ANNUAL REPORT

OF THE

Government Bacteriologist

AND

DIRECTOR LABORATORY,

PIETERMARITZBURG,

FOR THE

Year ended December 31st, 1903.

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Annual Report of the Government Bacteriologist and Director, Laboratory, Pietermaritzburg, for the Year ended 31st December, 1903.

The Laboratory.

The Hon. the Minister of Agriculture.

SIR,—I have the honour to hand you herewith my Annual Report for the past year, during which time there has been a steady increase in all branches of the work undertaken by the Laboratory.

In addition to the work undertaken by my Department for the Department of Public Health and the Veterinary and other Departments of the Public Service—which, as you will see, has not been inconsiderable—the work of investigation into some of our most important diseases has been pushed forward as far as opportunity has permitted, and this work has not been devoid, I am pleased to say, of results which have broadened our knowledge on several important points. Thus, in the two chief enquiries which have occupied my attention, viz., Horsesickness and Bubonic Plague, you will see from the conclusions arrived at in the reports dealing with these enquiries, that original observations have been made which it is hoped will have value in their practical application.

The work in connection with the former disease is still in progress, but has suffered considerably from the delays inseparable from most researches. The excessive rainfall alone has put back the work of one particular branch of this enquiry by several months. For the experimental work carried on in this connection during the winter months, reference should be made to the Interim Report recently published, in which I was able to show the possibility of inducing a long-continued or chronic form of the disease at will, and free from the great danger of fatality usually attending the introduction of virus into the system of the horse. The production of a long-continued re-action to an inoculation with the virus was found to be productive of a considerable degree of immunity, and the work of the coming season will aim at devising the means of enhancing or increasing this immunity, in order that a high degree of protection may be—if possible—permanently confirmed. Such a line of work is hopeful, inasmuch as it is a departure from the well beaten track of sero therapy, or the endeavour to produce the desired result by the use of the serum of an animal highly fortified against the disease. This difficult problem, however, is at present engaging the attention of several workers in South Africa, and it seems permissible to hope that a method will be devised which will prove the solution of the long-vexed problem.

I was able last year to show the protection which could be secured against the disease by stabling horses in a smoky atmosphere, and my opinion as to the efficacy of such a practice remains unaltered. Numbers of horse-owners (particularly owners of large stables) have adopted the measures suggested—some with the best, and others with indifferent or bad results. This is as was expected.

I have caused an enquiry to be made into cases of reported failure and I am satisfied that failure, where it has resulted, has been the result of non-compliance with the suggestions as laid down. The fires for generating the smoke have often been left to a perfunctory kafir. Arrangements—entirely inadequate—for smoking large buildings have been made, doors and windows have been left open, and the rationale of the process entirely lost sight of in many instances. Cases of the disease have, not un-naturally, occurred, with the consequent calling in question (as was expected) of the efficacy of the process.

Where trouble has been taken and the arrangements have been good and carefully maintained for keeping the smoke in and flying insect out of the stable, the best results have ensued, and can always be assured to the careful horse-owner, with the exception of occasional cases in which animals become infected during the daytime by exposure in shady moist localities. Such instances, however, must always remain greatly in the minority.

During the past year the investigation of many points in connection with the existing outbreak of Bubonic Plague have come within my province as Government Bacteriologist. This work of research was taken up for the Department of Public Health, the Health Officer for the Colony —as the authority responsible for the repression of the outbreak—being desirous of more extended knowledge on certain points in connection with the bacteriology of the disease. My observations on this disease have already been communicated to the Health Officer, and will shortly appear embodied in the report on the Natal Outbreak. From an inspection of Appendix B, you will see the wide ground covered by this research alone. You will notice that many original observations were able to be made upon the life and habits of the bacillus, which I trust will prove of value in doing something towards assisting to suppress this much-dreaded human scourge for the future, both in Natal and in other parts of the world.

Certain statements as to the inefficacy of the Vaccine prepared against the disease Quarter Evil, led me to enquire from a large number of stock owners details as to their personal experience of the use of this preparation.

I beg to refer you to the accompanying Appendix "A" for the names of farms so visited, the numbers of inoculation made, and the opinions expressed. You will notice that there is a very gratifying general consensus of opinion as to the value of the preparation and its use in preventing the occurrence of the disease. Cases quoted have been taken promiscuously, and care has been taken to include all the adverse opinions obtainable, in order that a fair estimate of utility can be arrived at.

It would appear that in bad localities a bi-annual inoculation is useful, and that even upon the most dangerous veldt—where an inoculation is made each spring and autumn—the disease is rapidly suppressed, if not eradicated.

A preparation permitting of a double inoculation at a few days' interval, to take the place of the two half-yearly operations, can be issued if desired. Such procedure, however, does not give the same certainty of sustained immunity which attends the operation at a six month's interval.

That greater use is being made of this preparation is shown by the fact that over 5,400 doses have been issued in excess of last year's output, the exact numbers being 11,570 for 1902, as against 17,995 or almost 17,000 doses for the past year. This is encouraging. I would take this opportunity to remind those who use the preparation that I am always anxious to receive any information concerning its results, as I am obliged to depend in no small measure upon such reports for the maintaining of a high standard of efficiency in the Vaccine

Another preparation of which increased use has been made is the Serum (for the use of man or the lower animals) against the bite of venomous snakes. In several instances in which reports have been furnished the results attending the use of this preparation after the infliction of snake-bite have been very gratifying. The chief drawback to the use of the antidote has been the delay usually occurring between the infliction of the bite and the injection of the serum. I have endeavoured to render the preparation more readily available for emergencies by adapting the bottle containing the serum for use as the hypodermic syringe also. If this arrangement can be carried out with success the rapid application of the antitoxin, which is so important a factor in cases of snake bites, can be more often ensured.

The disease Blue Tongue of sheep has received as much attention as circumstances will permit. Much work has been done which will prove essential to the elucidation of the question, but for some months the urgency of increasing laboratory routine and the work of enquiring into more important diseases has so fully occupied the time of myself and staff that this sheep disease, and other maladies which I am anxious to investigate (such as the fatal diseases of calves, fowl sickness, &c.) must stand over until opportunity occurs for their being undertaken.

The field of disease-investigation is a large one, and it is not possible —however much one might wish to do so—to undertake the investigation of diseases of minor importance or individual cases of disease.

The routine work of my department has greatly increased within the last twelve months. Reports on morbid tissues and fluids for the Health and Veterinary Departments and for private Medical Practitioners, reports for the Criminal Investigation Departments, examinations for the diagnosis of Enteric Fever, Diphtheria, Malaria, &c., which help to form the routine laboratory work, has much more than occupied the usual hours of official work throughout the year.

As many as 22 analyses of various water supplies were undertaken within the last nine months of the past year. In addition to this the

usual manufacture and supply of the various laboratory preparations has been maintained. The issues from the institution have been as follows:—

Quarter Evil Vaccine ...	...	16,985 doses
Mallein ...	...	4,824 „
Tuberculin ...	...	2,465 „
Locust Fungus ...	...	480 tubes
Anthrax Vaccine ...	...	24 doses
Tetanic Serum ...	...	38 „
Antivenomous Serum ...	...	83 „
Rinderpest Serum ...	...	500 „

With the exception of the Anthrax Vaccine, Tetanus Antiserum, and Rinderpest Serum, all these preparations have been prepared at the Laboratory.

It will thus be seen that the general work of my department is rapidly increasing and making correspondingly increased demands upon my staff, and I should like to express my appreciation of the ready and zealous manner in which these duties have been carried out, entailing, as they very frequently do, work long after the usual official hours.

My professional assistant has been of the utmost service to me, especially in the general conduct of the routine work of the Institution.

I beg, in conclusion, to acknowlege with thanks the cordial support in the work of my department extended me by yourself.

I have the honour to be,

Sir,

Your obedient Servant,

H. WATKINS-PITCHFORD, F.R.C.V.S.

Appendix "A."—Quarter Evil Enquiry.

„ "B."—Bubonic Plague.

APPENDIX A.]

RESULTS OF ENQUIRY INTO THE EFFICACY OF THE PREPARATION, QUARTER EVIL VACCINE.

The first farm visited on the subject of Quarter Evil Vaccine was that of a Mr. LYTH, of Thornybush. Mr. Lyth, unfortunately, was not at home. His son was, however, able to furnish a certain amount of information. He could not state for certain how many years the Vaccine had been used on the farm. He remembers that the first season it was used they lost one yearling after the inoculation, the animal showing disease on the second day, and dying on the third. Beyond this they have experienced no loss since commencing to use the Vaccine. Only one and two year old animals are inoculated, their losses, previous to using the method, being principally confined to yearlings. Deaths, to his actual knowledge, however, have occurred on full grown oxen. They inoculate once a year as a rule, doing about forty head.

MR. CLARK (also residing near Thornybush) inoculates once a year only, doing about 40 calves on an average. Mr. Clark has only resided on the property about two years. During the first year he lost 15 (fifteen) out of 25, using at the time the "Seatoning" Method, as advised by Mr. Milborough. After the last death he inoculated with the Laboratory Vaccine, and since this, *he has had no losses* which can be attributed, truthfully, to Quarter Evil. Two deaths—six months after inoculation—which occurred within 10 (ten) days of one another, are likely to have been caused by the bite of a Black Mamba, which he afterwards discovered in his calf shed, and destroyed.

MR. H. SMITH (Vaalkop) uses about 60 doses yearly, making only one inoculation, and—being very careful in his methods of application—*he has had no losses since commencing to use the Vaccine.* He has also induced the natives to apply the method, to their benefit, and *entire satisfaction.*

MR. C. J. D. SCHEEPERS (Lange Hoek) was also kind enough to give the benefit of his experience.

His inoculations (which are made once yearly) averages about 30-40 head of young stock. *He has met with nothing but success.* He stated that Mrs. Scheepers (on the adjoining farm) and others, *had had equal success, and no failures.*

It will be noticed in the above cases, that actual figures are not always given. These were difficult to get at, with any degree of certainty. The district is, however, from all accounts, a bad one for the disease, and the results obtained from the information gathered cannot be regarded as anything but satisfactory.

MR. W. BAYNES', two farms, "Glenduthie" and "S ttle," were visited. Mr. Robinson—the manager of the former—was not able to

furnish information which would lead to any useful conclusion as to the benefits derived from the use of the vaccine, owing to the fact that his knowledge of the farm is only of two years standing. He is unable to state whether the disease has actually been known to occur there spontaneously or otherwise. The calves (55 in number) were inoculated last December *without mishap,* and removed to "Settle" in the following June, when Mr. Robinson was of opinion that some had broken down. This information—on visiting "Settle"—was not actually confirmed as the animals concerned were not particularly noted during the outbreak which subsequently occurred there.

Mr. Smith, the manager of "Settle," was able to furnish very complete tabulated information on the results of their inoculations :—

FARM "SETTLE."

Heifers.—Two years old, or rising two. Previous inoculation, January, 1902.

June 12th, 1903—1 died at Windy Hill (high stony ground).

June 16th, 1903—Inoculated with Laboratory Quarter Evil Vaccine. *Since then no further deaths.*

Young Oxen.—Two years old, or rising two. Previous inoculation, January, 1902.

March 23rd, 1903—Inoculated with Laboratory Quarter Evil Vaccine

July 18th, 1903—1 died, Quarter Evil (at Cibeleh).

Trek Oxen—Three-year-olds.

July 16th, 1903—1 died, Quarter Evil.

July 17th, 1903—*55 inoculated, and no further deaths.*

Trek Oxen.—Four-year-olds.

July 21st, 1903—1 died, Quarter Evil.

The remainder of oxen this age were not inoculated, and *no further deaths have occurred.*

General.—

July 2nd, 1903—1 four-year-old cow died, Quarter Evil.

No inoculation of remainder of this age, and no further deaths.

July 2nd 1903—1 two-year-old bull died, Quarter Evil. (This animal had previously been inoculated January, 1902.)

Yearlings.—or rising one.

Previous inoculations:—December, 1902, 73 ; January, 1903, 54 ; March and April, 1903, 47. Total, 174.

Among these (previous to the inoculations which were made on July 13th, 1903 :—

July 2, 1903—3 deaths.	July 7, 1903—2 deaths.
„ 3, „ 3 deaths.	„ 9, „ 1 death.
„ 6, „ 1 death.	„ 10, „ 2 deaths.

From the foregoing statement it may appear that the results, in some cases, have not always been quite as satisfactory as could be wished, but the reason for this appears to be that the inoculations are only made

once a year, whereas, on a farm carrying such gross infection, all the young stock should be inoculated twice at least—at intervals of six months. Mr. Smith intends to inoculate twice a year in future.

It will be observed that the majority of deaths occurred before the Vaccine was applied (July 17th, 1903), and the fact of the disease being in the different herds to such an extent accounts for the subsequent deaths. The introduction of the Vaccine into the animals' system tends, in many cases, to precipitate the disease if it be already latent, which shows the necessity for making the inoculation a matter of routine, and so anticipating the disease.

The inoculation should be made three weeks to a month before the time one's previous experiences in the locality warns one that the dangerous season will commence. In the ordinary course an animal which had become infected—say the day previous to inoculation - would not be safe until ten days after. The greatest mortality (including those done at "Glenduthie") was among those inoculated in December, 1902.

Of the 163 inoculated on July 17th, three died on July 18th, 20th, and 22nd respectively, and there have been no deaths since. Two animals, which were too wild to kraal, and were, consequently, not inoculated, died from Quarter Evil on July 14th and 16th respectively. This shows how rampant the disease was on the farm, and the *excellent results derived from the use of the Vaccine.*

An interesting fact with regard to the outbreak among the yearlings is that the greater number of deaths occurred among the progeny of one particular bull; whether from a high susceptibility of his strain, or from their being further advanced owing to the bull being put to the early cows, can only be a matter of conjecture, but cases pointing to the high susceptibility of certain strains are frequently met with.

A source of fallacy which should be borne in mind is the waiting for a case to occur before applying the method, instead of—in cases where the disease is already known to exist—anticipating and, at the same time, combating it.

The practice of allowing natives to perform the inoculations is bad, because the Vaccine is a very delicate agent, which, if carelessly applied, becomes an actual virus. The infinitesimal quantity used for a dose requires more scrupulous care and attention, which the ordinary native can hardly be trusted to observe.

Next in importance to the careful mixing of the Vaccine, is the constant agitation of the syringe between each injection. This is absolutely necessary to ensure good results.

Yet another point—often lost sight of—is the careful handling of the subject. All unnecessary roughness or noise, whereby the animal may become heated or excited, should be avoided. If the animal is over-heated it should stand for a time.

A strict attention to details may mean the saving of valuable animals.

In connection with the deaths among "Young Oxen," Mr. Smith stated that three other deaths, on August 6th, 18th, and 22nd respectively, had occurred among the same lot on the adjoining farm

(Mr. Renckin's) to which they had been removed. These animals had received two inoculations, viz., January 20th and March 23rd, 1903.

The disease appears prevalent on all parts of the farm. The high ground, on what is called "Windy Hill," is responsible for the death of the two heifers in June.

The last outbreak, in July, occurred among the yearlings on the flats.

The Laboratory Vaccine was used for the first time on June 13th 1899, 125 calves being inoculated, followed by four deaths on the 16th and one on the 17th of that month.

MR. KLUSSENER, of Port Shepstone, has used the Laboratory Vaccine for the past four years. He inocculates once a year, and *has never known an animal to break down*, except as a result of the inoculation, and that only among cattle where cases had already occurred.

He inoculates—indiscriminately—all animals over the age of three months. This, in that particular locality, is necessary, as deaths often occur among full grown, and often poor and aged animals.

During the past four years Mr. Klüssener has inoculated about 300 each year. He is extremely cereful in his methods of application, and allows nothing to interfere with it. He is very enthusiastic over the benefits derived by himself and owners in the district who have used the Vaccine, and *cannot say too much in its favour*. He quoted three recent cases of losses among full-grown cattle :—

1. Mr. Serran lost a 11-year-old ox in poor condition. He inoculated the remainder. One died three days after, but *no deaths since*.
2. Mr. Ringo—four years ago—was constantly losing full-grown span oxen, but *since inoculating has not experienced any loss*.
3. Mr. Bath, about four years ago, lost full-grown oxen, but *since inoculating (periodically) has had no losses*.

Mr. Klüssener does not rest any animals who may be working at the time of inoculation

He notices that an animal inoculated for the first time invariably has a local re-action, and that such does not occur after subsequent injections.

MR. W. F. WHITE, of Melmoth, has experienced *most satisfactory results* from the use of the Vaccine.

When he came to the district, sixteen years ago, his annual losses in calves and two-year-olds amounted to 75 per cent. For three years previous to the Vaccine being issued he used the French method, and reduced his losses to 1 per cent., but found it necessary to inoculate *every four months*. He has used the Laboratory Vaccine since its first issue, and *has never had* a *break-down*.

He inoculutes twice a year (March and August) and the only single instance during the past five years of a death from Quarter Evil was a calf, which—though inoculated in the previous March—was not done again in August. He inoculates calves over three months old and two-

year-olds only. He has induced many of the natives to adopt the method, with almost *equally satisfactory results.*

Mr. White says that by careful and systematic application of the Vaccine *he has banished the disease from the farm.*

MR. R. ARMSTRONG of Verulum, has used the vaccine since it was first issued, and *is delighted with the results.* He inoculates about 150 calves and yearlings once a year. The reason for inoculating once only, being, that a number of the young stock—principally oxen 18 months old—are sold to the natives.

These nearly always remain in the district, and Mr. Armstrong has *never heard of a single death.*

Previous to the use of the Vaccine his annual losses amounted to 60 per cent., including full grown animals, but since, only one death has occured, and that this year. This death, Mr Armstrong blames himself for, *i.e.* : not having suffered a single loss for so long a period, he allowed the season to pass without the usual inoculation, with the result that a calf 9 months old the case referred to – which had not been previously inoculated, died of Quarter Evil. Vaccine was immediately wired for and the usual inoculations made, and no other deaths have occured since.

Mr. Armstrong strongly affirms that *but for the benefits derived from the use of the Vaccine, it would be almost impossible to continue farming cattle in that locality.*

MR. C. H. MITCHELL, of Imbezana, says : *It is difficult to estimate the value of the Laboratory Vaccine. so absolutely perfect have been the results during the four years he has been using it.*

He inoculates once a year, averaging 120 head and every beast over three months old. Before the Vacciue was used, the greater number of deaths occurred among full-grown cattle. He has, at different times, inoculated a large number of animals for others.

Three years ago an outbreak of a very serious nature occurred among the native cattle in his district, when Mr. Mitchell *inoculated some hundreds with the Laboratory Vaccine, and, thereby, effectually stopped the outbreak.* In connection with this a circumstance happened which—but for the subsequent enquiry—might have been attributed to the failure of the Vaccine. A batch of 100 were inoculated for a neighbouring owner. Five months afterwards, four deaths from Quarter Evil occurred among them. The history of the animals was at once gone into, and it was found that the two first to die *had not been inoculated,* while the two others were the last to receive injections, and—owing to the Vaccine running out—*they had only received a very small dose.* This case is interesting, showing, as it does, the great necessity for strict attention to details, and for every animal receiving the prescribed dose. The four animals referred to were all full-grown.

Mr. Mitchell says that for years, previous to using the Vaccine, his annual losses amounted to 20 per cent., the greater number of deaths occuring among full-grown stock.

MR. J. F. DE JAAGER, of Eshowe, testified to the good results obtained by the use of the Laboratory Vaccine. Four years ago his losses were very considerable. He now has stock running on the farm

of Mr. Kritzenger, all of which have been inoculated, and *is not experiencing any losses.* He also stated that Mr. Kritzenger has *had very great success.* Mr. Kritzenger was, unfortunately, too ill to accord an interview.

Mr. de Jaager spoke of a disaster which happened to him a short time ago, which helps to again emphasise the necessity for most scrupulous care in Quarter Evil localities, especially when castrating. He operated on 65 young oxen. The day following one of the batch displayed signs of suffering from Quarter Evil and died. This death was followed in quick succession by 60 others out of the remaining 64. The obvious explanation of this is found in the fact that the same knife was used to operate on all these animals.

Mr. J. Moon, of Manderston, has used the Laboratory Vaccine for the last two years *with absolute success.* For four years previously he had used the Pasteur Vaccine. He commenced to use it (Pasteur) on the occasion of an outbreak during which he lost 45 out of 120 head. He says that, having experienced such perfect results from its use, he still places greater confidence in it, and only uses the Laboratory Vaccine because the Pasteur is locally unobtainable. He acknowledges, however, that *the results have been equally satisfactory.*

Mr. Baker, of Manderston, has used the Vaccine for three seasons, and *is highly pleased with the results, having had no losses.*

Mr. Turnbull, of Pietermaritzburg, occupying a farm at Manderston, has not been very successful, but admits it being the result of faulty application of the method. He has used the Vaccine for two years. Last year's inoculation was followed by four deaths, but it should be mentioned that two deaths from Quarter Evil had occurred just prior to the inoculation. It is, therefore, only reasonable to say that the cattle must have been already infected. This year's operation was followed by two deaths, but the method of application left much to be desired, no care having been taken to grind the Vaccine, in consequence of which the needle was constantly getting blocked. Mr. Turnbull has only occupied the farm for two years, and so is unable to give the history of the disease there.

Mr. Moon stated that a Mr. F. Harrison, recently living near Manderston, had also experienced bad results; but it appears that this gentleman was even more careless in his method, for he did not even take the trouble to grind the Vaccine.

Mr. F. F. Churchill, M.L.A., late of Hill Crest, *has always experienced good results from the use of the Vaccine.* In 1898 he inoculated with the Pasteur Vaccine, and the following year he used the Laboratory Vaccine. Six months after using the latter a death from Quarter Evil occurred. Acting on the advice from the Laboratory, he inoculated twice a year, viz., November and January, without further loss. He was in the habit of inoculating from young calves, and previous deaths had occurred among these only.

His actual knowledge of the history of the disease dates back to 1897 only, but he knows that it existed in the district for many years.

Having given over the farm to the South African Constabulary, he does not now own any cattle.

Mr. T. M. McKENZIE, of Cramond, was not very successful at first, and his experience was also that of his brother (Mr. Geo. McKenzie, of the adjoining farm "Buccleuch"). The explanation probably lies either in an undue susceptibility to the disease among animals, or to the want of greater care in the application of the method.

Up to 1900 Mr. T. M. McKenzie had used the Pasteur Vaccine with success, but in that year, it being unobtainable, he had recourse to the Laboratory Vaccine. Inoculating in the spring, two deaths occurred the following autumn. He now inoculates twice a year, with *perfectly satisfactory results*. He inoculates all calves from three to fifteen or eighteen months old. Each animal, therefore, gets two (and in a few cases three) injections.

MRS. MCKENZIE, SENR., testifies to the disease having existed for at least 45 years, and was probably present before that. She remembers some serious outbreaks, but was, unfortunately, unable to furnish actual figures showing the annual losses before the Vaccine was used.

MR. GEO. MCKENZIE has experienced *the most satisfactory results* from the use of the Laboratory Vaccine since 1899. Previous to that year his annual losses amounted to 10 per cent. in calves and yearlings (principally the latter). The worst month was usually June. Calves only are inoculated once a year (April 17th), the number varying from 70 to 100. Thus, each animal receives but one injection, *but Mr. McKenzie has not experienced a single loss from Quarter Evil since the commencement*. Mr. McKenzie's experience of the farm is of 18 years' standing. The former owner, Mr. R. Scott, now of Kokstad, was in former years a heavy loser from the disease.

MESSRS. E. & C. COMINS, of York, have a *very high opinion of the Laboratory Vaccine*, which they have used since 1899 (previous to which they had used Pasteur's). By careful application they *have had most satisfactory results*. They inoculate in February every year, doing about 50 calves, and this is the only inoculation they receive.

Before inoculating, the annual losses amounted to 20 per cent. (the losses were confined to calves). March and April were the worst months, and the disease often re-appeared in the spring (September).

Natives in the vicinity still continue to lose cattle every year, and, bearing in mind the small number they own, it points to the fact of the disease being still in existence. *It would be well, therefore, if owners of cattle*—after satisfying themselves as to the efficacy of the Vaccine—*were to use their influence to induce the Native to use it also*. By that means the disease might eventually be stamped out.

MESSRS. MOE BROS. have used the Laboratory Vaccine since 1899 with *uniformly complete success*.

Two deaths occurred after inoculating last December from a disease of a lingering nature, which at the time they were inclined to attribute to some effect of the Vaccine, because it appeared to start just after the inoculation had been made; but they say—out of fairness—that they cannot positively assert such was the case, particularly as the

two calves were examined at the time by the District Veterinary Surgeon. It was only the coincidence which caused them to be suspicious.

Messrs. Moe's experience of the farm dates back to 1882, at which time the annual losses from the disease amounted to about 30 per cent. of calves. Only one inoculation a year is made (calves .

Their experience points to January, February, and March as being the worst months. On one occasion the usual annual inoculation was omitted, with the result that they lost two or three beasts from Quarter Evil. Messrs. Moe express themselves as being *entirely satisfied with the results* they have had from the use of the Laboratory Vaccine.

MR. T. BRAITHWAITE, of Haartebestefontein, Seven Oaks, has used the Vaccine for four years.

He inoculates once a year (usually in the month of March) calves only, each animal—as in the preceding cases—receiving but one dose. When the Vaccine has been properly applied *he has never had a loss* from Quarter Evil or any bad results following the inoculations. He had previously been using the Pasteur method for two or three years, and had had several losses. The yearly losses, previous to using the Vaccine, were about 15 per cent. He has been in occupation of the farm for 23 years. During the first seven years he did not experience any losses from Quarter Evil.

The disease started just after purchasing a cow and calf at Greytown. Shortly after arrival the calf died of Quarter Evil, the cow dying a few days after it from the same cause. Unfortunately, the carcases were allowed to be cut up and skinned, thereby enabling the disease to get a firm footing.

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MR. DEANE, M.L.A., when using the Vaccine of 1899 issue, lost one animal, which he attributes to the result of the inoculation, but another animal which actually took the disease, recovered. Since then *the results have been entirely satisfactory*,

He inoculates once a year only, doing about 30 young calves. The disease existed on the farm when he first took it over—15 years ago ; a few cases only occuring at the outset, but yearly increasing.

The average yearly loss before using the vaccine amounted to 15 per cent. The neighbouring natives still continue to lose cattle from the disease every year.

MESSRS. REICHE BROS. have used the Government Vaccine during the last two seasons with *signal success, and are very pleased with the results obtained.* They find it advisable to inoculate every animal over six months old, irrespective of age, as their neighbours often lose full-grown animals.

The number inoculated last September was 70, and in October of this year, 150. They know the disease to have existed on their farm for

the last 26 years, *i.e.*, ever since their occupation, but cannot give the percentage of losses. The last deaths occurred three years ago, when Vaccine was not being used.

MESSRS. HOLLEY BROS. used the Vaccine first in 1899, but no inoculations were made the two following seasons through the absence at the front of both the partners, and they cannot say whether or not any deaths from Quarter Evil occurred during that time.

Inoculations are made once—the early calves in September, and the latter calves in the following spring.

The number inoculated annually average about 30, and *no mishaps or breakdowns have occured.* In former years the death rate from Quarter Evil was occasionally very high among the young stock, reaching at times to nearly 100 per cent. The deaths were usually among the 2-year olds, with an occasional 3-year old; only one death of a full-grown animal has been known to occur—a cow imported from another district. To the best of their knowledge the disease has always existed on the farm.

MR A. FYVIE states that on taking over the farm four years ago, he was told by Mr. Rosenbrock, a former owner, that he would never be able to raise any young stock on account of the prevalence of Quarter Evil. He at once began to use the Laboratory Vaccine, with the very satisfactory result that among his young stock *he has never had a loss*, and his cattle have considerably increased in numbers. He inoculates once a year (usually in September), doing all animals from 10 months to 3 years old.

Mr. Fyvie had an interesting, but unfortunate, experience this year before inoculating. He had purchased two 3-year olds from Mr Geo. McKenzie, of Buccleuch, and these animals were running on a lower part of his farm near a native kraal. The native lost three beasts from Quarter Evil, and the two in question belonging to Mr. Fyvie also succumbed. Mr. Fyvie immediately inoculated the remainder of his young stock and has had no further loss. It will be remembered that Mr. Geo. McKenzie inoculates his calves *once only,* and, using the Vaccine for the last five years, has never had a death from Quarter Evil, but the fact that his natives have occasional losses is a proof that the disease still exists on the farm. The probability is that the two cattle were rendered more susceptible by the change of locality and grazing.

MR. GORDON. In former years the disease appeared very frequently on the farm. One year, perhaps, only three or four deaths would occur, while in another there would be quite an epidemic, when the death rate would reach from 60 to 100 per cent. among the young stock, and occasionally a few old cattle. The last outbreak of this description occurred four years ago.

For the last three years Mr. Gordon has used the *Laboratory Vaccine without loss or mishap.* Two inocculation were made annually—May and September—the age of animals done varying from three months to two years old.

Last year a yearling died which had received an inoculation the previous May. This happening a full month after the expiration of the six months immunity, shows the necessity for two half-year inoculations.

Many users of the Vaccine, however, continue to inoculate, but once only *with, so far, every success.*

Owners gain their own experience in this matter; but it should be borne in mind that perfect safety is only obtainable by two half-yearly injections. Mr. Gordon's yearly inoculations average about 35 head.

MR. GIFFORD has used the Vaccine for four years *with perfect results.* He inoculates in May and September, the inoculation being regulated by the time the animal was calved, some getting two and some three inoculations, the animals done varying from six months to two years old. Previous to taking preventive measures by the use of the Vaccine, small losses occurred (over a period of 16 years) annually. Before Mr. Gifford's occupation Quarter Evil was very bad.

Two deaths occurred this year, a three-year-old heifer and a yearling; but these were strange cattle which had been sent to the farm for grazing purposes, *and had not been inoculated.* Natives continue to have losses among their few head.

MR. A. MCKENZIE was away, but his manager (Mr. Culverwell) was able to furnish a few facts. Mr. McKenzie *has used the Vaccine with success* for the last five years.

He inoculates about 50 head of calves and yearlings, usually in March and September. On both occasions this year he overran the usual time, and in consequence lost a yearling in April which had been inoculated the previous September, and also a calf which had received an injection on the 4th of October. These are the first deaths which have occurred since using the Vaccine, and might have been avoided. There have been no further deaths.

MR. PERFECT experienced unfortunate results from his only trial of the Vaccine. He believes it to be due to the fact of its being a single instead of a double Vaccine, like the preparation issued by the Pasteur Institute. Mr. Perfect, however, has an open mind on the matter, and does not dissuade others from using the Laboratory Vaccine, knowing, as he does, that *many farmers in his district are obtaining good results from its use.*

MESSRS. FANNIN BROS. have used the Vaccine since 1899 *with perfect results* until this year, when they lost a yearling eight months after inoculation.

They have only been inoculating once a year, but have now decided to make two half-yearly injections, viz, in May and August. They have hitherto inoculated calves only, from six months old, but future inoculations will probably include everything up to two years old.

In former years the farm had a very bad reputation for Quarter Evil, the owner being quite unable to rear any young stock on account of it. Messrs. Fannin began by using the Pasteur method.

MR. J. W. MCKENZIE, of Richmond, has used the Vaccine since 1899, and *has had no bad results.* He inoculates yearlings only, averag- about 50 head, and they receive but one inoculation.

MR. T. W. J. HALL, of Mooi River, has used the Laboratory Vaccine for four years, inoculating about 80 calves and yearlings annually. As Mr. Hall generally waits for a case to appear before inoculating,

nothing much is to be gained from his experience, but he expresses himself as *being perfectly satisfied with the results after inoculation.* Mr. Hall could not remember the actual losses before the Vaccine was used, but in 1888 an outbreak occurred, resulting in the deaths of between 30 and 40 animals.

The experiences of DR. BREWITT and MR. J. COOK (of Estcourt) are of considerable interest. The cattle—including 80 head of calves and yearlings—were running together on winter thorn veld. In October of this year seven deaths from Quarter Evil occurred. They then obtained a supply of the Laboratory Vaccine and inoculated the remainder, *and no further deaths occurred.*

Mr. Cook, who inoculated the animals, says that three *were showing signs of Quarter Evil when they were injected, but all recovered.* No losses from Quarter Evil had been reported for seven years.

MR. R. DOUGLAS, of Estcourt, has applied the method since 1899, and inoculates everything from one month to two years old. Up to the present year *he has had every success.* In July of this year three died of Quarter Evil, but Mr. Douglas says that he cannot positively attribute these to the failure of the Vaccine, as there is the possibility of their not having been inoculated the month previous to death when the others were done. The average number inoculated amount to 50, and are increasing annually. In former years the death rate was not very heavy—about 2 per annum. Nine years ago he lost 30 head, and it is since that time inoculations have been regularly made. Mr. Douglas used the Pasteur method for the first four years, and the Laboratory Vaccine since.

MR. ROBERT WOODS of Willowford, Estcourt, used the Vaccine for the first time this year.

Towards the end of June about 100 head of young stock were inoculated and one death occurred two days afterwards. Mr. Woods does not blame the Vaccine for this, as he was rather late in making the inoculations and thinks that the disease was already present.

MR. C. HARDING used the Vaccine for the first time this year after losing ten head, and *no further deaths occurred after inoculation.*

These were the first deaths which had occurred on the farm for 15 years. He has occupied the farm for 23 years and, according to the evidence of natives, the disease then existed, but Mr. Harding did not experience any loss till eight years later. He is *very pleased with the results obtained by the use of the Vaccine*, and intends in future to inoculate regularly and also to recommend it to his nephew in King-williamstown, Cape Colony, who has had much difficulty in rearing young stock in consequence of the ravages of Quarter Evil.

MR. A. STUART of Estcourt. The evidence obtained in this case shows the great necessity for carefully observing the instructions for the use of the Vaccine. Had these instructions been properly carried out Mr. Stuart would not in all probability have lost seven animals this year Mr. Stuart's young stock has been inoculated for him since 1889, but this year he made the injections himself. The seven deaths referred to extended over a period of four months, and are as follows :—

1 Four-year-old Ox,	1 Four-year-old Heifer,
3 Yearlings,	2 Calves.

The calves had not been inoculated, the yearlings not since the previous September, and the two-year-olds had not been injected for three years. These casualties cannot be attributed to failure of the Vaccine, but rather to the false security of the four previous years, when, by good luck, the single inoculation had proved sufficient, owing probably to the absence of local infection during that period. This system of a single inoculation is, unfortunately, very prevalent among farmers, notwithstanding all warnings, and it can only be hoped that it will not be persisted in.

With regard to the farm's history of the disease, Mr. Stuart has been in occupation of the place for eleven years. Arriving in the spring, he lost a large number of cattle from Quarter Evil, including full-grown animals.

After that, and right up to the time of using the Vaccine, two or three deaths occurred every year. The average number inoculated amount to about 50 head. *This is the first time any deaths have occurred since using the method,* and Mr. Stuart is *very pleased with the results obtained.*

MR. E. KEMP, of "Selbourne," Estcourt, has used Vaccine since its first issue, and until this year *without loss or mishap.* Only one inoculation a year is made, usually in June, and then all animals from three to eighteen months old, the average number amounting to about 140 head. On January 28th of this year, a two-year-old beast died, which is supposed to have been inoculated on the 11th of September last, *i.e.*, a little over four months after the date of the injection. On May 27th, a yearling heifer died which should have been inoculated on September 11th, but which, Mr. Kemp thinks, may possibly have been overlooked. On October 10th a yearling died which had been inoculated on June 4th, four months after inoculation. On November 4th a yearling died which had been inoculated on June 4th (five months after). A beast belonging to a Native on the farm died three days after being inoculated. The disease having been very bad this year, Mr. Kemp thinks it more than likely that this animal was already infected before inoculation.

Mr. Kemp is *quite satisfied with the results he has always had from the use of the Vaccine,* and does not think the deaths referred to would have occurred but for the exceptional season, and *his faith in the method remains unshaken.* He has lived on the farm for 25 years. Previous to adopting the method his losses were at times very great. Mr. Kemp thinks of inoculating twice a year in future.

REV. J. SCOTT used the Vaccine on his own cattle for the first time this year, but the natives on his mission have *used it with every success* for the past two seasons, and *have every confidence in it.*

MR. H. BROWN, of Springfield, has used the Vaccine for two years, inoculating in January, and doing about 50 head of yearlings on each occasion.

Last year he *had every success,* but this year he lost two head after inoculating in January; the first died four, and the other seven, days after the injection. *Mr. Brown is very satisfied with the Vaccine.*

MR. F. E. KING has used the Vaccine for three years. He inoculates once a year only, usually in March, doing about 80 head of yearlings. The year before last he lost two yearlings 14 days after the

inoculation. *Mr. King has great faith in the method, and is very pleased with the results obtained.* In former years—*i.e.*, before using the Vaccine—he had losses all the year round, the deaths, as a rule, occurring among the yearlings, with, occasionally, a two-year old.

MR. H. L. FRANCIS has used the Vaccine for five years. The first year he made two inoculations, but since then he has only inoculated once every twelve months. He does all young stock up to two years old.

In 1899 he lost two head a few days after inoculating, but cannot remember what his losses were before using the Vaccine. Since 1899 he *has met with every success.* His yearly inoculations average 90 head.

MESSRS. MAPSTONE BROS., of Thornville Junction, have used the Laboratory Vaccine since 1899 *with every success.* The inoculations are not made regularly; some years two, and others only one. Should a death of an un-inoculated animal occur—say a calf born after the last injections were made—the whole are again inoculated. As a rule only calves and yearlings are done, but occasionally some 2-year olds, averaging about 100 head in all. In former years the losses were occasionally very heavy, but did not average more than 5 per cent on the whole. In October of this year a sucking calf died of Quarter Evil and all young stock were immediately inoculated, and no further deaths occurred. Messrs. Mapstone *have never lost an inoculated animal.*

MR. FRANK NICHOLSON, of Arnolds Hill, has used the Laboratory Vaccine for four years *with every success.* His inoculations are usually made in March, once a year only. This year, however, the young stock have not been done at all, and, so far, there have not been any losses. As a rule he only inoculates the young calves, averaging about 100.

In former years the natives on the farm have at times lost very heavily. Mr. Nicholson has known the farm all his life and says the disease has always been in existence.

MR. R. NICHOLSON has never used the Laboratory Vaccine for his own cattle, but has inoculated with it for others; among them being Mr. Newlands, of Sight Hill, Mr. F. Nicholson, Mr. Hammond, and Mr. Hall.

The inoculations for Mr. Newlands were made in October, after three deaths from Quarter Evil. Calves, yearlings and 2-year olds, were done and no further deaths occurred. In the other cases deaths from Quarter Evil had occurred before inoculation, *but none after.* Mr. Nicholson has always adopted the Pasteur method, but *having seen such good results he intends trying the Laboratory Vaccine.*

MR. R. A. MCKENZIE, of Richmond, has used the Vaccine for two years, making one inoculation only—in March or April. Last year—before he had time to inoculate—three deaths from Quarter Evil occurred, but no further casualties afterwards. Yearlings only are done, about 100 each time. In 1896 sixty deaths from Quarter Evil were recorded among the young stock.

MR. T. W. FLETT, near Richmond, used the Vaccine for three years, but has not inoculated at all this year, and, so far, *has escaped loss.* Mr. Flett runs two adjoining farms, and, while on one he has never known

Quarter Evil to exist, on the other the deaths from the disease have reached 50 per cent per annum; a year never passed without experiencing heavy losses. The worse experience he has had was some few years ago —before the introduction of Vaccine—when the method of resorting to heavy bleeding was in vogue. On that occasion nearly every young animal on the farm perished, possibly through the use of an infected phleam as no antiseptic precautions were taken. Mr. Flett inoculates once a year only, doing everything under 12 months old, the average number being about 25.

MR. P. FLETT has resided on his farm for 23 years, and during the first eight years he never lost a beast from Quarter Evil. The disease started with the death of a young heifer 15 years ago. For 11 years he used the Pasteur Vaccine and the last four years the Laboratory *Vaccine with every success.* Two deaths occurred subsequent to the usual inoculations which were made in September, 1902. Mr. Flett is unable to give the exact date of these deaths, but believes they occured within six months of inoculation. Only the young calves are inoculated, averaging 20 per annum. Mr. Flett intends inoculating twice a year in future

MR. J. W. P. MARWICK has used the Vaccine since 1899 but is irregular in the application of it. He inoculates in September and sometimes again in April, doing calves and yearlings. The disease existed when he first took over the farm 23 years ago. Previous to 1899 he applied the Pasteur method, and his losses, before inoculating, amounted to 25 per cent., which has been reduced to four per cent. The application of the method is probably responsible for these.

Last year five animals died soon after inoculation, one being within the six months' limit. The first was a calf which had not been previously inoculated, dying three months after. On the seventh day two yearlings and a three-year old heifer and her calf died. The heifer had been inoculated on two previous occasions. Later on, within the six months' period, another calf broke down and succumbed. These inoculations were made about the end of September, 1902. During the time Mr. Marwick has used the Laboratory Vaccine he has had one or two losses from the disease, *but among animals whose period of immunity conferred by the Vaccine had expired.*

Mr. Marwick usually inoculates calves and yearlings, but last year he also did the three-year olds. The average number done is about 50.

MR. A. H. COCKBURN has used the Vaccine for four years. He inoculates once a year only.

In former years the deaths from Quarter Evil amounted to 35 per cent. The disease still exists, as the natives continue to have losses. He inoculates about 80 head of calves and yearlings. *No deaths have occurred from Quarter Evil since using the Vaccine.* For some years previously Mr. Cockburn had used the Pasteur method with equal success.

Mr. Cockburn's brother (M. A.) was unfortunately away from home, but the former stated that the experiences of his brother tallied with his own. Their farms adjoin.

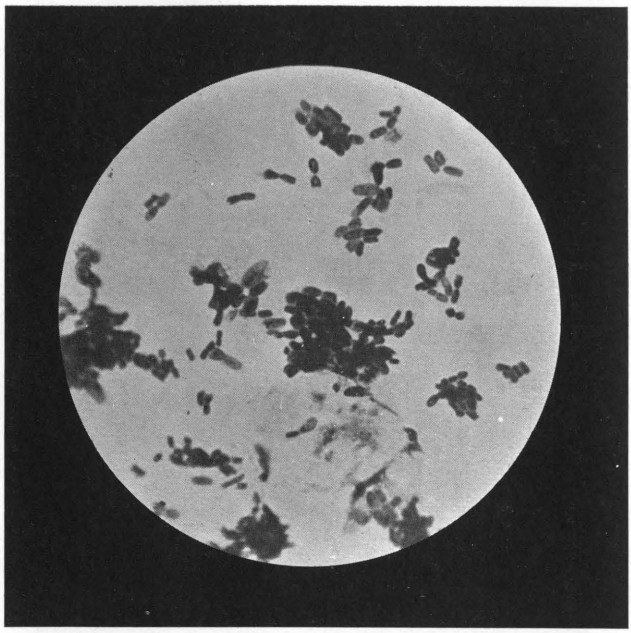

FIG. 10.

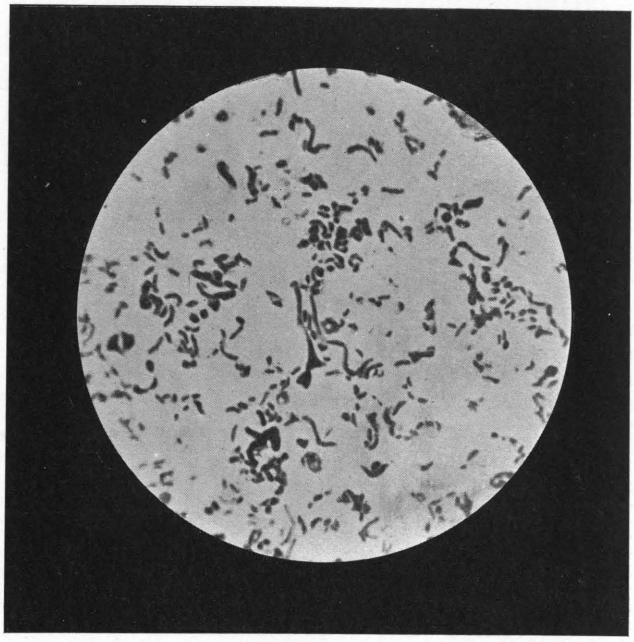

FIG. 11.

Another point of interest in connection with this medium is that where the organisms grew at Laboratory temperatures they remained in a fair state of preservation even after 100 days. One transplantation to same medium when 75 days old resulting in appearance as shown in Fig. 11.

Face p. 21.

MRS. AITKEN, of Sunnyside, cannot remember how long she has used the Vaccine, but belives it to be from the commencement of the issue of the Vaccine from the Laboratory. Calves only are done—usually in the month of February, those born after that time being done in the following July. Mr. Aitken lost an animal two years ago, but cannot say whether within the six months' period Two uninoculated animals which had been brought on to the farm died at the same time. The mortality from Quarter Evil was very heavy in former years, sometimes reaching to nearly 100 per cent.

MR. ANDREW AITKEN *has not had any losses from Quarter Evil among inoculated animals* since using the Vaccine, which he has done for the last four years, doing about 40 to 50 calves under 12 months of age. The losses previous to using any Vaccine averaged 50 per cent. per annum. September is the usual month for inoculating, but last year four deaths occurred before the inoculation. Mr. Aitken had previously used the Pasteur Vaccine. Seeing that only one inoculation a year is made, and taking into consideration the heavy losses experienced in bygone years, Mr. Aitken may consider himself fortunate.

MR. W. COMRIE cannot say for certain how long he has used the Vaccine, but thinks four years. He inoculates twice a year—in February and August. The calves being done when young, receive their second injection when nearly 12 months old. The average number done is about 30 each time. Mr. Comrie had adopted the Pasteur method for six years previous to using the Laboratory Vaccine. The yearly losses before inoculating were very heavy, and very few few cattle could be raised on the farm. *Mr. Comrie is delighted with the results he has obtained, and cannot remember ever losing an inoculated animal.* Two years ago 20 head of cows and calves were brought down from Ipolela, and four cows died from Quarter Evil soon after arrival. The remainder were immediately inoculated, and *no further deaths occurred.*

MR. J. MORTON, of Tweedie Hall, has used the Vaccine for the past two years *with every success.*

The inoculations are carried out in October, calves of from four to six months old only being done.

Mr. Morton stated that Mr. Hyslop—on whose farm his (Mr. Morton's) cattle had been in the habit of grazing—had suffered severely from Quarter Evil.

MR. MCLEAN, near Dargle Road, is *very pleased with the results* he has had from the use of the Laboratory Vaccine. When he purchased the farm, some seven years ago, he was told by the former owner and neighbours that he would have great difficulty in raising any young stock *owing to the ravages of Quarter Evil.* His first year's experience ed him to believe they were right, as, out of the eight calves he had, six died from the disease, and the losses in the following years—until using Vaccine—were in the same proportion. He has been inoculating for four years, and does about 40 head, from young calves to two-three-year-olds, once a year only, in January and February. Mr. McLean remembers losing an inoculated animal, but cannot say whether it was within the six months limit ; even if such was the case, he would not put

it down to any shortcomings of the Vaccine, but rather to some mistake on his own part, having had such success in the past.

MR. ARMSTRONG, of Dargle Road, has used the Vaccine for four years, making two injections yearly at the beginning of June and again in September, doing all young stock. June has proved the worst month. Mr. Armstrong believes he lost an inoculated anim l, but cannot say whether it was within the six months.

MR. NESBITT, whose farm adjoins that of Mr. Armstrong's, has used the Laboratory Vaccine for two years, after many years use of the Pasteur. His injections are made in January and once a year only. He has *had every succe s* with both Vaccines. About 50 head are done each time. The losses before using either method averaged 20 per cent.

MR. C. W. LANGE, of Chakas Kraal, used the Vaccine for the first time last October, after losing four full-grown beasts. 40 head of all ages were done, and the disease stopped immediately. At the same time 10 were done for a neighbour, and only one—which was very far gone with the disease—died. Mr. Lange intends inoculating twice a year in future.

MESSRS. ESSERY AND SONS, of Riet Valley, *have successfully used* the Vaccine for two years. About 100 head were done each time, and Messrs. Essery and Sons intend inoculating twice a year in future.

MR. F. CULVERWELL, near Mooi River and Noodsberg, has used the Vaccine for four years, inoculating twice a year—in May or June, and November—all young stock from six months to two years old. It is mostly on the Noodsberg farm that the disease appears, very seldom on the Mooi River farm.

The losses from Quarter Evil in former years were very heavy, and averaged 50 per cent. Mr. Culverwell is *very pleased with the results* he has had from the use of the Vaccine.

MR. DOUGLAS CAMPBELL, of Summersford, Ixopo, has inoculated for two seasons, mo e as a matter of precaution, owing to the prevalence of, and losses from, Quarter Evil on the adjoining farm "Seaforth" Calves only are inoculated, and the only death which occ rred was an animal which had just been brought up from the Umzimkulu with others, two of which died at the river two days before.

Mr KIRKMAN states that last year one of his Natives lost a calf from Quarter Evil. He at once inoculated his own cattle, and *although they were running on the same veld, no death occurred. He has recently inoculated again with success.*

Mr. Kirkman mentioned the case of a former neighbo r (Mr. Elliott). This gentleman during one season lost 15 head from Quarter Evil, but *after inoculating had experienced no further loss.*

Mr. IVINS inoculated for the first time in January, 1903, after losing five head—four of them being imported Argentine yearlings. *No further deaths occurred after inoculation.*

MR. BEWS inoculated in February of last year for the first time, after losing a calf. 40 head were done, and *no further deaths occurred.*

MR. JAMES COMRIE stated that last year Mrs. P. Comrie had lost three calves from Quarter Evil. He immediately inoculated with the Laboratory Vaccine the remaining 21, and *no further deaths occurred.*

MR. W. F. DAVENHILL, of Dronk Vlei, states that in November, 1901, eleven deaths from Quarter Evil occurred, including two 4-year olds; but after inoculating the remainder of his cattle, there were *no further losses* that season.

About the same time in the following year, however, another beast died. He immediately inoculated the remainder, and *no fresh cases occurred.* In 1903 he inoculated earlier, and *did not lose a single animal.* About 50 head are done each time.

MR. TOM WILL, of Dronkvlei, has used the Vaccine for four years, inoculating once a year only. The year previous a beast had died from Quarter Evil, which he allowed to be skinned and cut up. Two others died immediately afterwards, followed by two more, making five in all. Since inoculating regularly *there have been no further losses.*

MR. MARRIOTT, of Dronkvlei, has used the Vaccine since it was first issued in 1899, doing, for himself and others, about 100-120 head of calves and yearlings every year.

With the exception of a death, *before inoculation*, in 1902, Mr. Marriott *has not experienced any losses, and cannot recall a breakdown or bad result.*

MR. E. BURD has used the Vaccine for four years, doing about 50 to 60 head of calves, from six months to two or three years old. Two inoculations are made yearly—July and November. The only death which has occurred on the farm since inoculating was in February last year. This was a two-year old which had been inoculated in the previous November.

Mr. Burd *considers the results to be eminently satisfactory*, and accounts for the one death by saying it was probably owing to high and exceptional susceptibility. The losses before inoculating were very heavy. The disease usually appeared at the beginning of September, and lasted through the summer

Mr. W. K. ANDERSON, of Maxwell, Ixopo, has used the Vaccine for two years *with every success,* previously applying the Pasteur method. Inoculations are made once a year only, and the average number done is about 50 head of calves.

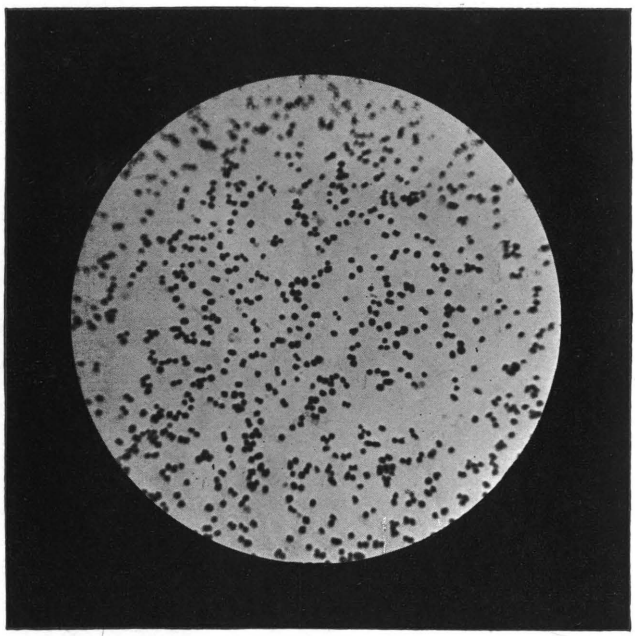

FIG. 18.
PLAGUE ORGANISM GROWING AT TOP OF AGAR SLOPE.

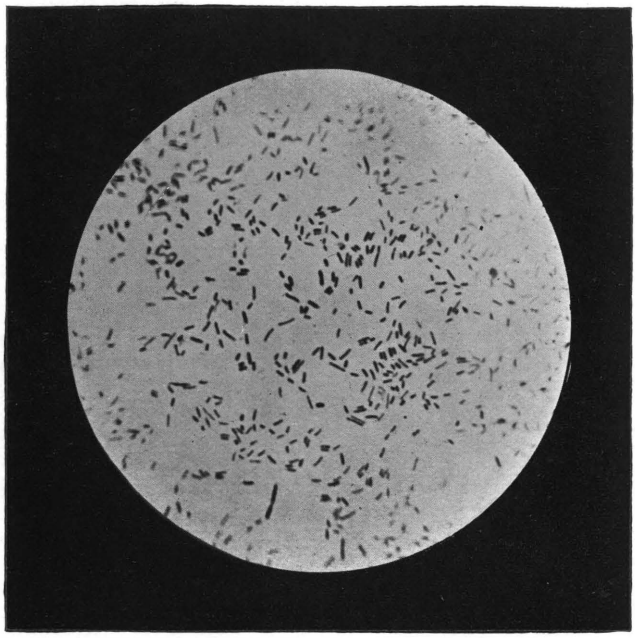

FIG. 19.
SAME ORGANISM GROWING AT LOWER END OF SAME SLOPE.
Face p. 26 (d).

BACTERIOLOGICAL REPORT

ON THE

PLAGUE IN NATAL

1902-3

BY

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F.R.C.V.S., GOVERNMENT BACTERIOLOGIST

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1904

THE LABORATORY, MARITZBURG.

January 19th, 1903.

TO THE HEALTH OFFICER FOR THE COLONY..

SIR,—

As the general details of the recent epidemic have been dealt with fully elsewhere, the subjoined report is intended to be restricted simply to details appertaining to the bacteriology proper of the outbreak without more than a passing reference where necessary to such points of clinical and epidemiological interest as seem directly connected with the matter under consideration.

One item, however, of bacteriological as well as general interest in connection with the history of the outbreak which seems in place here is the undoubted value of precautionary measures for prompt bacteriological action which were assured before the advent of the disease. The establishment of a small laboratory for the routine examination of rats found dead or sick in the neighbourhood in which it was considered the chief danger of introduction lay was attended by the best results, and it is probable that the certainty with which the commencement of the epizootic was detected through this means rendered possible that prompt detection and suppression of those earlier cases of the disease so important in combating an epidemic of Plague.

I propose, therefore, in the present Report to con-

fine my observations to such points of bacteriological interest in connection with the Bacillus Pestis Bubonicæ as its morphology, vitality, and virulence, and to amplify these points with clinical or other evidence only where such seems necessary.

The pressing demands made upon my time in my official capacity as the Director of the Research Laboratory, Maritzburg, made it imperative to ask for assistance in a work which it was hoped would embrace a broad field of inquiry. The necessity for such provision was readily concurred in, and accordingly the assistance of Dr. Haydon, who had previously been engaged by the Government of Bombay in connection with Plague work, was secured, and he assumed duty on December 18th, within a few weeks of the outbreak, and at once undertook the routine duties of the small Laboratory established at the Point, Durban.

Those duties—which, though routine, were none the less onerous and responsible—consisted chiefly in the examination of the rodents found dead or dying in various localities, and as the arrangements for detection and collection of rats were of an ample and efficient nature, it became necessary daily to subject the organs and tissues of a large number of rats to bacterioscopic examination. This, together with the constant observation and maintenance of cultures of organisms (made during life or from the cadaver both of man and the rat) in cases where the clinical history required confirmation or support, necessitated constant and assiduous application.

With the extension of the epidemic to Pietermaritzburg, and the possibility of making other provisions for carrying on the routine work, I thought it advisable to utilise the services of Dr. Haydon at Pietermaritzburg upon work of a less routine nature, and

this suggestion meeting with your concurrence, he was accordingly transferred to my Laboratory from Durban.

In the subjoined report, therefore, I have had the assistance of Dr. Haydon, who has worked with me with untiring assiduity for the past ten months, and to whom I am indebted for many suggestions and a hearty co-operation.

One of the earliest difficulties encountered in the bacteriology of the Natal outbreak was that of arriving at an exact diagnosis from the microscopical appearances available.

This point (which, as will be readily recognised, is one of prime importance where prompt repressive steps are necessary) does not seem to have been recorded in the history of outbreaks of the disease elsewhere, with the exception, perhaps, of the recent epidemic in the Cape Colony.

The difficulty, however, in Natal has occurred upon quite a number of occasions in both human and animal tissues, and has shown beyond dispute the unreliability of the usual simple microscopic procedure in deciding upon a case of suspected Plague.

It has been a matter of repeated observation that organisms isolated from various cases, other than Plague, submitted for bacteriological opinion have appeared identical with the Bacillus Pestis Bubonicæ as regards size, shape, and the different staining property possessed by this organism. This difficulty arising in connection with the accurate and prompt diagnosis of the disease has been dealt with in the following pages, as it is felt that the reliance which it has been customary to place generally upon bacterioscopic appearances in the diagnosis of Bubonic Plague, apart from those derived directly from bubo-juice, etc., cannot for the future be given full weight, except where supported by

a clinical history of the most unequivocal nature, tending to confirm the indications of the microscope.

The validity of this objection to placing full reliance upon the bacterioscopic appearances will appear from an inspection of the photomicrographs which elucidate this point, from which it will be seen that close similarity may exist on comparison with the typical or text-book bacillus of Kitasato, and other alien organisms of similar morphology.

That this difficulty of diagnosis should have been one of not infrequent recurrence seems more remarkable when the limited number of classified organisms capable of being mistaken microscopically for the Plague bacillus is considered.

The liability of confusing the Bacillus Pestis with other organisms than those hitherto recognised as possible causes of confusion—such as the germs of Chicken Cholera, Rabbit Septicæmia, Swine Plague, Pneumo-pleurisy of Calves, etc.—must be considered, therefore, as increasing the limitations attending the use of the microscope alone.

I propose to deal briefly in the first place with the normal, cultural, and microscopical appearances met with in the Plague Bacillus during the Natal outbreak. Such observations can have in most instances the value of merely corroboratory evidence, though it is hoped that some of the details in this connection (hitherto undescribed) may prove of value to subsequent workers in the same field.

After dealing with the morphology of the organism, I propose to discuss the important question of its vitality and virulence under varying conditions of environment, and lastly its pathogenicity for the lower animals.

Finally, I shall hope to illustrate briefly the difficulties

sometimes encountered in arriving at a definite diagnosis for clinical purposes, etc., by quoting details of some of the instances in point and their differential features.

It has been thought advisable to illustrate the text as amply as possible.

Apart from the many curious instances of pleomorphism exhibited by the bacillus, simulating the morphology of almost all forms of bacterial life, I have thought it advisable to bring forward evidence as conclusive as might be of the typical nature of the organisms used in the endeavour to produce the disease in pigs, fowls, etc. The magnification of all photomicrographs is constant throughout—viz. 950 diameters.

I have the honor to be, Sir,

Your obedient Servant,

H. WATKINS-PITCHFORD, F.R.C.V.S.

(*Government Bacteriologist*).

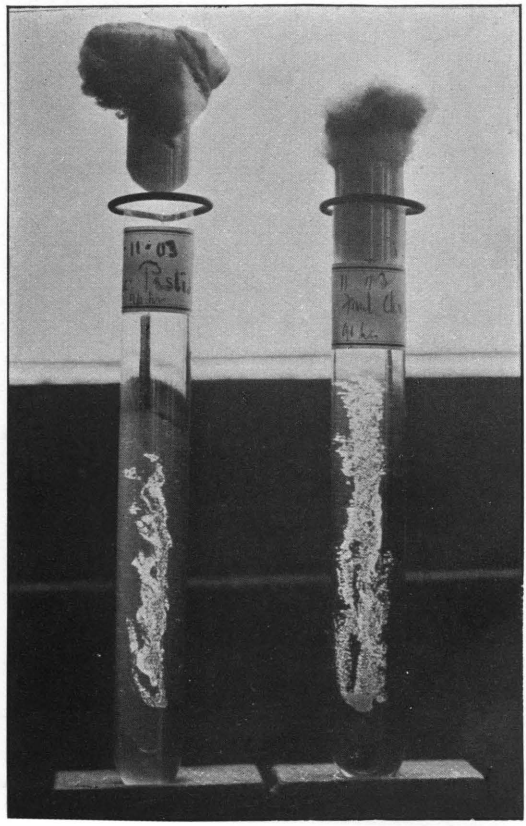
SECTION II.

OPTIMUM CONDITIONS OF TEMPERATURE, AND CULTURAL OBSERVATIONS.

OPTIMUM CONDITIONS OF TEMPERATURE.

A SERIES of observations undertaken with the object of determining the above point has shown, while the growth is perhaps slightly more vigorous and pronounced in the warm incubator at 37° C. during the first 48 hours, that after this period the tubes maintained at normal and subnormal temperatures rapidly outstrip those grown at 37° C. This is very noticeable after the expiration of one month, when it will be noticed that the tubes maintained at from 15° to 18° C. show evidences of much more copious and vigorous growth than those grown either at 20° or 37° C., while the difference between the growths from hot and cold incubators is still more marked. This point bears out the evidences of rapid degeneration noticed in the morphology of organisms cultivated at 37° C. for 10 days before being taken from the incubator.

The above observation is not without its significance in considering the seasonal influences connected with the spread of Plague epidemics, as the ability of the Bacillus Pestis to grow vigorously between so wide a range of temperatures as that existing between 15° and 40° C. would seem to show the ease with which this organism can adapt itself to the varying seasonal temperature.



CULTURAL OBSERVATIONS.

The growth even in early cultures presents varying appearances according to the amount of material sown, whether taken from tissues or from another culture, and the temperature at which cultivation is made. Thus with spleen pulp containing large numbers of the organisms the resulting growth on solid media will be a transparent slimy streak (*vide* Photo No. 2*d*), while if heart blood or a bouillon culture be used the early appearances will consist of discrete dew-drop-like colonies (*vide* Photo No. 1*a*).

In all the following observations a recent bouillon culture from a rat's spleen was used.

On *agar agar*, incubated at 37° C. for 24 hours, the growth can often be seen of a fairly granular character by the naked eye, and can always be detected by the use of a hand lens.

In 48 hours, at 37° C., growth is always apparent, and consists of minute discrete colonies, colorless, or of a delicate grey color, well raised above the surface of the medium.

These vary in diameter from .25μ to 2μ. By transmitted light, these minute droplet colonies, viewed with a lens, display a translucent opalescence.

After 72 hours the discrete appearance of the colonies tends rapidly to be lost by confluence, so that a 7 to 9 days' culture presents an irregular whitish growth, tending in its gradual coalescence to opacity, but presenting always well-elevated edges. Variations from the normal cultural type, such as described by Yersin, Klein, and others, have also been met with, and will be found on referring to paragraph 1, Appendix A.

Gelatine.—The growth upon nutrient gelatine is without any marked points of difference except such

as would result from the use of a medium of greater transparency (Fig. 1, *a*). Thus the discrete droplet colonies appear more translucent, and the opacity of their central parts less marked. No great differences in rapidity of growth subsist between this medium and agar agar. Where discrete colonies of any age exist the firmness of their attachment to this medium is noticeable, due probably to the depth to which the growth penetrates.

Glucose Agar.—Very similar to, but less abundant than on glycerine agar.

Litmus Agar.—Alkalinity commences to be observable in about 6 days.

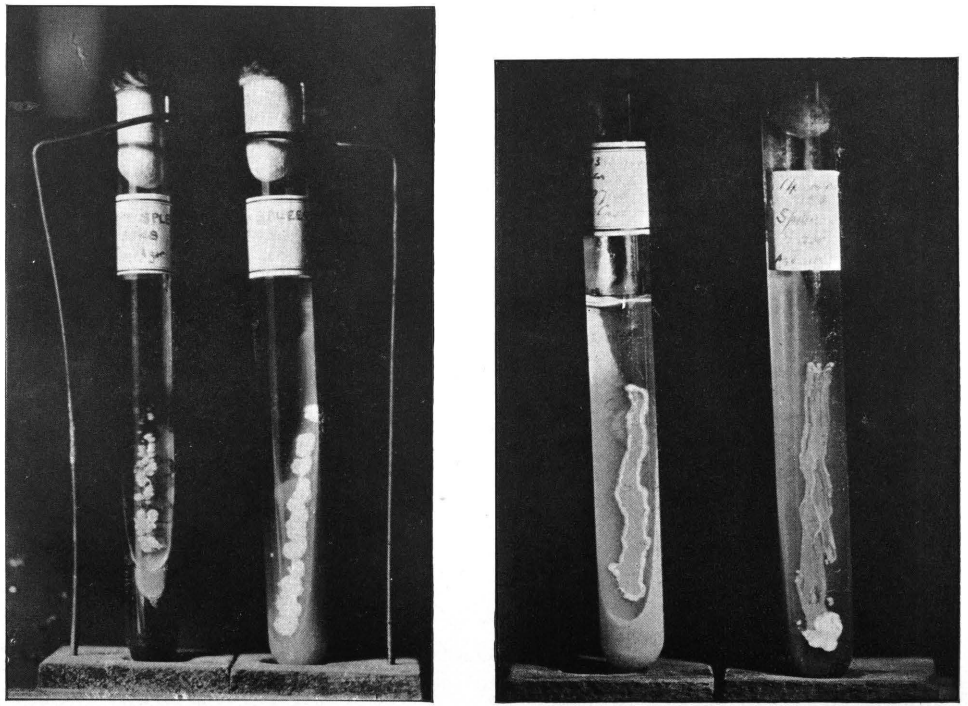
Glycerine Agar (3 per cent. glycerine).—There are no very distinctive points between this medium and plain agar. Growth is less rapid, and has less color.

Salt Agar (2·5 per cent.).—The growth is somewhat less abundant than on other solid media, presenting no distinctive appearances.

In Rat Agar.—Growth was more abundant than on any other solid media used (*see* Fig. 2, rat agar *b* and *c* compared with plain agar *a* and *d*).

Rabbit Agar.—Little difference from plain agar.

On Ox Serum.—In 48 hours appears a thin opaque film, with a very finely granular surface which contrasts with the smooth iridescence of the rest of the slope. Touching with the öse makes little difference to the appearance of the film. As the age of the culture increases, little change takes place except that the film becomes thicker, more raised, and its surface becomes nodulated. On this medium a stickiness and toughness of the minute colonies are more often noticed than on other media. The growth on this medium is never so copious and abundant as on agar and glycerine



a *b* *c* *d*

FIG 2.

Care was taken to sow these tubes with equal quantities of material, and the growths are of equal age.

Face p. 10.

agar. This observation seems at variance with those of previous describers.

In Beef Bouillon.—At 48 hours a scanty deposit rises on agitation and faintly clouds the liquid—less viscid than in glycerine bouillon.

Tends to become more floccular as age of culture advances.

A film appears on the surface and adheres to the glass in about 72 hours.

Beef Bouillon (with glycerine 3 per cent.).—After the lapse of 24 hours a scanty, viscid, yellowish white whorl arises on agitation of tube.

Later flocculi are always to be found on the surface adhering to the sides of the tube.

If kept at rest after agitation the medium becomes clear again in 48 hours.

In *Glucose Bouillon* the growth is less abundant, but displays much the same characteristics as in plain bouillon.

Salt Bouillon (2·5 per cent. Na Cl).—Very little growth was obtained at any time. The medium at rest remains clear, but after a few days a faint cloudiness on agitation is noticed.

When sown profusely, however, in flasks in 5 per cent. salt bouillon-ghee, an abundant stalactitic formation is noticeable in 72 hours.

Rat Bouillon.—A more rapid and abundant growth is noticed than on beef bouillon, other characteristics being identical.

Milk.—Does not coagulate, and presents no particular characteristics.

Potato.—Growth slow, and without particular characteristics. After 20 days at normal temperature, the growth is dry, creamy-white, with irregular elevated edges (*see* Fig. 3).

Stalactitic Growth.—Much stress has justly been placed by previous observers on this cultural characteristic as a means of differentiation (*see* Fig. 4).

The best method of observing this interesting phenomenon would appear to be by sterilising small discs of cork, and when quite cold inoculating these fragments with a recent culture of Plague. These infected discs of cork are then transferred under aseptic precautions to flasks of ordinary bouillon or bouillon containing up to 3 per cent. salt. The appearances are to be noted best in an Erlemmeyer flask, the base of which, being considerably wider than the apex, allows detached fragments to fall clear to the bottom of the flask without risk of lodgment on the sides, with consequent obscuration of the interior of the flask. The formation of the growth which depends from the lower surfaces of the cork can then be readily observed, such growths being able, by reason of the buoyancy of the cork fragments, to attain to considerable dimensions before becoming detached and falling to the bottom of the flask. It is of interest to note that an infection of the flask with several organisms will not interfere with the production of this appearance beyond the general opacity or cloudiness of the medium, and this fact might possibly be of advantage as a point in the technique of diagnosis.

Before sowing such fragments of cork, after infection of the same, it is well to give the contents of the flask a gentle rotary motion, in order to prevent the adhesion of the cork to the sides of the vessel.

No special peculiarities are observable in the microscopical examination of portions of the stalactitic growth; oval or coccal forms are not so frequent as in many other media.

Anaërobiosis.—Growth appears to be almost in-

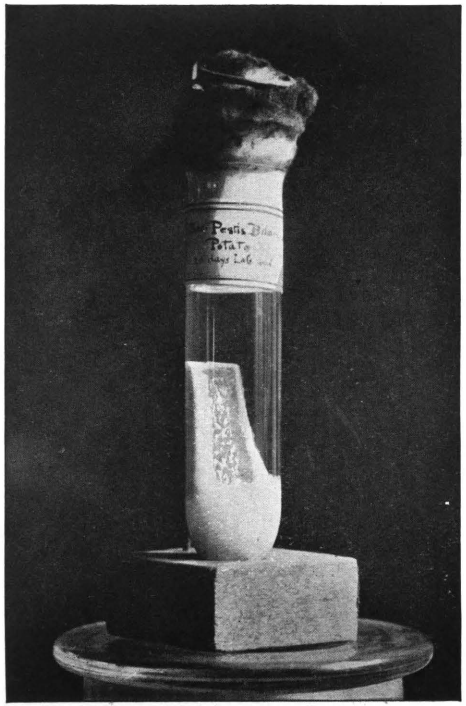


FIG. 3.

FIG. 4.

Face p. 12.

hibited in CO_2. Bouillon shows no growth after a month. The statement that the vitality of the organism is increased when developing in earth, cow-dung, floors, etc., owing to the presence of excess of CO_2 in ground air, would not seem to be borne out.

The reaction of the organism is distinctly alkaline. After 36 hours' growth in neutral bouillon, this condition is marked and increases with age, being marked at eight months and beyond.

SECTION III.

MORPHOLOGY OF THE BACILLUS PESTIS BUBONICÆ.

THE organism, which may be looked upon as typical, is that to be isolated from the spleen, bubo, or blood stream. Its general description is well known—viz. that of a short ovoid bacillus staining more deeply at either pole. This characteristic of bipolarity can probably be produced in all bacillary forms of the true Plague organism by alteration in the technique of staining, and it will be further found that with the increase of age this characteristic tends to be lost. Although this bacillary form is to be looked upon as typical, it will generally be found that a large percentage of the organisms in any given preparation consists of coccal or spherical forms in which no bipolarity can be observed.

It may be noted here that considerable modifications in contour and differential staining properties appear to take place *in corpore* with advancing age of the bacillus and advent of putrefactive changes.

This observation is not without its value in examination of tissues of uncertain age, as it is probable that the typical form above alluded to tends to become less marked with the advance of time; and it should be borne in mind that these typical appearances cannot be certainly expected in tissues after a certain lapse of time.

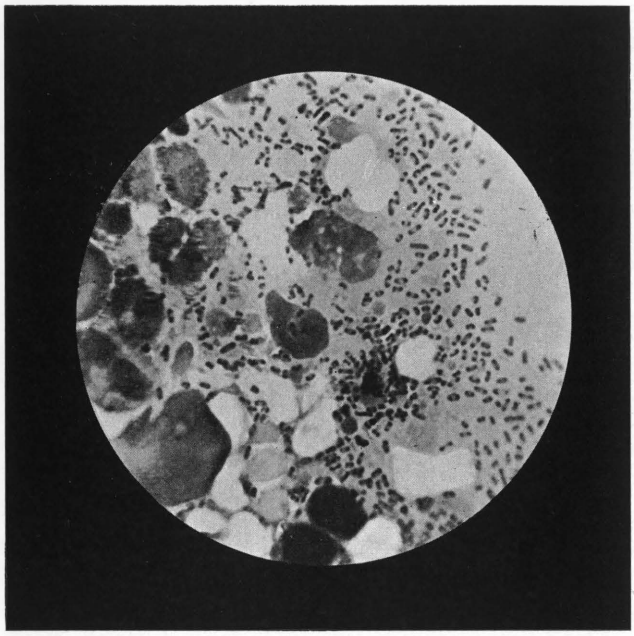


FIG. 4A.

NORMAL TYPE OF BACILLUS IN THE RAT. *Face p.* 14.

This typical form of the organism will be found to obtain with constancy in the tissues of most of the animals to which it proves pathogenic. The only point of variance is a slight difference in measurement. The size of the Bacillus Pestis Bubonicæ taken from the human spleen has been found to average in length 1·64 microns, and in breadth ·82μ; while in the organism taken from the bubo itself the size is slightly smaller, averaging 1·448μ in length and ·82μ in breadth. The above average was arrived at by 60 separate estimations, the extremes of length and breadth being respectively 3·4μ and 1·28μ.

Calculated from 120 estimations in spleen tissue taken from the rat, the organism gave a length of 1·584μ and a breadth of ·816μ, while in 50 estimations in guinea-pig tissue the length was found to be 1·311μ, and the breadth ·717μ, being thus of less size generally than that found in the human tissues. It may be stated, therefore, that the size of the Bacillus Pestis Bubonicæ in man and the lower animals averages about 1·5 microns in length by ·8μ in breadth.

In cultures grown upon artificial media the microscopic appearances will be found to vary considerably. The various media used were as follows :—Agar, gelatine, glucose agar, litmus agar, glycerine agar, salt agar (NaCl 2·5 per cent.), rat agar, rabbit agar, ox serum, beef bouillon, beef bouillon with glycerine 3 per cent., glucose bouillon, salt bouillon 2·5 per cent., rat bouillon, milk, potato, etc.

On these media, although observations were commenced upon a 24 hours' growth in each case, it was decided to pass these over in consequence of the growth being so extremely slight that a risk was run of removing from the surface of the medium some of the organisms actually implanted there. The following

observations therefore are made upon growths 48 hours old and upwards.

(1) *Agar agar.*—After 48 hours' incubation the organism is seen as a short deeply staining bacillus varying in length from ·62 to 1·7μ, the average of a number being 1·066μ and the average breadth ·708μ. Practically no differential staining is observable, or only a slight trace.

After 72 hours at 37° C. on this medium the bacillus tends to lose its property of staining intensely, while some of the individual organisms have lost their rounded coccal forms, and tend in some cases to an irregular truncated or even cubical shape. This appearance tends to become more marked in 96 hours, and the failure to stain becomes more noticeable.

After 144 hours the tendency to irregularity of contour is marked, while a number of degenerate forms hardly discernible are intermingled with above.

After 240 hours bacillary forms are rare, the field consisting chiefly of faintly stained large coccal forms, the diameter of which ranges from ·4μ to 1·2μ, contrasting with organisms of an equal age when cultivated on glycerine agar. Scattered throughout the field are occasional deeply stained coccal forms.

After lapse of a month the organism has lost all its affinity for the stain, but is still visible in carefully prepared preparations as a faintly stained, irregularly shaped microbe, the transverse axis almost equalling the longitudinal.

In 50 days the field consists of very faintly stained débris, and what appears to be organisms which have lost all characteristic shape. There is, however, a good sprinkling of well-stained forms, chiefly of a coccal shape, though occasional rod forms with slight differential staining exist here and there.

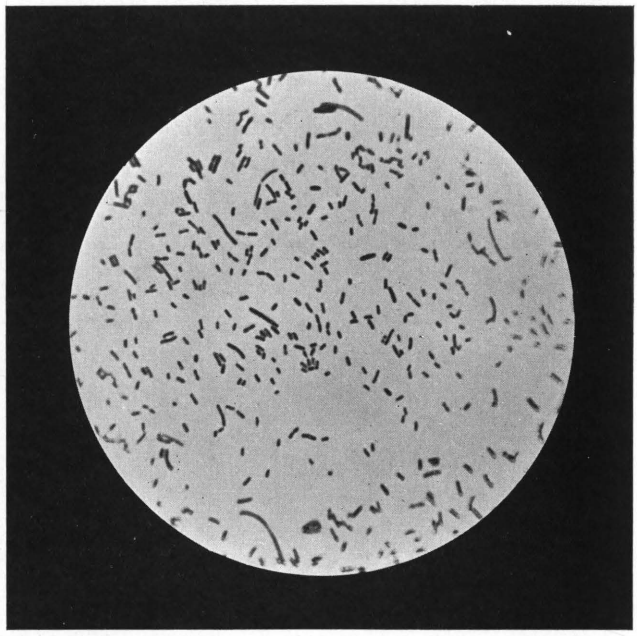


FIG. 5.

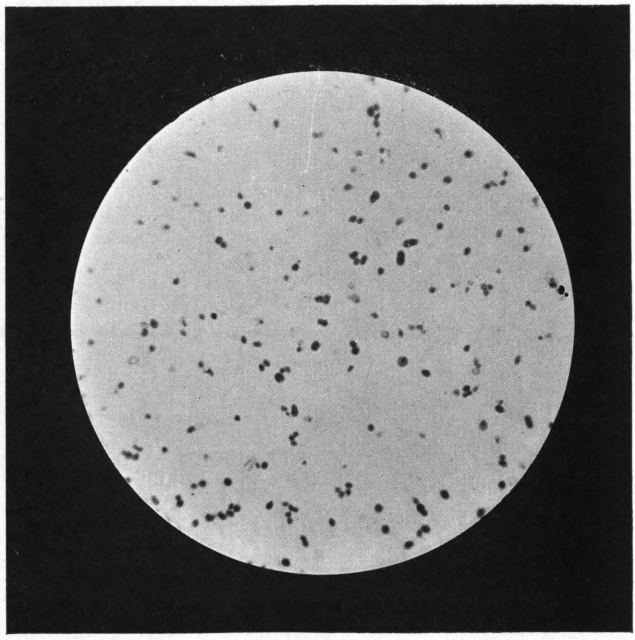


FIG. 6.

Face p. 17.

On subculture of this 50-day organism, and even after the lapse of 75 days, the original typical form reappears in 48 hours.

Gelatine.—After 48 hours' incubation at 22° C. the tendency to formation of long curved threads is marked, while a general tendency to elongation of the individual bacilli is noticeable, with corresponding decrease in the proportion of coccal forms. The average length of the organism after 48 hours' incubation is about 1·75μ (*see* Fig. 5).

In 96 hours thread forms are less noticeable, while the more normal coccal forms are general. No signs of degeneration or involution are present at this period, and the individual bacilli stain vigorously.

In 12 days the long thread forms are noticeably decreased, and the organism has less affinity for the stain. Elongated slender forms, however, are still frequent.

In 30 days all thread forms have disappeared, and the field is filled with small coccal growths, staining faintly, with frequent spherical bloated involution shapes (*see* Fig. 6).

(3) *Glucose Agar.*—In 48 hours a very scanty field exists. Normal forms occur without differential staining, but the majority consist of small coccal forms.

In 72 hours the field is still very scanty, small coccal and diplococcal forms staining deeply, but showing very little tendency to bipolarity. In 96 and 144 hours the growth is still very scanty, and the field shows masses of faintly stained bacilli, giving the impression of disintegration and degeneration. In addition to these, well-formed, deeply staining organisms occur, which are separate and ungrouped. Occasional forms exist from 3μ to 4μ in length, but differential staining is absent throughout.

In 10 days at 37° C. disintegration is far advanced. Fields consist chiefly of aggregations of faintly stained small ovoid and bacillary bodies with an occasional thread.

In 20 days at laboratory temperature, however, the growth from the moist portion of tube consists of elongated bacilli, contrasting markedly with parallel growth on agar.

In 50 days the appearances are somewhat remarkable, the field consisting of slender, elongated forms of bacilli varying in thickness, becoming alternately thicker and thinner, the transition being gradual. The extremities of these forms are either bulbous or tapering. In parts of the field these thread forms will be found to form a tangled skein of much intricacy (*vide* Fig. 13). Coccal and ovoid forms have ceased to be visible, but all gradations in length exist in the bacillary forms. On a retransplantation of this 50-day abnormal form of the Bacillus Pestis to plain agar vitality is rapidly re-established, so that a 72-hour culture shows aggregation of elongated and well-stained bacilli, intermixed with masses of débris from the old growth.

It is further worthy of note that the 50-day growth on glucose agar, consisting of yellowish, raised, desiccated-looking colonies, resist disintegration by the platinum loop to a considerable degree.

On transplantation from this medium after 75 days no growth resulted.

Litmus Agar.—No special appearances are noteworthy. The tendency to alkalinity in old cultures (up to 8 months) has already been noted.

Glycerine Agar.—The general size of the organism is noticeably increased, while its contour is less defined. Differential staining in 48 hours is more marked than

upon agar ; coccal forms greatly predominate. Staining is not always bipolar, but seems in many organisms to be disposed irregularly on the short axis of the bacillus. The average length of an early culture on this medium is 1·49μ as against 1·06μ upon agar agar. The transverse measurement is also noticeably increased, being ·968μ as against ·708μ upon agar.

Tendency to capsulation has been observed in some specimens, but this appearance is inconstant and of doubtful value in consideration of the morphology of the bacillus. Tendency to thread formation is present. Occasionally, on this medium in early cultures, marked involution and irregular thread forms occur, as is often the case on rabbit agar (*see* Fig. 7).

In certain instances, the early 48-hour growth upon some samples of this medium gave the striking appearances as depicted in micrograph 8. Irregular branched and twisted forms, alternating with thick, elongated, or club-shaped forms appearing occasionally in this medium, tend to show the extreme polymorphism of the Bacillus Pestis.

Some of the twisted, branched forms as shown above appear distinctly segmented ; dichotomy proper, however, is always absent.

In 72 hours the appearances are not markedly altered. Occasional faintly stained large bacilli, three times the length and twice the breadth of the normal organism, are present. The majority of the field, however, is composed of coccal and diplococcal, with some thread forms which stain deeply (*see* Fig. 9).

In 96 hours coccal and bacillary forms predominate, showing marked bipolarity. The giant forms noted under 72 hours are increased in both diameters, and, while staining faintly, as a rule are occasionally deeply colored. Many leptothrix forms are present.

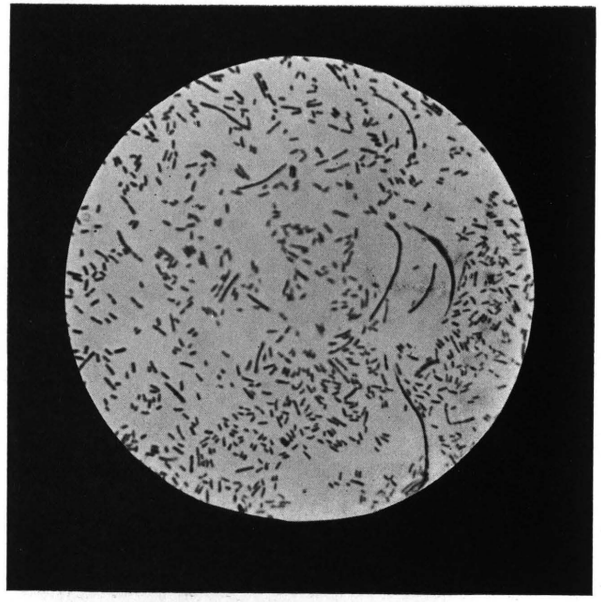


FIG. 7.

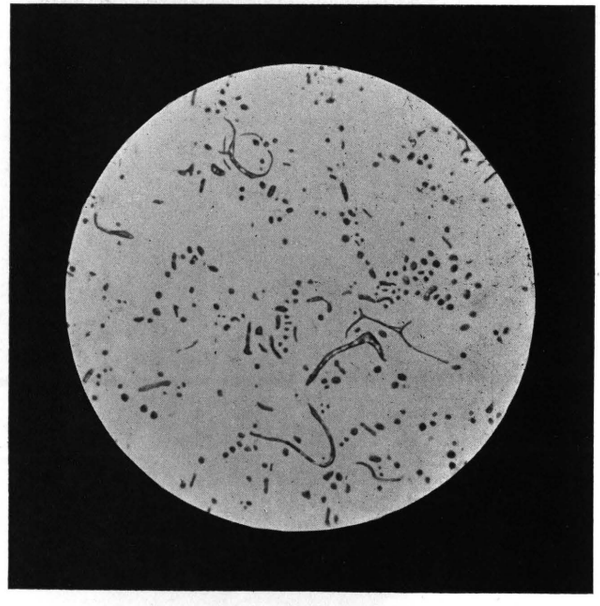


FIG. 8.

Face p. 19 (a).

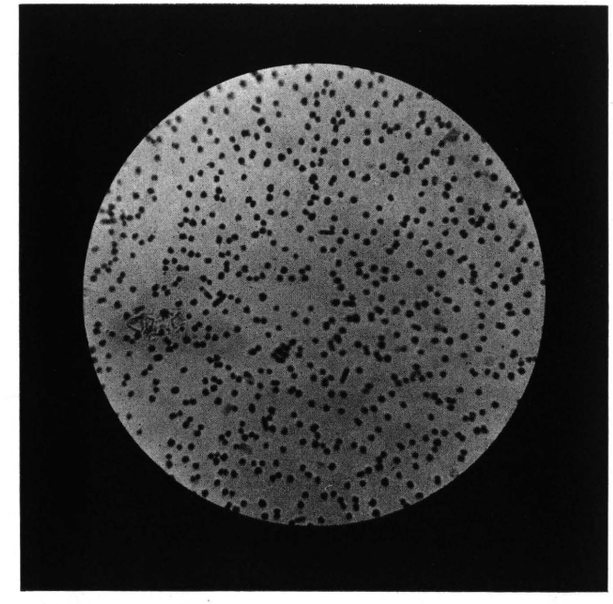


FIG. 9.

Face p. 19 (b).

In 144 hours polymorphism is more marked, club and baton forms are occasionally present, while the giant forms as above persist, and are irregular in their chromotism. Thread forms are frequent, forming involuted tangled masses, presenting a remarkable divergence of appearance from the normal type of bacillus.

In 240 hours the majority of forms consist of ovoid or spherical forms, these latter being as large as from ·5μ to 2·5μ in diameter. The staining is markedly deeper than in agar of corresponding age ; bacillary forms are also more numerous than in agar.

In 30 days well-stained bacillary forms again predominate, in some cases being elongated into threads of irregular thickness from 4μ to 6μ in length.

A groundwork or matrix of faint "ghost-like" forms exists, in which large, irregular, and pale or coccal forms predominate. In 50 days signs of degeneration of the bacillary bodies are general, but there is a good sprinkling of deeply stained bipolar organisms. These deeply stained apparently virile organisms are apparently embedded or in close relation with the disintegrating forms, and in many cases a fragment or end of some of these degenerate forms is deeply stained, giving the impression of the greater longevity or survival of the part of the organism perhaps in connection with its proliferation.

Long, slender, and curved forms are occasionally present. Retransplantation at this period rapidly produced typical appearances.

After 100 days upon this medium the evidences of degeneration are much more marked, although, as will be seen under the heading of vitality, the organism retains its pathogenic and proliferative powers.

Salt Agar (2·5 per cent. NaCl).—The involution forms upon this medium are marked from the earliest

incubative period. In 48 hours many long thick forms, seldom straight, are visible, showing no differential staining; many strange tadpole, pear-shaped, and irregular forms exist. Occasional normal forms are seen, showing differential staining. The aggregation of bacillary masses is noticeable.

In 72 hours involution forms are very frequent. All organisms stain well, and are firm, though very irregular in outline; large bloated dumbbell and spherical forms exist.

In 96 hours involution forms and aggregations of bacilli are not perhaps so marked. Many organisms assume the shape of a square or parallelogram, with the ends sharply truncated (dice box shaped), and often even concave (*see* Fig. 10).

In 144 hours little difference is observable.

In 240 hours disintegration far advanced, outlines of organism begin to be lost.

In 30 days an irregular, lightly stained, semi-granular mass results, showing very occasionally an organism faintly seen and very degenerate.

In 50 and 75 days these appearances are increased, appearing little more than an amorphous mass of faintly staining débris. Reference to the question of vitality, however, will show that even after 100 days the bacillus had not lost its power of propagation and pathogenicity.

Another point of interest in connection with this medium is that where the organisms grew at laboratory temperatures they remained in a fair state of preservation even after 100 days. One transplantation to same medium when 75 days old resulting in appearance as shown in Fig. 11.

Rat Agar.—A vigorous, deeply staining organism results from cultivation upon this medium. Thread

or leptothrix forms are frequent, measuring 20μ to 30μ in length or longer. The coccal forms chiefly present are slightly larger than those upon agar, being 1·164μ by ·756μ in breadth as against 1·066μ by ·708μ.

The vigour of growth of the organism upon this medium has already been referred to under cultural characteristics.

Rabbit Agar in 48 hours gives typical appearance. Occasionally, on freshly prepared moist rabbit agar, inoculated directly from gland of human being, marked thread forms (*see* Fig. 7) of irregular breadth result, or sometimes involuted twisted threads, not unlike the leptothrix forms seen in an actively growing culture of Bacillus Anthracis. This property of thread formation has been observed in a number of various media, both moist and partly dried out, and is therefore improbably connected with moisture available to growth of the bacilli. The thread formation has not been noticed to be restricted to individual colonies.

Ox Serum gives typical discrete organisms, small, deeply staining, but not differentiating in 48 hours.

In 72 hours there appears a slight tendency to degeneration, a number of the small coccal forms staining but faintly.

In 96 hours a distinct tendency to elongation is noticeable, while the whole field is stained but feebly.

At 144 hours the organism generally has lost all power of staining, with exception of a few typical young forms which are deeply colored and normal in appearance.

At 240 hours at 37° C. the degenerative appearance of those polymorphic forms so characteristic of some other media is noticeable. With a lengthy sojourn on the medium at laboratory temperatures, the organism appears to regain to some extent its power of staining,

and at the end of a month is seen fairly stained in the small coccal state.

At the expiration of 56 days some well-stained ovoid organisms stand out on a background of broken-down degenerate bacillary forms.

The organism on ox serum retains its vitality and typical appearance to some extent up to 100 days. Its virulence at this age proves absence of real degeneration, and when retransplanted on to ox serum it rapidly assumes its usual small vigorous-looking and deeply staining appearance.

Beef Bouillon.—The characteristic appearance in 48 hours in this fluid medium is chain formation of from 4 to 6 elements, or more. These elements show bipolarity, and more nearly approach the typical organism isolated from tissues, being, however, slightly smaller.

After 240 hours' incubation involution forms appear occasionally amongst the elements composing the filaments which tend to zooglea formation ; but this feature is not well marked. Deeply stained chains can frequently be seen superimposed upon chains of pale, ghost-like elements, evidently degenerate, and contrasting markedly with the chains of younger growths.

After the expiration of 30 days the degeneration of the organism is more advanced. Elements swollen and irregular in shape coalesce, forming a network of chains faintly stained, and entangling other chains of well-stained elements.

The bacillus in this medium retains its vitality up to 100 days, though the microscopic appearances show excessive bacillary degeneration.

Glycerine Bouillon.—The organisms are larger and plumper in this medium than in plain bouillon, with a tendency to bipolarity. Clusters occur. Chain formation occurs (*vide* Fig. 15). Evidences of degeneration

occur at 72 hours, when certain elements of a chain lose their staining power, while other bacilli of the same chain remain prominently colored. The terminal organism of a chain is frequently seen bloated and involuted. Decolorised chains of elements are frequent at 72 hours. After an incubative period of 96 hours involution forms are more frequent, occurring sometimes in the middle of a chain ; signs of degeneration at this advanced age, however, are not so pronounced as in plain bouillon.

In 240 hours at 37° C. the affinity of the elements of the chain for the stain appears almost lost. Masses of deeply stained material, however, can be seen adhering to these almost colorless networks of chains, giving an appearance to the field of large, rounded, or irregular masses of deeply stained material, connected by a faint tangle of colorless bacilli, the elements of which are slightly segmented.

After a further period of 20 days' incubation at normal temperatures the bacilli appear to regain their staining properties to some extent, so that the field shows a number of well-defined coccal forms ; such forms, however, are small and show no bipolarity. After 50 days at normal temperatures a tendency to elongation is noticeable. Leptothrix forms exist, and some involution shapes appear. The elongated elements become bulbous or pear-shaped. These appearances are increased after 75 days (*see* Fig. 16), and after the lapse of this time the bacillus can be recovered with ease by retransplantation into glycerine bouillon, in which many thread forms can be seen (*see* Fig. 17). The occurrence of these thread forms in liquid media is noticeable, as the suggestion has been made that such forms are directly connected with the dryness which ensues with the lapse of time on solid media.

Glucose Bouillon.—Chain formation is marked, the elements appearing smaller than in other liquid media. After 10 days appearances of degeneration are marked; occasionally, small, deeply stained, single coccal forms are observable. In 50 days the large irregular masses noticed in beef bouillon are present in great numbers, and faintly stained chains of organisms with occasional elements more deeply stained here and there. The appearances of these masses often suggest that they are composed of a close agglutination of degenerated bacillary bodies, as frequent microbic forms can be detected, forming apparently part of the mass.

Salt Bouillon (2·5 per cent. NaCl).—A frequent point of remark in this medium is the clear-cut squareness of the organism, a chain of elements with sharp transverse divisions frequently resulting, recalling somewhat the appearances of the Bacillus Anthracis. Faint transverse striation shows a disposition to differential staining. After 30 days in 5 per cent. salt-ghee-bouillon at normal temperature, the organism shows but slight sign of degeneration and but little tendency to involution forms; but when incubated at 37° C. in tubes containing small quantities of the medium the growth is much slower, and degenerative changes and involution forms become rapidly well marked.

On Rat Bouillon.—The growth is rapid and copious (as remarked under cultural observations), the microscopic appearances are those of beef bouillon, with better-filled fields.

In connection with the subject of the characteristics of growths at varying ages and under varying conditions of culture, it should be noticed that growths which, after lengthened periods of incubation, show upon examination organisms advanced in apparent degeneration are still able upon transference to fresh

media to produce vigorous cultures within the usual period.

Thus reference to the accompanying micrographs will show the vigorous growth of the Bacillus Pestis Bubonicæ produced by a single subculture from the old and degenerate organisms (*see* Figs. 12 to 17).

It will be also observable that prolonged culture upon a medium may result when retransplanted in a marked divergence from the shape of the organism as originally sown. Thus, in the above case of glycerine bouillon, the young growth as originally obtained from this medium, and the young growth as obtained by retransplantation from a 75-day culture, show distinct differences, the power of chain formation being apparently lost and giving place to a thread or leptothrix formation, besides other points of minor difference. In the case of beef bouillon this power of chain formation is still retained, however.

Another point of considerable importance in observing the morphology of this organism is the extreme difference observable in the bacilli growing upon the top of the nutrient slope (*vide* Fig. 18), and those taken from the depth of the same tube (*vide* Fig. 19), where the medium is thickest. The accompanying photomicrograph illustrates this fact, which is connected probably with the amount of moisture available to the bacterial growth.

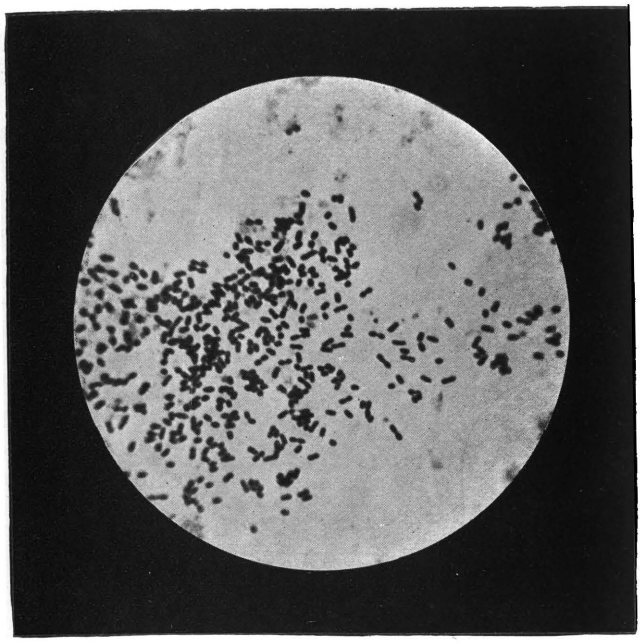


FIG. 12.
ORIGINAL GLUCOSE-AGAR GROWTH.

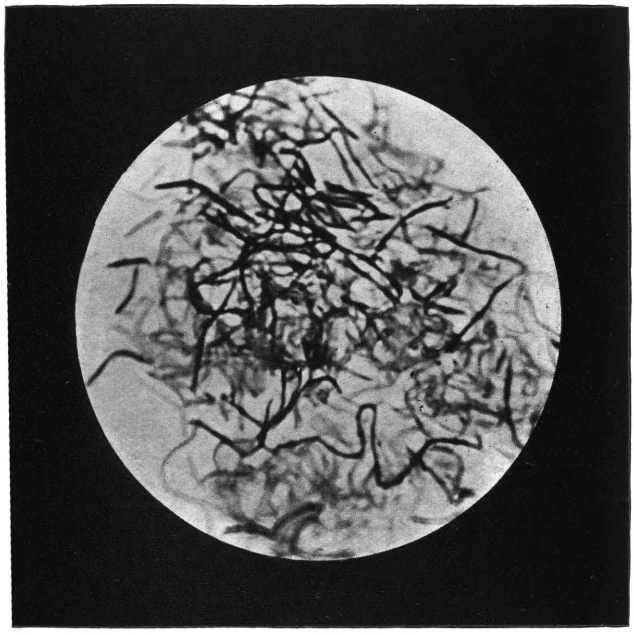


FIG. 13.
50-DAY GLUCOSE-AGAR GROWTH.

Face p. 26 (a).

SECTION IV.

VITALITY AND VIRULENCE OF BACILLUS PESTIS BUBONICÆ.

In endeavouring to estimate the period during which the Plague organism retains its vitality under the conditions of normal laboratory temperatures, media, etc., it was found that after 50 days' culture in bouillon with 2·5 per cent. NaCl the organism was incapable of further growth when retransplanted upon other media.

After the lapse of 75 days the sowings from glucose agar and glucose bouillon also proved to be sterile when inoculated upon other fresh media.

In the estimation of the virulence of these growths it was found that after 100 days' culture glycerine agar, ox serum, salt agar, glycerine bouillon, plain bouillon proved fatal to guinea-pigs inoculated with them, while agar at this period had lost its virulence.

Reference to the following table of relative virulence will show the various periods of incubation, weights of animals and quantity of infective material being rendered as uniform as possible.

In the endeavour to estimate the degrees of difference existing in organisms isolated from man and the lower animals, no great points of variance were observable.

Mere sojourn in the animal system does not appear, however, to induce any pronounced degree of attenuation of virulence, as the following instance tends to show. A rat resisted inoculation with a virulent growth for

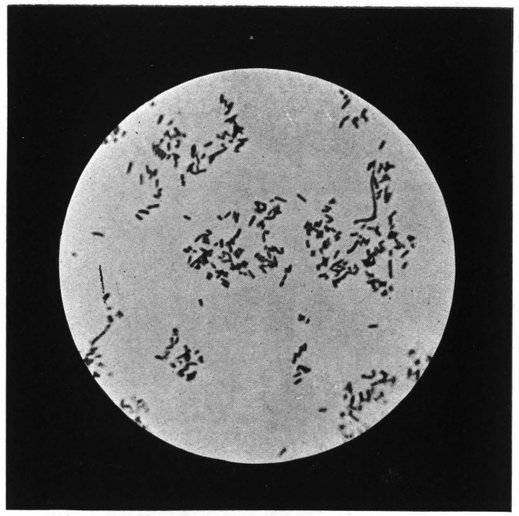


FIG. 14.
RETRANSPLANTATION FROM 13.

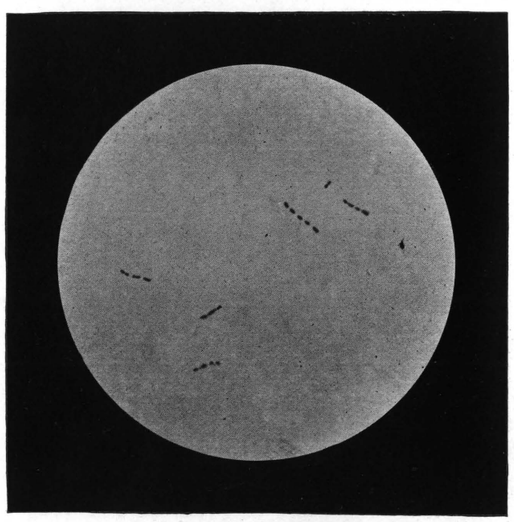


FIG. 15.
ORIGINAL GLYCERINE-BOUILLON GROWTH.
Face p. 26 (b).

FIG. 16.
75-DAY GLYCERINE-BOUILLON.

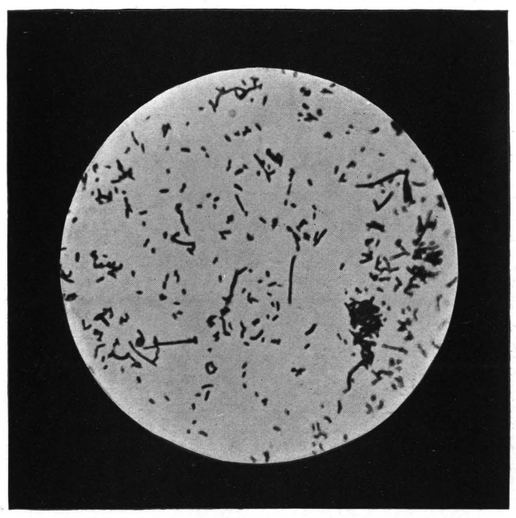


FIG. 17.
RETRANSPLANTATION FROM 16.
Face p. 26 (c).

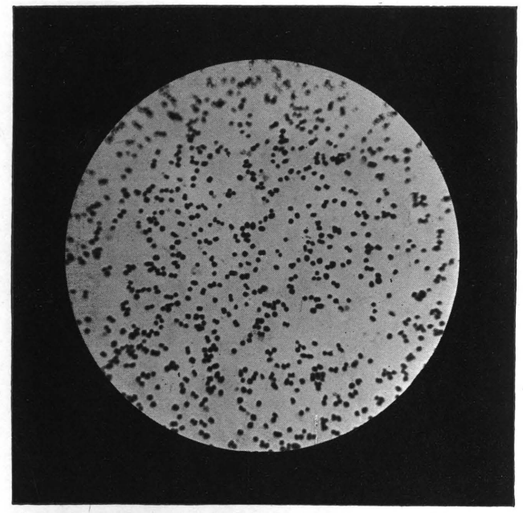


FIG. 18.
PLAGUE ORGANISM GROWING AT TOP OF AGAR SLOPE.

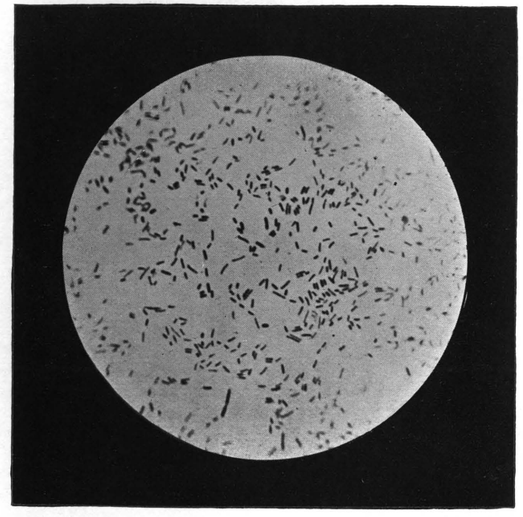


FIG. 19.
SAME ORGANISM GROWING AT LOWER END OF SAME SLOPE.
Face p. 26 (d).

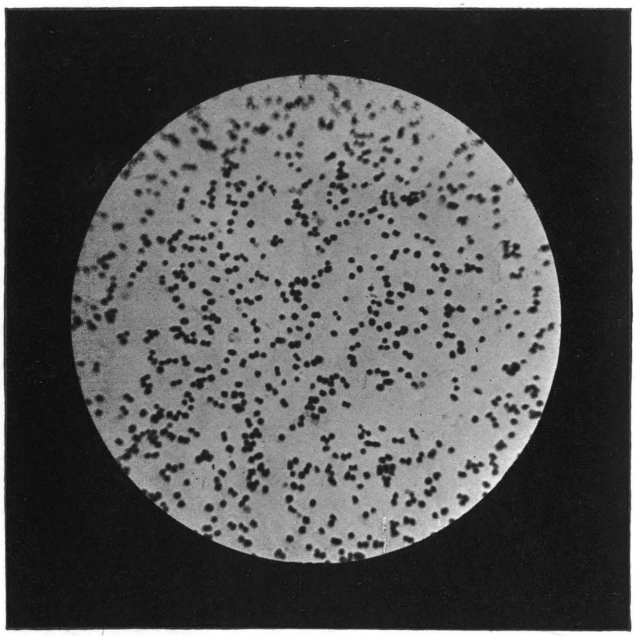


FIG. 18.
PLAGUE ORGANISM GROWING AT TOP OF AGAR SLOPE.

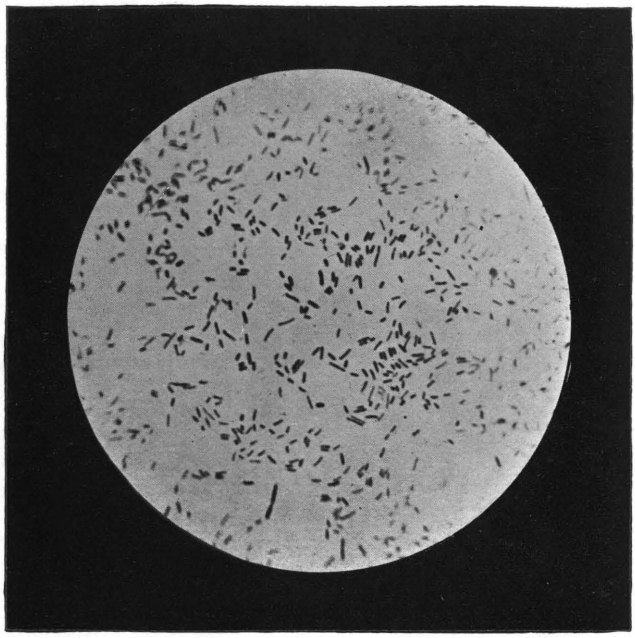


FIG. 19.
SAME ORGANISM GROWING AT LOWER END OF SAME SLOPE.
Face p. 26 (d).

23 days, and at the expiration of that time, while still in vigorous health, was killed and examined. No abnormality was observed to exist in the cadaver beyond the existence in the groin of a hard, enlarged gland, non-hæmorrhagic, and without peri-adenitis. Upon section it was found to contain a drop of thick pus, in which but few organisms could be detected.

VIRULENCE OF BACILLUS PESTIS AFTER LAPSE OF 100 DAYS WITHOUT RETRANSPLANTATION.

Medium.	*Dosage.*	*Date.*	*Result.*	*Duration.*
Agar	1 loopful	June 4	Recovered after temporary illness.	
Glycerine agar	,,	,,	Died June 11	158 hours.
Ox Serum ..	,,	,,	Died June 13	205 hours.
Salt agar ..	,,	,,	Died June 14	230 hours.
Glucose agar..	,,	,,	Survived, practically no illness.	
Salt bouillon..	1 c.c. after agitation	,,	Survived after practically no illness.	
Glyc. bouillon	,,	,,	Died June 9	113 hours.
Glucose bouillon	,,	,,	Recovered after mild illness.	
Bouillon ..	,,	,,	Died June 16	277 hours.
Control ..	Scratch in thigh, 1 loopful B. Bouillon	,,	Died June 11	158 hours.
Control ..	,,	,,	Died June 16	283 hours.
Control ..	,,	,,	Died June 9	118 hours.

Average Duration—old cultures—196·6 hours.
Average Duration—recent cultures—186·3 hours.

Upon cultivation, however, a typical growth resulted which proved extremely virulent for guinea-pigs. How long the organism would have maintained its vitality in its live environment is, of course, conjectural; the fact, however, remains that the animal resisted the infection, and, while still harbouring the virulent organism in a latent condition, remained itself in perfect health,

and capable under accidental conditions of spreading the infection.

Source of type of organism would seem to have little or no influence upon the question of its virulence, and reference to the table above will show that age within certain limits is also negligible in this connection.

The influence of burial upon the vitality and virulence of the bacillus is a subject of much importance from the point of view of the safe and efficient disposal of the bodies of persons succumbing to the disease. With a view of showing the length of survival of the Plague germ under conditions of ordinary interment, several experiments were undertaken. Two guinea-pigs were infected with virulent human Plague. Upon decease the body of one of these rodents was enclosed in a box made of half-inch deal, and the other cadaver was loosely wrapped in a piece of linen. These bodies were buried in a grave 5 feet deep in soil adjoining a burial-ground. The nature of the soil was sandy, with a substratum of fine gravel. After an interval of 108 days these bodies were exhumed and examined. The box was in good preservation, the wood being slightly damp. The decomposition of the cadaver was advanced, the limbs being detachable with a slight pull, while the viscera were found to be almost diffluent and recognisable with difficulty.

Two guinea-pigs were inoculated subcutaneously with an emulsion taken from viscera (probably the spleen). No illness resulted, and plates inoculated from same material showed no suspicious growths.

In the case of the guinea-pig buried in the cloth, the appearances of decomposition were very similar. Two healthy guinea-pigs inoculated with emulsion from spleen remained healthy ; but in plates made from the same an organism was isolated, somewhat resembling

Bacillus Pestis culturally and microscopically. This organism proved, however, to be actively motile and non-pathogenic, even in large doses.

A further opportunity arose for elucidating the question of the survival of the Plague bacillus in the case of a human cadaver, which it was found necessary to remove from the original place of interment after an interval of 190 days. In this case also the endeavour to isolate the organism from the viscera was unsuccessful, both by live inoculations and also plate cultures. It is, therefore, reasonable to infer that either the Plague bacillus loses its vitality after a period of a few months, or that it loses its morphology and virulence to such a degree as to become unrecognisable.

Further experiments were undertaken with the intention of showing the length of survival of the organism in soil.

A series of deep jars were filled with ordinary sifted loam, into the depth of which glass tubes of large calibre were plunged. Some of these jars were sterilised under a pressure of 1½ atmospheres for 3 hours on three successive days, while in others the contained earth was inoculated in an unsterilised condition.

The depth of the column of earth was approximately 25 centimetres. Ten cubic centimetres of a culture—the virulence of which was controlled by eight rodents—were introduced through the tubes into the column of soil at varying depths, or simply poured in some cases upon the surface of the soil. These jars were carefully closed with sterile paper caps, and remained at laboratory conditions. The soil contained sufficient moisture to admit of cohesion upon being squeezed in the hand.

At the expiration of varying periods small portions of the soil were withdrawn from the sterilised and unsterilised jars, both superficially and at a depth of

1 centimetre, and also from the depth of the columns of earth. These small samples of earth were ground down with sterile bouillon, and the supernatant fluid was inoculated subcutaneously into healthy guinea-pigs.

The results were as follows :—At the expiration of eight days after infection of the jars the first series of animals were inoculated, with the result that the bacillus was recoverable from all the jars, both from the surface and depth of the earth.

After the expiration of a month the effort was repeated, and it was noticed that no ill effects followed the inoculation of material from either surface or depth of the *sterilised* jars. In the case of the guinea-pigs inoculated, both from the surface and depth of the *unsterilised* jars, a lengthy illness followed the inoculation, eventuating in one case (that of the animal inoculated with soil from the surface) in recovery, but ending fatally in the case of the guinea-pig inoculated from the depth of the unsterilised earth. In this latter instance the illness was prolonged, and an abscess resulted at the point of the operation, and commenced to suppurate a week after inoculation.

The animal died after an illness of 20 days, and from the pus of the abscess the organism of Plague was isolated from amongst several other bacteria. This proved pathogenic to other guinea-pigs within nine days, the *post-mortem* and cultural appearances confirming the cause of death.

After a period of five weeks a further series of inoculations were made, with an entirely negative result.

It is, therefore, reasonable to conclude that the bacillus is incapable of extended life in soil either on its surface or at a depth of about 1 foot.

Association in earth with other soil organism would not appear to act inimically to the life of the bacillus,

which apparently fails to maintain its existence by reason of its unfavourable environment.

This fact would tend to be confirmed by the results about to be detailed in connection with the vitality of the organism in granaries, etc., and helps, therefore, rather to favour the theory that the bacillus maintains its existence in the bodies of living animals, probably rodents. That it is capable of existence beyond a normal period in the bodies of living animals is shown by the instance of the guinea-pig above mentioned, and also in that of the rat which was inoculated, and killed when healthy 23 days afterwards, from whose bodies the Bacillus Pestis Bubonicæ was isolated.

Color. — An endeavour was made to ascertain the influence of colored light upon the vitality of the organism. For this purpose a series of Petrie dishes were inoculated, such dishes being covered with glass squares of varying colors. It was found, however, that no difference existed in the vigour of the growths in the green, blue, ruby, and yellow dishes, the growths in which were as well developed as those in the control colorless dishes, and appeared to maintain their vitality as long.

Sunlight.—The bactericidal effect of direct sunlight was approximately ascertained on growths exposed on agar in Petrie plates. Such exposure was made at midday, the time of the year being mid-winter, the light being very actinic, maximum shade thermometer 78° F., barometer 28·43, with dry bulb 62°, wet bulb 56°. Dishes were tilted to the direct sun rays. A portion of each dish was covered with a strip of opaque paper, affording shade for a streak of normal growth, which acted as a control to the rest of the dish.

An active bouillon culture was used, and the dishes were exposed to the light directly after inoculation.

Loss of vitality began to be observed in gradations after 10 minutes' exposure, 5 minutes giving no perceptible result. Growths also resulted on the plates exposed for 15, 20, 25, and 30 minutes, but a distinct inhibition of growth was noticed after 40 minutes' exposure, the area shaded by the strip of opaque paper showing, after the usual period of incubation, a well-defined band of growth, contrasting with the feeble development of the rest of the dish.

An exposure of 60 minutes' duration to conditions as above proved sufficient in all cases to completely inhibit the growth on that part of the agar unprotected by shade.

Vitality.—The question of the vitality and virulence of this organism under natural conditions being one of the first importance, an extensive series of experiments was undertaken at your suggestion in the endeavour to elucidate this point.

For this purpose 10 small brick houses or cubicles were constructed, each being furnished with cement floors and a window and door, affording no lodgment or chance of escape to the rodents confined in them. The cubic capacity of these boxes was 264 feet each. Each box was ventilated by four air bricks, covered on the inside with wire gauze of sufficient fineness of mesh to be fly and vermin proof. They were numbered 1 to 10. Arrangements were made for excluding the light from certain of these compartments, while to others the light had free access. In these boxes were placed bags of grain of varying sorts, such as oats, bran, flour, mealies, and mealie meal, besides trusses of hay, etc., and an endeavour was made to simulate as far as possible the conditions obtaining in a granary or produce store.

These boxes were surrounded by a high fence of

corrugated iron, carefully constructed, while the whole compound thus formed was covered or roofed in with inch wire netting, to obviate the possibility of any rodents, which might by any accident escape from the boxes, leaving the premises and so spreading the infection.

It was found that the rats were capable of maintaining an absolutely healthy condition when such boxes remained uninfected. Thus, for many months one of the above boxes has served as a receptacle for healthy reserve or control rats, which have lived in large numbers and propagated under the above conditions.

The rodents—of which some hundreds were obtained both from town dwellings and also from the wharves and shipping at the port—were the usual black and brown rats (Mus rattus and Mus decumanus).

Each box was infected in the first place by the introduction of from 8 to 10 rats, which immediately prior to introduction were inoculated subcutaneously with a platinum loopful of virulent bouillon culture, or a hypodermic injection of the same. The technique of such inoculations was as follows :—The tail of a healthy rat was seized with a large pair of ratchet forceps known as " snake-tongs," used in the laboratory for the capture and manipulation of snakes in venom collection, and while held pendant by its tail the skin of the back of the neck was seized between the thumb and finger of one hand, while the other hand extended the body of the rat for the injection to be made. Some hundreds of rats were inoculated in this manner without serious accident. Such operations, however, were never devoid of exciting incident.

The endeavour of the above series of experiments was to show the length of time such repositories would

maintain their infectivity, whether conditions of light or darkness favoured the maintenance of the same, the efficiency of various processes of disinfection, and the infectivity from animal to animal.

It was hoped that the elucidation of such points would lead to an improved and more economical method of dealing with premises in which the contagium was known to exist.

The mortality occurring in these boxes was accurately observed daily from the time of the introduction of infected rats, such observations being either made through the glazed roof of each house, or, where necessary, by removal of the contained sacks, etc., after all possible precautions had been taken in such removal to avoid personal infection by dust, etc., in plugging of the nostrils.

When all the rats in one box had succumbed to the disease the boxes were closed for varying intervals, and at the expiration of such times fresh healthy rats were introduced into the boxes, and the infectivity or otherwise of such box decided by a lengthy observation of the occupants. A careful *post mortem* confirmed the cause of death in each case.

This method of judging as to the retention of vitality in the box was found to be reliable, as it was repeatedly observed that healthy rats introduced into a box in which the infection was comparatively recent rapidly succumbed.

Reference to Appendix B will show the details of this experimental work. In the present place a summary of these results is shown as follows :—

After the lapse of one month from the date of death of the last rat it was found that healthy rats introduced into the box contracted the disease ; this experiment was therefore positive at the expiration of 30 days.

After two months' lapse rats remained healthy when

introduced into the previously infected boxes, and the same negative result was found to attend those boxes which were shut up for more lengthy periods (up to four months). This experiment, extending over some six months, was conducted several times with different boxes, the rats so reintroduced remaining healthy. The result may therefore be considered conclusive, and it may be assumed that the infection of grain stores and other similar premises does not persist—in the absence of living rodents—over a period approximately of two months.

Influence of light on retention of vitality was practically negligible, and the exclusion of light did not tend to prolong the vitality of the organism (*vide* Appendix).

MEASURES FOR DISINFECTION OF BOXES.

Boxes which had been recently subjected to a thorough infection with many infected rats were chosen for the purposes of this experiment. It must be remembered in interpreting the results obtained that such boxes contained a number of small bags of grain, meal, etc., and some trusses of tightly baled hay, into which the rats had burrowed freely, presumably infecting the interior of such sacks and bales. These conditions, however, are such as obtain in general grain and produce stores, to which premises such results would seem applicable if similar methods of disinfection were adopted.

Sulphur Dioxide (5.3 per cent.).—The interior of the infected box and its contents was slightly damped with a fine spray of water before the SO_2 was generated. Sulphur was used in the proportion of 1 lb. to 200 cubic feet, and was consumed entirely by mixing into a paste with methylated spirit before ignition. The box was closed for 24 hours during the process, the

doors, window, and ventilators being carefully pasted over to prevent escape of SO_2 or access of fresh air. At the expiration of this time it was at once restocked with 6 healthy rats, which died of typical Plague within a few days (*vide* Appendix).

This experiment was repeated with a similar result in another instance.

Formic Aldehyde was generated in an infected box, the cubical contents of which were 264 feet. Five tabloids of paraform, an equivalent of 12 grammes of commercial formalin, were volatilised in this space by the Alformant lamp (the strength recommended by the manufacturers being 10 tabloids to 1,000 cubic feet). After complete closure of the box for 24 hours it was restocked with healthy rats, which rapidly succumbed to typical Plague.

It may therefore be safely assumed that the ordinary measures of disinfection with sulphur dioxide and formaldehyde are not efficient, even when in excess, in dealing with the conditions of contagion which are likely to obtain in granaries and other similar buildings where actual surface contact with the gaseous disinfecting agent is impossible or difficult.

Regarding the possibility of the direct transference of the infection from rat to rat, inconclusive evidence only was forthcoming, and the inquiry along this line was not very extended.

It was obvious, however, that the infection is capable of transference to the rat in the absence of other rats either sick or dead from the disease, and in the absence, as far as the closest scrutiny could show, of fleas and other vermin. When dead rats were allowed to remain in the boxes in which they had died, it was a matter of frequent observation that the rats surviving longest had consumed the bodies in whole or part of those

animals succumbing before them. This is probably a frequent, but cannot be a constant, factor in the spread of the disease from rat to rat. The agency of vermin in the transference of the disease from one rat to another must, however, be admitted as possible.

Time did not permit of exhaustive experiments in this direction. Two cases of transference of vermin which were floated off the bodies of dead rats, and at once placed in the coats of healthy animals, proved negative. Very many of the bodies of dead and dying rats were closely examined, and showed a remarkable freedom from vermin. In many cases no parasites at all were discoverable; seldom Pediculi, and quite occasionally rat-fleas (apparently *Pulex Pallidus*).

It seems, therefore, more probable, in view of the constancy with which healthy rats succumb when introduced, that infection is generally spread from animal to animal by the ingestion or inhalation of particles of infected matter, dejecta, etc., rather than by infection by means of vermin. This view seems strengthened when the short vitality of the bacillus (as shown in the box experiments above) is compared with the length of time fleas and their larvæ are capable of maintaining their vitality under favourable circumstances.

At the same time, the evidence of the agency of fleas as transmitters of the disease cannot be overlooked, and such instances of outbreaks following the removal of floors of infected premises where no precautionary measures for previous disinfection, damping, etc., have been taken, tend to confirm the flea-borne theory, which is further strengthened by the frequency with which the primary specific lesions in man are localised in the system of glands draining the lower extremities.*

* This argument could with equal force be employed in support of infection through the agency of dust on an abraded skin.—E. H.

Transmissibility from animal to animal.—The importance of the question as to the possible agency of the lower animals in the spread of the infection suggested the advisability of confirming the observations recently made on this point in Hong Kong. With this object in view, a series of experiments was made on the different domestic animals stated to have been found susceptible to Plague. The possibility of the contagium remaining latent, or in an incubative stage in the system (of, for instance, the pig or domestic fowl), and at the expiration of a lengthy period unexpectedly asserting its pathogenic properties, would appear to be so important a question in the epidemiology of the disease as to warrant careful confirmation of previous findings on this point.

A corroboratory investigation, therefore, was conducted on the lines of the recent inquiry of Professor Simpson in Hong Kong, and I may say at once that such investigation has failed in every instance to induce the fatal form of the disease which attended the experiments in China, to the results of which great importance has not unnaturally been attached.

Repeated endeavours have been made to induce a fatal form of the disease by the ingestion of infected material, and by the inoculation of virulent cultures and blood, as well as by close contact with an animal in a highly infectious condition (*vide* Appendix C).

The failure of the above inquiry to confirm the findings of the Report on Plague in Hong Kong cannot be attributed to any attenuation or loss of pathogenicity in the strain of bacillus used—at least, as far as its virulence for the human being and rodent is concerned—as all such inoculation experiments were conducted with cultures isolated direct from the cadaver, or, in the case of ingestion experiments, by the use of diseased tissues themselves.

The failure to confirm the transmissibility of Plague to the lower animals in a fatal form—as laid down in the Report referred to—can therefore only be explicable upon the assumption that the strain of bacillus used in the experiments in Hong Kong was possessed of a wider range of pathogenic properties, which, while being equally virulent with the Natal organism for men and rats, was possessed of additional or increased pathogenicity for other of the lower animals. Recent studies in immunity would seem to bring such a theory within the bounds of possibility.

That so considerable racial immunity should exist special to South African herbivora, pigs, and birds seems untenable in consideration of the ordinary susceptibility of rats and of man himself.

Insufficiency of data can, however, fairly be urged against the Natal observations, though it will be seen on comparison that the disparity in numbers of cases between the Hong Kong and Natal experiments is not very great. Thus, while of 31 hens subjected to experiment in Hong Kong 9 died (29 per cent.), of the 20 hens treated in Natal none succumbed or were ill; and similarly, while of the 15 pigs experimented upon by Professor Simpson 86 per cent. or 13 died, of the 10 pigs similarly treated in Natal none died, and but 1 showed a transient illness, due to manipulation. Reference to Appendix II. will show details of treatment in all cases coming under observation, which comprised pigs, calves, and fowls.

Even were the direct connection of such animals with the maintenance of an epidemic proved, it would seem easy to over-estimate the significance of a fact, which would be one rather of academic interest than practical importance.

The ingestion of human tissues by the pig, ox, or

even rat, is a sufficiently remote contingency to be dismissed, while the actual inoculation from or consumption of the flesh of Plague-stricken animals by the domestic animals of the large towns in which the Plague is most endemic, would seem also negligible in consideration of its epidemiology.

The conclusion, therefore, seems warrantable that, so far as Natal is concerned at least, these animals are not to be looked upon as factors concerned either with the spread of the disease or with its periods of latency.

SECTION V.

BACTERIOLOGICAL DIFFICULTIES ENCOUNTERED IN COURSE OF THE OUTBREAK.

As mentioned previously, considerable difficulty was encountered throughout the continuance of the epidemic in making a diagnosis from the microscopical appearances available. In fact, so grave did this difficulty become, that, even when supported by the clinical history, it was found advisable in many cases to defer a definite pronouncement until the cultural characteristics and virulency of the organism in question had been observed. A dependence upon the indications of the microscope simply in several important instances could not but have resulted in an erroneous positive pronouncement, with all its serious attendant consequences.

This difficulty does not appear to have occurred to any extent during outbreaks of Plague elsewhere.

The thoroughness of the means employed by the Health Department for the detection of early cases of Plague, and for the routine *post-mortem* examination of all cases in which the cause of death was not clearly apparent, has doubtless frequently accounted for the observation of microbic forms nearly allied morphologically to the Bacillus Pestis. Attention has been drawn in Appendix A to the possibility of the occurrence of forms of organisms in the tissues of rats indistinguishable microscopically from Plague bacilli (Fig. 24), so

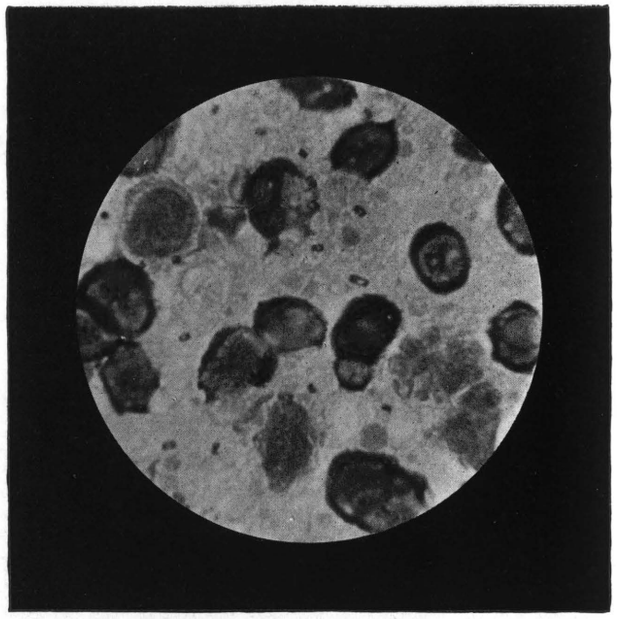


FIG. 20.

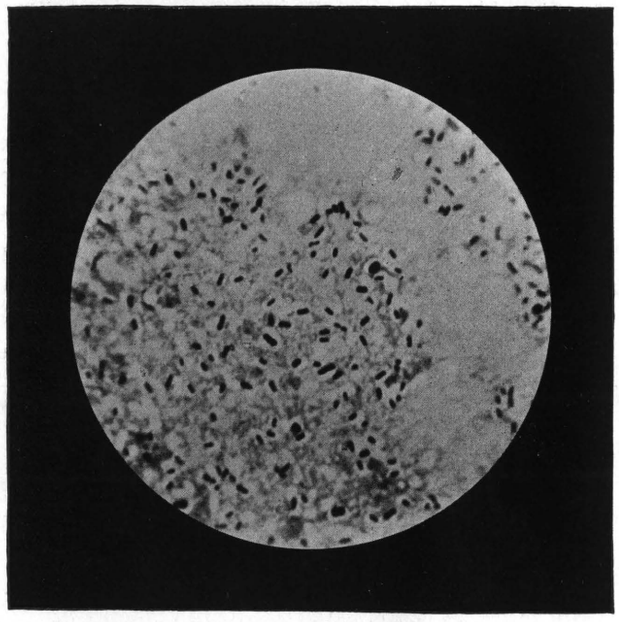


FIG. 21.

Face p. 43 *(a).*

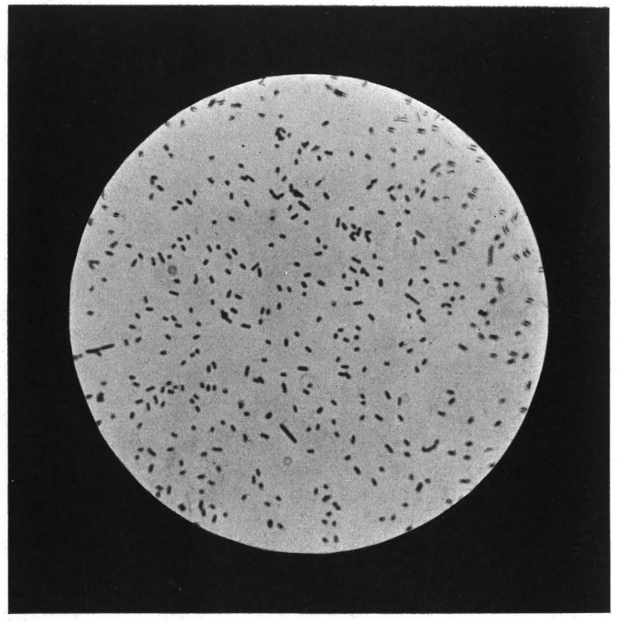


FIG. 22.

Face p. 43 *(b).*

that the same difficulty applies to the diagnosis of the disease in other than the human species.

A few instances of such cases may be quoted here, to exemplify the necessity of cultural observations being made to support bacterioscopic appearances.

Case No. 1.—The history of the case was as follows :— A Kaffir male was found dead in Pietermaritzburg during the prevalence of the epidemic. *Post-mortem* examination revealed pleuritic effusion and unilateral broncho-pneumonia. Portions of the internal organs in good preservation were forwarded at once to the Laboratory. Spleen smears showed an apparently typical Plague organism in pure culture (*see* Fig. 20), and upon this observation an affirmative diagnosis was at once made. Upon culture of the organism, however, points of difference became obvious, for while the bacillus showed bipolarity and was pathogenic for guinea-pigs, it produced also so rapid and diffuse a growth, and such atypical *post-mortem* appearances, as to lead to a re-decision as to the specific nature of the case (*see* Appendix A).

Case No. 2.—Tissues taken from a man were forwarded for examination without definite clinical history. The appearance of the organism in smears from the spleen where it appeared to exist in pure culture was highly suspicious (*see* Fig. 21). A guinea-pig was inoculated with an emulsion of organs, the bacillus proving pathogenic in 38 hours. Cultivations upon agar gave a rapid profuse growth, the organism tending to polar staining and appearing like a typical Plague cultivation on serum (*see* Fig. 22). Rapidity of growth and motility resulted in a negative opinion before a negative clinical history was received (*see* Appendix A).

In another case—that of a man taken suddenly ill upon premises in which previous cases had occurred—

the chief symptoms were referable to the lungs. Examination of the slightly blood-stained expectoration showed a highly suspicious organism apparently in pure culture, which, however, failed to produce death when injected into guinea-pigs and rats. This organism, therefore, was looked upon as being non-pathogenic, and proved to be so on more extended observation.

In another instance, a man was removed to the hospital in consequence of contracting typical Plague. Upon inquiry it was elicited that he was an assistant in charge of a poultry farm upon which the fowls, ducks, etc., had been dying in large numbers. Examination of tissues and blood from the bodies of these birds showed the existence of an organism with difficulty distinguishable from the Plague bacillus in appearance (*vide* Fig. 23), which proved upon culture to be the *Bacillus choleræ gallinarum*, pathogenic to guinea-pigs, but not to rats ; fowls readily succumbed to the organism, but not to control injections of virulent Plague cultures.

The above instances are examples of an often-recurring difficulty encountered throughout the continuance of the epidemic.

The fact that Plague is liable to be associated with diseases such as that just described, in which the pathogenic organisms are indistinguishable from those of Plague proper, should therefore be borne in mind. Outbreaks of Fowl Cholera, Swine Plague, etc., are thus liable to confusion with Bubonic Plague if reliance is placed solely upon microscopic appearances to the exclusion of other means of exact diagnosis.

Some of the early cultural appearances also of such organisms as Fowl Cholera simulate the growth of the Bacillus Pestis (*see* Fig. 1), but extended observations on pathogenicity, rapidity of growth, and other

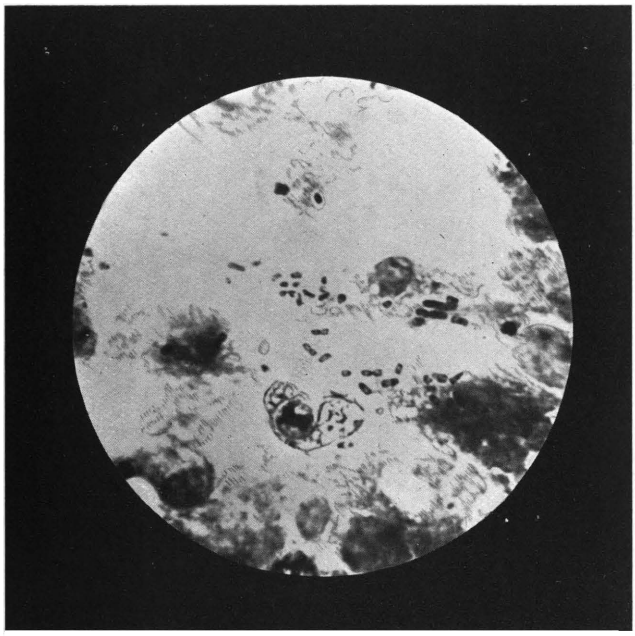
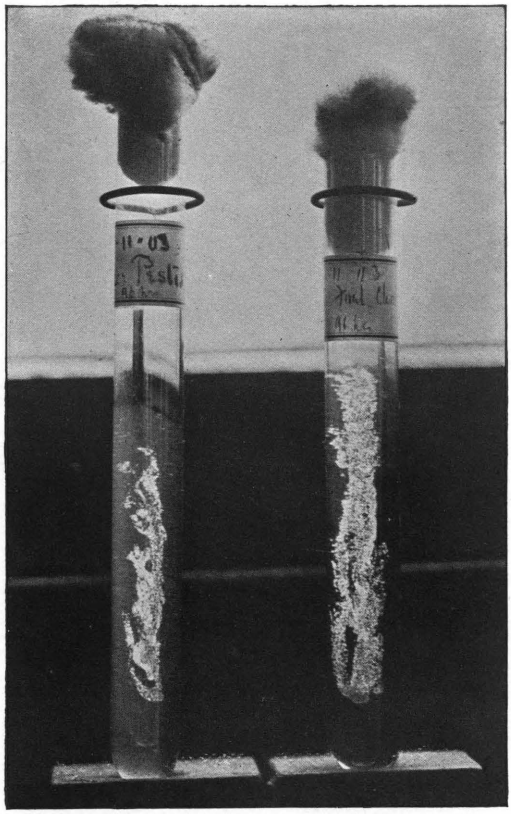


FIG. 23. *Face p.* 44.



a *b*

FIG. 1.

Face p. 44.

differences, cannot fail to afford means for exact determination.

The observations above embrace most of the important points in connection with the bacteriology of the disease. Consideration of time and other duties have prevented many of these points being dealt with in a more exhaustive manner.

I trust, however, that some of the above observations may not be without value in dealing with possible future outbreaks in our Colony and elsewhere of this universal scourge to mankind.

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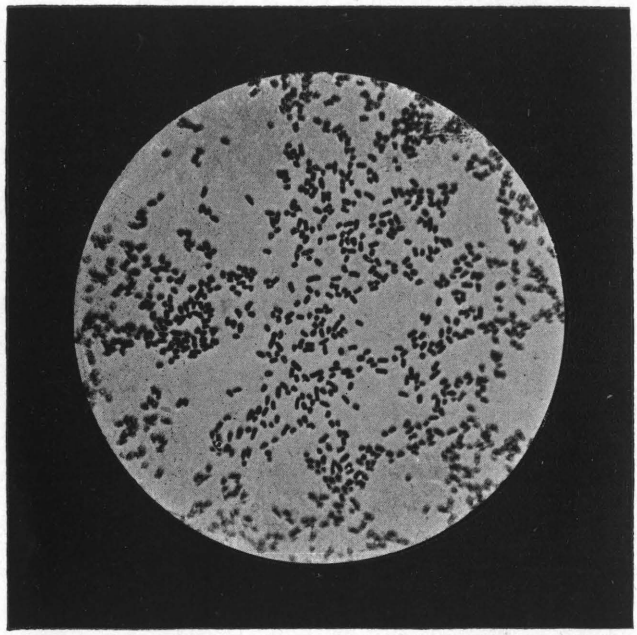


FIG. 24.

APPENDIX A.

I.

With reference to cultural appearances, Yersin mentions (and Tidswell of Sydney confirms the observation) that in many instances "small colourless and large cloudy colonies" are to be found growing side by side. Klein also, in the Centralblat for Bacteriology, July 10th, 1897, noticed the appearance of colonies contrasting markedly with their neighbours, being large, round or oval, consisting of longer or shorter straight or looped threads or bacilli, which he first regarded as contaminations, but subsequently considered such occurrence as a cultural characteristic of this organism. The same question has arisen in more than one instance in the present work.

In one instance—that of a culture made from a spleen of a rat within a few hours of its death from typical Plague—an appearance was found corresponding to the description above. In this instance, while the microscopic appearances of both growths were identical and typical (*see* Fig. 24), the cultural characteristics and pathogenicity as subsequently determined were widely divergent.

II.

Case I was that of a Kaffir male found dead. The *post-mortem* examination revealed some pleuritic effusion on one side and a condition of broncho-pneumonia. The spleen was friable and soft, and contained large numbers of bi-polar organisms. (*See* Fig. 20.)

Lung smears contained apparently the same organism. Agar cultures at 48 hours at 37° C. revealed a thick, raised, greyish shining growth, and microscopically a bacillus with rounded ends and with a trace of polarity (*see* Fig. 25.) In all probability some motility also existed.

This organism proved fatal to guinea-pigs in eight days, and the same organism was observable in the tissues at the seat of inocula-

tion (where an extensive tumefaction and necrosis existed) and in the liver in scanty numbers.

None were observed or were recovered from the spleen.

The liver cultures made from the infected guinea-pig were similar in all respects to the original human culture. (*See* Fig. 26.)

The organism did not produce stalactitic formation in ghee-bouillon, and this fact along with its slight motility, cultural appearances, and the lesions produced, led to a revision of the provisional positive diagiosis which was originally made.

Case 2 is that of a European whose clinical history and *post-mortem* appearances gave rise to little suspicion, but microscopical appearances in the spleen were somewhat similar to those of Plague. In this instance also the abundant growth of the particular bacillus in the spleen was probably *post mortem*, but the microscopical appearances were suspicious enough to warrant animal inoculation. (*See* Figs. 21 and 22.)

From subcutaneous inoculation of a few minims of the spleen pulp a guinea-pig succumbed in 38 hours, with an extensive phlegmon and gelatinous infiltration affecting the whole surface of the abdominal wall and extending into the limbs.

The exudate showed an organism in large numbers, varying from an ovoid form to a long straight bacillus. Bi-polarity of staining was present. Organisms of the same character were present in the liver and spleen, which were soft and congested, but not in the heart's blood.

The small intestines were slightly injected. No glandular enlargements were observed.

Cultures on agar incubated at 37° C. showed a profuse drab-coloured growth resembling cultures from the original spleen, which microscopically consisted of short, straight rods, with a tendency to polarity of staining and numerous threads. (*See* Fig. 27.)

This bacillus was found to be fairly actively motile, to curdle milk in 40 hours at 37° C., to be acid-producing in 20 hours at 37° C., to be non-liquefying and non-gas-producing and to present in anäerobic stab cultures in glycerine agar a curious ramified growth.

It was, moreover, found to be highly pathogenic for white rats and for guinea-pigs (death occurring in less than 48 hours), with an

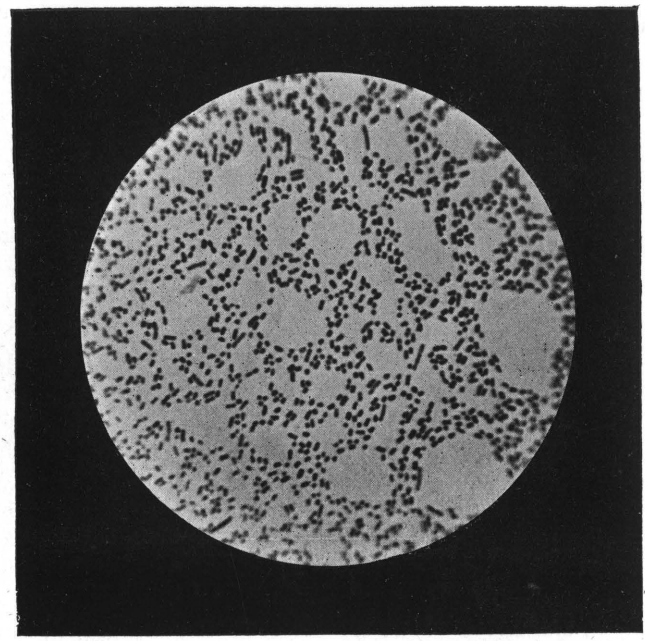


FIG. 25.

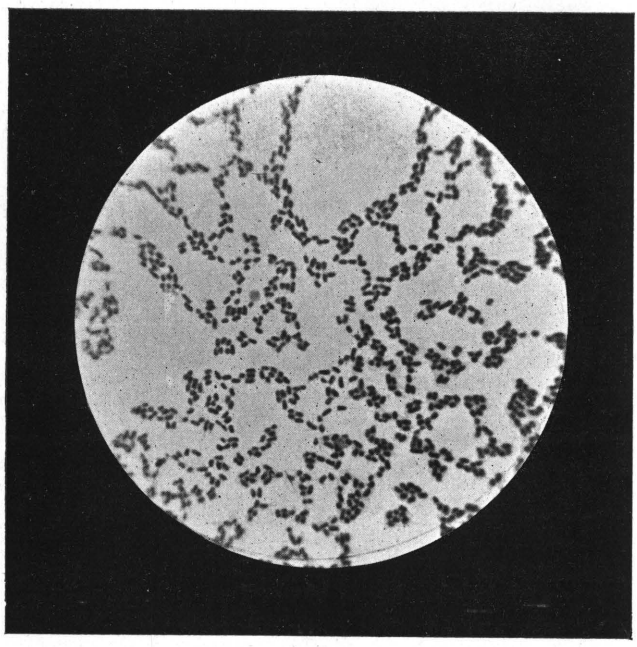


FIG. 26.

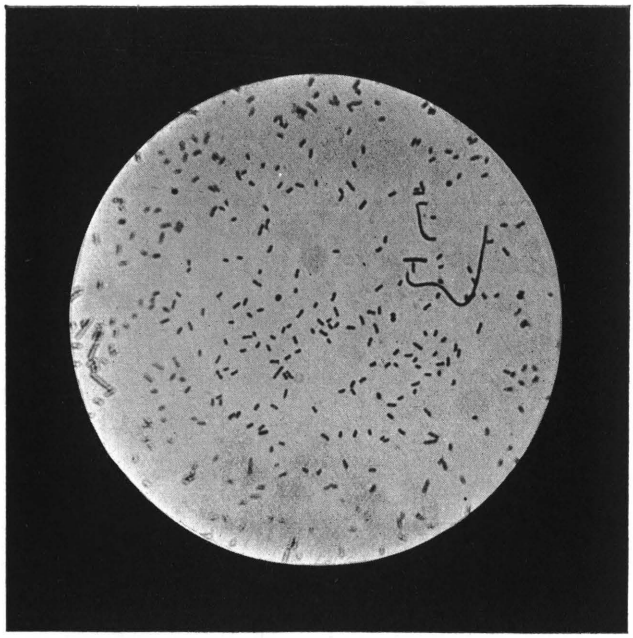


FIG. 27.

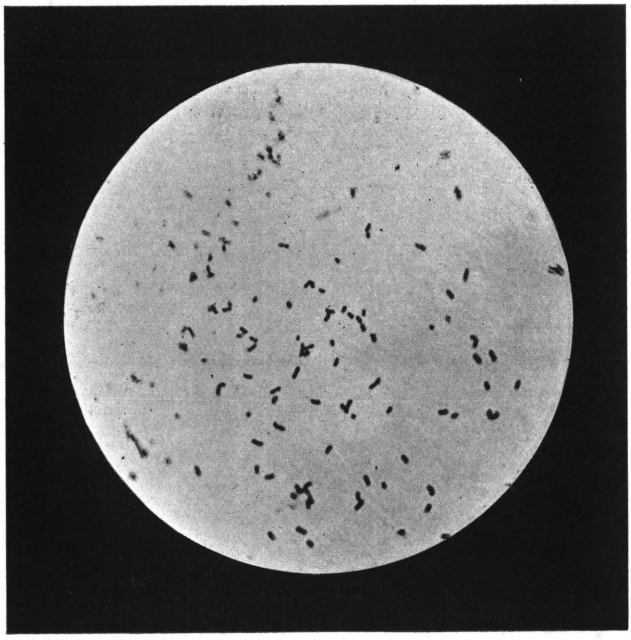


FIG. 28.

extensive phlegmon spreading from the point of inoculation.

It was not pathogenic for fowls.

The bacillus briefly described above was in more than one case the source of temporary delay in diagnosis, from its appearance in spleen smears and from its pathogenicity.

The haemorrhagic condition of tissues described above was, however, much more extensive and severe than the condition sometimes seen in inoculations with the Plague bacillus, and whereas in the latter case organisms are, as a rule, found in great numbers in the spleen, in the former the spleen and liver contain few organisms, and no enlargement of glands is noticed.

The organism isolated from fowls (referred to above—*vide* Fig. 23), which had caused some extensive mortality in ducks, fowls, geese, and pigeons (750 in number) on premises in which Plague had also occurred, was so alike in morphology (Fig 28) and cultural appearances (Fig. 1, *b*) to the Plague bacillus as to delay a definite pronouncement until differential points had been determined.

It was found, however, with this organism that the growth on agar appeared rather earlier at 37° C., that the growth is much less adherent to the medium, and can with ease be washed off by filling the tube with water (a phenomenon which does not occur with Bacillus pestis), that the organism is more slender, that stalactitic formation does not occur, that the pathogenicity is quickly lost by artificial culture, and, moreover, is widely divergent from the pathogenicity of Bacillus pestis. The latter was found to have no appreciable effect on fowls, although various strains were used and very large quantities administered, but to be constantly fatal in ordinary laboratory doses for rodents. The former was found to have a wider range of pathogenicity—namely, birds and rodents—and even to cause severe symptoms in pigs, but its virulence rapidly decreased when cultivated artificially.

APPENDIX B.

Granary Experiments.

I.

The conditions were very similar to those existing in an ordinary granary or grain store, the humidity probably being greater in the experimental granaries, water being constantly supplied and the doors kept closed.

Granary 1.—264 cubic feet. Well lighted. Infected by 8 inoculated rats; bodies left *in situ.*

On restocking four months after the last rat had died of Plague, the disease did no appear in the freshly-introduced rodents.

Granary 2.—264 cubic feet. Light excluded. Infected by 8 inoculated rats, and bodies left *in situ.*

Restocking four months after all were dead gave a negative result.

Granary 3.—264 cubic feet. Well lighted. Infected by 8 inoculated rats, and bodies left *in situ.*

On restocking two months after all were dead, the disease did not occur in the fresh rats.

Granary 4.—264 cubic feet. Light excluded. Infected with 8 inoculated rats and bodies not removed.

On restocking two months after, the disease did not appear in the fresh rats.

Granary 5.—264 cubic feet. Well lighted. Infected with 15 inoculated rats and the bodies left.

On restocking three months after, the disease did not re-appear in the fresh rats.

Granary 6.—264 cubic feet. Well lighted. Infected with 8 inoculated rats. At the time of inoculation, after careful handling and prolonged observation, only one flea was found (*P. pallidus*),

which was removed. On the decease of all the rats, the bodies were removed, and 8 healthy rats which had been under observation in a non-infected granary for a fortnight were turned in. At the expiration of eight days one of the fresh rats was found dead, and the examination yielded positive results, which were confirmed by cultural and re-inoculation methods.

All openings in the granary were then closed and pasted over, and the interior subjected to the action of SO^2, 5.3 per cent. for 24 hours, all rats having been removed before disinfection.

On restocking with 6 fresh rats (which had been under observation for a fortnight in a non-infected granary), 1 died from Plague in seven days (a finding confirmed by cultural and re-inoculation methods), and thereafter the other rats quickly succumbed.

The interior was then exposed to the action of Formic-aldehyde gas for 24 hours by means of an Alformant "A" lamp. All chinks were effectively closed. No smell of the vapour could be detected.

On restocking, the disease again appeared at the expiration of nine days, and the finding was confirmed in the usual way.

On the decease of all of these rats, the box was shut up for three months and then restocked. No disease occurred in the fresh rats, although under observation for a month.

Granary 7.—264 cubic feet. Well lighted. Infected with 8 inoculated rats and bodies left *in situ.*

On restocking at the expiration of three months, no disease occurred in the fresh rats.

Granary 8.—573 cubic feet. Well lighted. Infected by 8 inoculated rats, and bodies left *in situ.*

On restocking at the expiration of one month, one rat succumbed to Plague in 12 days. (The finding was confirmed by cultural and re-inoculation methods.) SO^2, 5.3 per cent. failed in this case also to disinfect the granary, as on restocking the disease reappeared in the fresh batch of rats.

Granary 9.—264 cubic feet. Light excluded. Infected with 8 inoculated rats, and bodies left *in situ.*

On restocking at the expiration of three months, no disease occurred in the fresh rats.

Granary 10.—Used as control box for keeping store of healthy rats. No epizootic occurred in this box, and the animals throve if kept well supplied with food, water, and bedding.

II.

A large number of *post-mortem* examinations of rats had necessarily to be undertaken for confirmatory purposes, and the most noticeable point was that certain lesions, such as enlargement and engorgement of spleen and liver, presence of glandular enlargements with periadenitis, either in axillae, groins, or mesentery, were more often absent than present. For purposes of comparison a number of healthy rats were examined, and measurements made of the spleen. Little difference was found to exist between the organs of these and of Plague-stricken rats.

Glandular enlargements were found to be rare, even in inoculated rats.

These observations are also borne out by notes on a large number of routine *post-mortem* examinations on rats during the course of the epidemic.

In two instances in subcutaneously-inoculated rats the principal seat of the disease was in the lungs, which were carnified and crowded with typical organisms (pure cultures being obtained), while the spleen and liver contained the bacilli in scanty numbers.

The *post-mortem* appearances noted in guinea-pigs vary considerably from those in rats.

The speckled spleen is generally not observed in those animals which have succumbed within three days of inoculation, as is generally the case when using young animals.

Local reaction, if inoculation is subcutaneous, is generally severe, and the nearest lymphatic glands usually show periadenitis with punctiform haemorrhages. A common feature is the presence of some amount of gelatinous infiltration of the subcutaneous tissues extending far beyond the point of inoculation.

Both lungs usually show patchy congestion.

White rats show similar lesions to brown and black rats.

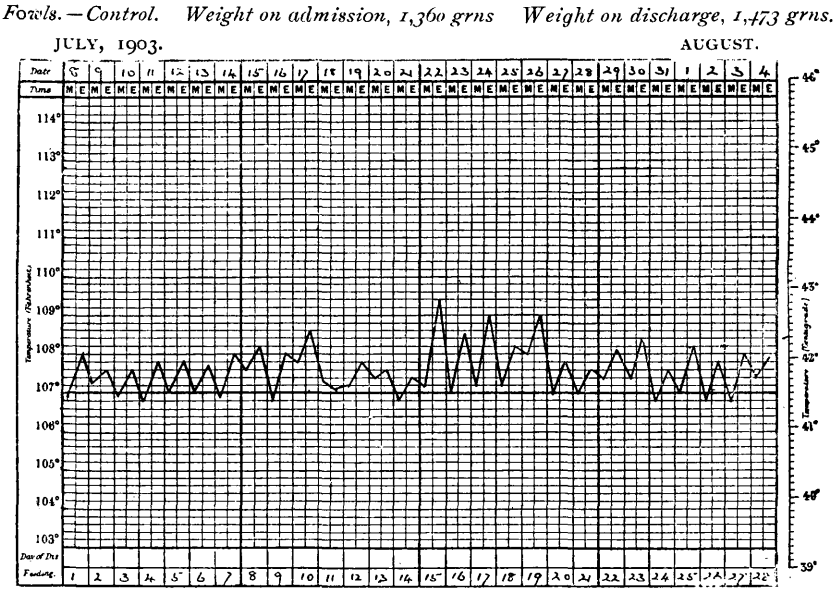
APPENDIX C.

Details of Animal Experiments.

I.—FOWLS.

The feeding and subsequent treatment of 20 fowls were carried out in the following manner:—

The organs of recently dead rats which had previously been inoculated with the Bacillus pestis (and placed in one of the



experimental granaries before described), were finely minced, mixed with rice grains, and placed in a large glass bowl. The bird was then gently held between the hands within reach of the bowl. In all cases the fowl ate greedily. The portions of the rat used for feeding were the spleen, liver, lungs, kidneys, heart, and buboes if any existed. The intestines were as a rule rejected. Each fowl generally ate approximately one-half the organs of a full-grown rat, although, as mentioned hereafter, in some case a fowl was given the whole of the organs. The beak was then wiped with a cloth moistened

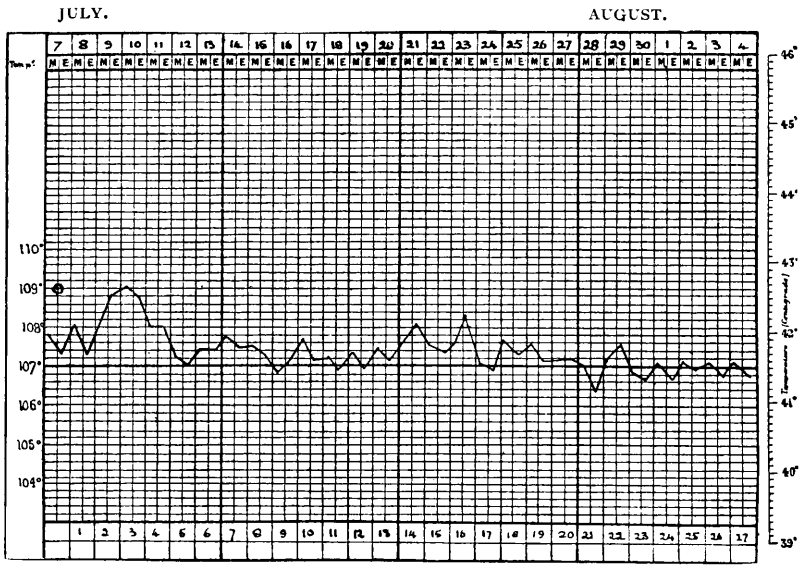
with disinfectant, and the bird placed in a zinc-lined cage standing over a large tray of disinfectant.

Control animals were inoculated, and the rat tissues were not used for feeding purposes unless found to contain large numbers of typical plague organisms.

Fowls 1 to 6 were fed and inoculated with the finely-minced organs of rats recently dead. The fluid part of the mixture contained

PESTIS.

Fowls. Case I.—Black Hen, grey neck. Weight on admission, 1,484 *grms.* ⊙ *Fed on July* 7, 1903, *with organs of Plague-stricken Rat. Excreta of this fowl innoculated on July* 24. *into Guinea-pig* G559. *Under observation until October* 12, 1903 *Hen-pecked by other birds. Weight on discharge,* 1,371 *grms.*



large numbers of organisms, *vide* photomicrograph of smear (Fig. 29), Section III.

Cultures on agar turned out to be typical both microscopically and macroscopically.

A control guinea-pig inoculated subcutaneously with three minims of the exudate at the same time died in three days, with a haemorrhagic condition of the groin glands and a finely mottled spleen that contained large numbers of typical organisms.

Cultures from the latter also proved in every way typical.

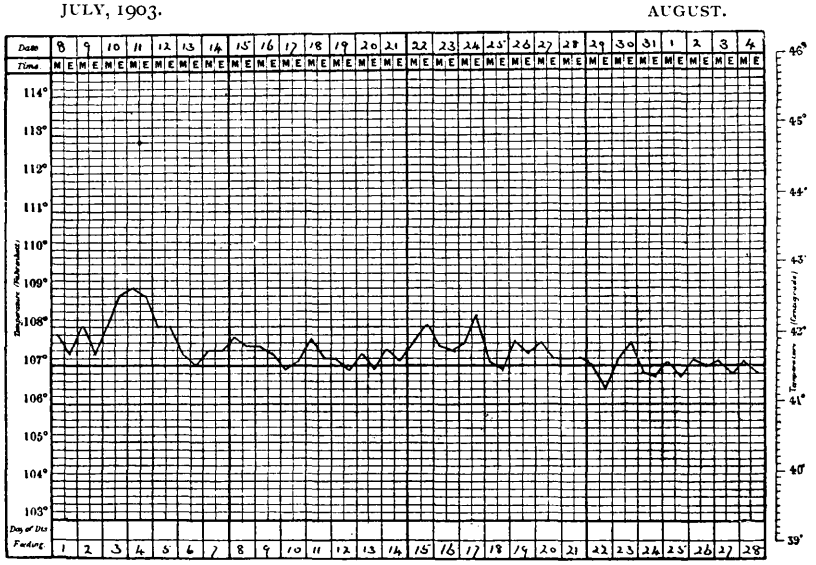
Fowl 1.—Under observation four days before the experiment.

Fed on July 7th with organs of recently dead rats, which four days previously had been inoculated with Bacillus pestis.

No signs of illness whatever were noticed, although the bird was under daily observation till October 12th, a period of 97 days. The comb remained bright pink in colour, the excreta constantly normal; the fowl gained weight.

PESTIS.

Fowls. Case II.—Black Hen grey neck. Weight on admission, 1.484 *grns. Fed, July* 7, 1903, *with organs of Plague-stricken Rat. Excreta of this fowl innoculated July* 24, *into Guinea-pig,* G559, *Weight on discharge.* 1.371 *grns. Under observation until October* 12. 1903. *N.B.—This bird was badly hen-pecked by other fowls in same cage.*



The excreta failed to induce Plague when introduced subcutaneously into guinea-pigs.

Fowl 2.—Under observation four days before the experiment. Fed on July 7th with organs of rats inoculated four days previously with Bacillus pestis.

No signs of illness observed at any time up to October 12th, when the bird passed out of observation. Comb remained bright pink in colour, and excreta were constantly normal, and when small quantities were injected into guinea-pigs only a local swelling resulted. The fowls gained weight, and laid eggs in captivity.

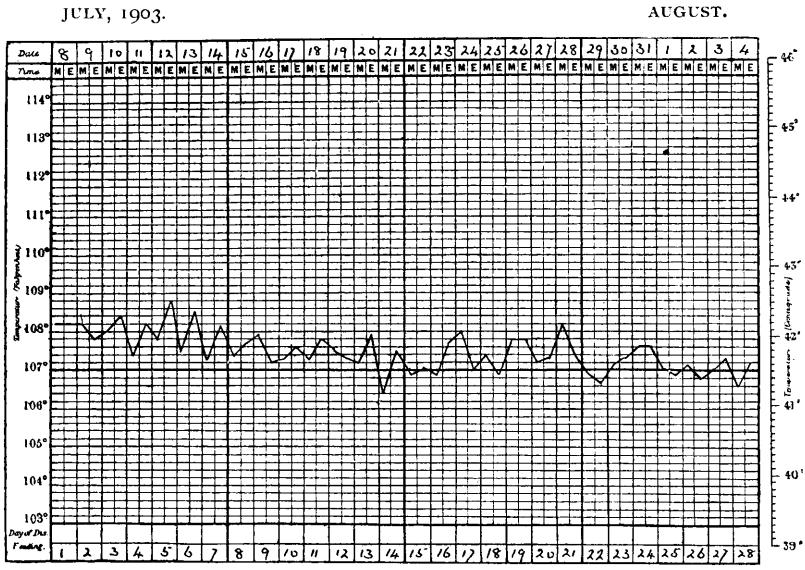
Fowl 3.—Fed on July 7th with organs of rats which were previously inoculated with Bacillus pestis.

No indisposition resulted, the comb remained bright pink, the excreta were constantly normal in appearance, and failed to induce plague when injected subcutaneously into guinea-pigs.

This fowl remained perfectly well, and gained in weight till it passed out of observation on October 12th.

PESTIS.

Fowls. Case III.—Grey Hen. Weight on admission, 2,447 *grns. Fed, July* 7, 1903' *with organs of Plague-stricken Rat. Excreta innoculated in Guinea-pig* 560 *on July* 24. *Weight on discharge* 1,360 *grns. Under observation until October* 12.



Fowl 4.—Inoculated subcutaneously in the flank with 2 c.c. emulsion of organs of rat recently dead which had previously been inoculated with Bacillus pestis. The emulsion contained large numbers of the organism.

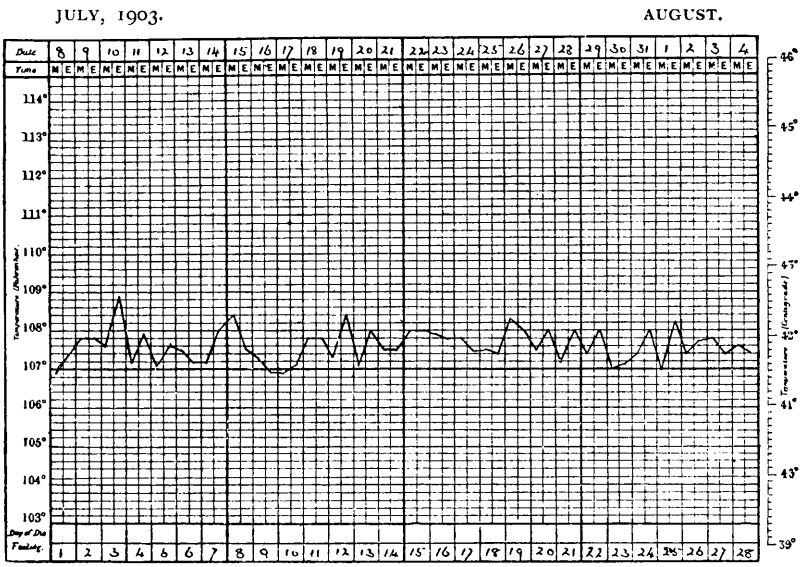
No signs of illness resulted up to October 4th, when observation ceased.

Fowl 5.—Inoculated sucutaneously in the flank with 2 c.c. of an emulsion of the liver and spleen of a rat recently dead of Plague.

The fowl was under observation for three months, but no indisposition was observed.

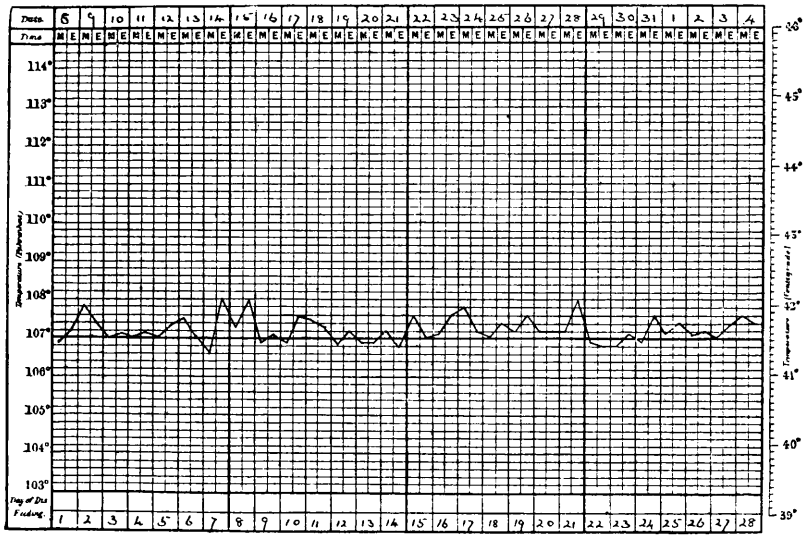
PESTIS.

Fowls. Case IV.—Young Cock. Weight on admission, 1.484 *grns. Inoculated, July* 7, *subcutaneously with* 5 *c.c. emulsion of liver and spleen of Plague-stricken Rat. Weight on discharge,* 1,710 *grns. Under observation until October* 4, 1903.



PESTIS.

Fowls. Case V.—Young Cock. Weight on admission, 1,484 *grns. Inoculated, July* 7, 2 *c.c. emulsion of organs of Rat. Weight on discharge,* 1.597 *grns. Under observation until October* 4, 1903.



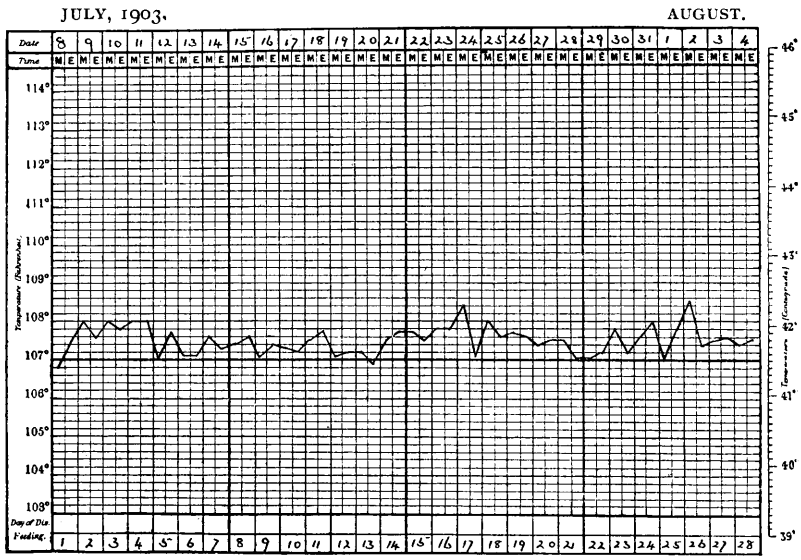
Fowl 6.—Inoculated in the flank on July 8th with 2 c.c. of an emulsion of the organs of recently dead rats, which had previously been inoculated with Bacillus pestis.

No illness whatever resulted in a period of three months.

Fowls 7, 8, 9, 10 (4 cocks) were fed on August 16th, each separately, with the finely cut up organs of rats which had previously been inoculated with Plague. The rats were freshly dead,

PESTIS.

Fowls. Case VI.—Black Cock. Weight on admission, 1,360 *grns. Inoculated, July* 7, 2 *c.c. emulsion of organs of Plague Rat. Weight on discharge,* 1,700 *grns. Under observation until October* 4, 1903.



and the material contained large numbers of typical organisms (*see* Fig. 30.

Cultures proved macroscopically and microscopically typical and highly pathogenic for rats and guinea-pigs.

Emulsion of the excreta of fowls 7 and 9 taken 48 hours after feeding only produced a temporary indisposition in guinea-pigs.

The fowls remained perfectly well from the day of feeding to November 30th, when observations were discontinued.

The combs were bright pink in colour, and dejecta were never observed to be otherwise than normal.

FIG, 30.

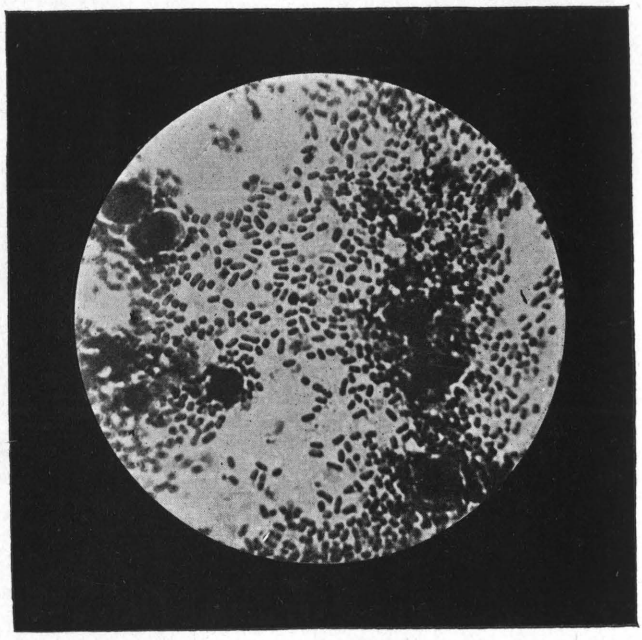


FIG. 31.

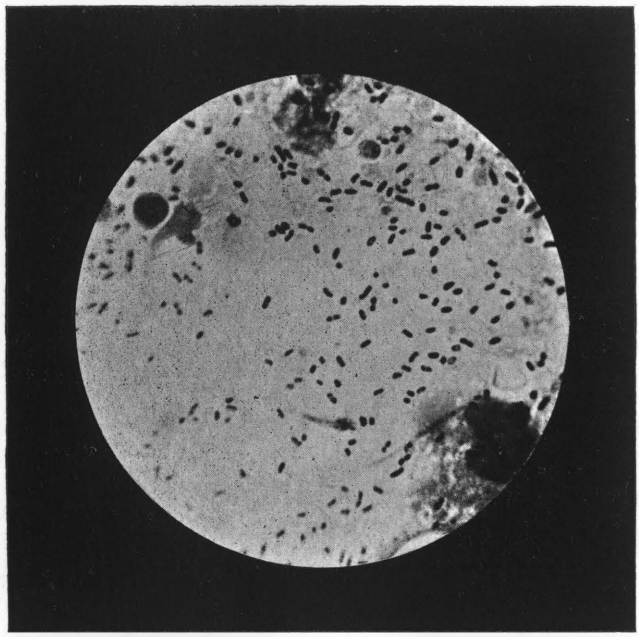


FIG. 32.

Fowls 11, 12, 13, 14, and 15 (3 cocks and 2 hens) were separately fed on the organs of rats recently dead, which had previously been inoculated with Bacillus pestis.

The material contained great numbers of organisms (Fig. 31), cultures on agar proved typical and virulent for guinea-pigs.

The excreta of Fowl 12 and Fowl 15 were collected 48 hours after feeding, and inoculated into two guinea-pigs. The latter showed only a local inflammation at the point of inoculation, which subsided without suppuration.

No indisposition whatever was subsequently noticed in the fowls. Several eggs were laid by the hens. Daily observations ceased on October 30th, when all seemed in better condition than at the beginning of the experiment.

Fowls 16, 17, 18, 19, and 20 (3 hens and 2 cocks) were separately fed on August 21st on the fresh organs of rats which had previously been inoculated with Bacillus Pestis. The material contained the typical bacillus in large quantities (Fig. 32).

Cultures proved typical and virulent for guinea-pigs. No indisposition was noticed, and on August 24th they were separately fed with the minced organs of a large guinea-pig, which had previously been inoculated with another strain of Bacillus Pestis. On September 1st Fowl 16 laid an egg.

On September 4th each fowl was again separately fed on the organs of another guinea-pig which had been inoculated for the purpose. On September 5th Fowl 16 laid an egg, and thereafter for some time laid on every alternate day.

On October 4th Fowls 16 and 17 ate the liver, spleen, lungs, heart, and buboes of a guinea-pig which had been inoculated for the purpose, and which had died with the speckled spleen, haemorrhagic glands and other typical appearances of Plague. The organs were crowded with bacilli, and cultures turned out to be also typical.

No indisposition resulted, and on the next day both laid eggs, and continued to lay on alternate days for a fortnight.

For three successive days after feeding with the Plague material, on October 4th, excreta was obtained from each fowl emulsified in bouillon and injected subcutaneously into guinea-pigs.

Some local inflammation at the point of inoculation and a slight rise of temperature for two days also resulted and the 6 guinea-pigs were eventually discharged well.

Fowls 16 and 17, which had thus received on four occasions large quantities of highly-infective material, as well as Fowls 18, 19, and 20, which had received infective material on three occasions, remained perfectly well, and gained weight after experiment.

The combs of all remained bright pink, the eyes bright, and the dejecta constantly normal. Observations were discontinued on December 10th.

II.—PIGS.

Pig 1.—Black Berkshire, 4 months old, and under observation five days before the experiment, was drenched on September 8th by means of a flexible canula with 100 c.c. of an emulsion of the liver, lungs, spleen, heart, kidneys, and bubo of a full-grown rat, which had been previously inoculated with Bacillus pestis. The emulsion contained large numbers of the typical organism, *vide* photomicrograph No. 33.

Considerable difficulty was experienced in passing the canula, and some force was eventually exerted. The operation caused considerable embarrassment of respiration, so much so that it was suggested at the time that the canula had entered the trachea. The emulsion was quickly administered; a streak of blood was observed on the end of the instrument. Immediately after release the pig suffered from dyspnoea, and showed alarming symptoms for about half an hour. Next day, however, he was much better, and remained so for three days. On September 12th respiratory symptoms reappeared; the breathing was labored, and moist râles could distinctly be heard.

This state continued till September 15th, when the pig began

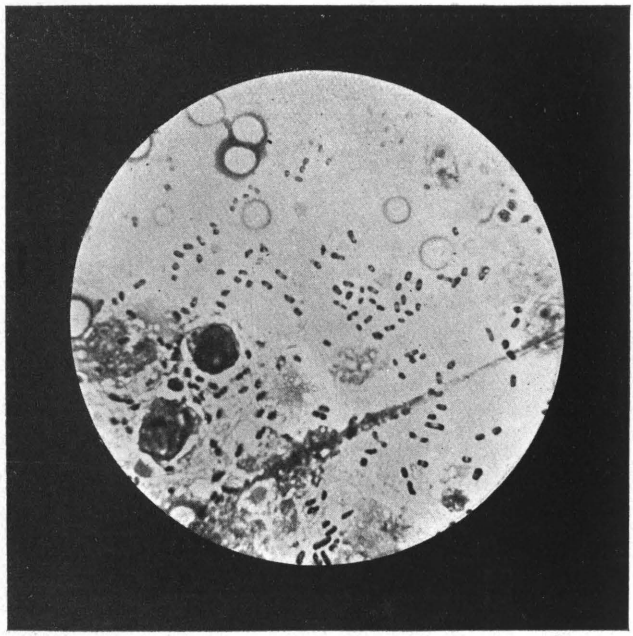
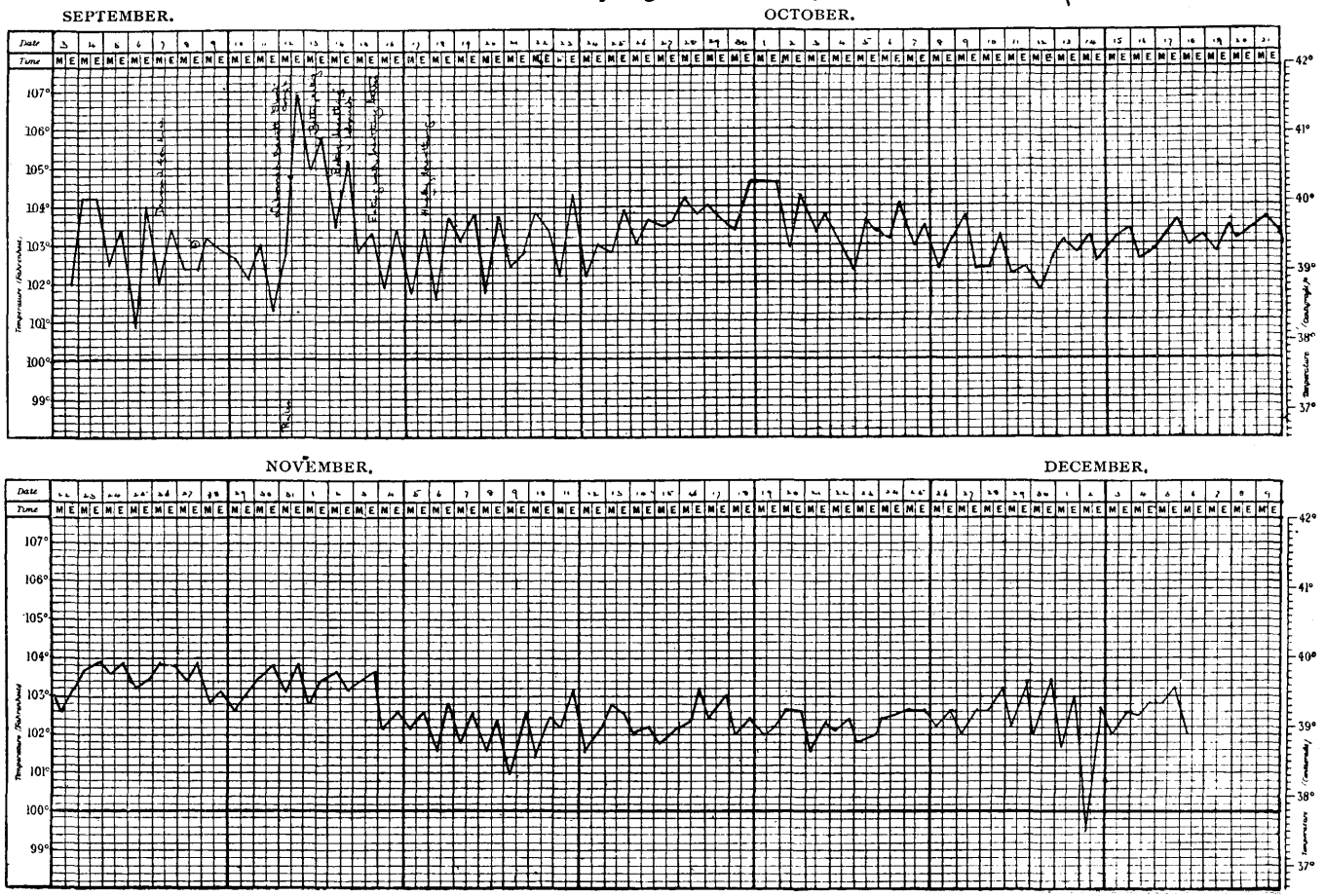


FIG. 33.

Pig. Number 1. *This Chart shows Lengthy Normal Record subsequent to Severe Infection* ⊙ *September* 8, *Drenched with* 100 *c.c. Emulsion of Organs. Rat Box*, 5.



to feed well, and the breathing gradually returned to normal. Glandular enlargements were searched for daily, but none occurred.

On September 14th some clear viscid mucus, of which there was a considerable discharge, was collected from the nasal passages, and .5 c.c. inoculated subcutaneously into a guinea-pig. On September 18th the guinea-pig died, and the *post-mortem* examination revealed the fact that death was due to Plague (*vide* photomicrograph 34). This finding was carefully confirmed by observation of the spleen cultures, stalactitic growth and re-inoculation into a guinea-pig with subsequent culture. All methods, however, proved positive. The Plague bacillus was also recovered by plating the pig's nasal discharge, and the resulting growths were subjected to the same tests.

Pig 1 remained well, and thereafter rapidly gained weght and condition.

It is, however, more than probable that the whole or part of the infective material was poured into the trachea or one of the larger bronchi.

Sixteen days after the administration of the material an attempt was made to again isolate the bacillus from the nasal discharge, which was by this time very scanty. The attempt failed, and Plague could not be induced in guinea-pigs by subcutaneous inoculation of the nasal defluxion.

The pig is still under observation on January 10th, but is improving in condition, and is in perfect health.

During the time of his illness, other pigs in the same stye were in close contact, but none showed a rise of temperature or indisposition, and a systematic attempt to isolate the bacillus from their nasal secretions failed.

Pig 2.—Black Berkshire, 4 months old, was fed on September 10th with an emulsion of the organs of a rat which had been inoculated with Bacillus pestis. The organs were crushed in a mortar, and soaked in 50 c.c. bouillon for half an hour. Large numbers of organisms were present, and cultures made at the time proved positive morphologically and pathogenic for guinea-pigs.

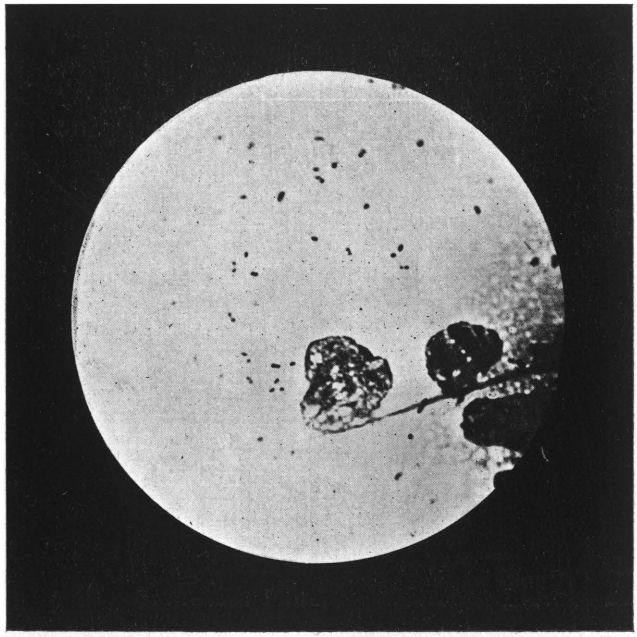
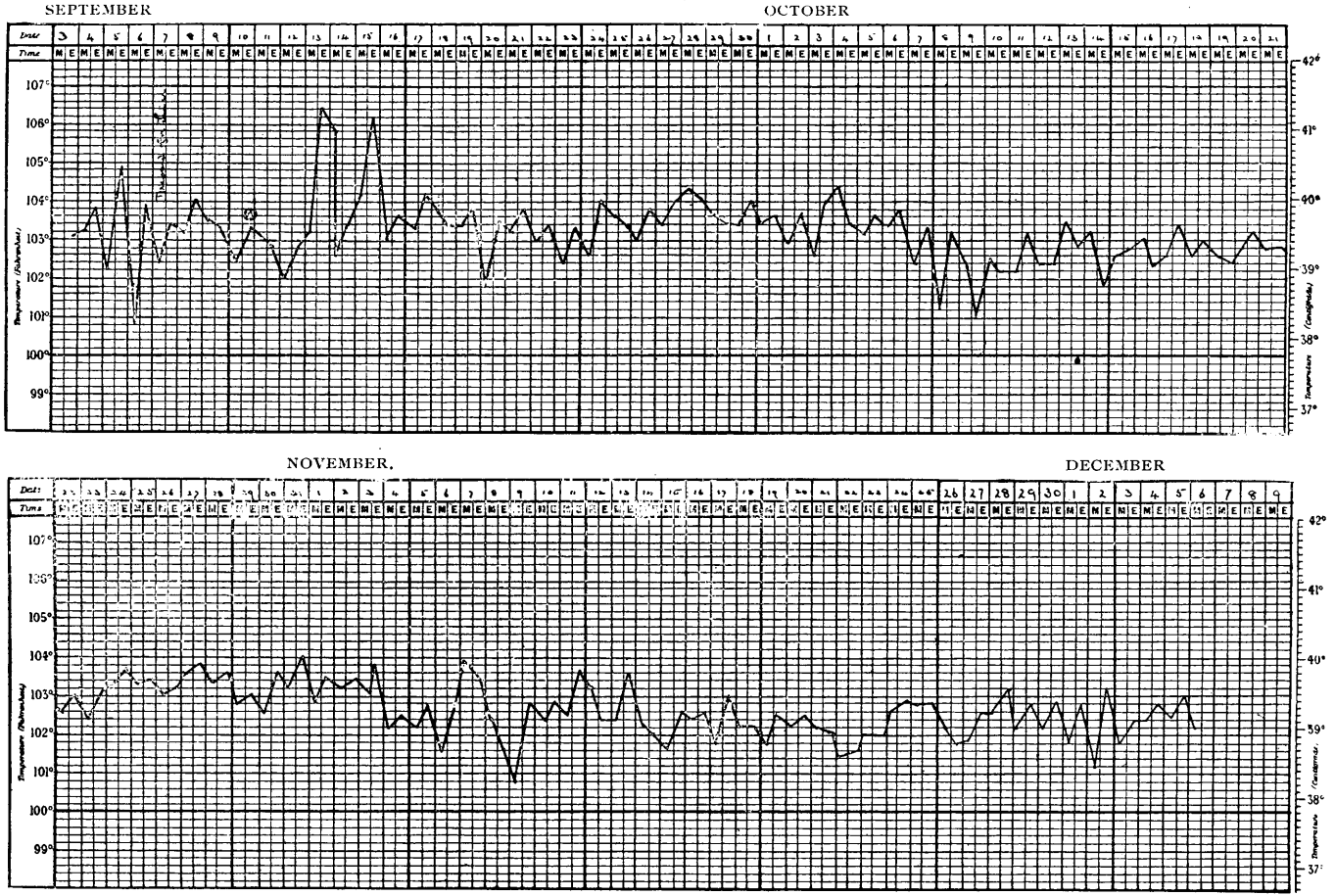


FIG. 34.

Fig. Number 2. This Chart shows lengthy Record subsequent to Infection (Rich Granary Record).



Except the transient rise of temperature, no ill-result whatever followed. Glandular enlargements were searched for daily, but none were discovered.

On January 10th the pig is still under daily observation, and is in prime condition.

Pig 3.—Black Berkshire, 5 months old, was fed on September 30th with an emulsion of the organs of a guinea-pig which had previously been inoculated with Bacillus pestis, and which had died in 110 hours with all the appearances of Plague. Cultures of the spleen proved typical and virulent for rats, and positive as regards stalactitic formation.

Beyond a transient rise of temperature, no ill effects were noticed, although the animal was under close observation. He ate greedily, and was as bright and lively as the control pigs. Twenty-four and 48 hours after feeding, the excreta were carefully obtained, and a small quantity of fœces emulsified in bouillon and injected into guinea-pigs. Local reaction was well marked in each case, but both recovered after indisposition lasting three days.

On January 10th the pig is fattening and in perfect health.

Pig 4.—Black Berkshire, about 6 months old, was on November 9th scarified by means of a scapel on the right flank over an area 1 inch in diameter, and with a wooden rod the spleen pulp of a guinea-pig which had been inoculated for the purpose with Bacillus pestis was well rubbed in. This guinea-pig had died with haemorrhagic groin glands and a speckled spleen containing a large number of typical organisms. Cultures proved subsequently positive morphologically, and were virulent for rats.

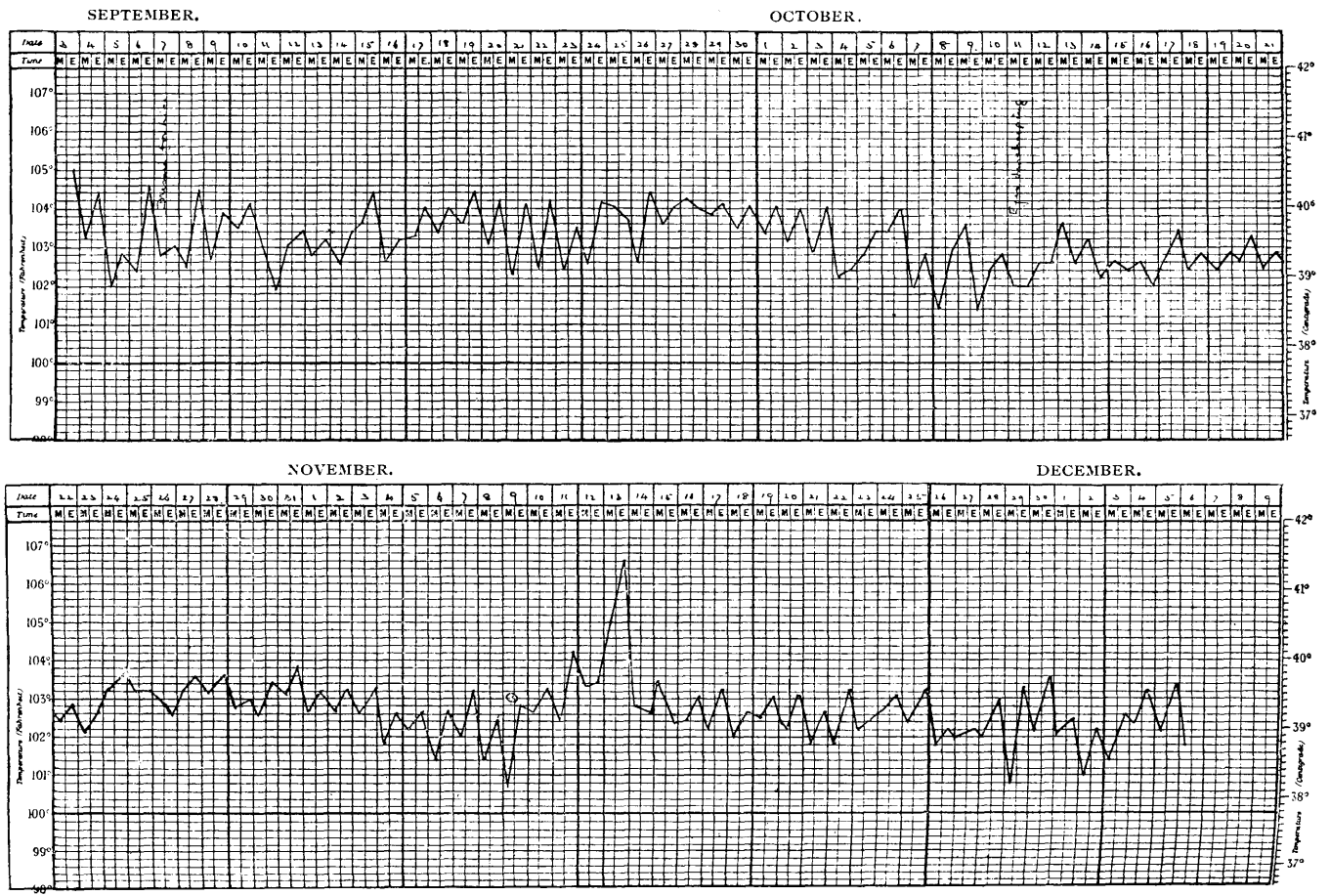
Every care was taken to make the vaccination effective, and the abraded surface was covered with dry cotton-wool over which were painted many layers of collodion.

On the third day a tense glazed appearance was observed around the area of scarification, and on the fourth day some tumefaction, which was not apparently tender.

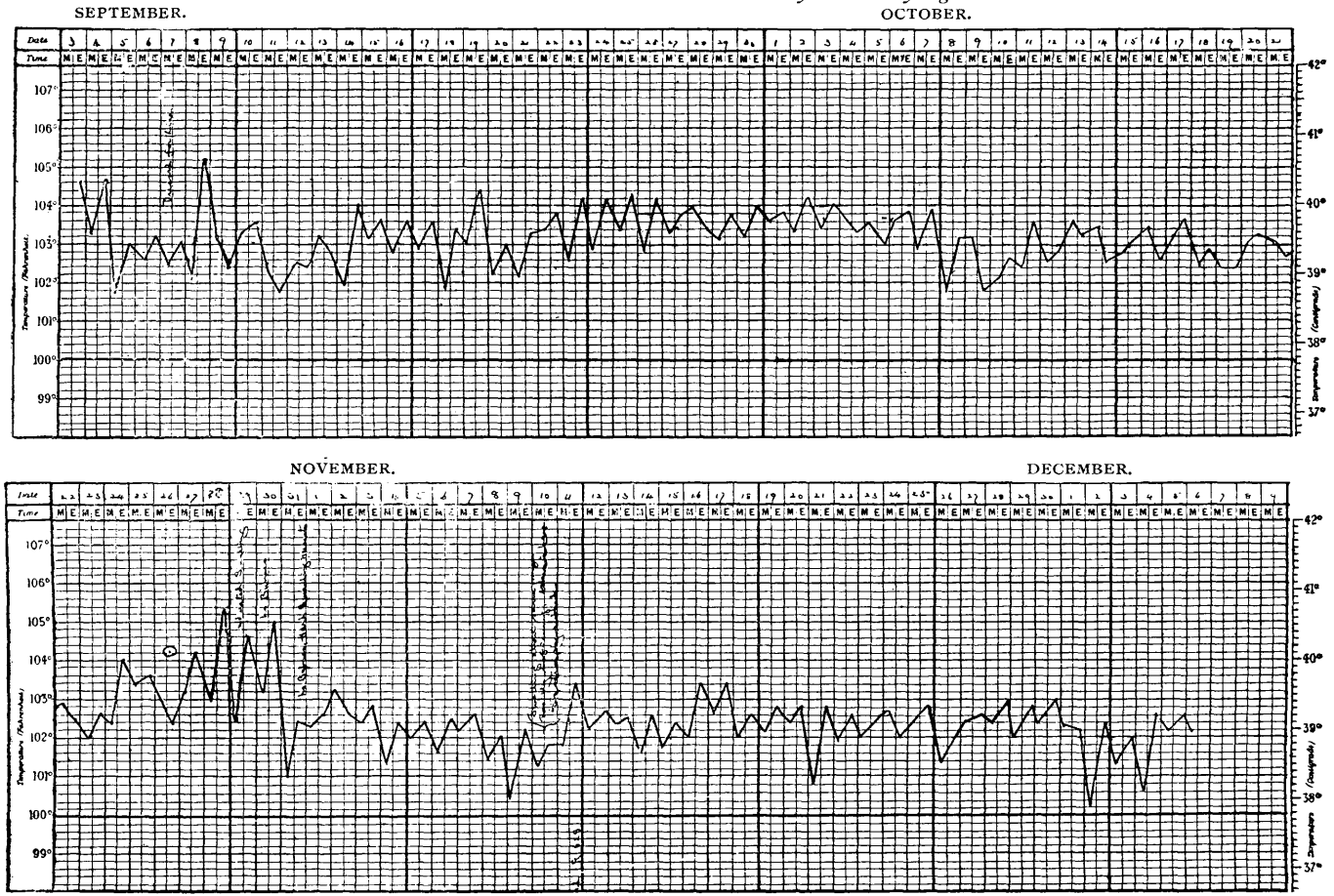
No glandular enlargements were discoverable at any time, and the pig showed no signs of illness. He was particularly keen on the

PESTIS.

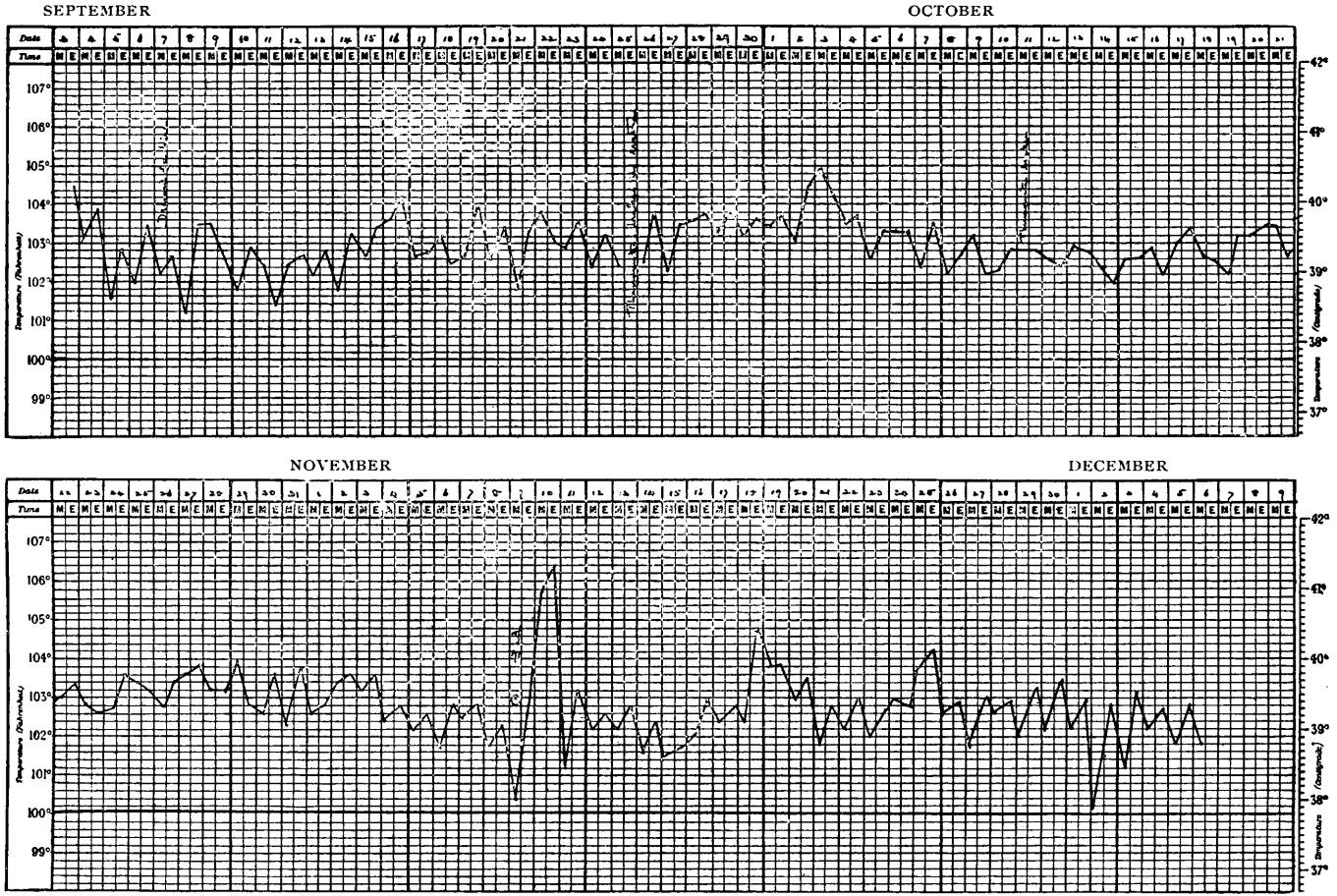
Pig. Number 4. November 9, 1903. ⊙ *Scarified, and Spleen of Guinea-pig rubbed in.*



Pig Number 5. Chart showing Lengthy Normal Record, during which time the Animal remained in contact with infected Pigs ⊙ October 26. Inoculated in the Flank subcutaneously with 6 day Agar Culture.



Pig. Number 6. This Chart shows a Lengthy Normal Record, during which time the Animal remained in Contact with infected Pigs. ⊙ *November 19, 1903. Fed on above date with Organs of Guinea-pig, No. 627.*



approach of food, and no difference could be observed between his condition and that of the control pigs.

On the fifth day the tumefaction increased, but thereafter it rapidly subsided, the abraded surface healing. On January 10th the pig is fattening.

Pig 5.—Black Berkshire, about 6 months old, was on the 26th October inoculated in the right flank with an emulsion of the whole of a six-day agar culture of Bacillus pestis. (Simultaneously a white rat was inoculated with a trace of the same culture as a control, and died with typical appearances. Culture of the spleen proved confirmatory.)

On the third day following the inoculation there was a patch of induration 2 inches in diameter at the point of inoculation not noticeable to the eye, but palpable and tender. The pig ate well, but seemed not quite so lively as the control pigs. On the following day, however, he seemed quite himself again, and ate greedily. The patch of induration was no longer tender, but was more raised, and apparently itched. Thereafter the pig enjoyed excellent health, and improved in weight and condition, although the swelling increased in elevation, and remained for a fortnight, when some fluctuation could could be made out.

Two days afterwards the abscess pointed and burst.

An attempt was made both by plating and by animal inoculation to recover the Plague bacillus from the discharge, but failed.

Small sloughs and thick yellow pus discharged for some days. The health of the pig seemed not to be materially deranged at any time, and a fortnight after inoculation the animal was approximately equal in weight to the control pigs.

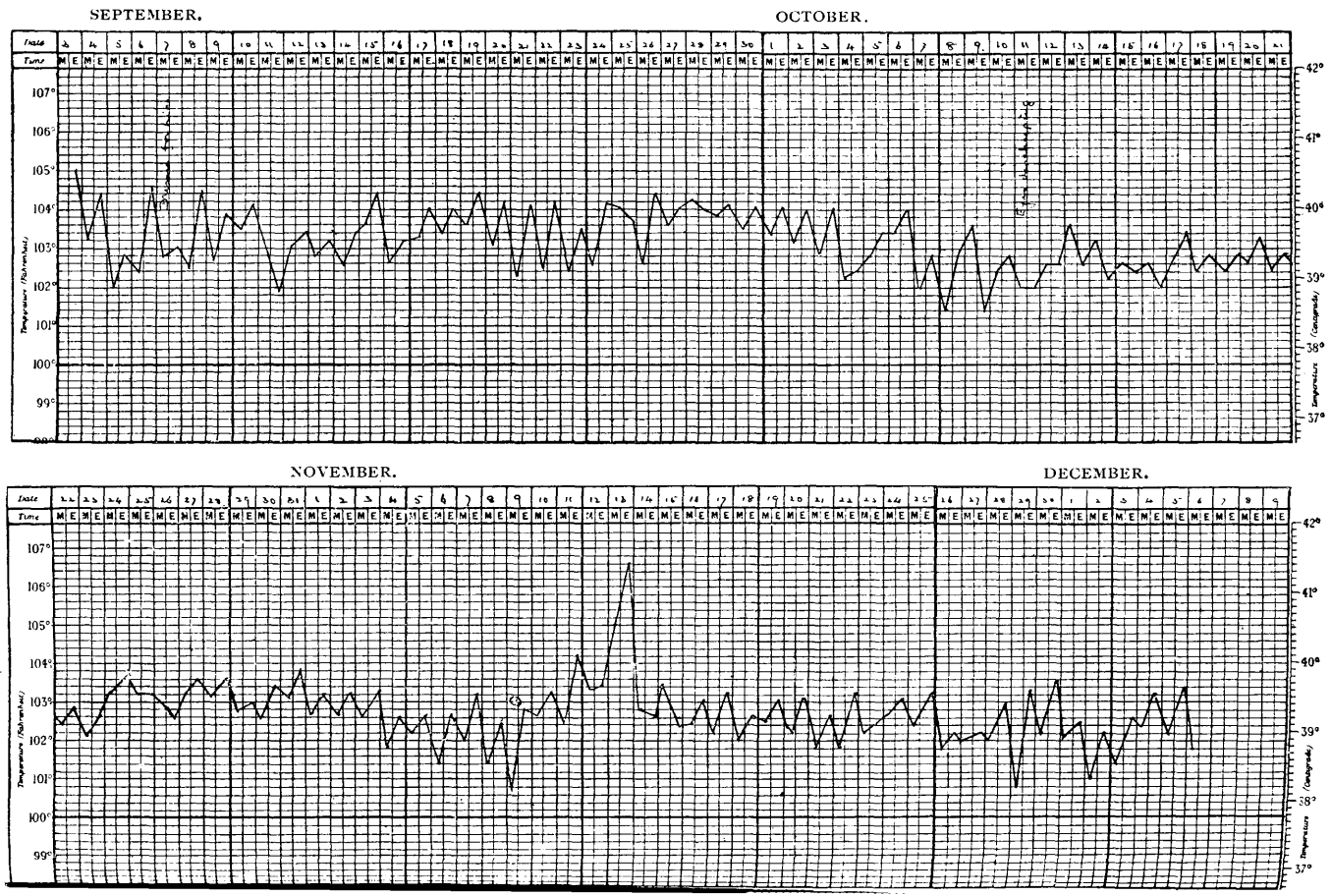
Daily palpations were made to discover glandular enlargements in the axillæ or groins, but none were made out.

On January 10th the pig is still in good condition.

Pig 6.—Black Berkshire, about 6 months old, ate greedily on November 9th, the liver, lungs, spleen, heart, kidneys, intestines, and abdominal walls with haemorrhagic groin-glands of a full-grown guinea-pig, which had been infected with Plague, and died in four days with the classical appearances.

PESTIS.

Pig. Number 4. November 9, 1903. ⊙ *Scarified, and Spleen of Guinea-pig rubbed in.*



The cultures of the spleen proved confirmatory and virulent.

This pig at no time lost appetite or showed any signs of illness or glandular enlargements, although the temperature rose to 105.7° F. the day after feeding. Thereafter the chart of this pig is similar to foregoing charts.

On January 10th the pig is in normal health and fattening.

Pig 7.—White, of an inferior type, known as Kaffir pig, about 4 months old, having shown a normal temperature for 5 days. The pig ate the liver, lungs, spleen, heart, kidneys, intestines with haemorrhagic glands of two full-grown guinea-pigs, which had died in three days with typical appearances from the inoculation of a few minims of spleen pulp of a native who had died of Plague. With this material was placed portions of the spleen, liver, and lungs from another case of Plague. It was desired to ascertain whether the administration of such a large bulk of infective material would cause any appreciable difference in the result obtained in former experiments. These tissues, mashed in a large bowl with a sprinkling of crushed maize, were eagerly eaten. The snout was then wiped with a cloth wrung out of disinfectant solution, and the animal kept under the closest surveillance.

The temperature rose to 106.2° F. on the third day, and on the succeeding day the pig was not quite so lively as the control pigs, but yet ate well. He brightened up on the fourth day, and thereafter seemed in excellent health.

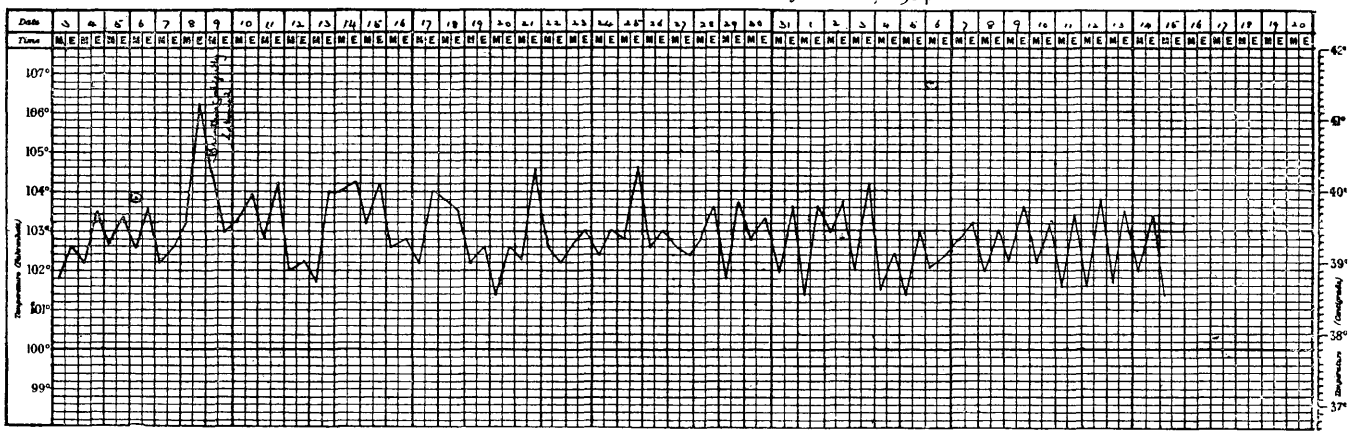
For some days, however, the temperature was slightly higher than that of the control pigs.

He gained in weight and condition constantly afterwards, and on January 12th is fattening.

The excreta collected at the moment of defaecation, 27 hours after feeding, failed to induce Plague in guinea-pigs when inoculated subcutaneously, and after local inflammation and threatened abscess the latter recovered.

The urine of the pig was obtained 53 hours after feeding, and ·5 c.c of the sediment was inoculated subcutaneously into the thigh of another guinea-pig.

Pig. Number 7. Lengthy Normal Record after receiving Large Quantities of Plague Tissues. Fed on December 6th.



The temperature rose, an abscess threatened, but the animal made a good recovery.

Pig 8.—Four months old, white, Kaffir variety. On December 18th ate greedily the spleen, lungs, liver, heart, kidneys, intestines, and the abdominal walls with groin glands of a full-grown guinea-pig, which had been inoculated with Bacillus pestis four days previously.

The spleen was crowded with typical bacilli; the other organs contained a fair number of the same. Culture proved confirmatory and pathogenic for guinea-pigs. A rise of temperature occurred on the fourth day, but no signs of illness were observed. The pig ate ravenously, and remained bright and lively.

On January 12th, 1904, the animal is thriving. The chart merely shows the slight rise of temperature on the fourth and fifth days.

Pig 9.—Black, about 5 months old, of the Kaffir variety. On December 18th fed with the organs of a guinea-pig, which had been infected for the purpose on December 14th, and which died with all the macroscopical and microscopical appearances of Plague.

Bacilli were in great abundance, and cultures proved confirmatory.

No subsequent indisposition whatever was noticed in this case, and on January 10th the pig continues to thrive.

The chart approximates closely to that of the control animal.

Pig 10.—Black, 5 months old, of the Kafir variety. Was fed on December 18th with about half the mashed organs of a guinea-pig which had been infected on December 14th, and which contained an abundance of bacilli. The usual surveillance was exercised, but no signs of illness was discoverable, and the temperature chart corresponds with those above.

Pig 11.—Black Kaffir pig, approximately 5 months old, was used as a control, and showed a normal record throughout.

III.—CALVES.

Calf 1.—A 7-months-old bull calf on November 14th received subcutaneously, behind left scapula, 5 c.c. of a 48-hours' beef bouillon incubated culture of Bacillus pestis. A control guinea-pig and a control brown rat were simultaneously inoculated with ·5 c.c. from the same tube, and a flask of bouillon containing a few drops of ghee was also sown.

The control animals both died on November 18. Each with typical appearances macroscopically and microscopically, and cultures from the spleen of each proved confirmatory. The bouillon ghee flask developed a good crop of stalactites in 72 hours.

On November 17th the calf developed what appeared to be an intercurrent attack of diarrhoea, which may have been due to the cement floor and general chilliness of his new quarters.

The control calf at the same time showed some indisposition with slight diarrhoea.

All attempts to isolate the Plague bacillus from the streaks of blood in the excreta failed.

By November 20th the coat was again sleek, and thereafter the animal throve.

Calf 2.—Bull, 6 months old, was drenched on November 19th with an emulsion of the thoroughly mashed-up organs of a rat which had been infected with Bacillus pestis five days previously.

The emulsion contained an abundance of the bacilli, and the strain used proved highly pathogenic to a control guinea-pig.

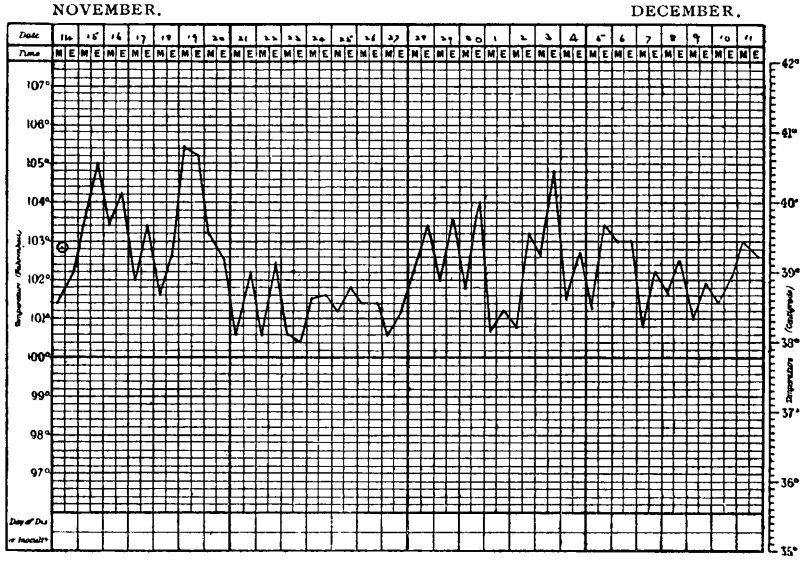
Confirmatory cultures proved positive.

This calf showed no signs of illness, fed well, and chewed the cud regularly.

At the end of the month he was discharged in improved condition.

PESTIS.

Calf. Number I.—Bull Calf, about 7 months old. ⊙ *November* 14. 1903, *received behind the left scapula subcutaneously* 5 *c cm. of* 48*-hour bouillon culture of Plague.*



PESTIS.

Calf. Number II.—Brown Bull, 6 *months old.* ⊙ *November* 19, *fed with emulsion of Rat organs (Box 3).*