing guinea-pigs or rabbits kept inside tin buckets and placed upon the floor of rooms where plague patients were dying or had just died. This method was chosen to avoid any possible error arising from the animals becoming infected through another channel than the air. Previous records show that in the arrangement of the experiments (except one), the animals were kept close to the patient, whereas our object was rather to ascertain the infectivity of the room *per se*. Toyoda and Yasuda[2] placed six guinea-pigs in a wire cage leaving it at different distances from a patient. None of the animals died after being exposed for 12 hours. Shibayama (1912)[3] referred to five guinea-pigs which were kept for 24 hours free and exposed "in such a way as to receive materials coughed outright in their faces" by a patient; these animals remained free from plague. Strong and Teague[4] could not produce plague in guinea-pigs exposed for two minutes at a distance of 5 cm. from dyspnoeic plague patients after having the abdomens of the animals shaved and extensively scarified. Raynaud (1912)[5] placed guinea-pigs in a plague-infected house but they remained healthy. Heiser (1913)[6] placed two guinea-pigs free from fleas and confined in a wire

[1] Zabolotny, p. 231.
[2] *Mukden Report*, p. 49.
[3] *Trans., Second Biennial Congress, Far East. Assoc. Trop. Med.* Hongkong, 1912, p. 131.
[4] *Loc. cit.* p. 154.
[5] *Rev. d'Hyg. et de Police Sanit.* 1912, pp. 861–867.
[6] *U.S. Public Health Rep.* 1913, XXVIII. No. 10, pp. 426–427.

Compound of the plague hospital, Harbin, 1921. Anti-plague staff on duty.

Photos of two pneumonic plague patients, 1921. Left one just admitted. Right one dying. Note the patch of red sputum on bench.

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cage on the sleeping mat of a plague patient (bubonic). One of the animals, found free from fleas, died of plague after four days. After disinfection (spraying with kerosene and washing with a larvicide), new guinea-pigs were exposed in the room but they did not die of plague.

The illness of Dr Yuan on February 17th–20th in our new Hospital block (steam-heated and maintained at 17° C.) enabled us to make the first investigation regarding the infectivity of the sick-room *per se* immediately after the death of the patient. For this purpose 12 guinea-pigs, two in each tin bucket were placed on the wooden floor of the room (12 by 12 by 10 feet with one large closed window), for periods ranging from ½ to 4 hours. Nothing was previously disturbed and the door was not opened except when the animals were removed at certain times. Four of the exposed guinea-pigs died. One of them exposed for 4 hours showed no plague infection, while the three others, exposed for ½, 1 and 4 hours respectively succumbed to septicaemic plague with congestion of lungs.

Eight guinea-pigs in lots of two were exposed on March 2nd to the air of the sick-room, ½ hour before, ½ hour after, 1 hour and 2 hours after death of patient. One animal died on March 17th (*i.e.* 15 days after exposure) showing no bacilli in organ-smears, though pure cultures from the blood were obtained.

Four guinea-pigs were allowed to stay in the plague-room from April 5th–9th where six patients had successively come in and died. One animal became sick, and on being killed showed lesions in the respiratory organs. No tonsillar or glandular infection was noticed. This experiment was performed on behalf of a Russian bacteriologist who at first believed the primary seat of infection to be situated in the tonsils.

Four other experiments were performed with 21 animals, but none succumbed to plague.

Similar experiments were also conducted in March at Dalainor by Dr Hsieh of our staff, who exposed 10 young rabbits at heights varying from 1–8 feet to the air of a room (10 by 10 by 12 feet) recently vacated by four persons who had died of plague. All animals survived.

Our series of experiments showed that out of 55 animals exposed for periods ranging from ½ to 96 hours, only five died of plague (two after ½ hour's exposure, one after 1 hour's exposure, one after 4 hours' exposure, the last one after 96 hours' exposure). Seven other animals exposed for 96 hours remained healthy and showed no plague when killed. It might appear therefore that the average sick-room by itself even when the floor was covered by sputum was not particularly dangerous. As in Yuan's case, conditions for infection appeared more favourable in modern fitted buildings. We must, however, remember that these experiments were carried out mostly with guinea-pigs, animals which may not be so easily infected by inhalation[1] "because they have a thick cluster of hair in their nostrils through which they breathe."

[1] Shibayama, *Experiments, etc.*, p. 131.

2. *Disinfection.*

Our experiments under this heading consisted in exposing plague sputum in open Petri dishes, sometimes on the floor and sometimes on the window-sill in an unheated and almost empty room, and then disinfecting the room with formalin gas or sulphur fumes. The rooms used were well suited for disinfection being part of our solidly built hospital buildings. Some previous experiences may be quoted: Simpson[1] stated that "formalin (gas) is destructive to the microbe in about 3 or 4 hours but has no penetrative powers and is accordingly insufficient." He regarded fumigation with sulphur as very uncertain and generally useless, and cited Hankin[2] who, in a disinfection experiment with sulphur after having sprayed the walls and the ceiling with water, found living plague bacilli though in diminished numbers.

In our 1921 Harbin experiments (Table XV) we found sulphur fumigation more satisfactory than formalin. Sulphur yielded negative bacteriological results in every case even after 12 hours' exposure. In three cases the plague bacillus survived 24 hours' exposure to formalin. The Clayton apparatus was not tested and there was no time to carry out further tests in sick-rooms with sputum on floors, beds and beddings, etc. Further experiments with the aid of animals should be carried out at the next opportunity, and it requires to be determined how soon an infected room loses its infectivity when left to natural influences after removal of the patient or corpse. We believe that such experiments will prove the effectiveness of nature in rendering an infected room innocuous apart from the directly contaminated objects. In that event, sanitary authorities will perhaps rely more upon nature's action than the vague and unsatisfactory methods of fumigation as at present practised[3].

C. Examination of Clothing and Money from Plague Patients and Cadavers.

Instances of plague infection by infected clothing have been observed. Simpson[4] cites a number of such instances. Therefore the possibility of infection through clothes was carefully considered at the Mukden Conference, 1911. Strong and Teague maintained that from a theoretical standpoint "the plague bacillus may be present (in articles of clothing) even though no particle of sputum may be visible upon them." Actual evidence was also brought forward of cases in which infection through such objects probably occurred[5]. The few bacteriological examinations recorded were negative[6], and other instances were mentioned where the clothes of plague patients were worn by healthy persons without causing infection. No instance of coins or banknotes causing infection was noted[7]. The Conference concluded that "There has been no positive epidemiological evidence to show that infection has been spread by clothing, merchandise or other inanimate objects[8]."

[1] Simpson, p. 390. [2] *The Plague of India*, 1896, 1897.
[3] *Practice of Medicine in the Tropics*, 1921, I. 221.
[4] *Loc. cit.* pp. 197–199. [5] *Mukden Report*, p. 226.
[6] *Loc. cit.* p. 228. [7] *Loc. cit.* p. 227. [8] *Loc. cit.* p. 389

From clinical and bacteriological observations made by us in the 1921 epidemic, we cannot quite agree with the above statement. Our clinical evidence in this regard will be supplied in another paper, the bacteriological data are as follows:

Pieces of cloth were cut from the coats on plague corpses and plated (Table XVI). Although the examination of the plates proved very difficult because of many contaminating colonies, positive or suspicious results were obtained in not a few instances. Owing to the scarcity of animals at the height of the epidemic, we were unable to confirm this evidence with animal tests. Towards the end of the outbreak we (*a*) inoculated some animals with emulsions from plate cultures and (*b*) confined rabbits and guinea-pigs in new coffins (partially closed) with clothes freshly removed from plague corpses. None of the animals thus exposed or of the inoculated animals died of plague (Tables XVI and XVII), the negative result being possibly due to the decreased infectivity of the clothes on account of warmer weather and the prevalence of a pulmonary (not pneumonic) type of infection.

Paper money and silver coins from the dead also gave negative results in cultures and one animal experiment.

D. Animal Experiments with Urine of Plague Cases (Table XIX).

Fujinami and ourselves have successfully cultivated *B. pestis* from the urine of pneumonic plague corpses[1]. The task of isolating *B. pestis* from the urine voided by *living* patients was very arduous. In the "Report on Plague Investigations in India"[2] a survey of previous investigations on this subject showed that the finding of *B. pestis* in such urine is exceptional; also that in India detection of the bacillus in autopsy cases was often impossible. Kasai[3] reported one positive result in six clinical cases examined. In the Indian Report, experiments performed by injecting the urine of plague patients directly into animals showed positive results in nearly 30 per cent. in one series and 19·3 per cent. in another. Our 1921 Harbin records indicated one successful *B. pestis* cultivation out of 30 specimens of plague urine examined. Of these, 23 were from living patients and 7 from corpses. The single positive finding was from a cadaver. Two guinea-pigs injected with urine (one from a living and one from a dead subject) gave negative results.

E. Observations upon Contacts and Plague Carriers (Table XX).

During epidemics of bubonic plague single instances have been observed where patients with secondary pneumonic plague infection showed *B. pestis* in their sputum for lengthy periods. One case was observed by the German Plague Commission, where the bacilli were present for 10 days and disappeared after 16 days. Gotschlich reported three instances[4] where the sputum con-

[1] *Mukden Report*, p. 149 and our autopsy findings in the present Report.
[2] *Journ. Hygiene*, VIII. 221.
[3] *Mukden Report*, p. 177.
[4] *Zeitschr. f. Hyg.* 1899, p. 402.

tained plague bacilli for 35, 41, and 76 days respectively. According to Mitchell[1], Rees "cited instances where *B. pestis* in virulent form was present in the sputum of pure *bubonic* cases for as long as two months after recovery." Similar cases were observed by Cayley[2] and others. Wilms found the bacillus in the urine 4–6 weeks after the cessation of the febrile symptoms. In view of this evidence, the Mukden Conference carefully considered the question of carriers and some bacteriological examinations were therefore undertaken. No positive result, however, was obtained, even in the remarkable case where a woman had doubtlessly infected several persons without contracting the disease herself[3].

As can be seen from Table XX, we examined in 1921 23 persons who had been living with plague patients, these contacts being examined soon after their admission into the isolation wagons. Among these 23 examinations, 20 proved negative for *B. pestis*; one case (No. 215) could not be counted as a genuine carrier inasmuch as the patient soon after examination was admitted into the hospital with plague pneumonia. The two remaining cases however had *B. pestis* in their sputum (in one case also on the tonsils) without subsequently showing or developing any clinical signs of the disease. These two cases are so important that detailed descriptions may be given:

(*a*) *Chang I*, aet. 27, motor-car driver, was found with 18 other men in a crowded inn, where on the same day (Feb. 2nd) a man had died under circumstances suspicious of plague. When we went into the inn to examine the dead body, all the contacts appeared well except Chang, who complained of headache with a temperature slightly above 37° C. His pulse was not fast, and he gave one the impression of fright rather than any serious illness. His sputum was of an ordinary salivary character. Chang was sent with the other contacts to the isolation hospital, because spleen puncture of the corpse had proved positive. He had no more fever and felt perfectly well during his whole stay there. Smears of Chang's sputum taken on Feb. 3rd showed no *B. pestis*. Agar cultures showed no characteristic naked-eye features, but plague-like organisms were observed under the microscope. On Feb. 7th a one-fifth slant of the culture was injected into the peritoneal cavity of a guinea-pig. The animal died 18 hours after injection. Smears from the heart, peritoneum and spleen showed *B. pestis* while cultivations from the first two organs gave pure colonies of the bacillus. On Feb. 6th, fresh agar cultures were made from the sputum and tonsils. All yielded *B. pestis* and a guinea-pig injected on Feb. 13th with a one-fifth slant of a sputum culture died after 24 hours showing *B. pestis* in smears. Cultivations from heart, spleen and peritoneum were positive. Chang escaped from isolation on the night of Feb. 7th and hence no further investigations were possible. This man was under close observation from Feb. 2nd to 7th, during which time he showed nothing abnormal. The specimens of sputum obtained on both occasions were by forcible coughing.

[1] *Journ. Hygiene*, xx. No. 4, 1922.

[2] *Indian Plague Commission*, Chap. III. p. 92 of Report.

[3] *Mukden Report*, pp. 226–228.

One of the 18 contacts, Wang died unexpectedly on the evening of Feb. 6th, *i.e.* five days after his last contact with one sick man in the inn. As the incubation period of pulmonary plague is usually 3–5 days, it is possible that Wang might have been infected by the carrier Chang who harboured the bacilli for at least six days. The remaining 17 contacts continued healthy and were dismissed well.

(*b*) *Chang II*, aet. 30, coolie, was one of four contacts examined on March 4th (No. 141), the other three giving negative results. His sputum was apparently normal and showed in smear preparations some plague-like organisms among cocci and other bacilli. Cultures looked suspicious. A one-fifth slant culture was injected subcutaneously into a guinea-pig on March 6th. The animal died after 18 hours. No plague bacilli could be detected in cover-glass preparations from heart, spleen and lungs, but the spleen culture showed one colony and that from the heart several colonies of plague-like bacilli. A guinea-pig injected intraperitoneally on March 8th with a one-fifth slant culture of this heart culture died the next day (19 hours) and showed *B. pestis* in smears and cultures from the spleen and peritoneum. Sputum and swabs from the tonsils of Chang were again obtained on March 9th and 13th but they proved negative. The serum of the man did not agglutinate *B. pestis* in a dilution 1 : 50 on March 18th. The tonsils in both cases were examined clinically, but nothing abnormal could be seen.

Here is then proof positive of the existence of healthy carriers in pneumonic plague epidemics. Should further observations be possible, it will be necessary to ascertain by bacteriological tests of both sputum and tonsils if the percentage of "carriers" among contacts is really as large as would appear from our limited observations.

Should such "carriers" be frequent their occurrence will have to be seriously borne in mind and it will be necessary to expose susceptible animals in proximity to carriers, or better still of contacts in general.

F. Experiments with Protective Masks (Table XXI).

During the first part of the epidemic we experimented with simple cotton and gauze masks of the pattern introduced by Wu Lien Teh and recommended by the Mukden Conference. When using them we always placed "cotton wool-plugs within the upper margin of the pad on either side of the nose to fill up the open angles[1]." Cultures were made from the outer layer of gauze and outer and inner layers of cotton of masks actually worn in the plague wards for various periods ranging from half to four hours. In only one instance did we cultivate *B. pestis* from the outer gauze of a mask, one worn continuously for three hours (Table XXI). We had to discontinue these experiments, because after Dr Yuan's death an additional precautionary measure was adopted in the form of a hood made of cloth with a square piece of silk (4 by 6 inches)

[1] *Mukden Report*, p. 465.

sewn on in front to protect the respiratory entrance. The hood had two apertures for the eyes, and was tucked inside the overall at the neck of the wearer. One culture taken on March 3rd from the outside of the rather moist silk-piece of a hood worn for one hour proved negative for *B. pestis*.

Masson[1] "holding gauze before the mouth of an infected patient and putting agar plates on the other side" obtained no growth of *B. pestis*. Barber and Teague[2], experimenting with *B. prodigiosus* by spraying, concluded that the Mukden cotton-and-gauze mask was inefficient because "it fails to conform to the configuration of the face and bacteria may pass directly through it." Dr J. W. H. Chun of our laboratory sprayed emulsions of *B. acidi lactici* upon masks mounted in metal frames and obtained similar results. When judging the protective value of the mask, stress should be laid upon the fact that in actual practice the mask is never put to such severe artificial tests as forcible spraying. Besides, in the plague wards one seldom stands within a shorter distance than three feet in the direct line of the patient's breath, and quiet breathing or an occasional cough is unlike the continuous spray used in the experiments.

IV. EXPERIMENTS WITH MIXED INFECTION AND WITH *PYOCYANEUS* VACCINE.

From past observations and actual experiments it has been established that the presence of other microorganisms is unfavourable to both the growth of *B. pestis* (*in vitro*, Yersin) and its virulence. Simonds[3] found that a plague culture accidentally contaminated by saprophytic organisms quickly lost its virulence. Such observations are familiar to laboratory workers. Daly reported[4] that in 1899 hundreds of plague corpses were stored in the mortuaries of Newchwang city and that "swarms of flies fed greedily on the juices exuding from these coffins. The caretakers lived in close proximity, their dwelling rooms were full of flies, and their food constantly fouled by them, yet not one of them contracted plague. Judging from this instance it would seem as if the putrefactive germs rapidly kill the pest bacilli." Simpson stated that the virulence of the *B. pestis* was enhanced by the presence of *Streptococcus*, while *B. coli communis*, *B. subtilis*, *Staphylococcus* and *Micrococcus prodigiosus* acted in a contrary manner. On the other hand, Bitter[5] found the *Streptococcus* inhibiting the growth of *B. pistis* (*in vitro*). Row placed *Staphylococci* upon scarified areas round the buboes of plague patients and carried out experiments to study the reason of the favourable results obtained. In a paper read in 1905 before the Grant College Medical Society, Bombay, he stated that animals infected with plague could be saved by a subsequent injection (after 24–28 hours) with *Staphylococcus* or with a mixture thereof with *B. pestis* or by a pure culture of *B. pestis* attenuated by "symbiosis" with *Staphylo-*

[1] *Mukden Report*, p. 90.
[2] *Philippine Journ. Sci.* Sec. B, VII. 255.
[3] Simpson, p. 90.
[4] *Mukden Report*, p. 309.
[5] *Rep. Egypt. Plague Com.* Cairo, 1897, cited in Hiss and Zinsser's *Textbook of Bact.* p. 31.

coccus. In a second paper read in 1906 before the Bombay Medical and Physical Society, he described the degenerative morphological changes in *B. pestis* in "symbiosis" with *Staphylococcus* and stated that "there is strong experimental proof that marked benefit ensues in guinea-pigs infected with virulent *B. pestis* when they are treated 24 hours after infection by one or the other of the following methods: (*a*) A cutaneous scarification with live staphylococci in the vicinity of the pest infection. (*b*) A similar operation with a mixture of *B. pestis* and excess of staphylococci grown in symbiosis." Row found that "the absence of *B. pestis* in the suppurating bubo is only apparent and not real, and the general failure to detect the bacillus in bubonic pus cultures is due to the fact that we get such an abundance of staphylococci in one or two days' agar growths that a slide smear shows no evidence of *B. pestis*. Nor is it possible to isolate the *B. pestis* by infecting guinea-pigs by the cutaneous method of infection described by Kolle, for, as will be pointed out later on, it seems to me that even were the plague bacilli present, they are in a state of attenuation—this being either *per se* or at all events when in association with staphylococci." He was however able to recover virulent *B. pestis* by scraping the growth of staphylococci from the 7–10 day old cultures and setting aside for 24–28 hours. He thus obtained minute colonies on the surface of the agar and found in the smears staphylococci as well as degenerated bacilli, which when isolated in subcultures proved to be morphologically and experimentally true *B. pestis*. It should be emphasised that in experiments with mixed infection complete reliance should not be placed on agar but that bouillon cultures should be employed because occasionally *B. pestis* can only be recovered from the latter.

MacConkey[1] immunised guinea-pigs and rats by inoculation of cultures of *B. pseudotuberculosis rodentium* or filtrates of such for several months. Rowland[2] obtained the same results for guinea-pigs but not for rats. In 1921–1922 we conducted three sets of experiments on mixed infection. While experimenting upon the action of disinfectants upon plague sputum we noticed occasionally that on agar slants contaminated with spore-bearing organisms no plague bacilli could be detected. We isolated two such spore-bearing bacilli, one of which corresponded to *B. subtilis*, the other appeared to be new. We are indebted to Dr Edgar Tsen of the Union Medical College, Peking, for information regarding the latter bacillus. Its chief cultural and other characteristics are as follows:

Motility. Non-motile. *Staining properties.* Gram positive. *Broth.* Growth in form of a granular precipitate; small amount of granular and stringy sediment but no surface growth. *Agar slant.* Abundant viscid growth, smooth and moist. Isolated colonies round, convex and opaque. *Agar stab.* Good spreading surface growth. Slight growth along upper part of stab; no growth along lower part of stab. *Agar colonies.* Surface colonies round, yellowish, convex and viscid. Edges made of slightly curved parallel strands. Deep colonies small and irregular in shape. *Gelatin stab.* Growth along upper part of stab with funnel-shaped liquefaction. *Gelatin colonies.* Round colonies with fimbriate edges. Liquefaction starts

[1] *Journ. Hygiene*, VIII. No. 3, 1908, p. 335. [2] *Loc. cit.* 1912, pp. 350–357.

early. *Potato.* Abundant non-pigmented wax-like growth. *Loeffler serum.* Yellowish growth with slight liquefaction. *Litmus milk.* Gradual reduction of litmus. Peptonization sets in slowly. *Fermentation tubes.* Dextrose, mannite, maltose, lactose, and saccharose broths in fermentation tubes not fermented. (Incubation period 8 days.) *Indol reaction.* Negative.

This bacillus is referred to as *Bacillus H.* in the text.

While cultivating *B. pestis* in combination with *B. subtilis* or *B. H.* on agar and bouillon, we obtained growths of both organisms, but the first appeared to grow somewhat slowly. Therefore, we conducted the following animal experiments:

Series I. Three guinea-pigs were injected intraperitoneally with mixtures of one-fifth slant of a virulent plague culture and one-fifth slant of the new *B. H.* Animal 1 succumbed quickly in 15 hours. At autopsy nothing unusual was found macroscopically. No bacteria could be detected in the films from heart, spleen or peritoneum. The heart culture remained sterile while cultures from spleen and peritoneum showed only *B. H.* (only agar cultures were used in this instance). Animals 2 and 3: one had received one-fifth slant *B. H.* two days earlier. Both animals survived for an unusually long time (50 and 144 hours). Both showed at autopsy a circumscribed peritonitis near the diaphragm with a fibrino-purulent coating of the surrounding organs especially liver. Both *B. pestis* and *B. H.* were recovered.

Series II. Two guinea-pigs were injected with mixed cultures of *B. pestis* and *B. subtilis* after having received previous injections of an emulsion of the latter alone. Again death occurred after a considerably longer interval (72 and 120 hours). The same localised peritoneal reaction was noted in both animals, although the second one had been injected subcutaneously and not intraperitoneally. Both *B. pestis* and *B. subtilis* were found in cultures.

One rabbit was injected subcutaneously with 0·20 c.c. of a 9 day old mixed bouillon culture of *B. pestis* and *B. subtilis* (1922). The animal died after 96 hours, surviving no longer than animals which had been infected with plague alone. Again an incipient circumscribed peritonitis was found although the animal had received subcutaneous injection. The heart culture proved negative but cultures from the other organs gave a positive result with bacilli.

Series III. From a tarabagan contact (inhalation experiment) we obtained cultures of *B. pestis* associated mostly with *B. pyocyaneus*. Thirteen rabbits were inoculated with these mixed cultures (Table XXIII). Two (R. 62 and R. 63) survived for a month and at autopsy showed chronic changes traceable to the infection. Two more (R. 44 and R. 60) yielded no *B. pestis* in cultures, only very doubtful ones in smears, so that they cannot be considered as having succumbed to a mixed infection. Four other rabbits died not long after the injection (24–120 hours) and showed at autopsy more or less marked signs of localised reaction. Their report is as follows: (*a*) Rabbit 45 had a small subcutaneous abscess in the left inguinal region away from the site of the (subcutaneous) injection; pus from this showed some *B. pestis* in both smears and cultures; no growth from heart or lungs. (*b*) Rabbit 47 had a small sub-

cutaneous abscess on the chest (from which *B. pestis* and *B. pyocyaneus* were recovered), subcutaneous oedema of abdomen, haemorrhages in the fascia beneath, and a circumscribed peritonitis in region of ascending colon. Cultures taken from here showed both organisms. Doubtful growths of *B. pestis* were obtained from heart and lung. No changes could be detected at site of subcutaneous injection. (*c*) Rabbit 49 showed abscess at the site of injection, the peritoneum being almost perforated with commencing peritonitis. Heart culture was sterile and a few *B. pestis* were cultivated from the lungs. (*d*) Rabbit 48 showed oedema of the subcutis on right side of abdomen. The site of injection (subcutis of back) was not oedematous; only congested vessels were present and traceable to the oedematous area.

Note. The presence of abscesses in the neighbourhood of the peritoneal cavity, even in the four cases where the site of injection was in the dorsum, may be explained by the quiet somnolent and squatting attitude of the sick animals, which tended to produce abscesses by gravitation.

Five out of the 13 rabbits injected with these mixed cultures succumbed to the infection after a long period (9–29 days).

(i) Rabbit 46 was used 8 days after the mixed infection for another experiment as it was suffering from chronic skin disease, and had to be disposed of quickly. This animal died a day after the second injection. A big abscess was found in the subcutis of abdomen and was apparently due to the earlier injection.

(ii) Rabbit 58 died 29 days after the mixed infection. Macroscopically, only congestion of the spleen could be noted. Smears from heart, spleen and lung showed few plague-like bacilli. Heart culture remained sterile; that of spleen showed one small colony and in subcultures only a few plague-like bacilli. Sections of spleen showed bipolar stained plague-like bacilli.

(iii) Rabbit 59, dying after 29 days, showed at autopsy a pelvic abscess most probably centred in the vagina. Smears from heart and spleen, cultures from spleen and sections of abscess showed *B. pestis*.

(iv) Rabbit 57 died after 20 days, having been inoculated with three-quarters loopful of a trachea subculture. Autopsy: found considerable swelling of spleen studded with yellow nodules on surface and section; spleen cultures sterile; a few *B. pestis* recovered from heart culture.

(v) Rabbit 65, injected with a mixture of *B. pyocyaneus* (three parts), and *B. pestis* of lessened virulence (one part), died after 13 days, showing changes in spleen similar but much less marked than in Rabbit 57. Only a few *B. pestis* were recovered from liver culture. Heart culture was sterile; spleen only showed non-plague organisms.

Note. Some interesting points may be gathered from the above experiments: (*a*) the long interval between infection and death in 5 of the 11 animals; (*b*) the chronic changes observed in the organs; (*c*) the scanty findings of *B. pestis* (heart cultures often sterile). The apparent attenuation in virulence may be due either to the influence of *B. pyocyaneus* or a lessened virulence of the *B. pestis* recovered from the tarabagan (T. 12).

The two last experiments in this category seem to support the view of a lessened virulence of the *B. pestis*. (*a*) Rabbit 90, injected with 1 c.c. of trachea subculture mixed with 1 c.c. of a highly virulent *B. pestis* culture, died after three days. Autopsy: haemorrhagic oedema of the subcutis of the

chest similar to R. 48. Cultures from heart, spleen and liver showed few *B. pestis* but plentiful *B. pyocyaneus*. A control animal injected with double the amount of the same virulent *B. pestis* culture (R. 92) died 24 hours later. (*b*) R. 100, injected with 1 c.c. of 24-hour mixed culture of virulent *B. pestis* and *B. pyocyaneus* died in 27 hours. Cultures from the heart remained sterile; those from the liver and spleen showed *B. pyocyaneus* (plenty) and *B. pestis* (few). A control animal (R. 101) injected with the same amount of pure plague culture, died in four days. A rabbit injected with *B. pyocyaneus* alone survived.

Conclusions. From these experiments it will be seen that cultivation of *B. pestis* with *B. pyocyaneus* does not diminish virulence of the former. The alternative, namely a lessened virulence in the strain of the *B. pestis* used, is therefore probable, and our earlier hope of effective immunisation with some such organism as *B. H.* or *B. pyocyaneus*, has not been realised. Nevertheless, in view of the evidence brought forward it seems to us that further research upon mixed infections should be prosecuted.

Experiments with Pyocyaneus Vaccine (*vide* Table XXV).

Preliminary tests *in vitro* showed that pyocyaneus vaccine had no retarding influence on the growth of *B. pestis*. Our experience with living pyocyaneus bacilli led to our experimenting with plague culture and pyocyaneus vaccine (*vide* Table XXV).

In the first of these experiments, the rabbit injected first with plague culture and pyocyaneus vaccine and then on two successive days with pyocyaneus vaccine alone (R. 98) died two days later than control R. 97, which succumbed to an injection with plague alone after three days. As, however, this vaccine contained 0·50 per cent. of carbolic acid, which could act upon the plague bacilli 15 minutes previously, this favourable result could not be considered as due to the influence of the vaccine itself. Two more animals treated with a combination of plague culture and pyocyaneus vaccine (without carbolic acid) died in four days. This result conformed with those generally obtained in the experiments performed at this time[1].

Experiments with Adrenalin.

Adrenalin has been tried in plague on a large scale[2] without favourable results. In 1922, our staff tested adrenalin in combination with plague vaccine and with antipest serum because of good results reported by Renaud[3] when treating serious cases of pneumonia with a combination of adrenalin and anti-

[1] One experiment was performed with *B. pestis* mixed with *B. acidi lactici* (*vide* Table XXIV). We cultivated the first in milk and contaminated it three days afterwards with *B. acidi lactici* and incubated the mixture. After five days, 0·20 c.c. of this mixed culture was injected into R. 108, which died of plague in four days and showed both kinds of bacteria in films and cultures. The control animal, R. 110, injected with *B. pestis* cultivated in milk alone, died in six days.

[2] Powell in the discussion of Row's paper, 1906, *loc. cit.*

[3] *Bull et Mem. Soc. Med. des Hôp. de Paris*, June 23rd, 1921.

pneumococcic serum. In the first experiment (Table XXVI) three animals were inoculated with plague. One served as control; the second was then reinjected on three consecutive days with plague vaccine; the third was treated in the same way as the second, except that adrenalin was given 20 minutes before each vaccine injection. All the animals died of plague, the control animal last. The animal treated with the vaccine-adrenalin combination was the first victim. Nothing unusual was observed at autopsy.

R. 127, infected with plague and treated afterwards for four days with combined antipest serum and adrenalin, died in five days of plague. This agreed with results usually obtained by us at that time. It may be necessary to test this treatment by intravenous injections (as did Renaud) to see if it yields a quicker or better immunisation of healthy animals.

V. EXPERIMENTS UPON NON-SUSCEPTIBLE ANIMALS (Table XXXVI.)

Most investigators have not succeeded in infecting pigs and birds with plague. Both the German and Austrian Plague Commissions in India failed to produce plague in oxen, pigs and poultry. Negative were also the results of London[1], who used various birds, and of Watkins-Pitchford[2], who inoculated pigs and hens with virulent cultures and blood. Flu[3] found none of his chickens succumb to the infection; in one a local reaction (extended necrosis) with no *B. pestis* was noticed. Bannerman and Kapadia[4] investigated pigs, fowls and ducks by feeding them with material from dead plague rats as well as by percutaneous and subcutaneous inoculation with the same material. None of the animals succumbed. Slight general and local reactions were observed in a pig infected percutaneously, while another pig showed a localised abscess. The German Plague Commission observed similar reactions in sheep, goats and cows, pus from sheep giving positive findings of *B. pestis*. Bannerman and Kapadia concluded that the positive results reported by Simpson[5] and Wilm[6] were due, not to a true infection of *B. pestis*, but possibly of a similar bacteria, which is only pathogenic to these animals.

Our own 1921 experiments were performed upon two young pigs, three chickens and three ducks, which received subcutaneous injection (Table XXXVI). The pigs and chickens received big doses of *B. pestis* cultivated two days before from human P.M. 22. As the animals remained absolutely well for two weeks, they were reinjected with fresh human plague blood from P.M. 27. The ducks were also inoculated with one-third slant of an 11-day culture from a human case (P.M. 33). There can be no doubt that our experiments fulfilled the requirements of Simpson, inasmuch as they were performed during the epidemic with material of high virulence. Yet none of the animals died of plague!

[1] *Arch. d. Sc. Biolog.*, Inst. Imp. de Med. Expér. St Petersb. VI. 67.
[2] Hill, *Rep. Plague Natal*, 1902–3.
[3] *Med. uit het Gen. Lab., Weltevreden*, 3rd Series A, 1919.
[4] *Journ. Hygiene*, VIII. 209.
[5] Simpson, p. 106, etc.
[6] Wilm, *Hygien. Rundschau*, 1897, p. 291, and *Rep. to Sanit. Bd., Hongkong*, 1903.

No general reactions were observed in any of our cases. Both pigs showed localised abscesses (size of a walnut) five days after the second attempt. Two examinations of the greenish pus obtained by puncture revealed no plague bacilli, other organisms growing. It may be added that at the first puncture one pig struggled so fiercely that its peritoneal cavity was probably pierced through the abscess. No reaction occurred even after this accident. In one of the chickens, a small swelling was seen five days afterwards at the site of injection. Thickish blood was obtained by puncture, the smears showing no microorganisms and the cultures only one minute colony (Pneumococcus).

One of the ducks died accidentally three months after the first experiment. The other birds survived for six months and were then killed by a weasel. The pigs were under observation for a year. The swellings in the pigs and chicken disappeared eventually.

SUMMARY OF CONCLUSIONS.

(1) We found that the *B. pestis* present in plague sputum, although more resistant than *in vitro*, was killed within nine hours by direct sunlight at a winter temperature (– 3° C.).

(2) Mere drying of plague sputum, irrespective of other factors, *e.g.* temperature, humidity, is not a sufficient test of the killing of *B. pestis* under all circumstances. We have cultivated *B. pestis* from seemingly dry sputum in 40 per cent. cases after exposure in Petri dishes to sunlight, and in 60 per cent. cases when exposed upon wood or surgical gauze.

(3) Disinfectants and antiseptics, even in strengths above those usually recommended, have not the generally expected results upon plague sputum. For instance, carbolic acid lotion, 1:10, requires five minutes to prevent growth of *B. pestis* in sputum. Concentrated alcohol (methylated spirit) is the surest means of sterilising the hands and gloves in plague work.

(4) Rooms where patients have died of pneumonic plague do not seem particularly dangerous. A modern-built, steam-heated room, with tightly fitting windows and protected from draughts, appears more dangerous than old-fashioned native houses.

(5) The disinfection of grossly contaminated articles, like floors, walls, etc., is necessary. The problem of fumigation of the air-contents remains an open one.

(6) The infectivity of clothing as a means of propagating pneumonic plague cannot be neglected.

(7) The existence of plague carriers has been proved in the 1921 epidemic.

(8) The Mukden cotton-and-gauze mask, when properly applied, is the best means of personal protection against infection by inhalation. For those in constant and immediate contact with patients, we would advise the wearing of an additional hood with silk-piece sewn on in front, besides the use of goggles.

(9) Pigs and birds were found to be non-susceptible to highly virulent fresh material.

PART II.

THE RÔLE OF THE TARABAGAN IN THE EPIDEMIOLOGY OF PLAGUE.

I. NATURAL PLAGUE IN TARABAGANS.

The question of the rôle which the tarabagan played in the origin and spread of the first Manchurian outbreak, was widely discussed during and shortly after that epidemic. While much circumstantial evidence was brought forward and experiments were carried out to determine the susceptibility of the animal to infection with *B. pestis* and thus to establish the importance of the tarabagan factor in an indirect way, definite evidence of animals sick with plague was rather scanty notwithstanding many endeavours made in this direction.

While the Chinese-Russian Expedition in the summer of 1911 could not detect animals suffering from or succumbed to plague[1], some suspicious sick marmots and corpses were detected by Russian investigators[2]. But in only one of these animals "complete evidence of plague was found" by Zabolotny[3].

In the summer and autumn following the second Manchurian Plague Epidemic (1921), new findings were made by Russian physicians working in Transbaikalia near the Manchurian frontier. Medical Officer Kwan of our Service stationed at Manchouli was instructed to procure, if possible, material for bacteriological and histological confirmation in our own laboratory. He proceeded to Transbaikalia on the 21st September, 1921, and submitted a report of this journey, from which we quote the following data: At Soktu station a small laboratory was installed in a railway car and waggon, where the tarabagan question was investigated by Drs Zokoff, Klotkoff and Shunoff and four students. Dr Shunoff, seemingly an expert in laboratory work, had joined the laboratory only a few days ago from Chita.

Since the last summer daily expeditions on horseback had been made to find tarabagans suffering from plague. No plague was discovered among living tarabagans, but on the 16th September a student, Pavloff, found the dead body of such an animal on the hills south of Soktu, which had largely been eaten by the eagles; on the following days he found two more of them. All those three tarabagans had inguinal buboes and appeared to have succumbed to plague as was proved at post-mortem and by cultural tests. The macroscopic findings were: enlargement and inflammation of the affected glands (no suppuration was detected), enlargement of the spleen, congestion (to a dark blue colour) of the lung without apparent pneumonia. Plague bacilli were found

[1] Wu Lien Teh, "First Report of the North Manchurian Plague Prevention Service," *Journal of Hygiene*, XIII. No. 3, 1913, p. 239 *et seq.*

[2] Jasienski and Chmara-Borshevski, "Plague Report of the Chinese Eastern Railway, Harbin, 1912," and Wu Lien Teh, *loc. cit.* pp. 253–254.

[3] *Conference Report*, p. 192 and Wu Lien Teh, *loc. cit.*

in all these organs and pure cultures of *B. pestis* were obtained from the affected organs. On the 20th September two guinea-pigs were injected intraperitoneally with pure cultures obtained from a bubo. The animals died after three days and showed *B. pestis* in smears. Two other guinea-pigs injected the same day (20. 9. 21) were surviving up to the time of departure of our Medical Officer from Soktu.

Finally, the Russian doctors claimed to have found *B. pestis* in one flea of a tarabagan and to have proved that it could carry infection to man. Thus the case of the station-master of Dauria on the 1st August was claimed to be due to this mode of infection and not to skinning or eating of tarabagans. Medical Officer Kwan had occasion to see this flea specimen under the microscope. He was given a cover-glass preparation of another flea to be examined at our Harbin Laboratory. Our Medical Officer suggested to the Russians to make animal experiments on this important question by exposing guinea-pigs to the bites of tarabagan fleas. Kwan sent to our Harbin Laboratory the following specimens:

Five stained cover-glass preparations.
Two agar cultures from the tarabagans.
Pieces of spleen, lung and bubo of the tarabagans for histol. examination.

The examination of these specimens in our Harbin Laboratory showed in two of the slides (from bubo of a tarabagan and spleen of a guinea-pig) bipolar stained bacilli similar to plague bacilli. Only few bacilli of the same appearance could be seen in a poorly stained smear from a culture of one of the tarabagan buboes. The slide marked "bubo of guinea-pig after inoculation" was not very well stained either, but it showed bacilli similar to the *B. pestis* and others. No bacteria could be detected in the specimen of the tarabagan flea; the specimen was incomplete and it was not possible to determine to which variety the flea belonged. Smears taken from both cultures showed *B. pestis* in rather impure form. Experiments performed with these cultures (*vide* Table XXVII) gave in one case (rabbit) negative results, while a guinea-pig succumbed four days after it had received a whole agar slant of the other culture. No pure cultures were obtained from this animal, and only doubtful results were seen in a rabbit which had been injected with its blood.

Histological examination of the specimens of organs sent showed only few plague bacilli. The piece marked bubo of tarabagan displayed small necrotic areas amidst the lymphoid tissue, while sections from the lungs were marked by signs of pneumonic infiltration.

Apart from the above-mentioned specimens sent by Dr Kwan, it was not possible to obtain further confirmatory evidence regarding tarabagan plague from Transbaikalia.

There is no doubt, however, that at least one of the tarabagans found in the autumn of 1921 suffered from plague. Although this is the second occasion within the last ten years on which the existence of natural plague among tarabagans has been scientifically established, one should be chary about

over-emphasising its *practical* importance in the origin and spread of human plague. In the case of the rat, it has repeatedly been observed that numerous cases of plague among them precede an epidemic among human beings, making a rat epizootic a vital factor in the etiology of human plague. In the case of the Siberian marmot no such chain has as yet been satisfactorily established. In spite of long and careful observations in the marmot regions during the past ten years, only isolated instances of tarabagan plague have been found. One is aware of the close relation of the domestic rat as compared with the wild tarabagan to man, so that an epizootic among *Mus decumanus* is at once evident, whereas an epizootic among *Arctomys bobac* in the Siberian wastes may well be over before a scientific expedition reaches the spot. To this circumstance is probably due the fact that the finding of plague-infected tarabagans in both instances was only made after human cases had been observed in the vicinity. Until the existence of plague among free-living tarabagans is scientifically established before a human outbreak or independently of it, the relation of tarabagan epizootic to human plague will have to remain an open question[1]. It is hoped that the second expedition which the Chinese Government is sending out in 1922 to the marmot regions may shed further light upon this interesting problem.

II. EXPERIMENTS UPON TARABAGANS.

A. Introduction.

Experimental infection of the tarabagan has been performed by previous observers by different methods. Thus, Strong and Teague[2] infected three tarabagans percutaneously and three by thrusting in a plague contaminated hypodermic needle. Five of these animals died of acute plague; the sixth, however, infected subcutaneously and killed a fortnight later, showed small necrotic areas in the subcutis near the site of inoculation and a similar area in the anterior portion of the abdominal muscles, swelling of the inguinal glands, numerous yellowish nodules in the liver, a few in the spleen and one hyperaemic area in the right lung. Plague bacilli in small numbers could be demonstrated in abscesses and nodules. Two animals of their second series killed after approximately the same interval, showed similar changes of a chronic nature. Strong and Teague pointed to the similarity of these changes with those found in chronic rat plague. The two authors performed also two inhalation experiments with tarabagans. One of the inhaled animals died after three days and showed a primary lymphatic infection and secondary

[1] Occurrences of apparently *secondary* plague infection have been observed not only in domestic but also in free-living animals. Thus, Simpson (*loc. cit.* p. 129) cites instances of plague among monkeys and squirrels in India, and among different animals confined in the Zoological Gardens, Sydney. Berdnikow (*Centralblatt f. Bakter.* LXIX. 258) considered this possibility for the plague-infected rodents of the Astrakhan Steppes but came finally to the conclusion that they suffered from primary plague.

[2] *Mukden Report*, pp. 237 and 385 and *Phil. Journ. Science*, VII. Sec. B, No. 3, p. 224.

septicaemia apparently due to accidental laceration of the mucous membrane of the mouth (the animal bit several times at the sharp metal nozzle of the spray); the second animal died of primary plague pneumonia. Dujardin-Beaumetz and Mosny[1] inoculated three hibernating marmots with *B. pestis*; one of these died soon after, the two others survived for 61 and 115 days respectively, showing enormous numbers of *B. pestis* in their organs. Zabolotny and Tschurilina[2] reported similar results in tarabagans during the hibernating period. Their animals after inoculation with plague survived for 10–12 days, while the controls died after 3–4 days.

In order to obtain further proof of the rôle played by the tarabagan in plague, we decided to continue, and improve wherever possible, the experiments first started by us in Mukden 1917 upon the small marmot (*Spermophilus citillus*)[3]. For this purpose we utilised over fifty of the large variety (*Arctomys bobac*) caught in the neighbourhood of Manchouli and sent to our central Harbin Laboratory.

B. Inhalation Experiments.

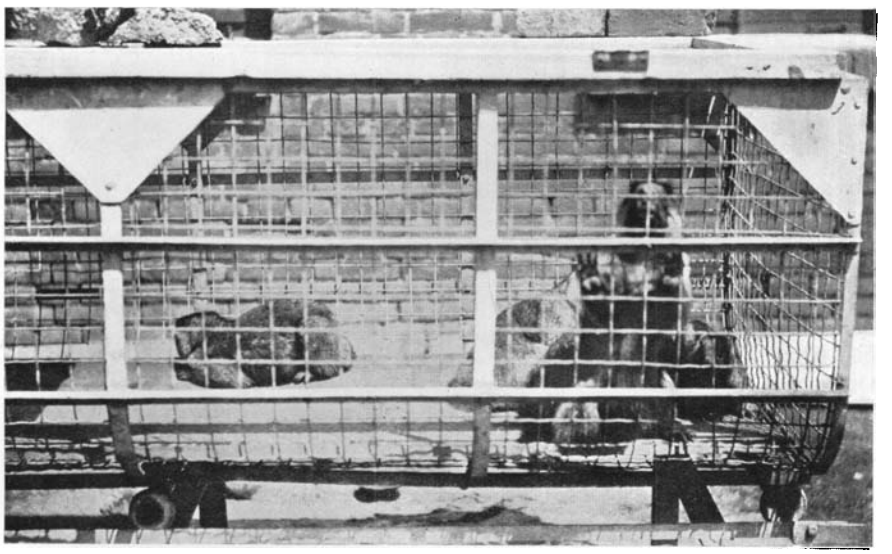
In both summers of 1921 and 1922 inhalation experiments were performed, summer being chosen because of the greater facilities afforded in the open air. These experiments will be continued in the coming winter, when we expect the new, better equipped animal laboratory to be completed. The results obtained up to date may with advantage be herewith recorded.

We used in the first series of experiments, 1921, cultures and subcultures from a purely pneumonic and a pulmonary human case, while in the second series cultures recovered from the tarabagans of the first series were used. In March 1922 a third series of inhalation experiments were started in which were employed cultures: (*a*) from a tarabagan of Series I, dying after six months of chronic plague, (*b*) from a rabbit killed by culture (*a*), and (*c*) from the 1910 epidemic kept virulent by recent passage through animals. When comparing the results obtained with these different strains, it must be acknowledged that in 1921 the best results were obtained with the strain from the pulmonary case. There was no marked difference between the cultures and subcultures. In 1922 the strains from the tarabagan with chronic plague case gave very satisfactory results, while the two contacts kept with the animal infected with the 1910 strain survived up to July 1922 (four months).

As can be seen from Tables XXVIII and XXXI nine animals were altogether infected by inhalation, six of which succumbed within eight days, while two died spontaneously of plague after about three weeks. The ninth animal showed, when killed 17 days after the inhalation, positive findings. Of the 26 contacts nine died spontaneously. In the P.M. of four of these animals succumbing after intervals ranging from 20 days to six months, plague infection was clearly present. The five other animals died some months after their exposure as contacts, two apparently not of plague, while in the other three

[1] *Compt. Rend. Acad. Sci.* 1912, CLV. No. 4, pp. 329–332.
[2] Berdnikow, p. 257.
[3] *American Journal of Infectious Diseases*, XX. No. 2.

Tarabagans congregated in a corner.



Tarabagans kept in a large wire cage. They are very vicious and have sharp claws. Medium sized iron bars are easily bent by them.

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the results of the P.M. examination were more or less suspicious. Four more of the contacts were killed after a period from two to three weeks after exposure. In the post-mortems similar suspicious findings were obtained. The rest of the contacts are still surviving up to the end of June 1921 and are apparently healthy with one exception—a small tarabagan, which seems thinner and more apathetic than the other animals.

Series 1921.

Tarabagans inhaled	6	Deaths	6
Contacts exposed	21	Positive plague deaths	2
		Suspicious plague deaths	7

N.B. None of the contacts died before 14th day.

Series 1922 (up to end of June).

Tarabagans inhaled	3	Deaths	3
Contacts exposed	6	Positive plague deaths	2
		Suspicious plague deaths	0

N.B. None of the contacts died before 19th day.

The following inhalation technique was employed in 1921:

The animal was firmly strapped upon the prepared stage with the nose held inside an iron muzzle. It was then covered with an oblong metal box without bottom and having a small circular aperture at the head end for the introduction of the nozzle of a spray. An emulsion of the culture to be employed was made with the aid of 10 c.c. of saline solution and sprayed from a graduated cylinder fitted with a fine atomiser. All precautions were taken against a possible infection by the operators. The actual experiment was performed by doctors only, the assistants standing at some distance.

After inhalation the animals were kept in cages measuring two by two by two feet with iron bars on the top and covered with fine galvanised wire gauze. In two of the experiments the contacts were separated from the inhaled animals by a partition made of similar material while in the three remaining experiments they were herded together. The results obtained with the animals placed separately differed in no marked degree from the second lot.

In the 1922 experiments, the same technique was employed except that we dispensed with the muzzle. In the former experiments it was found extremely difficult to fix the head of the animal firmly in the muzzle. Repeated attempts to do so resulted sometimes in slight bleeding from nose and mouth of the animals which we wished to avoid in the new experiments. The disadvantage of this new procedure, however, was that the nozzle of the spray could not be kept accurately directed against the respiratory entrance, so that in one instance the fur on the chest and abdomen was wetted with droplets from the emulsion. This point will be referred to again.

In future experiments we shall place the animals in an air-tight box with an iron lid, into which the indiarubber tubing of the spray will lead, thus obviating accidental lesions to any part of the respiratory entrances.

C. Findings in Inhaled Animals.

The details of the P.M. *findings in the inhaled animals* are set forth in Tables XXIX and XXXII. All five inhaled animals of the 1921 category which died spontaneously, showed in their respiratory tract more or less marked signs of primary plague infection similar to the changes in human pneumonic plague. T 7, which was killed 17 days after inoculation, showed besides enlargement and congestion of two cervical glands, slight congestion of larynx and trachea and a semipurulent discharge in the bronchi, many abscesses of the size of half a lentil in the hyperaemic lungs. Few plague bacilli were seen in blood smears (taken from the heart and periphery) or in films from the congested lung tissue and a cervical gland, while in the smears from the contents of the bronchi, pus of the lung abscesses and the spleen no *B. pestis* were detected. Cultures taken from one of the affected cervical glands and the bronchi were positive but impure. Only a few plague colonies were recovered from the lung. Hence this case forms in every respect a striking contrast to the others of this category, and inasmuch as the animal proved to be very strong and resistant when it was chloroformed there can be no doubt that we have to deal here with a subacute or even chronic form of pneumonic plague, comparable to the cases with chronic symptoms observed by Strong and Teague after subcutaneous infection.

Two of the inhaled animals of the 1922 series showed lung changes similar to, though in a lesser degree than, those encountered in the 1921 series. This seems remarkable as the animals did not die quickly but only 25 and 27 days respectively after inhalation. This phenomenon cannot be explained by a lessened virulence of the cultures employed, because one of them had recently been passed through rabbits, while the other culture proved to be of normal virulence when injected subcutaneously. In both instances, *B. pestis* grew moderately in the cultures.

The P.M. findings in the third animal of this category (T 107) which died five days after inhalation, seemed puzzling. The tongue was found congested, some of the cervical glands were swollen and hyperaemic. Haemorrhagic effusion was noted in the front cervical tissues and also in the upper larynx. The haemorrhagic patches present in the left lung appeared to be due not to an inflammatory process but to aspiration. There were ecchymoses on the epicardium. The spleen was big, but not soft; the liver showed fatty degeneration; the kidneys were congested. Ecchymoses were noted beneath the serosa of the stomach; its contents seemed normal and there were petechiae beneath the mucosa of the pylorus. The ileum was of blue-black colour; when opened it was found to be filled with reddish-black bloody masses; the mucosa showed a dark reddish brown colour, but no ulcerations or other pathological changes. Large intestine and contents appeared normal. Almost pure cultures of *B. pestis* were obtained from the heart; spleen culture was contaminated. Liver culture positive, but impure; from the ileum an almost pure culture

was obtained, which in animal experiments exhibited unusual features but nevertheless proved to be *B. pestis*. We will deal with these experiments as well as with faeces cultures from T 114 and 117 under a separate heading.

Summarising the findings of T 107, it may be possible that the death of this animal was due to accidental causes. It was very strong, struggled fiercely when strapped upon the stage and when removed to its cage. Hence the haemorrhagic effusions in the musculature and upper larynx as well as the bloody contents of the ileum might be due to this struggle, but it is difficult to explain definitely the source of the blood in the ileum. If the cause of death be an accidental one, then we may trace the growths of *B. pestis* to the inhalation five days previously with virulent bacilli. On the other hand, we may have to deal in this instance with a case of intestinal plague. It is therefore necessary to settle the matter by histological examination.

D. Findings in Contacts.

The P.M. results of *the contacts* in both 1921 and 1922 series are detailed in Tables XXX and XXXIII.

The four contacts showing plain plague manifestations will be treated later. In the meantime, only the seven tarabagans with slight evidence of *B. pestis* infection are dealt with here. These animals showed macroscopically either no changes which could be considered due to a plague infection (T 11, T 18, T 115), or only slight changes connected with such an infection. Among these (T 6 and T 36) slight changes in cervical glands were noted; T 36 showed also small yellow nodules in the normal-sized spleen. Another (T 20) was emaciated, the thymus seemed bigger than normal, and spleen was acutely swollen. Finally T 132, which was apparently killed by the other tarabagans, had a small grey spot on the epicardium which was surrounded by a hyperaemic zone and whose nature must be investigated histologically. The changes in the respiratory tract of T 6, T 18, and T 36 might be considered as caused by chloroform narcosis by which these animals were killed.

In the three animals which died spontaneously months after exposure (T 20, T 115, T 132), the cultures were sterile or negative for *B. pestis*, so that only in the smears, especially those taken from the spleen, a few bipolar stained bacilli resembling the *B. pestis* could be detected.

One of the four killed animals (T 11) showed in spleen cultures a few *B. pestis*. T 6 had a few suspicious bacteria in the cultures from heart and spleen, more such in its trachea culture; T 18 very few plague-like organisms in its heart culture and some rather doubtful bacteria from the trachea; T 36 only very few plague-like bacilli in the culture from the trachea, while those from heart and spleen were sterile.

We do not lay undue stress upon these slight and not very definite findings. On the other hand, the results of these P.M.s should not be considered as negative, and every endeavour should be made to get more definite evidence upon this point. This question will be mentioned later on.

Two contacts of the 1922 experiments died spontaneously, one after 20 days, the second after 42 days. Both succumbed to plague infection, as proved not only by films and cultures but also by animal experiments (Table XXXIII). Both animals showed small hyperaemic patches in the lungs and their spleen was bigger than normal. One animal had in addition some swollen and slightly congested cervical glands. It is noteworthy that contact T 112 died one week *before* the inhaled T 117 from which it was separated by a wire screen.

Two contacts of the 1921 experiments which died spontaneously displayed rather remarkable P.M. findings. In T 12, dead 35 days after exposure, two swollen and congested cervical glands were noted, the tonsils were swollen and slightly congested, a small abscess was seen on the pharyngeal mucosa. Larynx and trachea were congested, the latter containing slightly pinkish froth. The lungs were so congested that they looked blue-black. The liver was congested; the spleen—swollen and softer than normal—showed two small abscesses. Some haemorrhages were noted in the mesentery, the Peyer's patches in the ileum seemed somewhat swollen. In smears from the bronchi *B. pestis* were present mixed with other organisms. In the films from other organs a few *B. pestis* were detected. Cultures from the heart remained sterile, those from the spleen showed few plague-like bacilli among other kinds (no pyocyaneus bacilli present). In cultures from the other organs *B. pestis* were found mixed with others, mostly *B. pyocyaneus*. The experiments performed with these cultures have already been reported (Table XXIII); they led to the conclusion that the virulence of the *B. pestis* appeared somewhat lessened after passing through the tarabagans.

A particularly interesting case was that of T 8 which died six months after exposure. At P.M. its thymus seemed slightly swollen. Haemorrhagic spots were noted in the left lung. The right lung was firmly adherent to the pericardium. The pericardial sac was markedly distended with much fibrino-sanguineous exudate. The parietal layer of the pericardium was markedly thickened, while the visceral layer showed the appearance of the cor villosum. The spleen was acutely swollen and very soft. There was fatty degeneration of the liver. On the mesentery few haemorrhages seemed evident. Some bloody contents were noted in the stomach.

Culture of the spleen proved sterile; from the liver only few *B. pestis* were recovered among other microorganisms. Cultures from the pericardium gave a pure growth of bipolar-stained Gram-negative bacilli, which through numerous experiments performed by both subcutaneous injection and inhalation (T 114 and T 117 with their contacts) proved to be virulent plague bacilli.

E. Experiments with Faeces of Tarabagan.

In the cultures from the faeces of T 12, *B. pestis* seemed to be present together with *B. pyocyaneus*, and in one of the two experiments performed with them (Table XXIII, R 44 and R 49) both kinds of microorganisms

were recovered from the cultures. From the bloody masses contained in the ileum of T 107, bacilli similar to *B. pestis* were also obtained in almost pure culture. Four experiments were done with this culture (Table XXXIV). The first rabbit (R 119) injected with two loopfuls of the culture and reinjected after 17 days with the same dose, died 24 hours after the second injection. At P.M. the lungs were found slightly hyperaemic. Some cloudy liquid was noted in the pericardium. There was a circumscribed fibrino-purulent peritonitis present near the diaphragm. The spleen was very big (see photo) and showed many solid yellow nodules (measuring up to the size of half a lentil) on its surface, where the nodules projected, as well as on section. The mesenteric glands were partly swollen. The significance of these findings will be mentioned later on. Suffice it to state here that smears from the spleen showed a few suspicious bacteria among other organisms, while cultures from the heart and liver proved sterile and those from the spleen gave a few colonies of suspicious bacilli. R 122, which was injected with one loopful of this culture, died after four days, showing some subcutaneous abscesses in the thoracic region, an acutely swollen spleen and positive cultures of *B. pestis* from heart, spleen and liver. In two other experiments with the ileum culture from T 107 (R 126 and R 130) death occurred after five days, and no evidence of infection with *B. pestis* could be established. R 135, however, died six days after having been injected with a subculture from the tarabagan culture, showing *B. pestis* in spleen smears and heart cultures.

These positive results particularly attracted our attention, so we made in all later P.M.s of tarabagans cultivations from stomach, ileum and sigmoid. Cultures which appeared positive or suspicious were tested by animal experiments. As can be seen from Table XXXIV, we only obtained positive results with cultures from the intestinal tract of tarabagans showing plague findings in their other organs as well. Thus R 116, R 118 and R 120 died after inoculation with faeces cultures from tarabagans which had evident plague. R 125 died after having been injected with the stomach culture of such a tarabagan, and R 128 after infection with a culture from the ileum. It should be expressly stated that in no case did macroscopical evidence reveal the tarabagans, from which these cultures were taken, as having suffered from an intestinal form of plague. These facts appear to coincide with the view of Zabolotny regarding the positive findings of *B. pestis* in the intestine of plague patients. ("That these observations did not, in his opinion, prove at all that there had been cases of primary intestinal plague. These experiment only showed that plague bacilli passed through the intestinal passages or came perhaps from haemorrhages of the mucous membranes of the bowels[1].")

The presence of *B. pestis* in the intestines of these tarabagans cannot be explained by the supposition that the animals licked their fur which at times glistened with droplets of *B. pestis* emulsion after the inhalation, because one contact separated by wire screen had also positive findings of *B. pestis* in its intestines.

[1] *Mukden Report*, p. 185.

No positive results have so far been obtained in the experiments performed with intestinal cultures from two tarabagans with doubtful evidence of plague infection (T 115 and T 132) and from another with negative bacteriological findings (T 133). This point will be discussed later on.

F. Feeding Experiments upon Tarabagans (Table XXXV).

It has been proved that certain species of rodents similar to the tarabagan can be successfully infected with plague-contaminated food. Thus Wu Lien Teh and Eberson had four positive results in an experiment conducted with six marmots of the species *Spermophilus citillus*[1]. Schurupoff[2] also stated that the infection for *Spermophilus guttatus* could be conveyed in food.

The question of the infection of the tarabagan (*Arctomys bobac*) *per os* was considered at the Mukden Conference, where Zabolotny suggested the possibility of its becoming infected by feeding on human plague corpses[3]. According to the same observer human bones were found in holes of some tarabagans (p. 241). But as far as we are able to ascertain no definite evidence has been brought forward. Only one indirect feeding experiment has been carried out with tarabagans by Wu Lien Teh[4], who unsuccessfully fed a starving tarabagan with the corpse of another. To investigate the matter further we started a series of feeding experiments with tarabagans. So far we have not tried to change the regular vegetable diet of the animals except by adding large quantities (25 c.c. bouillon) of virulent plague cultures. Up to date the control rabbits have died after a short interval (5–8 days), showing only slight macroscopic changes at P.M. though giving positive bacteriological findings of plague as verified by culture and experiment (Table XXXV). One tarabagan still survives (40 days after commencement) and is apparently quite well. An attempt to recover *B. pestis* from its faeces proved negative[5].

The second tarabagan died after 19 days and showed at P.M., besides some swollen and hyperaemic cervical glands and pneumonic patches in the lungs, bloody mucus in the distended ileum where the mucosa was congested. No signs of ulceration were present. The rest of digestive tract showed no macroscopical changes. In smears from the spleen no definite plague bacilli were seen; from the contents of the ileum plentiful *B. pestis* were obtained. Culture from heart, liver and spleen remained sterile; from stomach and faeces only few *B. pestis* could be recovered. From the ileum considerable growths (impure) of *B. pestis* were obtained. Infection of the tarabagan by the alimentary canal thus seems possible. It is true that the succumbing animal showed changes

[1] *The Amer. Journ. Infectious Diseases*, XX. No. 2.

[2] *Central. f. Bakter.* 1912, LXV. Nos. 4–5, pp. 243–256.

[3] *Loc. cit.* p. 240.

[4] *Journ. Hygiene*, XIII. No. 3, p. 256.

[5] The animal died later, showing at P.M. besides enlarged and congested cervical glands, marked congestion of the respiratory tract and acute swelling of spleen, meteorism (extension of colon), and haemorrhagic areas (perhaps round mesenterial glands) in the mesentery. Cultures especially from the intestinal tract seem suspicious for *B. pestis* but no definite results were obtained so far in the experiments.

in the lungs as well as in the intestines, but the former appeared of secondary importance.

These experiments will be continued so as to comprise feeding with infected meat also—possibly from carcasses of plague stricken animals.

We have seen in exceptional instances the heads of corpses (T 106, T 132) bitten off and even partly eaten up by their bed fellows. By nature these animals are vegetarian, and it is doubtful if such investigations would add much to our knowledge of the epidemiology of the disease.

In the same way that lung plague has been proved to occur in the tarabagan possessing all the features of human pneumonic plague, so the finding of intestinal plague among tarabagans may be said to pave the way for our belief in its existence also in man. There is substantial, but only clinical, evidence of the existence of an intestinal form of plague in man [1,2] but this has been much controverted by such observers as Kitasato, Strong, Zabolotny, etc.[3] With such clear pathological evidence, which we have brought forth as a result of our Mukden and Harbin experiments upon marmots, it would be idle to deny the possibility of a similar intestinal infection in man. Surely, its mere absence in 25 autopsies out of nearly 5000 plague deaths in Mukden does not justify the attitude taken up by Strong in denying the existence of Intestinal Plague in man.

G. Conclusions from Experiments upon Tarabagans.

Our experiments prove that, once started, plague among tarabagans may lead to two, possibly three, degrees, of the disease. These are:

(*a*) An acute form, with all the familiar features.

(*b*) A chronic localised form, showing quite unusual manifestations.

(*c*) Possibly a "carrier" form, marked by slight deviations from the normal found during and after life.

Of the acute form, no more need be said as its nature and character are well known. In the chronic localised form, we have seen an animal (T 8) survive for six months and then die spontaneously showing at death marked pericarditis. Another animal (T 7) surviving for 17 days and then killed by choloroform, displayed several abscesses in both lungs and would certainly have lived longer if we had allowed it. These two animals obviously harboured virulent plague organisms and under favourable circumstances could have communicated the disease to man. Regarding the third category, plague-like bipolar-stained bacilli were found in small numbers in the blood and organs but we were not able to satisfy ourselves about the cultures or their infectivity. The fact remains, nevertheless, that this type of animal might serve, besides the chronically diseased tarabagans, as hosts for the plague bacillus. Moreover, it appears not impossible for this supposed carrier to "light up" under favour-

[1] Simpson, pp. 291–292.

[2] Osler and McCrae, *Practice of Medicine.*

[3] *Mukden Report*, pp. 185–186.

able conditions, and become actively infective. The Indian Plague Commission[1] twice stated that the chronic (resolving) form of rat plague could not become acute, but other observers, *e.g.* Swellengrebel and Otten[2] admitted this possibility for chronic animal plague. Clinical evidence tends to support the view of the existence of such a "carrier type." As late as September 1921, a localised outbreak of Bubonic plague causing four deaths occurred in Transbaikalia from the skinning and eating of some apparently healthy tarabagans by two Russians. Most Russian investigators believe in direct tarabagan infection as a cause of the almost yearly outbreak of plague among the people of these regions.

We propose to perform in future direct injections with emulsions from the seemingly most affected organs of supposed carriers (spleen, cervical glands, thymus and sputum). Just as the gall-bladder seems to harbour the *B. typhosus* in carriers of that disease, so perhaps the organs mentioned above may have an affinity for the plague bacillus. Judging by our experiments upon the faeces in tarabagans, it appears that the intestinal tract does not possess such an affinity for the plague organism in "carriers."

III. ADDENDA.

A. Fleas and Ticks in Tarabagans.

In a former Report we have described the flea infesting the tarabagan to be *Ceratophylus silantievi*, and the tick on the same animal to be a variety of *Rhipicephalus* (possibly *R. haemaphysaloides*). Our experimental work has hitherto been confined principally to the fields of inhalation and feeding, and hence the presence of these insects on their hosts has not apparently influenced our findings. At an early opportunity, we hope to study the rôle they play in conveying infection.

B. Tonsillar Infection in Tarabagans.

In every P.M. done upon the tarabagan we have carefully investigated the mouth and fauces, but, whether the cervical glands be affected or not, we have failed to locate any lesion in the tonsil or vicinity, which may suggest a possible entrance of the infection. This fact supports the view that infection through the tonsils in human plague is unusual, and lends additional weight to our post-mortem findings. (See separate article.)

C. Chronic Plague Changes met with in our Experiments.

It is difficult without histological examination to reach a definite conclusion regarding the changes occurring in animals which succumbed slowly to plague infection, whereby lesions of a chronic character developed. These results will be given in a future contribution.

[1] *Journ. Hygiene*, VII. 470, and X. 345.

[2] *Archiv. f. Schiffs- und Tropenhyg.* 1914, XVIII. No. 5, pp. 149–159.

The macroscopic changes due to chronic infection with *B. pestis* are varied. We met quite insignificant changes as well as marked lesions. Among the latter, the findings in the spleen of some rabbits (R 57, R 65, R 119) and tarabagans (T 36) were noteworthy, as they seemed to correspond to the descriptions by Strong of chronic plague in the tarabagan[1]. It will also be interesting to see how far they resemble the lesions described as "resolving" rat plague[2]. The changes observed in R 59 might likewise be compared to the pelvic buboes in "resolving" rat plague[3], while R 139 also showed nodules with necrotic centres in its omentum and mesentery.

SUMMARY OF CONCLUSIONS.

(1) The existence of sporadic cases of natural plague among tarabagans has again been established.

(2) The tarabagan is easily susceptible to pneumonic plague produced by the inhalation of *B. pestis* in spray form, and may contract, besides the acute infection, chronic type of the lung affection.

(3) Our inhalation experiments have proved contacts to be liable to pulmonary plague infection in both acute and chronic forms. The existence of carriers among tarabagans seems also probable.

(4) Tarabagans can be infected with plague by feeding upon artificially infected food, contracting an alimentary type of the disease. The *B. pestis* is easily cultivated from the intestinal tract of animals suffering from plague.

(5) Our evidence shows that subacute or chronic plague may exist among tarabagans in Mongolia and Siberia, and thus form a connecting link in the epidemiology of plague in those regions.

[1] *Mukden Report*, p. 439.

[2] *Journ. Hygiene*, VI. 530; VII. 359 and 457; X. 335; Plague Supplement 2, 1912, pp. 266, 287.

[3] *Ibid.* VII. 463–464.

APPENDIX.

Table I. Exposure of Plague Sputum to Direct Sunlight.

Hours exposed	Date	Time	Temp. C.	Barom.	Petri dish open	Petri dish closed	Condition sputum	Cult. of *B. pestis*	Remarks. Colonies
½	March 1	11½–12	0	748·7	.	1	App. unch.	p	
½	,, 3	4 –4½	–3	747·0	.	1	,,	p	
½	,, 4	12 –12½	3	748·3	1	.	,,	p	
1	,, 3	11½–12½	–1	746·6	.	1	,,	p	
1	April 3	12 –1	15	748·7	.	1	St. moist	p	Few
1	March 4	12 –1	3	748·3	1	.	App. unch.	p	
1	April 3	12 –1	15	748·7	1	.	Alm. dry	?	
2	March 4	12 –2	3	748·3	.	1	App. unch.	p	
2	April 3	12 –2	15	748·7	.	1	St. moist	?	
2	March 4	12 –2	3	748·3	1	.	Dry	n	
2	April 3	12 –2	15	748·7	1	.	Alm. dry	p	Filmy gr.
3	March 4	12 –3	3	748·3	.	1	App. unch.	p	
3	April 3	12 –3	15	748·7	.	1	Dry	?	Contam.
3	March 4	12 –3	3	748·3	1	.	App. unch.	p	Few
3	April 3	12 –3	15	748·7	1	.	Dry	n	
4	,, 3	12 –4	15	748·7	.	1	,,	?	
4	,, 17	12 –4	9	744·1	.	1	App. unch.	p?	
4	,, 3	12 –4	15	748·7	1	.	Dry	?	
4	,, 8	12 –4	8	746·5	1	.	,,	n	
4	,, 12	12 –4	19	738·5	1	.	,,	p?	Few
4	,, 15	12 –4	10	749·1	1	.	,,	p	
4	,, 17	12 –4	9	744·1	1	.	,,	p	Few
5	,, 4	12 –5	3	747·0	.	1	App. unch.	p	
5	,, 17	12 –5	9	744·1	.	1	St. moist	p	
5	,, 8	12 –5	8	746·5	1	.	Dry	n	
6	,, 8	x	3	746·6	.	1	,,	p	Few
6	,, 10	x	9	745·2	.	1	App. unch.	n	
6	,, 15	x	5	748·5	.	1	St. moist	n	
6	,, 17	x	4	744·6	.	1	,,	p	
7	,, 12	x	13	739·2	.	1	Alm. dry	n	
8	March 9	x	10	754·0	.	1	App. unch.	p	
8	,, 9	x	10	754·0	1	.	Dry	p	Few
9	,, 16	x	5	748·0	.	1	St. moist	n	
9	,, 12	x	2	752·5	1	.	App. unch.	n	
9	,, 13	x	–1	748·4	1	.	Dry	n	
10	,, 16	x	5	748·0	.	1	St. moist	n	

NOTE. Number of experiments 36. 'p' means positive; 'n' means negative; 'x' sputum was exposed to sun as long as any available. At sunset, Petri dish was closed and stored in cool place. Next morning it was again exposed to the sun for required number of hours. 'app. unch.' means apparently unchanged. Therm. readings are average, always Centigrade. Barometer readings also average.

Table II. Exposure of Sputum to Diffused Daylight.

The covers of the Petri dishes containing sputum were separated from the lower part by wire and the whole left on the window sill of a room. Room was either heated to av. temp. of 10° C. or left cold.

Hours exposed	Date	Time	Room heated	Room unheated	Outside temp.	Barom.	Condition of sputum	Cult. of *B. pestis*
7	March 11	11 a.m. to 6 p.m.	1	.	–8	752·4	Dry	p
8	April 2	11 a.m. ,, 7 p.m.	1	.	4	749·2	St. moist	p
12	,, 2	11 a.m. ,, 11 p.m.	1	.	4	749·2	,,	p
14	March 9	8 a.m. ,, 10 p.m.	1	.	–8	754·0	,,	n
23	April 2	11 a.m. ,, 10 a.m.	1	.	4	749·2	Dry	p
24	March 11	11 a.m. ,, 11 a.m.	1	.	–8	752·4	,,	n
24	April 6	,, ,, ,,	1	.	–1	752·5	,,	n
24	,, 7	,, ,, ,,	.	1	–1	749·8	App. unch.	p
30	March 11	11 a.m. ,, 6 p.m.*	1	.	–9	752·4	Dry	n
48	April 6	11 a.m. ,, 11 a.m.*	1	.	–1	749·6	,,	p
48	,, 7	x	.	1	+1	748·2	App. unch.	p
96	,, 7	x	.	1	3	746·7	Dry	n

* Next day.

NOTE. 'x' means time continued until next day or days. Twelve experiments were made.

Table III. Exposure of Sputum to Artificial Cold.

Plague sputum was collected in covered Petri dishes and secured in round tins, which were then placed in a biscuit box and placed in the ice-cellar.

Date exposed	Date begun	Date finished	Condition of sputum	Culture of *B. pestis*	Remarks
26	March 15	April 10	App. unch.	p	
33	April 26	May 29	„	n	
33	„	„	„	p	
33	„	„	„	n?	
33	„	„	„	p	
33	„	„	„	n	
40	Feb. 14	March 26	„	p	
69	„	April 24	„	p	
69	„	„	„	p	
99	April 26	Aug. 3	Alm. dry	p	During the warmer period (June 1 – Aug. 31) sputum was kept in cold storage room (Temp. −3° C.)
99	„	„	„	n	
208	„	Nov. 20	Dry	n	

Table IV. Exposure of Sputum on Wood and Gauze.

Pieces of wood and gauze with plague sputum were placed in Petri dishes in the shady corners of a slightly heated room (Temp. 10° C.). The dishes were uncovered in the series March 5 and 11. In the latter, they were covered with white sterile gauze.

Hours exposed	Date begun	Gauze	Wood	Outside temp.	Barom.	Condition of sputum	Cult. of *B. pestis*	Remarks. Colonies
24	March 5	1	.	−8	748·7	Dry	p	
24	„ 5	.	1	−8	748·7	„	p	
30	„ 11	1	.	−9	752·4	„	n	
30	„ 17	1	.	−5	747·2	„	p	
30	„ 11	.	1	−9	752·4	„	n	
30	„ 17	.	1	−5	747·2	„	p	
48	„ 5	1	.	−7	748·0	„	n	
48	„ 11	1	.	−10	751·1	„	p	
48	„ 17	1	.	−3	745·6	„	p	
48	„ 29	1	.	−4	748·8	Almost dry	p	Few
48	„ 5	.	1	−7	748·0	Dry	n	
48	„ 11	.	1	−10	751·1	„	n	
48	„ 17	.	1	−3	745·6	„	p	
48	„ 29	.	1	−4	748·8	Almost dry	p	
54	„ 29	1	.	−4	748·8	„	p	
54	„ 29	.	1	−4	748·8	„	n	
70	„ 29	1	.	−1	747·8	Dry	p	Few
70	„ 29	.	1	−1	747·8	„	p	One
72	„ 29	1	.	−1	747·8	„	p	Few
72	„ 29	.	1	−1	747·8	„	p	
96	April 7	1	.	5	747·2	„	n	
96	„ 7	.	1	5	747·2	„	n	Contaminated

Note. Twenty-two examinations were made altogether.

Table V. Plague Sputum deposited upon Earth.

Plague sputum was deposited upon fresh earth in Petri dishes and placed in the shady corner of a slightly heated room (mean Temp. 10° C.). Petri dishes covered in earlier experiments; in later ones some were covered with pieces of sterile gauze, others with lid.

Hours exposed	Date	Outside temp.	Barom.	Condition of sputum	Cult. of *B. pestis*	Remarks. Colonies
1	April 9	7	746·6	.	p	Few; lid closed
2	March 12	−10	752·5	.	n	
2	April 9	7	746·6	.	n	Lid closed
4	March 11	−9	752·4	Dry	n	
6	April 12	13	739·2	.	p	Few
7	March 11	−9	752·4	Dry	n	
7	April 15	5	748·5	.	p	
8	March 9	−8	754·0	Dry	n	
24	March 5–6	−8	748·7	„	n	

Note. Altogether nine experiments were made.

Table VI. Plague Sputum covered by Earth.

Sputum was placed in Petri dishes and covered with earth, either fresh or partially sterilised. Dishes were closed in series of March 14 and of April 9, and covered with white paper in the later series. These experiments were done in our main laboratory (Temp. 17° C.).

Hours exposed	Date	Earth fresh	Earth sterilised	Barom.	Cult. of *B. pestis*	Remarks. Colonies
½	March 21	1	.	743·7	p	Few
½	,, 21	.	1	,,	p	x
1	,, 14	1	.	748·3	n	
1	,, 14	.	1	,,	n	
1	,, 18	1	.	748·4	?	
1	,, 18	.	1	,,	p	
1	,, 21	1	.	743·7	p	Few
1	,, 21	.	1	,,	p	x
1	,, 29	1	.	747·4	p	Few
1	,, 29	.	1	,,	p	
2	,, 29	1	.	,,	p	Few
2	,, 29	.	1	,,	p	
3	,, 14	1	.	748·3	n	
3	,, 14	.	1	,,	n	
3	April 9	1	.	746·6	p	Few
4	,, 9	1	.	,,	p	,,
5	,, 13	1	.	733·2	p	,,
6	,, 17	1	.	744·6	p	,,
12	,, 24	1	.	746·7	p	

NOTE. Altogether 19 experiments were made with only four negatives. 'x' means earth was almost sterile, only a few non-spore-bearing colonies growing in controls.

Table VII. Plague Sputum mixed with Earth.

Sputum was *mixed* with earth. Technique as in Table VI.

Hours exposed	Date	Earth fresh	Earth partly sterilised	Barom.	Cult. of *B. pestis*	Remarks. Colonies
1	March 14	1	.	748·3	p	
1	,, 14	.	1	,,	p?	
1	,, 18	1	.	748·4	n	
1	,, 18	.	1	,,	n	
2	,, 18	1	.	,,	p	
2	,, 21	1	.	743·7	p	
2	,, 21	.	1	,,	p	
3	,, 14	1	.	748·3	n	
3	,, 14	.	1	,,	p	
3	,, 18	1	.	748·4	p	Few
3	,, 18	.	1	,,	n	
3	,, 21	1	.	743·7	p	
3	,, 21	.	1	,,	p	
3	,, 29	1	.	747·4	p	Few
3	,, 29	.	1	,,	p	,,
4	,, 18	.	1	748·4	p	,,
4	,, 21	1	.	743·7	p	
4	,, 21	.	1	,,	p	
4	,, 29	1	.	747·4	p	Few
4	,, 29	.	1	,,	p	
5	,, 29	1	.	,,	p	Few
5	,, 29	.	1	,,	p	,,
6	,, 29	1	.	,,	p	,,
6	,, 29	.	1	,,	p	

NOTE. Twenty-four experiments were done with only four negatives.

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Table VIII. Action of Liquid Disinfectants.

The sputum was subjected to the action of solutions of different disinfectants in varying strengths. For technique see text.

Disinfectant	Sol.	30 min.	20 min.	10 min.	5 min.
Ac. carbol.	1 : 10	.	.	n (1)	n (1)
	1 : 20	? (5)	? (5)	? (6)	? (10)
	1 : 40	? (7)	? (7)	p (7)	p (3)
	1 : 80	p (5)	p (5)	p (4)	p (3)
Hg. perchl.	1 : 500	.	n (2)	p (2)	p (2)
	1 : 1000	n (3)	? (5)	? (5)	p (4)
	1 : 2000	n (3)	? (4)	p (5)	p (4)
	1 : 4000	p (4)	p (3)	p (4)	p (4)
Lysol	1 : 50	n (4)	n (4)	? (4)	? (3)
	1 : 100	p (5)	? (5)x	p (5)	p (5)
	1 : 200	p (4)	p (4)	p (4)	p (4)
	1 : 400	p (5)	p (4)	p (4)	p (4)
Phenoid	1 : 50	? (4)	p (4)	? (3)	p (3)
	1 : 100	p (3)	p (3)	p (3)	? (3)
	1 : 200	p (3)	p (3)	p (3)	p (3)
	1 : 400	p (3)	p (3)	p (3)	p (3)
Pot. permang.	1 : 500	? (3)	p (3)	p (3)	p (3)
	1 : 1000	p (1)	p (1)	p (1)	p (1)
	1 : 2000	p (1)	p (1)	? (1)	p (1)
	1 : 4000	p (1)	p (1)	p (1)	p (1)
Hydrog. peroxide	1 : 3	p (1)	p (1)	n (1)	p (1)
	1 : 5	p (1)	p (1)	p (1)	p (1)
	1 : 10	p (1)	p (1)	p (1)	p (1)
Izal	1 : 50	? (2)	? (2)	? (2)	? (2)
	1 : 100	? (2)	p (2)	p (3)	p (3)
	1 : 200	p (1)	p (1)	p (1)	p (1)
	1 : 400	p (1)	p (1)	p (1)	p (1)
Lysoform	1 : 50	p (1)	p (1)	p (1)	? (2)
	1 : 100	p (1)	p (1)	p (1)	p (2)
	1 : 200	p (1)	p (1)	p (1)	p (1)
	1 : 400	p (1)	p (1)	p (1)	p (1)
Antiform.	1 : 10	p (1)	? (1)	p (1)	p (1)
	1 : 20	? (2)	p (2)	p (2)	? (2)
	1 : 40	p (2)	p (2)	p (2)	p (2)
	1 : 80	p (2)	p (2)	p (2)	p (2)

Explanation. 'p' means growth obtained in all or majority of experiments; 'n' means no growth obtained in experiments; '?' means growth obtained in minority of experiments, the rest being sterile or contaminated; figure in bracket means number of experiments performed; 'x' means positive result obtained in animal experiment.

Table IX. Action of Alcohol and Lime.

Technique as in Table VIII.

Time mins.	Alcohol concentrated	Alcohol diluted $\frac{1}{2}$	Lime water 1 : 10	Slaked lime
30	n (1)	p (1)	n (1)	n (2)
20	n (1)	p (1)	n (3)	? (3)
10	n (1)	p (1)	? (4)	? (3)
5	n (1)	p (1)	? (4)	? (3)
4	n (3)	p (1)	p (3)	? (2)
3	p (10)	p (1)	? (3)	n (2)
2	p (10)	p (1)	p (2)	n (2)
1	p (2)	p (1)	? (2)	n (2)
$\frac{1}{2}$	p (1)	p (1)		

Note. Absolute alcohol and concentrated methylated spirit gave same results. Signs same as in Table VIII.

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Table X. Action of Lime.

Plague sputum was placed in Petri dishes either on slaked lime or covered with it. Experiments done in laboratory (Temp. 17° C.).

Hours exposed	Date	Placed on lime	Covered with lime	Barom.	Cult. of *B. pestis*
¼	April 24	.	1	746·7	n?
½	,, 24	.	1	,,	n
1	,, 17	.	1	744·6	n
2	,, 17	1	.	,,	p?
4½	,, 13	1	.	733·2	n
5	,, 13	.	1	,,	n

Table XI. Feeding Experiments with Plague Sputum.

Two sets of animals, each consisting of one rabbit and one guinea-pig, were fed with plague sputum mixed with ordinary vegetable food.

Days fed	Date begun	Date finished	Died	P.M. result
3	April 11	April 13	April 14	No plague
5	,, 24	,, 28	May 13	,,

NOTE. One rabbit and one guinea-pig survived.

Table XII. Experiments upon Coughing.

Plague patients were allowed to cough on agar plates held perpendicularly and directly in front of their mouths at varying distances.

Date	Outside temperature	Barom.	Plague ward	Open air	Result: perpendicular distance in feet ½	1	2	3	4	5	6
March 7	−4	747·5	1	.	.	.	n?	n	.	n	n
,, 8	−5	748·7	.	1	.	.	n	n	.	n	n
,, 8	,,	,,	1	.	.	.	.	.	.	n	.
,, 9	−8	754·0	.	1	.	.	n	.	n	?	.
,, 29	−4	747·4	.	1	p	p	.	.	.	.	.
April 2	4	749·2	.	1	p	p	p	?	.	.	.
,, 5	−1	750·3	.	1	.	n	n	?	n	.	.

Table XIII. Infectivity of Sick Rooms. Agar Plates.

Uncovered agar plates were placed in the sick rooms of plague patients at heights of 1 ft., 3 ft. and 6 ft. as far away from patient as possible (not in direct line of breath). Room 10 by 10 by 10 ft.

Hours exposed	Date	Barom.	Culture of *B. pestis*	Remarks
½	Feb. 28	747·9	n	
½	,,	,,	n	Room chosen where one patient had died and
½	March 3	747·0	n	been removed an hour before. Plates were placed
½	,, 4	748·6	n	on floor, bed and window-sills
1	,, 13	748·4	n	

Table XIV. Infectivity of Sick Rooms. Animals.

Guinea-pigs and rabbits were placed in rooms where plague patients were dying or had died immediately before. Animals were kept in pairs in tin buckets (diam. 11 ins. and height 11½ ins.) placed on floor. At stipulated times, the pairs were removed from room. Experiment of Feb. 20 was performed in steam-heated main building (Temp. 17° C.) in a room 12 by 12 by 10 ft. with one outside window; the others were in plague wards.

In another experiment a rat was used.

(*a*) GENERAL TABULATIONS.

Date begun	½ hr. b. d.	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
Feb. 20	1	a	a	1	1	b	.	.	.	.	.
March 2	1	a	1	1	.	.	.	.	.	.	.
,, 21	1	1	1	1	.	.	.	.	.	.	.
April 5	.	.	.	.	.	.	.	.	.	.	c
,, 7	.	.	.	.	1	.	1	1	1	.	.
,, 12	.	.	.	.	.	.	.	.	.	.	d
,, 21	.	.	.	.	.	.	.	.	.	e	.

EXPLANATION. '½ hr. b. d.' means ½ hr. before death. All others after death. 'a' means death of one of the pair; 'b' means death of both; 'c' these guinea-pigs (4) were killed by a Russian colleague to whom they belonged; 'd' two guinea-pigs and two rabbits exposed; 'e' rat exposed.

Table XIV—*continued.*

(*b*) Table of Post mortems (Animals).

Hours exposed	Date of exposure	Animal died	Interval	Result	Bacteriological findings
½	Feb. 20	Feb. 27	7 days	p	*Smears.* Heart, lung, spleen, pos. *Cult.* Lung, pos.
½	March 2	March 17	15 ,,	p	*Smears.* Heart, lung, spleen, neg. *Cult.* Lung, neg.; heart, spleen, pos.
1	Feb. 20	Feb. 24	4 ,,	p	*Smears.* Heart, neg.; spleen, pos. *Cult.* All pos.
4	Feb. 20	Feb. 25	5 ,,	p	*Smears.* Heart, spleen, pos. *Cult.* All pos.
4	Feb. 20	Feb. 25	5 ,,	n	
84	April 21	May 1	7–10 ,,	n	(Rat used)
96	April 5	April 9	4 ,,	p	Other three guinea-pigs showed no signs of plague

Table XV. Action of Formalin Gas and Sulphur Fumes.

Plague sputum in open Petri dishes was exposed in an unheated room (10 by 10 by 10 ft.) to formalin gas or sulphur fumes. Formalin gas was generated with 400 g. formalin, 200 g. pot. permang. and 200 g. water. Sulphur (2 lbs. for each 1000 c. ft.) was burnt in earthenware bowl surrounded with water in basin. Walls and floor moistened with water. Windows sealed by paper.

Hours exposed	Date	Formalin gas	Sulphur fumes	Outside temp.	Barom.	Condit. of sputum	Cult. of *B. pestis*	Remarks. Colonies
4	April 1	1	.	7	744·5	.	p	
4	,, 1	.	1	7	,,	.	p	
12	,, 14	1	.	6	735·1	App. unch.	p	
12	,, 16	1	.	4	744·9	,,	p	Few
12	,, 14	.	1	6	735·1	Still moist	n	
12	,, 16	.	1	4	744·9	Dry	n?	
12	,, 16	.	1	4	,,	,,	n	
15	,, 10	1	.	8	743·8	Almost dry	p	
15	,, 10	.	1	8	,,	,,	n	Contam.
18	,, 18	.	1	7	744·5	Still moist	n	
20	,, 6	1	.	−1	751·1	Almost dry	?	
20	,, 9	1	.	8	745·9	,,	p	
20	,, 6	.	1	−1	751·1	Dry	n	Contam.
20	,, 9	.	1	8	745·9	Almost dry	n	
20	,, 9	.	1	8	,,	,,	n?	Contam.
23	,, 8	1	.	5	746·6	.	n	
23	,, 26	.	1	7	748·2	Dry	n	
24	,, 14	1	.	6	741·8	App. unch.	n	
24	,, 18	1	.	7	744·5	Still moist	n	
24	,, 18	1	.	7	,,	Almost dry	p	Few
24	,, 24	1	.	8	744·3	Dry	p	Few
24	,, 24	1	.	8	,,	,,	n	
24	,, 24	1	.	8	,,	,,	p	Few
24	,, 25	1	.	8	746·0	,,	p?	
24	,, 25	1	.	8	,,	,,	n	
24	,, 25	1	.	8	,,	,,	n	
24	,, 30	1	.	11	750·0	,,	n	
24	,, 30	1	.	11	,,	,,	n	
24	,, 14	.	1	6	741·8	Still moist	n	
24	,, 18	.	1	7	744·5	,,	n?	
24	,, 20	.	1	7	744·8	Dry	?	
24	,, 20	.	1	7	,,	,,	n	
24	,, 20	.	1	7	,,	,,	n	
24	,, 21	.	1	10	740·6	Almost dry	?	
24	,, 21	.	1	10	,,	Dry	?	
24	,, 21	.	1	10	,,	,,	n	

Note. Thirty-six experiments were made altogether.

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Table XVI. Examination of Plague Infected Clothing.

Pieces of cloth cut from the external coats of plague corpses were gently stroked on agar plates. Care was taken not to employ any visible sputum-soiled cloth.

Hours dead	Date	Outside temp.	Barom.	Coat front	Coat back	Cult. of *B. pestis*	Remarks: Colonies
2	April 2	4	749·2	1	.	p	Few
4	,, 7	−1	749·8	1	.	p	,,
4	,, 7	−1	,,	1	.	p	,,
4	,, 7	−1	,,	1	.	p	,,
4	,, 7	−1	,,	1	.	n?	Contam.
4	,, 13	12	733·2	1	.	p	
4	,, 13	12	,,	.	1	p	
4	,, 13	12	,,	1	.	p	Few
4	,, 13	12	,,	.	1	n	
4	,, 17	4	744·6	1	.	n	
4	,, 17	4	,,	.	1	?	
4	,, 17	4	,,	1	.	p?	
4	,, 17	4	,,	.	1	p	Few
6	,, 24	7	746·7	1	.	p	,,
6	,, 24	7	,,	.	1	p?	
24	,, 9	5	746·6	1	.	p	Few
24	,, 9	5	,,	.	1	p	,,
24	,, 9	5	,,	1	.	p	,,
24	,, 9	5	,,	.	1	p?	
24	,, 9	5	,,	1	.	p	Few
?	March 5	−8	747·9	1	.	p	
?	,, 12	−10	752·5	1	.	n	
?	,, 16	−7	748·0	1	.	n	
?	,, 21	−6	743·7	1	.	n	x
?	May 9	14	741·5	1	.	n	
?	,, 9	14	,,	.	1	n	
?	,, 14	13	744·6	1	.	p?	
?	,, 14	13	,,	.	1	n	
?	,, 14	13	,,	1	.	n	y
?	,, 14	13	,,	.	1	n	y

NOTE. Altogether 30 experiments were made. 'x'=another piece of same coat soiled with visible dry sputum was negative; 'y'=animal experiments proved negative.

Table XVII. Animal Experiments with Infected Clothing.

Sets of two guinea-pigs and two rabbits were kept in new coffins (partially closed), where outer clothes recently removed from dead were placed.

Hours exposed	Date begun	Date finished	Animal died	Result	Remarks
12	April 26	April 26	May 5 (rabbit)	n	
24	,, 26	,, 27	.	.	Alive
24	,, 28	,, 29	May 25 (rabbit)	n	
72	May 20	May 23	,, 23 (rabbit, guinea-pig)	n	x
144	April 28	,, 4	,, 18 (rabbit)	n	y

NOTE. 'x,' 'y'=clothes were replaced by another set after two and four days respectively.

Table XVIII. Experiments with Paper Money and Coin of Plague Cases.

Technique was same as in Table XVI. Cultivations much contaminated.

Date	Paper money	Silver coin	Cult. of *B. pestis*	Remarks
April 13	1	.	n	
,, 13	1	.	?	
,, 17	1	.	n	
,, 17	1	.	n	
,, 24	.	1	n	x
,, 25	1	.	n	
,, 25	1	.	n	
,, 27	1	.	n	y

NOTE. 'x'=plentiful non-plague colonies obtained. Two controls done with silver coins of healthy persons showed fewer colonies. 'y'=rabbit inoculated with emulsion remained healthy.

Table XIX. Animal Experiments with Urine of Plague Cases.

2 c.c. of urine from plague cases were injected subcutaneously into guinea-pigs.

Date	Sick	Dead	Animal died	Cult. of *B. pestis*	Remarks
April 24	1	.	n	.	Survived
„ 24	.	1	n	.	„

Table XX. Observations upon Contacts and Plague Carriers.

Contacts immediately after admission were investigated for carriers.

Case no.	Date	Sputum	Tonsil	Result	Date of animal experiment	Animal died	Result	Remarks
2	Feb. 2	1	.	p	Feb. 7	Feb. 8	p	Positive case
2	„ 6	1	.	p	„ 13	„ 14	p	
2	„ 6	.	1	p	.	.	.	
123	March 1	1	.	n	.	.	.	
126	„ 2	1	.	n	.	.	.	
141	„ 4	1	.	p	March 6	March 7	p	Positive case
141	„ .	.	.	.	„ 8	„ 9	p	x
141	„ 9	1	.	n	.	.	.	
141	„ 9	.	1	n	.	.	.	
141	„ 13	.	1	n	.	.	.	
141	„ 18	.	.	.	.	.	.	y
142	„ 4	1	.	n	.	.	.	
143	„ 4	1	.	n	.	.	.	
144	„ 4	1	.	?	March 6	March 10	n	
191	„ 14	1	.	n	.	.	.	
192	„ 14	1	.	?	March 19	.	n	
192	„ 19	1	.	n	.	.	.	
193	„ 14	1	.	n	.	.	.	
194	„ 14	1	.	n	.	.	.	
195	„ 14	1	.	?	March 19	.	n	
195	„ 19	1	.	n	.	.	.	
196	„ 14	1	.	n	.	.	.	
209	„ 22	1	.	n	.	.	.	
210	„ 22	1	.	n	.	.	.	
215	„ 26	1	.	p	.	.	.	z
220	„ 28	1	.	n	.	.	.	
220	„ 30	1	.	n	.	.	.	
221	„ 28	1	.	n	.	.	.	
222	„ 28	1	.	n	.	.	.	
223	„ 28	1	.	n	.	.	.	
223	„ 30	1	.	n	.	.	.	
226	„ 30	1	.	n	.	.	.	
227	„ 30	1	.	n	.	.	.	
228	„ 30	1	.	?	.	.	.	
228	April 2	1	.	n	.	.	.	

Note. Thirty-five examinations were made, thirty upon sputum and three upon tonsils. 'x'=an emulsion of heart culture (March 7) was injected; 'y'=serum of this case 1 : 50 did not agglutinate *B. pestis*; 'z'=not counted as carrier because case developed plague soon after.

Table XXI. Experiments with Protective Masks.

Cultures were taken from (*a*) outer layer of gauze, (*b*) outer layer of cotton wool, and (*c*) inner layer of cotton wool of masks actually worn in plague wards.

Hours exposed	Date	(*a*)	(*b*)	(*c*)	Outside temperature	Barom.	Cult. of *B. pestis*
½	March 3	1	.	.	−11	747·0	n
½	,, 3	.	1	.	−11	,,	n
½	,, 3	.	.	1	−11	,,	n
3	,, 3	1	.	.	−11	,,	n
3	,, 3	.	1	.	−11	,,	n
3	,, 3	.	.	1	−11	,,	n
3	,, 5	1	.	.	− 8	747·9	p
3	,, 5	.	1	.	− 8	,,	n
3	,, 5	.	.	1	− 8	,,	n
3	,, 9	1	.	.	− 8	754·0	n
3	,, 9	.	1	.	− 8	,,	n
3	,, 9	.	.	1	− 8	,,	n
4	,, 12	1	.	.	−10	752·5	n
4	,, 12	.	1	.	−10	,,	n
4	,, 12	.	.	1	−10	,,	n

Table XXII. Experiments with Mixed Infection*.

Series I and II.

Animal	Date of infection	Date of death	Protocol
Gp. I	17. iii 21	18. iii (15 h.)	Recd. intrap. 1 c.c. BP. and 1 c.c. BH. *S.* Neg. *C.* Ht. ster.; Sp. and Pe. BH.
Gp. K	24. iii	26. iii (50 h.)	Recd. intrap. 1 c.c. BH. on 22. iii, then 1 c.c. BP. and 1 c.c. BH. At pm. peritoneal abscess. *S.* Ht. and Bl. neg.; Sp. and Abs. BP.; Pe. cavity, BP. and BH. *C.* Ht. BP.; Sp. BH.; Pe. BP. and BH.
Gp. M	21. iii	27. iii (144 h.)	Recd. intrap. 1 c.c. BP. and 1 c.c. BH. At pm. peritoneal abscess. *S.* Ht. neg.; Pe. BP. *C.* Ht. and Pe. BP. and BH.
Gp. O	24. iii	27. iii (72 h.)	Recd. intrap. 1 c.c. BS. on 22. iii, then 1 c.c. BP. and 1 c.c. BS. At pm. peritoneal abscess. *S.* Ht. and Abs. BP. *C.* Ht. and Abs. BP. and BS.
Gp. P	1. iv	6. iv (120 h.)	Recd. subc. 1 c.c. BS. daily 25–31. iii, then 1 c.c. BP. and 1 c.c. BS. on back. At pm. peritoneal abscess. *S.* Ht. ? BP.; Sp. BP. *C.* Ht. BP.; Sp. and Pe. BP. and BS.
R. 109	27. iii. 22	31. iii (96 h.)	Recd. subc. on back ⅕ c.c. 9 day *C.* BP. and BS. At pm. Sp. covered by pus. *S.* Sp. BS. and ? BP. *C.* Ht. ster.; Lv. and Sp. BS. and BP.

* Abbreviations used in Tables XXII–XXXV:—Abs. = abscess. ac. carbol. = carbolic acid. BH. = spore-bearing bacillus. Bl. = blood. BP. = *Bacillus pestis*. br. = bronchus. BS. = *B. subtilis*. *C.* = culture. congest. = congestion. contam. = contaminated. cult. = culture. exp. = experiment. Gp. = guinea-pig. h. = hours after inoculation. Haem. = haemorrhage. Ht. = heart. imp. = impure. inguin. = inguinal region. inocd. = inoculated. intrap. = intraperitoneal. isol. = isolated. Lg. = lung. Lv. = liver. macro. = macroscopic. neg. = negative. Pe. = peritoneum. pm. = post-mortem examination. pos. = positive. Py. = *B. pyocyaneus*. PyV. = *B. pyocyaneus* vaccine. Recd. = received. rt. = right. *S.* = smear. subc. = subcutaneous. subcult. = subculture. Sp. = spleen. T. = Tarabagan. tarabs. = Tarabagans. Tr. = trachea.

Table XXIII. Experiments with mixed Infection.

Series III. (*B. pestis* with *B. pyocyaneus* (B.Py.).)

Animal	Date of infection	Date of death	Protocol
R. 45	20. ix	21. ix (24 h.)	Recd. subc. whole slant bronchus *C.* from T. 12 plus loop BP. At pm. congest. Larynx and Tr. Haem. Lg. subc. abs. inguin. rg. *S.* Pos. BP. *C.* Ht. and Lg. ster. Inguin. abs. BP. impure.
R. 46	13. ix	22. ix (9 d.)	Recd. subc. whole slant trach. *C.* from T. 12. Survived for 9 days and then used for another exp. At pm. big subc. abs. abdomen. TB. nodules in lungs, confirmed histol.
R. 44	13. ix	19. ix (6 d.)	Recd. subc. whole slant faeces *C.* from T. 12. At pm. no apparent macro. changes. *S.* Some BP.-like in all organs. *C.* Ht. and Lg. ster. Trach. contam.
R. 47	23. ix	25. ix (2 d.)	Recd. subc. whole slant trach. subcult. from T. 12. At pm. perit. abs. and subc. abs. in chest. *S.* Some BP. in all organs. *C.* Ht. BP. impure. Perit. and subc. abs. BP. and Py. Lg. ? BP. and Py.
R. 49	3. x	8. x (5 d.)	Recd. subc. on back 2 loops faeces subcult. from T. 12. At pm. abscess site inject. Early peritonitis near liver. Abs. kidney. ? Right pneumonia. *S.* Some BP. in all organs. *C.* Ht. ster. Lg. BP. imp. Bladder, perit. faeces, pus BP. and Py.
R. 58	3. x	1. xi (29 d.)	Recd. subc. 1 loop Lg. cult. from T. 12. At pm. Sp. congest. *S.* Ht. Sp. Lg. some BP.-like. *C.* Ht. ster. Sp. ? BP. Histol. some bipolar BP.-like in Sp.
R. 48	3. x	4. x (24 h.)	Recd. subc. on back 2 loops trach. subcult. from T. 12. At pm. oedema rt. subc. abdomen. *S.* Some BP. in all organs. *C.* Some BP. imp. in all organs.
R. 59	4. x	2. xi (29 d.)	Recd. subc. on back $\frac{1}{4}$ loop subcult. trach. from T. 12. At pm. abs. pelvis (? vagina). *S.* Ht. and Sp. some BP. *C.* Ht. ster. Sp. BP. imp.
R. 63	12. x	19. xi (38 d.)	Recd. $\frac{1}{2}$ loop subc. trach. subcult. from T. 12. Survived 32 days, then used for another exp.
R. 62	12. x	18. xi (37 d.)	Recd. subc. $\frac{1}{2}$ loop trach. subcult. from T. 12. Survived 30 days, then used for another exp.
R. 57	12. x	1. xi (20 d.)	Recd. subc. $\frac{3}{4}$ loop trach. subcult. from T. 12. At pm. Lg. and Lv. congest. Sp. enlarged and full of nodules. *S.* Ht. and Lg. ? BP. Sp. and Pe. some BP. *C.* Ht. BP. imp. Sp. ster.
R. 65	8. xi	21. xi (13 d.)	Recd. subc. $1\frac{1}{2}$ loops Py. (isol. from T. 12 and still containing some BP.) At pm. Lg. petechiae. Lv. fatty. Sp. swollen with miliary nodules. *S.* Some BP.-like in all organs. *C.* Ht. ster. Sp. contam. Lv. some BP.-like imp.
R. 60	8. xi	13. xi (5 d.)	Recd. subc. $1\frac{1}{2}$ loops trach. subcult. from T. 12. At pm. Lv. fatty. Sp. acute swollen. *S.* Some BP.-like in all organs. *C.* No BP.
R. 90	9. ii. 22	12. ii (3 d.)	Recd. subc. 1 c.c. bouillon subcult. trach. from T. 12 plus 1 c.c. BP. At pm. oedema in subc. tissue chest. Small abs. Lv. Sp. acute swollen. *S.* Lv. some BP. Other organs ? BP. *C.* Ht. Sp. Lv. some BP. and plentiful Py. Control R. 92 inocd. with BP. alone died in 4 days.
R. 100	5. iii. 22	6. iii (27 h.)	Recd. 1 c.c. of 24 h. culture of Py. and BP. subc. At pm. ? Rt. pneum. Sp. acute swollen. *S.* Ht. ? BP. Sp. some BP. Lv. BP. and Py. *C.* Ht. ster. Lv. and Sp. Py. and some BP. Control R. 101 inocd. with BP. alone died in 4 days. Control R. inocd. with Py. alone survived.

Table XXIV. Experiments with Mixed Infection.

Series IV. (*B. pestis* with *B. acid. lact.*, BL.)

Animal	Date of infection	Date of death	Protocol
R. 108	27. iii. 22	31. iii (4 d.)	Recd. subc. $\frac{1}{8}$ c.c. of 5 day cult. BP. and BL. mixed in milk. At pm. Lg. congest. esp. in rt. lower lobe. Lv. congest. and fatty. *S.* Some BP. and BL. *C.* Ht. and Lv. plenty BP. some BL. Control R. 110 inocd. with BP. in milk alone died in 6 days.

Table XXV. Experiments with Pyocyaneus Vaccine (PyV.).

Animal	Date of infection	Date of death	Proctocol
C. R.	23–25. ii. 22	Surv.	Recd. subc. on 3 days PyV. preserved in $\frac{1}{2}$ per cent. ac. carbol. Survived.
R. 97	23. ii. 22	26. ii (3 d.)	Recd. subc. 1 loop BP. (control). At pm. pos. BP.
R. 98	23. ii. 22	28. ii (5 d.)	Recd. subc. 1 loop BP. mixed with $2\frac{1}{2}$ c.c. PyV. (preserved as above); on 2 following days 2 c.c. PyV. daily. At pm. Sp. swollen. Lv. fatty degeneration. *S.* Sp. shows BP. *C.* Sp. Lv. Ht. BP. imp.
R. 104	8. iii. 22	12. iii (4 d.)	Recd. subc. 2 loops of 2 day BP. cult. grown in fresh PyV. (no carbol). At pm. animal pregnant. No apparent changes. *C.* Ht. Lv. Ut. BP. imp. Fetus sterile.
R. 105	8. iii. 22	12. iii (4 d.)	Recd. subc. 2 loops of BP. cult grown in fresh PyV. (another sample, no carbol). At pm. pos. BP.

Table XXVI. Experiments with Adrenalin mixed with Vaccine or Serum.

Animal	Date of infection	Date of death	Protocol
R. 96	14. ii	18. ii ($4\frac{1}{2}$ d.)	Recd. subc. $\frac{1}{6}$ slant BP. (control). At pm. pos. BP.
R. 95	,,	18. ii (4 d.)	Recd. subc. $\frac{1}{6}$ slant BP. and on 3 following days 1, 2 and 2 c.c. anti-pest vaccine. At pm. Sp. soft and swollen. Lv. fatty. *S.* Ht. ? BP. Lv. and Sp. BP. *C.* Ht. Lv. Sp. BP.
R. 94	,,	18. ii ($3\frac{1}{2}$ d.)	Recd. subc. $\frac{1}{6}$ slant BP. and on 3 following days 1 c.c. adrenalin followed after 20 minutes by 1, 2 and 2 c.c. anti-pest vaccine. At pm. Sp. acute swelling. Lv. fatty degeneration. *S.* Ht. Sp. Lv. BP. *C.* ,, ,, ,,
R. 127	29. iv	4. v (5 d.)	Recd. subc. 1 loop BP. and on 4 following days 1 c.c. adrenalin and 1 c.c. anti-pest serum. At pm. Rt. Lg. congest. Sp. slightly swollen. Lv. fatty degeneration. *C.* Ht. Sp. Lv. BP.

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Table XXVII. Cases of Natural Plague in Tarabagans.

Animal	Date of infection	Date of death	Protocol
T. X	?	16–18. ix. 22	Materials from 3 dead tarabs. found in Transbaikal. *S.* Bubo pos. BP. Cultures obtained from these tarabs. were sent to us in Harbin and proved pos. for BP.
Gp. X	20. ix. 22	23. ix. 22 (3 d.)	Materials from this gp. inocculated in Transbaikal were sent to us in Harbin and showed BP in smears from spleen and bubo.
R. T	11 and 14. x. 22	17. x. 22 (3–6 d.)	Recd. subc. on 11 October one loop of culture (*a*) from T. X. *S.* Ht. and Sp. ? BP. Re-inocd. whole slant on 14 October. *C.* Both neg. BP.
Gp. 50	,,	18. x 22 (4–7 d.)	Recd. subc. on 11 October one loop of culture (*b*) from T. X. Re-inocd. whole slant on 14 October. At pm. visceral pleura petechiae. Lv. congest. ? abs. Sp. covered with pus, acutely swollen, ? abscesses. *S.* Sp. BP. Ht. neg. *C.* Ht. and Sp. pos. BP. imp.
R. 53	18. x. 22	22. x 22 (4 d.)	Recd. subc. ¼ c.c. blood from gp. 50. At pm. some pus at site injection. Lower pt. tr. congest. Visc. pleura petechiae. Rt. lg. ? pneum. Lv. congest. Sp. swollen. *S.* Some BP. in all organs. *C.* Ht. neg. Sp. and Lg. ? BP. Trachea some BP. imp.

Table XXVIII. Diagram of Tarabagan Experiments, 1921.

Original culture of pneumonic case	Subculture of pneumonic case		
T. 1 inhaled 8. viii: d. 16. viii. pos. P. Contacts: T. 2 exposed 8. viii T. 3 ,, 8. viii T. 13 ,, 15. viii T. 14 ,, 15. viii	T. 4 inhaled 8. viii: died 13. viii. pos. BP.	Contacts: T. 5 exposed 8. viii T. 6 ,, 8. viii x T. 15 ,, 15. viii T. 16 ,, 15. viii	⋮ means wire screen of cage | ,, solid partition of cage x = animal killed 1 Sept. ? BP.

Original culture of pulmonary case	Subculture of pulmonary case		Tarabagan cultures
T. 7 inhaled 8. viii: k. 25. viii: PB. Contacts: T. 8 exposed 8. viii: d. 6 months: BP. T. 9 exposed 8. viii T. 17 ,, 15. viii T. 18 ,, 15. viii: k. 1. ix: ? BP.	T. 10 inhaled 8. viii: died 13. viii pos. BP.	Contacts: T. 11 exposed 8. viii: k. 25. viii: ? BP. T. 12 exposed 8. viii: d. 12. ix: BP. T. 19 exposed 15. viii T. 20 ,, 15. viii: d. 6 months: ? BP.	T. 31 inhaled 22. viii: d. 28. viii: BP. T. 32 inhaled 22. viii: d. 29. viii: BP. Contacts: T. 33 exposed 22. viii T. 34 ,, ,, T. 35 ,, ,, T. 36 ,, ,, k. 6. ix: ? BP. T. 37 born 4. ix

On 11 March, 1922, surviving animals were placed together. More died.

T. 106 on 12. iii: no BP.
T. 105 ,, 12. iv: ? BP.
T. 132 ,, 11. v: ? BP.
T. 133 on 15. v: no BP.
T. 147 ,, 1. vii: ? BP.
T. 148 ,, 3. vii: no BP.

Table XXIX. Results of Inhalation Experiments with Tarabagans, 1921.

No.	Inhaled	Date of death	Protocol
T. 1	8. viii	16. viii (7½ d.)	Recd. orig. cult. from kidney pm. 6. At pm. cerv. and postpharangeal glands swollen. Tr. and bronch congested, some blood in br. Pneum. patches both Lgs. Lv. fatty. Sp. acutely swollen. Stom. and intest. petechiae. *S.* Bp. in all organs. *C.* Bronchi BP. imp. Ht. BP. imp. Control re-inocd. with BP. died in 36 h.
T. 4	8. viii.	13. viii (4½ d.)	Recd. subcult. from kidney pm. 6. At pm. cervical glands enlarged. Tongue and epiglottis small haemorrhage. Tr. congested. Lgs. haemorrhage, no marked pneumonia. Perit. haemorrhage. Sp. swollen. *S.* BP. in all organs. *C.* Ht. and Lg. BP. imp. Control re-inocd. with BP. died in 40 h.
T. 7	8. viii	25. viii (k. after 17 d.)	Recd. orig. cult. from spleen pm. 34. At pm. cervical glands swollen and congested. Larynx and Tr. slightly congested. Br. mucus. Lgs. congested with many abs. *S.* Ht. Lg. and cervical gland, some BP. Br. Sp. Abs. neg. *C.* Lg. some BP. Cervical gland and Br. BP. imp. Control re-inocd. with BP. died in 50 h.
T. 10	8. viii	13. viii (5 d.)	Recd. subcult. from spleen pm. 34. At pm. bloody froth nostrils. Cervical glands enlarged. Tongue haemorrhage. Epiglottis and Tr. congested. Pink sputum in bronchi. Lg. haemorrhagic patches. Sp. swollen and congested. *S.* BP. in all organs. *C.* BP. imp. in all organs. Control re-inocd. with BP. died in 50 h.
T. 31	22. viii	28. viii (6 d.)	Recd. orig. cult. from heart T. 1. At pm. cervical and bronchial glands swollen and congested. Epiglottis and Tr. haemorrhage. Tr. red full of pink froth. Lgs. haemorrhage esp. left upper lobe. Epicardial haemorrhage. Lv. fatty. Sp. acute swelling. Omentum haemorrhage. Upper jejunum congested. *S.* BP. in all organs. *C.* Tr. and Ht. BP. imp. Control re-inocd. with BP. died in 4½ d.
T. 32	22. viii	29. viii (6½ d.)	Recd. orig. cult. from lung of T. 10. At pm. cervical glands swollen. Tr. congested full of bloody froth. Rt. Lg. haemorrhage. Lt. Lg. congested. Lv. fatty. Sp. acutely swollen. Intestine slightly congested. *S.* BP. in all organs. *C.* Ht. and Peric. BP. imp. Control inocd. with BP. died after 5½ d.

Table XXX. Results of Tarabagan Contacts, 1921.

No.	Exposed	Date of death	Protocol
T. 11	8. viii	k. 25. viii (17 d.)	At pm. nil macros. *S.* Ht. some BP. Sp. neg. *C.* Ht. ster. Sp. some BP. imp.
T. 6	8. viii	k. 1. ix (24 d.)	At pm. one cervical gland slightly congested. Oedema glottis and Lgs. (? narcosis). *S.* Some BP.-like in all organs. *C.* Ht. and Lg. ? BP. Tr. BP.-like imp. Rabbit inocd. with Tr. cult. still alive after 17 days.
T. 18	15. viii	k. 1. ix (16 d.)	At pm. Lgs. congested. Sp. slightly congested. *S.* Some BP.-like in all organs. *C.* Ht. very few BP.-like. Lg. neg. Tr. ? BP.-like imp.

Table XXX—*continued.*

No.	Exposed	Date of death	Protocol
T. 36	22. viii	k. 6. ix (14 d.)	At pm. cervical gland swollen and congested. Bl. froth nostrils, larynx, Tr. Slight oedema glottis (? narcosis). Sp. miliary yellow nodules. *S.* Some BP.-like in all organs. *C.* Ht. and Lg. ster. Tr. very few BP.-like imp.
T. 12	8. viii	d. 12. ix (35 d.)	At pm. cervical glands swollen and congested. Tonsils swollen and congested. Pharynx small abs. Larynx and Tr. congested with pink froth. Lgs. almost black. Lv. congested. Sp. acutely swollen with 2 small abs. Mesent. haemorrhages. *S.* Some BP. in all organs. *C.* Ht. ster. Sp. BP.-like imp. Other organs BP. mixed with plenty Py. Animal experiments with cultures recorded in Table XXIII.
T. 8	8. viii	6. ii. 22 (6 months)	At pm. Lt. Lg. haemorrhagic patches. Rt. Lg. adherent to pericardium. Pericarditis with fib.-sanguineous exudate. Lv. fatty. Sp. acutely swollen. Stom. some bloody contents. *S.* Pericard. Lv. Sp. BP. *C.* Sp. ster. Lv. some BP.-like imp. Pericardium BP. Several exps. with rabbits and other tarabs. by inhalation and injection all positive.
T. 20	15. viii	14. ii. 22 (6 months)	At pm. emaciated. Thymus slightly swollen. Sp. acutely swollen. *S.* Ht. neg. Sp. and Lv. some BP.-like. *C.* Ht. Sp. and Lv. ster.
T. 106	? viii	12. iii. 22 (7 months)	At pm. head bitten off by other tarabs. Nil macros. *S.* Ht. and Sp. neg. *C.* Sp. ster. Ht. and Lv. *Staphylococcus albus.*
T. 115	? viii	12. iv. 22 (8 months)	At pm. Sp. enlarged but no acute changes. *S.* Sp. some BP.-like. *C.* Ht. Sp. and Lv. ster. Faeces ? BP.-like imp. Rabbit inocd. with faeces cult. neg. BP.
T. 132	? viii.	11. v. 22 (9 months)	At pm. head bitten off by other tarabs. Lgs. greyish. Lt. Lg. hyperemic areas. Epicardium small grey spot surrounded by hyperemic zone. Lv. fatty infil. (?). Other organs apparently normal. *S.* Larynx and Sp. some BP.-like (?). *C.* Sp. and Lv. ster. Alim. canal neg. BP. Anim. exp. with faeces cult. neg. BP.
T. 133	? viii	15. v. 22 (9 months)	At pm. Lgs. congested with scattered hyperemic areas. *S.* Lgs. and Sp. neg. *C.* Ht. Lg. and Sp. ster. Stom. and ileum ? BP. Faeces BP.-like imp. Rabbit inocd. with faeces cult. neg. BP.

Table XXXI. Diagram of Tarabagan Experiments, 1922.

Subcult. peric. T. 8	Cult. spleen R. 102		Cult. spleen R. 103
T. 114 inhaled 17. iii: d. 11. iv: P. Contact: T. 124 exposed 17. iii: d. 28. iv: P.	T. 117 inhaled 17. iii: died 13. iv: pos. P.	Contacts: T. 112 exposed 17. iii: d. 6. iv: P. T. a exposed 17. iii T. b „ „	T. 107 inhaled 17. 3: d. 22. iii: P. Contacts: T. c exposed 17. iii T. d „ „

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Table XXXII. Results of Inhalation Experiments with Tarabagans, 1922.

No.	Inhaled	Date of death	Protocol
T. 107	17. iii	22. iii (5 d.)	Recd. 1910 strain BP. passed through one gp. and 3 rabbits. At pm. cervical tissues haemorrhagic effusion. Cervical glands swollen and congested. Larynx haemorrhagic effusion. Lt. Lg. 3 haemorrhagic patches (? aspiration). Epicard. petechiae. Lv. fatty. Sp. enlarged. Stom. petechiae. Ileum filled with bloody masses, no ulcer. Large intestine and faeces normal. *S.* Some BP. in all organs. *C.* Ht. Lv. and ileum BP. Sp. contam. Exps. with Ht. and ileum cult. pos. BP. (Table XXXIV.)
T. 114	17. iii	11. iv (25 d.)	Recd. subcult. pericard. T. 8. At pm. Rt. Lg. small haemorrhagic patches. Sp. acute changes. *C.* All organs BP. imp. Exps. with faeces cult. pos. BP. (Table XXXIV.)
T. 117	17. iii	13. iv (27 d.)	Recd. cult. Sp. R. 102 (obtained from strain T. 8). At pm. Lgs. congested especially right upper lobe. Pleura petechiae. Lv. fatty degeneration. *C.* Ht. ster. Other organs BP. imp. Exps. with faeces cult. pos. BP. (Table XXXIV.)

Table XXXIII. Results of Tarabagan Contacts, 1922.

No.	Exposed	Date of death	Protocol
T. 112	17. iii	6. iv (20 d.)	At pm. cervical glands swollen and congested. Lgs. hyperemic patches. Sp. acutely swollen. *C.* All organs BP. imp. Exps. with urine cult. neg.; with faeces cult. pos. (Table XXXIV.)
T. 124	17. iii	28. iv (42 d.)	At pm. Lt. Lg. hyperemic patch. Sp. sl. enl. *C.* All organs BP. imp. Exps. with cult. from Lv. Stom. and ileum pos. (Table XXXIV.)

Table XXXIV. Experiments with Cultures from Alimentary Tract of Tarabagans, 1922.

No.	Inoculated	Date of death	Protocol
R. 119	1. iv 18. iv	19. iv (? 1–18 d.)	Recd. twice 2 loops ileum cult. T. 107. At pm. Lgs. sl. congested. Perit. abs. near diaphragm. Sp. enlarged. Mesent. glands swollen and studded with nodules. *S.* Sp. some BP.-like. *C.* Ht. Lv. ster. Sp. some BP. imp. Rabbit 122 inocd. with one loop Sp. cult. died in 4 days of plague.
R. 126	28. iv	3. v (5 d.)	Recd 2 loops of ileum cult. T. 107. At pm. Sp. acutely swollen. *S.* Some BP.-like in Sp. *C.* Ht. Sp. and Lv. bipolar unlike BP.
R. 130	4. v	9. v (5 d.)	Recd. 2 loops of ileum cult. T. 107. At pm. Lt. Lg. hyperemic areas. Sp. sl. acute change. *S.* ? BP. in Sp. *C.* All neg.
R. 135	11. v	17. v (6 d.)	Recd. 2 loops ileum subcult. T. 107. At pm. nil macros. *S.* Sp. BP. *C.* Ht. BP. imp. Sp. and Lv. contam.
R. 116	8. iv	13. 4 (5 d.)	Recd. 1 loop faeces cult. T. 112. At pm. Sp. acute ch. *C.* Ht. Sp. and Lv. BP. imp.

Table XXXIV—*continued.*

No.	Inoculated	Date of death	Protocol
R. 118	12. iv	14. iv (2 d.)	Recd. 2 loops faeces cult. T. 114. At pm. oedema subcutis chest. Sp. acute ch. *C.* Ht. and Lv. ster. Sp. BP. Rabbit 121 inocd. with 1 loop Sp. cult. died in 6 days of plague.
R. 131	18. iv 28. iv	10. v (12–22 d.)	Recd. 1 and 2 loops faeces cult. T. 115. At pm. Lgs. hyperemic areas. Sp. acute ch. *S.* Sp. ? BP. *C.* All ster.
R. 120	18. iv	22. iv (4 d.)	Recd. 1 loop faeces cult. T. 117. At pm. Lgs. congested. Lv. and Sp. covered with pus. *C.* All BP. pos.
R. 125	29. iv	1. v (2 d.)	Recd. 1 loop stomach cult. T. 124. At pm. Sp. acute ch. *S.* Sp. ? BP. *C.* Ht. Lv. ster. Sp. BP. imp. Rabbit 134 inocd. with 2 loops Sp. cult. died in 8 days of plague.
R. 128	2. v	8. v (6 d.)	Recd. 1 loop ileum cult. T. 124. At pm. Lgs. hyperemic areas. *S.* Sp. BP. *C.* Ht. and Lv. BP.
R. 139	12. v	4. vi (23 d.)	Recd. 2 loops faeces cult. T. 132. At pm. perit. abs. near Lv. Mesent. and oment. necrotic nodules. *S.* Ht. neg. Sp. and Pe. ? BP. *C.* Ht. and Sp. ster. Lv. ? BP. All 4 animal exps. with Lv. cult. proved neg.
R. 136	16. v	18. v (2 d.)	Recd. 2 loops faeces cult. T. 133. At pm. Sp. acute. *S.* Ht. and Sp. neg. *C.* ? BP. in all organs. All 5 animal exps. with cult. proved neg.
R.?	27. v	—	Recd. 3 loops faeces cult. T. 133. Remained healthy.

Table XXXV. Feeding Experiments with Tarabagans, 1922.

Two sets of animals, each consisting of one tarab. and one rabbit were fed for five days with their usual food (beans and cabbage respectively) mixed for each animal with 25 c.c. virulent BP. bouillon. Animals of first set received no fresh food on 6th and 7th days; on 8th day dishes were changed. In second set, dishes were changed on 6th day.

Animal	Feeding period	Date of death	Protocol
T.?	22–28. v	29. vii	Still healthy (1 month).
R. 138	22–28. v	30. v (2–8 d.)	BP. positive in smears, cult. and exps.
T. 146	12–16. vi	1. vii (13–18 d.)	At pm. cervical glands swollen and congested. Lgs. congested with haemorrhagic patches. Ileum bloody mucus, mucosa congested, no ulcer. *S.* Sp. ? BP. Ileum BP. *C.* Ht. Lv. and Sp. ster. Stom. and faeces some BP., ileum more BP.
R. 144	„	17. vi (1–5 d.)	BP. pos. in smears, cult. and exps.

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Table XXXVI. Experiments upon Non-Susceptible Animals.

Date	Pig	Duck	Chicken	Material injected	Quantity	Survived	Remarks
April 29	1	.	.	Sp. C. (pm. 22)	Slant	14 days	Re-injected 13. v
„ 29	1	.	.	„	„	„	„
„ 29	.	.	1	„	½ slant	„	„
„ 29	.	.	1	„	„	„	„
„ 29	.	.	1	„	„	„	„
May 13	1	.	.	Fresh blood (pm. 27)	5 c.c.	1 year	a
„ 13	1	.	.	„	„	„	a
„ 13	.	.	1	„	2 c.c.	4 months	b
„ 13	.	.	1	„	„	„	.
„ 13	.	.	1	„	„	„	.
„ 28	.	1	.	Sp. C. (pm. 33)	⅓ slant	„	.
„ 28	.	1	.	„	„	„	.
„ 28	.	1	.	„	„	„	.

NOTE. Altogether experiments were made on two pigs, three ducks and three chickens. a = abscess developed at site of injection. Pus showed no BP. May 18 and 22. b = haematoma developed at site of injection. Blood showed no BP. May 18.

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