The relation of tetanus to the hypodermic or intramuscular injection of quinine / by Sir D. Semple. --.

(NEW SERIES.) No. 43.

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OF THE

GOVERNMENT OF INDIA

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BY

LIEUTENANT-COLONEL SIR D. SEMPLE, *Kt.*, M.D.,
Director, Central Research Institute, Kasauli

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Price annas 12 or 1s. 2d.

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CONTENTS.

The Relation of Tetanus to the Hypodermic or Intramuscular Injection of Quinine.

CHAPTER I.

Introduction.

EVERY now and again we are confronted with the observation that cases of tetanus occur after the hypodermic or intramuscular administration of quinine, even in circumstances where every possible care has been taken to ensure sterility of the syringe and fluid injected. When an accident of this nature occurs it is only natural that the patient's friends and the public should feel inclined to throw the blame on the operator, and reproach him with having used a contaminated syringe, or contaminated solution, or with not having sufficiently cleansed the skin before introducing the needle.

In some cases it is possible that one or the other of these precautions may have been overlooked; but there are facts which clearly account for cases of tetanus supervening after quinine injections, where none of the precautions mentioned has been omitted.

At first sight it would seem impossible when given a sterile syringe, sterile quinine solution, and sterile skin at the site of inoculation, to produce tetanus, and so it would be except for the fact that there are people who harbour in their bodies tetanus spores, which may lie dormant either in a recently healed up wound or abrasion, or possibly in an old healed up injury long since forgotten. There are also many healthy individuals who harbour tetanus germs in their intestinal tracts. Tetanus spores being extremely resistant and capable of surviving under very adverse conditions for many years, it follows that they are not likely to give up their disease-producing qualities by remaining in a resting stage when stowed away in the tissues, and as a matter of fact we know from experiments on animals (see table XIII) that they can remain alive and retain their virulence in the tissues for seven months at least, and very possibly for years. Tetanus spores have also been proved to remain alive and virulent on a rusty nib for as long as 18 years. Compared with this test of their vitality, the shelter which they find in the tissue of an animal would not be likely to reduce their pathogenic capabilities should conditions for germination become favourable.

In a latent tetanus-spore infection there are many conditions, in addition to the hypodermic injection of quinine, which would favour the growth of the spores,

but as these conditions have no direct connection with quinine as a favouring agent it will be necessary to mention only a few of them.

It has long been known that in campaigns where soldiers are subjected to great fatigue coupled with extremes of heat and cold many cases of tetanus occur. Here the depressing influences of fatigue, in conjunction with great heat or great cold, would diminish the resistance in those who harboured infection.

Fournier Pescay[1] states, "On several occasions when men had marched all day under a blazing sun in Spain, a number were attacked on the following day with generalised tetanus."

Baron Larrey[2] states, "In the Austrian Campaign in 1809, the wounded who happened to be most exposed to the cold and damp air of the freezing nights in Spring, after having endured different degrees of very high temperature during the day were almost all attacked with this disease which was prevalent only at this season when the thermometer fell at night to half its daily maximum. The day on which the battle of Bautzen was fought was very hot, and the following night very cold; next day there were 110 cases of tetanus. After Austerlitz, and Eylau and during the retreat from Russia, there was scarcely a case of tetanus, the temperature was very low but uniform."

Seidillot[3] states, "Our wounded at Constantine in 1836, who were placed in narrow corridors, and who had to endure hot days and frosty nights, were attacked by tetanus in very large numbers."

It will be seen from the few quotations given that extremes of heat, cold, and fatigue, were clearly recognised as factors conducive to tetanus, and at a time when nothing whatever was known about the causal micro-organism. Possibly some of the wounded mentioned by the observers here quoted may have contracted tetanus from the ground on which they lay, but the fact of such an acute observer as Baron Larrey associating the disease with extremes of heat and cold is significant, and in the example given by Fournier Pescay the men were not wounded at all.

Quinine, when given hypodermically to a spore-infected person would produce favouring conditions for the production of tetanus in two ways—

(1) By a paralysing effect on the phagocytes when given in large doses and for some time.

(2) By destroying tissues at the seat of injection, and by this means producing a suitable local anaerobic focus where a stray phagocyte carrying tetanus spores might get stranded.

It will be seen from the experiments in this paper, that a hypodermic injection of quinine in animals invariably destroys tissue at the site of injection, and dead subcutaneous tissue would act in every way as a suitable medium for the germination of tetanus spores irrespective of how they found their way there.

In 1904, Vincent[4] brought forward observations which proved that the physiological action of quinine can prevent phagocytes from successfully coping with tetanus spores ; and that quinine, heat and other depressing influences, diminish the resistance to tetanus in animals inoculated with spores.

Small doses of quinine would increase the leucocytes, and probably also increase phagocytosis, but when large doses are given the paralysing effect of the drug on leucocytes has to be taken into account. Further, there is another condition to be taken into account when quinine is given hypodermically, *viz.*, the malarial infection for which it is given; this would lower resistance to most infections, tetanus included.

Many hearsay records might be mentioned in which tetanus is said to have followed the hypodermic administration of quinine, and no doubt many of these are authentic, but in the absence of sufficient authority to vouch for their accuracy one would not be justified in treating information of this kind with the respect due to established facts.

It is a pity that medical men who have had to deal with such cases have not been more eager to publish all details connected with them. Possibly the fact of tetanus following any operation being looked upon as a misfortune which ought not to have happened, may account for little being said on the subject, and for obvious reasons one can understand this being the case.

There are, however, a number of cases recorded.

Maclean[5] mentions six cases as having occurred in India, the details of which were brought to his notice. These cases evidently made a deep impression on his mind, for he adds, " There is something revolting in a death brought about directly or indirectly by a remedy intended to cure."

Manson[6] issues a note of warning when he states, " Not only abscesses, sloughing and chronic painful indurations have sometimes followed the hypodermic injections, but also tetanus." Manson evidently believed that the quinine had nothing to do with tetanus for he adds, " In these latter unfortunate cases it was not the quinine that caused the tetanus; it was the tetanus bacillus, and this tetanus was introduced either in a dirty needle, or in a fouled solution."

Vincent[7] mentions that the French in Madagascar had eleven cases of tetanus following upon injections of quinine ; and he also draws attention to a number of cases reported by other observers.

In India 10 cases occurring after injections of quinine have been brought to my notice. In one of these cases the tetanus bacillus was isolated by me from a sample of the distilled water in which the quinine was dissolved, so that in this particular case the quinine was evidently not altogether to blame, but it may have been conducive to infection by establishing favourable local conditions when the

same amount of tetanus-infected water might not have produced the disease given with a morphia or cocaine injection.

We know that hypodermic injections of morphia, cocaine, strychnine, or digataline are rarely (if ever) followed by tetanus. I cannot remember ever hearing of a case of morphia injection giving rise to tetanus, and I am sure it is a method of treatment more generally adopted than quinine injections. The syringes used for morphia, cocaine, etc., are just as likely to be septic as those used for quinine, so it cannot be altogether the cleanliness of the syringes used which accounts for the difference.

Quinine being a protoplasmic poison is not likely to harbour living tetanus spores before being dissolved, and the acidity of the tabloids used now-a-days would not admit of any tetanus spores remaining alive in them. On numerous occasions I have made anaerobic cultivations from quinine tabloids, but have never succeeded in isolating tetanus bacilli.

Before entering into the details of the subject matter of this investigation, it is necessary first of all briefly to describe the nature of a tetanus infection, and to mention a few leading points connected with tetanus bacilli, their spores, and toxine; then to place on a sure footing the effects of quinine on the animals (guinea pigs) chiefly used in the experiments which follow.

After this has been done the influence of quinine in bringing about infection in the varying conditions in which tetanus spores and tetanus cultures were used in guinea pigs and monkeys will be discussed in the order in which these experiments are given in the various tables, and also the remaining experiments, including those in which quinine was not given.

Finally the uses of tetanus antitoxin as a remedy in averting tetanus when it is necessary to give quinine hypodermically will be considered, and a summary of conclusions arrived at will be given.

CHAPTER II.

The Nature of Tetanus Infection.

When a wound becomes infected with tetanus it means that tetanus spores have been introduced; and in the majority of cases it also means that other micro-organisms have been introduced at the same time.

The kinds of wounds most likely to be followed by tetanus are those in which the tissues have been crushed and soiled with earth, or dust and dirt from roads; also deep punctured wounds produced by contaminated spikes of wood, metal, or other instruments.

The tetanus bacillus, which is the causal organism of tetanus, requires anaerobic conditions before it can germinate and produce the disease. These anaerobic conditions are more likely to be found below the surface of contaminated crushed wounds, than in any other class of surface wound; but there are no wounds, no matter how superficial and trivial, in which conditions favourable for the development of tetanus may not become established.

When other bacteria besides tetanus spores have gained entrance to a wound anaerobic conditions are more likely to be produced, because many of the germs that commonly contaminate wounds use up the oxygen in their vicinity, and this would favour the growth of any tetanus spores introduced at the same time. In such a wound, crushed and dead tissue would act as a suitable medium for the multiplication of bacteria (including tetanus germs), whereas in punctured wounds anaerobic conditions would certainly be found immediately below the surface from the outset.

A complete description of the tetanus bacillus will be found in all standard text books on bactericlogy, and on this account it will be necessary to enter into only a few of the details connected with its life-history, especially those details which have a direct bearing on the experiments given further on, carried out with tetanus spores and quinine.

The tetanus bacillus is a spore-forming germ which produces extracellular toxines; and it is to these toxines that all the symptoms of tetanus are due. It was first described by Nicolaier in 1884, and is often referred to as Nicolaier's bacillus. This observer produced tetanus in mice and guinea pigs by injecting them with garden earth, and when symptoms set in he was able to communicate the disease to other animals by using pus from the site of injection.

About the same time Carle and Rattone proved that the pus and secretions from tetanus wounds in man would cause tetanus when inoculated into animals. These experiments were the first proofs of the infectious nature of tetanus.

Kitasato, in 1889, was able to obtain the bacillus in pure culture. He took advantage of the fact that the spores of tetanus are very resistant to the action of heat, and can withstand boiling for from 3-5 minutes. When a mixture containing tetanus spores and other germs (whether spore-forming or not) is boiled in a water bath for 2 or 3 minutes, or in many cases only just heated up to boiling point, and then removed, in the majority of cases the tetanus spores are not injured, and all other contaminating bacteria are killed off. When these tetanus spores are transferred to a suitable medium, and placed under anaerobic conditions at a temperature of 37°C., they germinate and a pure culture is the result.

In shape the bacillus resembles a small pin or drumstick, which means that when fully grown it is a small thin rod in which the spore forms at one end, and gives it a characteristic shape. During the first day or two of its life in a suitable medium it is a thin motile rod, then after from 24 to 48 hours spores begin to form. In an old culture the rod part of the germ disappears and only the small round spores remain. In this spore condition tetanus germs are widely distributed in nature. They are found in almost every sample of rich garden earth, and in the manure of many animals, especially the herbivora. It is quite the exception to find them absent from the intestinal tracts of horses. On this account one would expect to find them on roads and streets soiled with the excreta of horses and cattle, and especially on the floors of stables, and in manured garden soil, and in the soils of almost every inhabited locality. Tetanus spores have also been found in commercial gelatine, and in ligatures made from the gut of sheep, both of which are prepared from animals which harbour tetanus spores in their intestinal tracts. The fact of their occasional presence in gelatine has led to fatal results in the use of this material as a subcutaneous injection to control obstinate hæmorrhage.

On numerous occasions I have isolated tetanus bacilli from garden earth, stable floors, and stable manure, and in four cases out of ten examined, from human fæces. Some years ago when visiting a well-known horse repository in London I picked up a little earth and dust from the yard where the horses were shown for sale, and from this material I had no difficulty in obtaining a rich growth of tetanus bacilli.

The spores of tetanus when swallowed by horses, cattle, and other animals, (and probably also by man on uncooked vegetables and salads, which have been grown on manured soils) find in the intestinal tract anaerobic conditions where they can germinate, and are then passed out with the excreta, and distributed in the spore stage here, there, and everywhere.

It is not known whether tetanus spores can find in the soil conditions suitable for germination, or whether it is absolutely necessary in order to prevent extinction that they should pass through a stage of development in the intestinal tract of animals. Possibly there are localities in which they can develop, as

for instance, in the swamps of some of the Pacific islands, where the natives are said to poison their arrows with tetanus by dipping them in the mud of these swamps. We know that there are other spore-forming anaerobic germs which germinate in earth, and possibly tetanus bacilli may do so in some particular soils.

Irrespective of whether tetanus bacilli can germinate in the soil, or in other situations not connected with man or animals, the fact remains that the spores can survive for many years under most adverse conditions. Probably there are no other known pathogenic spores which have the same resisting powers, and as the disinfection of tetanus means the destruction of spores, it is necessary to know something about their resisting powers. Some strains are more resistant than others. The less resistant strains are destroyed in a Koch's steam steriliser at 100°C in 5 minutes; but on the other hand there are strains which require 2½ hours in steam at 100°C, before they are destroyed.

According to Theobald Smith[8], the spores of some strains resist boiling for 40 to 70 minutes, and steaming in a Koch's steriliser for 2½ hours, but not for 3 hours.

Roseneau,[9] from experiments which he has carried out, states that it is necessary when sterilising tetanus-infected solutions to boil for not less than 2 hours, or to steam in a Koch's steriliser for a similar time. He also advises steam under pressure as the most reliable disinfectant for tetanus spores.

In an autoclave the spores are destroyed in 15 minutes at a temperature of 120°C., and in 20 minutes at a temperature of 115°C., 5 per cent. carbolic acid requires 15 hours, and 2 per cent. tricresol, or lysol, 2 hours. A solution of corrosive sublimate of the strength 1 in 1000 requires several hours, but when hydrochloric acid is added to the solution to the extent of ½ per cent. it kills tetanus spores in 30 minutes.

As an example of what one has got to expect when dealing with tetanus-infected material, the following history of the life of tetanus spores on two rusty nibs is interesting and instructive.

In 1891 two steel nibs were dipped in a tetanus culture in Paris, and then placed in a sterile test tube, which was plugged with cotton wool and fitted with a rubber cap. The test tube containing the two nibs came into my possession at the Pasteur Institute of India, Kasauli, in the Spring of 1900, and had not in the meantime been tampered with, but presumably kept in a dark cupboard, screened from light. In 1902 I happened to require a tetanus culture for experimental purposes, so I removed one of the nibs from the test tube, placed it in a tube of sterile broth under anaerobic conditions, and grew a pure culture of tetanus. This culture was virulent for guinea pigs in small doses.

The other nib remained in the original test tube, plugged with cotton wool only, and in a cupboard which was often open, until December 1909, when I took it out, placed it in a tube of broth, and under anaerobic conditions grew a pure

growth of tetanus bacilli from it. This growth was also virulent, and killed guinea pigs in small doses. (0. 1 c.c. of a four weeks' growth).

Both nibs, when taken out of the original test tube, were rusty, and on this account I was rather surprised to obtain a growth of tetanus bacilli from either of them, and I was also surprised to find that both cultures were virulent. The spores on the first nib had been eleven years in a dried condition, and those on the second nib 18 years, and no doubt they would have remained alive and virulent for several more years.

Cases are on record where splinters of wood infected with tetanus spores and kept for 2½ and 11 years were still capable of conveying the disease, but I have no knowledge of any other authentic case in which the spores in a dried condition remained alive and virulent for 18 years.

In the face of these facts it is only reasonable to infer that in the soil tetanus spores might have an equally prolonged life, provided they are not directly exposed to the sun's rays. From earth they find their way into the intestinal tracts of horses, cattle, sheep, goats and other animals, attached to food, such as grass, hay, roots, etc., and evidently in some cases into the intestinal tracts of man adherent to uncooked vegetables. In these various intestinal regions the spores find anaerobic conditions where they can germinate, and are then passed out with the excreta, and again find their way to earth, roads, streets, and stables, etc. In a vicious cycle of this kind their vitality and virulence would depend on whether the conditions for development were favourable or the reverse.

It is not to be wondered at, then, that tetanus germs are plentiful in nature. The wonder is that cases of the disease are as rare as they are. This can be accounted for only by the comparative non-virulence of the majority of strains if not connected with a case of tetanus.

When tetanus germs are isolated from garden earth, stable floors, and other places, as a rule it requires a large dose to produce even local tetanus in a susceptible animal such as a guinea pig; but it sometimes happens that a virulent strain can be obtained from the soil and other sources. As examples, I may mention that on one occasion I found that it required 2 c.c. of a 10 days' culture to produce local tetanus in a guinea pig; and on another occasion ¼ c.c. of a 10 days' culture proved fatal to a guinea pig. Both cultures had been isolated from earth but from different localities. I have also worked with a tetanus culture of such virulence that it was only necessary to dip the point of a surgical needle into it, and then prick a guinea pig's skin to give the animal fatal tetanus. It will be seen from these examples that there is a very wide variation in the virulence of different strains of tetanus bacilli.

As far as indirect infection is concerned the horse is probably the most fertile source. Some time ago a case was brought to my notice in which lint and a bandage

which had been for some days on a shelf in a stable were used to dress an open boil on a man's arm, with the result that fatal tetanus set in a few days afterwards. The lint and bandage used in this case were what remained from a dressing applied to a horse's leg some time before, and after remaining exposed in the stable, where they doubtless got contaminated with tetanus, they were applied to the patient's arm without the knowledge of his medical attendant.

In this connection I might also mention that, when immunising horses with snake venom in order to prepare antivenene, abscesses at the seat of injection of the venom are of common occurrence. These abscesses, when opened, are likely portals of entrance for the tetanus germs which horses harbour in their intestinal tracts. I have had practical experience of four cases of tetanus in horses contracted in this way. After these accidents I adopted the routine practice of giving antivenene horses an occasional dose of antitetanic serum, and since then I have not had a single case of tetanus in those horses.

In a tetanus-infected wound the spores which have gained an entrance and found suitable local conditions germinate at the seat of infection. The bacilli resulting from the growth of the spores remain localised at the seat of infection, or in its immediate vicinity, and produce an extra-cellular toxine which is the direct cause of the symptoms of tetanus.

The disease is what is known as an "intoxication process," *i.e.*, it altogether depends upon an extracellular poison elaborated by the causal micro-organism, and in this respect it resembles diphtheria. When tetanus bacilli have been grown in pure culture for a month (or less) at a temperature of 37°C., and the toxine separated by filtration through a Pasteur Chamberland filter, every symptom of tetanus can be produced in animals by injecting the germ-free toxine.

According to Ehrlich, tetanus toxine is made up of at least two toxine bodies, *viz.*, tetanospasmin and tetanolysin. Of these two bodies tetanospasmin is the more important, as it has a strong affinity for nerve tissue, especially nerve centre cells, and gives rise to the muscular spasms characteristic of the disease. Tetanolysin has some effect in breaking up red blood corpuscles, but it has not been proved that this is of much account in a case of tetanus; it is a negligible side issue.

Meyer and Ransome[10] have demonstrated that the transmission of the toxine to the central nervous system takes place by way of the motor nerves only. It is absorbed by the end organs of the motor nerves at the seat of infection, and travels by way of the axis cylinders to the susceptible nerve centres. These observers also give it as their opinion that "the greater part of the period of incubation is the expression of the time occupied in the conveyance of the toxine from the periphery along the motor nerves to the susceptible centres, and that the actual movement of the toxine takes place not in the lymphatics, but in the protoplasm of the nerves."

Within the last few years several experiments have been carried out which go to prove that part of the toxine may be absorbed by the lymph and blood channels, and as a proof in this direction, generalised tetanus can be produced in animals by injecting toxine into a vein.

Henri and Cernodeannu[11] are of the opinion that the toxine is conveyed by the blood and lymph streams in all cases.

In any case it is certain that no symptoms of the disease appear until the toxine produces its effects upon the cells of the nerve centres, brain, or spinal cord, or both.

In acute generalised tetanus the brain centres are always affected, but in local tetanus, which is common in experimental tetanus in guinea pigs, the toxine has only reached to the section of the spinal cord supplying motor nerves to the seat of infection, and when this is the case the disease is likely to become chronic and last for weeks.

Tetanus toxine is easily destroyed by heat. A temperature of 55°C. for 1½ hours renders it inert. A shorter time at 60°C., produces the same effect ; and a temperature of 75° C. removes every trace of its toxic properties in 5 minutes or less.

The pure spores of tetanus when freed from every trace of toxine are known as "washed spores" and in this condition they can be injected into animals without causing tetanus.

"Washed spores" can be obtained by filtering a pure tetanus culture, so as to separate the toxine, and then washing the precipitate on the filter several times with sterile normal saline solution, in order to remove every trace of toxine. Another method is to heat a pure bouillon culture of tetanus in which the spores are well formed at a temperature of 75°C. for 5 minutes.

Pure "washed spores" when obtained by either of these methods fail to convey tetanus when injected into guinea pigs or other susceptible animals.

The phagocytes at the seat of injection are capable of dealing with pure tetanus spores, and any which may escape phagocytosis get stowed or hidden away in the local tissues, to remain there alive for months, or years, until conditions become favourable for them to germinate, and grow out into toxine-producing bacilli.

When virulent "washed tetanus spores" are injected with any materials such as sterile sand, sterile powdered charcoal, or bacteria, which injure the tissues and handicap phagocytes, the spores grow out and tetanus is the result. A non-lethal dose of toxine mixed with washed spores produces fatal tetanus. In an experiment of this kind the toxine acts by producing a negative chemiotactic influence on the phagocytes, and the spores get an opportunity of growing out into bacilli. A tetanus infection can only take place when the spores germinate.

When quinine or lactic acid is mixed with washed tetanus spores and injected into animals, tetanus sets in rapidly, and is more certainly fatal, because both these substances destroy tissue at the seat of injection, and so favour the growth of tetanus bacilli.

A few tetanus spores gaining entrance to a wound which had not been badly contaminated with dirt and other micro-organisms would very likely get destroyed by the phagocytic action which would immediately set in; but in a badly contaminated wound, or one in which tissues were destroyed, or where anaerobic conditions and feeble phagocytosis prevailed, tetanus would result.

In wounds infected with tetanus spores another result is possible, *viz.*, a condition in which the spores remain latent or dormant, having escaped destruction by the phagocytes, and at the same time not finding suitable conditions for germinating into bacilli—a latent infection in which for some reason or other the spores for the time being failed to grow out into bacilli, and at the same time escaped phagocytosis.

In experimenting with tetanus spores, it is absolutely necessary to use pure cultures, and it is advisable to use cultures which have had ample time to form spores, and the strain should be virulent. A virulent strain grown at a temperature of 37°C., for 3—4 weeks, and under strict anaerobic conditions should answer well.

Young bacilli with little or no toxine might not produce the disease, although they would contain toxine locked up in their bodies (intracellular toxine). The addition of dilute lactic acid or chloroform water would extract the toxine from young bacilli or their spores, and when otherwise harmless young bacilli had been steeped in either of these fluids the mixture would be toxic, and produce tetanus.

In a virulent strain of tetanus, the toxine produced when a culture has been grown under the most favourable conditions, and for a month or six weeks, is fatal to susceptible animals such as mice or guinea pigs in extremely minute doses.

I have grown a tetanus culture in which $\frac{1}{1000}$ c.c. of the toxine free from bacilli was sufficient to kill a medium sized guinea pig, and cases are on record where a millionth of a c.c. was sufficient to kill mice, and weight for weight mice are not so susceptible as guinea pigs.

Burnet[12] states that weight for weight the animal most sensible to tetanus is the horse. He also gives the following comparison of sensibilities culled from the works of other authors: "If we express by the figure 1 the quantity lethal to a given weight of horse, the quantity which kills the same weight is 2 in the case of the guinea pig; 4 in that of the dog; 13 in that of the mouse; 2,000 in that of the rabbit; and 200,000 in that of the fowl; that is to say, the fowl is 200,000 times more resistant to tetanus than the horse."

The earliest symptoms of tetanus in the horse are lockjaw, stiffness of the neck, and difficulty in swallowing; and in man, and monkeys, lockjaw is also one

of the earliest symptoms. We know that the horse is extremely susceptible, probably one of the most susceptible animals to tetanus, and lockjaw being one of the earliest symptoms in man and the horse, we might infer from this that man is also very susceptible to tetanus.

Metchinkoff,[13] some years ago, demonstrated that alligators and tortoises are insensible to tetanus toxine. He injected large quantities into tortoises and found that these animals remained well and retained the toxine in their bodies for months.

The fowl can be rendered sensible to tetanus by placing it in a cold chamber, or making it stand in iced water; and some cold-blooded animals, such as frogs, can be rendered susceptible by placing them in a chamber heated to a temperature of 37°C.

When the symptoms of tetanus set in acutely, the death-rate is very high, over 90 per cent., but when symptoms set in slowly, and the disease becomes to a certain extent chronic, there are about 30 per cent. recoveries.

A form of tetanus known as "hydrophobic tetanus" following wounds on the face has been described. In this form the incubation period is short, owing to the fact that the toxine has only got a short distance to travel along the motor nerves before it reaches the cells of the brain. Spasm of the throat, resembling the spasms of hydrophobia, is an early symptom, hence the name "hydrophobic tetanus."

Another form known as "visceral tetanus" has been described and recognised by the French. In this form the infection originates in one of the internal organs. It is extremely fatal and the disease runs a very rapid course, the symptoms being somewhat masked and obscure, owing to the fact that the great splanchnic sympathetic nervous system is involved. Cases of uterine tetanus which have been known to follow criminal abortions would come under this heading.

McFarland[14] refers to a case of tetanus reported by Kamen in which the intestine of a patient dead of tetanus was found to be rich in tetanus bacilli. He gives this as an example of cases in which tetanus may possibly occur without a wound by absorption of the bacilli through abrasions in the mucous membrane. It is conceivable that a few tetanus spores absorbed from the intestinal tract, and carried by phagocytes to a part of the body where there was dead tissue (*e.g.*, the site of a quinine injection), would give rise to tetanus. Would this method of infection account for some of the cases in which quinine had been given?

CHAPTER III.

The effects of large doses of Quinine on small animals such as Rabbits and Guinea Pigs.

In these experiments no attempt was made to follow up the excretion of quinine from the system, or to enter minutely into its physiological effects. The principal objects were—

(1) to arrive at a dose, which, without proving fatal, would produce well marked visible effects;

(2) to arrive at some conclusion as to what the local effects of quinine are when given hypodermically;

(3) to prove whether the local and general effects of quinine when given hypodermically have any influence in favouring tetanus infections;

(4) to obtain information as to whether animals can withstand larger doses by the stomach than hypodermically;

(5) to gain some information on the effects of quinine when given intravenously.

The salt of quinine used in almost all cases was the bihydrochloride, and in the form of tabloids as prepared by Burroughs, Wellcome & Co., London.

The tabloids were dissolved in sterile normal saline solution in the proportion of 1 gr. to 1 c.c., a solution the acidity of which corresponds to 1·3 per cent. combined sulphuric acid, or 1 per cent. combined hydrochloric acid. A solution of this strength was chosen in order that the escharotic action might not be too severe when injected hypodermically, or into muscles. The solution used when the drug is administered hypodermically to malarial patients, is, I understand, invariably much stronger, and on this account the local effects on the tissues at the seat of injection must be very pronounced.

It will be noticed that in a few of the experiments (see Tables I and VII) the dilutions used were stronger, and in a few weaker than 1 gr. to 1 c.c. The objects in using a stronger dilution were to ascertain whether absorption would be slower or quicker—and whether the local effects would be to any appreciable extent more pronounced than when weaker dilutions were used; and in the few intravenous experiments in which weaker dilutions were used the object was to ascertain whether more quinine could be tolerated when given by this method in weak dilutions.

Rabbits and guinea pigs were the animals used. Both are susceptible to the action of quinine, whether given hypodermically, intravenously, or by the stomach;

but they can stand fairly large doses without any apparent injury other than the local effects when given hypodermically.

(a) Quinine hypodermically.

When large doses of quinine are given hypodermically to rabbits and guinea pigs, they rapidly come under its influence, and death takes place in a few hours. A large dose for a guinea pig would be 1 grain per 150 grammes weight of animal.

One grain per 150 grammes of guinea pig is a certain lethal dose; but I have known 1 grain per 233 grammes to prove fatal in some cases and not in others, so there is evidently an individual factor difficult to account for. On this account it would be impossible to arrive at a minimum lethal dose for all cases, and to express it in grains per grammes of guinea pig, and to say that this amount would kill with absolute certainty.

Some years ago, when it was the practice to standardise antityphoid vaccine by arriving at the minimum lethal dose for a guinea pig of a certain weight, a similar individual factor interfered with its accuracy, and to such an extent that the method had to be abandoned and other more accurate methods of standardising, such as counting the baciili, adopted.

On the other hand it is possible to come somewhere near a minimum lethal dose of quinine, which, without leaving much of a margin, would never fail to kill, and such a dose is 1 grain per 150 grammes of guinea pig. I have never known guinea pigs to survive this amount when given hypodermically, and in some cases smaller doses have proved fatal.

It is easy to arrive at a dose which will produce well-marked visible effects without killing the animal, and such a dose is 1 grain per 350 grammes of guinea pig. This amount can be given hypodermically for several days in succession without proving fatal, except perhaps in a few cases indirectly by the production of severe sloughs at the seat of injection and the suppuration which accompanies them.

When large doses are given to guniea pigs, symptoms set in within 10 minutes. The symptoms take the form of convulsive muscular spasms, which set in gradually and soon become more pronounced and severe, and the animal dies asphyxiated. For some time before death the animal lies in its cage, but slight twitching of the muscles and limbs are apparent, and when disturbed these become more severe. The rapidity and severity with which symptoms set in and prove fatal depends on the amount of quinine given. When 1 grain per 150 grammes of weight is given symptoms set in within ten minutes, and a fatal result may be expected any time between 4 and 24 hours. When larger doses are given symptoms appear a few minutes earlier, and death takes place in all cases under 24 hours.

A glance at the experiments detailed in Table I will serve to explain all that is necessary on the subject.

In Table II the results of four experiments with the bihydrochlorate of quinine are given, and it will be seen that they differ in no material point from those of the bihydrochloride used in all the other experiments, and on this account it was deemed advisable to use only the latter salt of quinine, as it is the one most generally used for hypodermic injections in cases of malaria.

The local effects were in all cases very evident. When the animals died before a slough had time to form, the tissues at the seat of injection presented the appearance of having been burnt with pure carbolic acid. A white escharotic patch was never absent, and one could trace exactly the extent to which the fluid had been distributed. In those experiments in which the animals survived for a few days or survived altogether, it was quite the exception for a local slough not to form. These sloughs included the true skin, subcutaneous tissue, and deep fascia, and exposed the muscles; and in animals which survived it required weeks before healing was complete.

In the few experiments recorded in Table III, in which the quinine was given intramuscularly, the general and local effects differed in no way from the subcutaneous experiments, except that the animals appeared to suffer more pain. I believe that the experience of malarial patients who have had intramuscular injections of quinine is to the effect that the subsequent pain and discomfort are worse than when given hypodermically.

In rabbits 6 grains per kilogramme of body weight is a certain lethal dose when given hypodermically, and in some cases less would suffice. Here again there is an individual factor difficult to account for. In Table IV it will be seen that in one experiment a 1,410 gramme rabbit survived for 4½ hours after receiving 10 grains; whereas another rabbit weighing 1,500 grammes which received the same amount of quinine died after 30 minutes. In another experiment a 1,500 gramme rabbit died 2½ hours after 8 grains, and a rabbit of the same weight showed practically no symptoms after 6 grains.

Six grains per kilo. is a lethal dose for a rabbit when given hypodermically, and in many cases 5 grs. per kilo. would suffice. Three grains per kilo. produces symptoms of excitement without ever proving fatal.

When a lethal dose is given to a rabbit, symptoms set in after 10 or 15 minutes. These symptoms always commence by quickened respiration, and, soon after, some of the animals develop muscular twitchings and spasms, but not so marked as in the case of guinea pigs. Before death they lie helpless for a variable period, and die asphyxiated, the heart continuing to beat for some minutes after respiration has ceased.

The local action on the tissues is the same as in guinea pigs, and when a non-lethal dose is given a slough invariably forms, but much earlier than in the case of guinea pigs.

(b) Quinine by the mouth.

Rabbits and guinea pigs can survive larger doses introduced into the stomach than hypodermically. In the case of rabbits the lethal dose by the stomach would at least be double the hypodermic lethal dose, but in the case of guinea pigs a lethal dose would prove fatal when increased by $\frac{1}{5}$ to $\frac{1}{4}$ its amount and given by the stomach. (See Tables V and VI.)

In guinea pigs the symptoms produced resemble those following hypodermic injections, and set in with the same rapidity, but are not quite so severe unless very large doses are given; whereas in rabbits the symptoms are hardly noticeable, except when large doses are given, when they lie down on their stomachs, stretch out their legs, and respire with difficulty.

The results of the experiments recorded on Tables IV and V indicate a marked difference between the lethal doses for a rabbit hypodermically and by the stomach. Twelve grains is a certain lethal dose for a 2,000 gramme rabbit when given hypodermically, but a rabbit of the same weight can survive 25 grains when given by the stomach. Compared with rabbits, the difference in guinea pigs is only slight. A 500 gramme guinea pig can survive 4 grains by the stomach, when $3\frac{1}{2}$ grains given hypodermically kills an animal of the same weight.

Evidently weight for weight these small animals are far more tolerant of quinine than man.

A guinea pig which, roughly speaking, weighs only 1lb. (500 grammes) can survive 4 grains by the stomach, and if man, weight for weight, could withstand the same amount, this would mean that a man weighing 11 stone could survive a dose of 616 grs. of quinine by the stomach, which is impossible; even a quarter of this amount would almost be a poisonous dose for a man.

(c) Quinine intravenously.

The intravenous method of giving quinine was tested on rabbits only. In these animals a small dose given into a vein kills quickly. One grain in 1 c.c. normal saline solution will in 9 cases out of 10 kill a rabbit weighing from 1,700 to 2,000 grammes, and any slight increase in this amount would prove fatal within one minute.

0·5 grain of quinine per kilogramme represents about the lethal dose for a rabbit when given intravenously.

Rabbits weighing from 1,200 to 1,500 grammes can bear $\frac{1}{2}$ grain dissolved in $\frac{1}{2}$ c.c. normal saline solution, without showing any very noticeable symptoms. When large doses such as 0·75 grains per kilo. are given, the animals commence to die immediately the injection is completed, and in most cases they are dead within one minute. Respiration suddenly ceases, but the heart goes on beating for some minutes.

A more dilute solution of the quinine salt and a proportional diminution of its acidity makes no difference in its killing properties. (See the experiments detailed in Table VII and compare experiments 4 and 6 with 7 and 8.)

From this fact one would infer that the lethal effects are due to the quinine and not to the acidity of the solution.

In order to make this point certain, the series of experiments detailed in Table VII were devised. As I have already mentioned, the quinine was used in the proportion of 1 grain to 1 c.c. normal saline solution, and the acidity of a solution of this strength corresponds to 1·3 per cent. H_2SO_4, or 1 per cent. H.Cl.

Rabbits weighing from 1,400 to 1,500 grammes can withstand intravenously 1 c.c. of normal saline solution containing either of these mineral acids in the percentages given (1·3 per cent. H_2SO_4 or 1 per cent. H.Cl.) They can also withstand 2 c.c. of normal saline solution containing 1½ per cent. of either acid and show no symptoms of any kind, and this is an acidity much stronger and in a larger bulk of fluid than the lethal quinine dose in a dilution of 1 grain in 1 c.c.

Stronger dilutions of H_2SO_4, such as 1½ c.c. of a 5 per cent. dilution kill very quickly, but the same amount of 5 per cent. dilution of H.Cl. gives rise to no apparent symptoms. (See the experiments detailed in Table VIII.)

Any blood changes which may occur after the intravenous injection of quinine in rabbits are not apparent when rapidly fatal doses are given, but when several non-lethal doses are given traces of hæmolysis are to be found in the supernatent fluid after centrifuging a sample of blood with an equal volume of 1½ per cent. citrate of soda in normal saline solution; when another sample is taken and allowed to clot, clotting takes place as usual, and the serum is tinged red.

Stained specimens appear normal, and leucocytes are present in the proportion found in normal rabbits.

When ½ grain doses are given intravenously every 10 minutes, symptoms of quinine poisoning set in after the second dose and become more pronounced after every subsequent dose, and the animal dies asphyxiated after the eighth or ninth dose. In an experiment of this kind (No. 11, Table VII) there were no apparent blood changes after the sixth and seventh doses, but 30 minutes after the ninth dose, the animal died, and evidence of hæmolysis was apparent in specimens taken from the heart immediately after death. The blood clotted like a normal specimen, but the serum was tinged red, and so was the supernatent fluid of another specimen after centrifuging with an equal volume of 1½ per cent. of citrate of soda in normal saline solution, specimens taken at the same time and stained appeared normal. See Table VII for results of intravenous injection.

When large hypodermic doses are given to rabbits, traces of hæmolysis will be found after a few hours, should the animals survive for so long; and in those that succumb similar changes will be found immediately after death, but nothing abnor-

mal appears in stained specimens. See Table IX for blood changes after large hypodermic doses.

Is there any tolerance to large doses of quinine ?

A non-lethal dose of quinine given hypodermically to a rabbit one day, does not establish any tolerance to what would, in the ordinary course of events, prove a lethal dose given the next day. When the interval between the first and second doses is extended to a fortnight a similar absence of any tolerance is evident. (See Table IX.)

Table I.

Experiments on Guinea Pigs to test the effects of Quinine when dissolved in normal saline solution and given hypodermically.

No. of experiment.	Weight of guinea pig.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1	500	Bihydrochloride.	3	3	Muscular spasms after 10 minutes, severe and continued for several hours. Appeared well next day.	Died after 9 days.	Skin and tissues sloughed at seat of injection.
2	500	Ditto.	5	5	Severe muscular spasms after 7 minutes.	Died after 4 hours.	Escharotic action on tisses at seat of injection.
3	500	Ditto.	3	3	Muscular spasms after 8 minutes ; looked like dying after 5 hours ; began to improve after 6 hours ; seemed well next day.	Died after 38 days.	General wasting.
4	500	Ditto.	3	3	Ditto ditto .	Died after 36 days.	Ditto ditto.
5	500	Ditto.	3½	3½	Muscular spasms after 15 minutes. Lay helpless in cage with twitching of muscles after 6 hours.	Died after 9 hours.	Escharotic action on tissues at seat of injection.
6	320	Ditto.	3	3	Muscular spasms after 7 minutes.	Died after 4 hours.	Ditto ditto.
7	350	Ditto.	2	3	Muscular spasms after 10 minutes.	Died after 17 hours.	Ditto ditto.
8	300	Ditto.	2½	2½	Ditto ditto .	Died after 14 hours.	Ditto ditto.
9	390	Ditto.	1	1	Muscular twitching and excited after 10 minutes. Apparently well after 2 hours.	Survived .	Oedema and tenderness at seat of injection.
10	350	Ditto.	1	1	Ditto ditto .	Ditto .	Skin and tissues sloughed at seat of injection.

Table I—*contd.*

Experiments on Guinea Pigs to test the effects of Quinine when dissolved in normal saline solution and given hypodermically—*contd.*

No. of experiment.	Weight of guinea pig.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
11	350	Bihydrochloride.	1½	1½	Convulsions after 10 minutes, severe for 3 hours.	Died after 20 hours.	Marked escharotic action on tissues at seat of injection.
12	300	Ditto.	2	2	Ditto ditto .	Died after 3¼ hours.	Ditto ditto.
13	350	Ditto.	1	½	Muscular twitching after 10 minutes.	Survived .	Skin and tissues sloughed at seat of injection.
14	350	Ditto.	1½	¾	Convulsions after 10 minutes ; severe for 4 hours.	Survived .	Local sloughing.
15	350	Ditto.	2	1	Severe convulsions after 10 minutes.	Died after 24 hours.	Severe escharotic action at seat of injection.

It is evident from this series of experiments that 1 grain of quinine bihydrochoride per 150 grammes of guinea pig is a certain lethal dose when given hypodermically ; and in some cases a smaller amount would suffice.

Table II.

Experiments on Guinea Pigs to test the effects of Quinine when dissolved in normal saline solution and given hypodermically.

No. of experiment.	Weight of guinea pig.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1	500	Bihydrochlorate.	4	4	Severe muscular spasms after 10 minutes, tetanic in character and continued for hours ; seemed well next day except for pain and thickening at the seat of injection.	Died after 4 days.	Sloughing took place at seat of injection.
2	350	Ditto.	5	5	Muscular spasms after 8 minutes. Severe in character and lasting until death.	Died after 7 hours.	Tissues at seat of injection necrosed.
3	340	Ditto.	2	2	Muscular spasms after 10 minutes, severe in character.	Died after 2 days.	Ditto ditto.
4	440	Ditto.	1	1	Excited after 10 minutes, no spasms.	Survived .	Ditto ditto.

It will be seen that the results recorded in this table in which bihydrochlorate of quinine was used, differ in no material point from those in which the bihydrochoride was used.

Table III.

Experiments on Guinea Pigs to test the effects of Quinine when dissolved in normal saline solution and given intramuscularly.

No. of experiment.	Weight of guinea pig.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1	440	Bihydrochloride.	3	3	Severe muscular spasms after 10 minutes.	Died after 24 hours.	Marked swelling in muscles of thigh at seat of injection, and gelatinous exudation.
2	430	Ditto.	2	2	Muscular spasms after 8 minutes.	Died after 16 hours.	Ditto ditto.
3	400	Ditto.	1	1	Muscular twitchings after 8 minutes. Seemed well next day.	Survived .	Pain and swelling in muscles of thigh at seat of injection for several days, a sloughing sore on the 5th day.

The general effects after intramuscular injections resemble those produced by hypodermic injections, and set in with the same rapidity.

Table IV.

Experiments on Rabbits to test the effects of Quinine when dissolved in normal saline solution and given hypodermically.

No. of experiment.	Weight of rabbit.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1	1,320	Bihydrochloride.	3	3	Refused its food for a day; no other symptom.	Survived .	Local tenderness and thickening at seat of injection.
2	1,400	Ditto.	5	5	Ditto ditto . .	Ditto .	Ditto ditto.
3	1,410	Ditto.	10	10	Quickened respiration commenced 10 minutes after dose. Lay helpless in cage after one hour.	Died after 4 hours.	Escharotic action at seat of injection.
4	1,500	Ditto.	10	10	Quickened respiration after 10 minutes, then lay helpless in cage.	Died after 39 minutes.	Ditto ditto.
5	1,500	Ditto.	8	8	Excited after 10 minutes, quickened respiration after 30 minutes.	Died after 2½ hours.	Ditto ditto.
6	1,500	Ditto .	6	6	Excited after 30 minutes; apparently well after 2 hours.	Survived .	Slough at seat of injection.

Six grains per kilogramme of rabbit is a certain lethal dose, but in some cases less would suffice.

Table V.

Experiments on Rabbits to test the effects of Quinine when dissolved in normal saline solution and given by the stomach.

No. of experiment.	Weight of rabbit.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1	2,060	Bihydrochloride.	30	30	After 20 minutes lay down in cage, and breathing difficult. Apparently well one hour after dose.	Died after 12 hours.	Sloughy patch of mucous membrane near duodenal end of stomach exposing a local submucous hæmorrhage.
2	2,050	Ditto.	15	15	After 20 minutes, quickened respiration. Seemed well after one hour.	Survived.	
3	2,000	Ditto.	20	20	Ditto ditto . .	Ditto .	After three weeks wasting set in and death took place at the end of a month. *P. M.* disclosed no apparent disease.
4	2,000	Ditto.	25	25	Ditto ditto . .	Ditto .	

15 grains per kilogramme of rabbit is a certain lethal dose when given by the stomach; less would suffice in some cases.

Table VI.

Experiments on Guinea Pigs to test the effects of Quinine when dissolved in normal saline solution and given by the stomach.

No. of experiment.	Weight of guinea pig.	Salt of quinine used.	Amount given.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1	470	Bihydrochloride.	3	5	Slight spasms on handling after 2½ hours, but these soon disappeared. Progressive emaciation set in after the 2nd day.	Died after 10 days.	General wasting.
2	500	Ditto.	5	5	Severe spasms after 10 minutes (convulsions).	Died after 4½ hours.	Stomach normal.
3	500	Ditto.	4	4	Excited after 20 minutes, slight spasms after 1 hour. Seemed well after 3 hours.	Survived.	
4	500	Ditto.	3	3	Excited after ¾ hour. Seemed well after 2 hours.	Ditto.	

1¼ grs. per 150 grammes of guinea pig is a certain lethal dose when given by the stomach; but in some cases less would suffice.

Table VII.

Experiments on Rabbits to test the effects of Quinine when dissolved in normal saline solution and given intravenously.

No. of experiment.	Weight of rabbit.	Salt of quinine used.	Amount given.	Amount of fluid in which dissolved.	Symptoms produced.	Result.	Remarks.
1	grms. 1,300	Bihydrochloride.	grs. 3	c.c. 3	Respiration suddenly ceased.	Died within one minute.	*P. M.* immediately after death. Blood appeared normal. No clots in heart or large vessels. No hæmolysis. Stained specimens of blood normal.
2	1,400	Ditto.	2½	2½	Ditto ditto . .	Ditto .	Ditto ditto.
3	1,990	Ditto.	2	2	Ditto ditto . .	Ditto .	Ditto ditto.
4	1,740	Ditto.	1	1	Ditto ditto . .	Ditto .	Ditto ditto.
5	1,970	Ditto.	½	½	Slight difficulty in breathing for a few minutes after injection. Seemed well after 15 minutes.	Survived	A capsule of blood gave normal coloured serum on clotting. A stained specimen of blood appeared normal. No hæmoloisis.
6	1,760	Ditto.	1	1	After a minute it rolled over and struggled for breath and looked like dying; began to recover after 5 minutes but breathed rapidly for 20 minutes; seemed well after 30 minutes.	Recovered	Tested blood 5 hours after quinine. Stained specimen normal. Clotting and serum normal. Traces of hæmolysis on centrifuging with citrate of soda solution.
7	1,750	Ditto.	1	2	Slight difficulty of respiration for a minute after injection but no other symptom.	Ditto .	Blood same as experiment 6.
8	1,570	Ditto.	1	2	Respiration suddenly ceased.	Died within one minute.	*P. M.* immediately after death. No clots in heart or large vessels. Blood normal in appearance. Stained specimens normal. Clotted normally. No hæmolysis.
9	2,170	Ditto .	2	6	Struggled for breath for 30 seconds, then breathing suddenly ceased.	Died within one minute.	*P. M.* and condition of blood same as experiment 8.
10	1,450	Ditto.	2½	1½ given in ½ c.c. doses (½ gr.) at intervals of 5 minutes.	Slight difficulty of breathing for a minute or two after each injection; otherwise no symptoms.	Survived	A capsule of blood gave normal coloured serum on clotting. A stained specimen of blood appeared normal. No hæmolysis.

Table VII—*contd.*

Experiments on Rabbits to test the effects of Quinine when dissolved in normal saline solution and given intravenously—*contd.*

No. of experiment.	Weight of rabbit.	Salt of quinine used.	Amount given.	Amount of fluid in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
11	1,760	Bihydrochloride.	4½	4½ c.c. given in ½ gr. doses every 10 minutes.	None after first dose, but respiration quickened, and marked respiratory difficulty after all the other doses, and lasting from 5 to 8 minutes. These symptoms increased after the 9th dose (last dose), and continued for 30 minutes, when death suddenly took place.	Died 30 minutes after last dose.	Blood tested after the 6th and 7th doses appeared normal, as regards clotting, serum and stained specimens. No hæmolysis. *P. M.* immediately after death. No clots in heart or large vessels, and blood normal in appearance and in stained specimens. A sample taken in a glass capsule clotted in the usual time, but serum slightly tinged red (hæmolysis). On centrifuging a small quantity in 1½ per cent. citrate of soda in normal saline, the supernatent fluid was slightly tinged red, showing evidence of hæmolysis.

¾ gr. per kilogramme of rabbit is a certain lethal dose when given intravenously; but in some cases less would suffice.

Table VIII.

Experiments on Rabbits to test the effects of Sulphuric or Hydrochloric Acid when given intravenously.

No. of experiment.	Weight of rabbit.	Dilution of sulphuric or hydrochloric acid in normal saline solution.	Amount given.	Symptoms produced.	Result.	Remarks.
	grms.		c.c.			
1. Sulphuric acid.	1,450	5 per cent.	1½ into vein of ear.	Breathing suddenly ceased.	Died within one minute.	*P. M.* immediately after death. Blood in jugular vein leading from ear-vein injected was thick, black, and tarry looking. Heart's blood was dark and contained some loose dark clots.

Table VIII—*contd.*

Experiments on Rabbits to test the effects of Sulphuric or Hydrochloric Acid when given intravenously—*contd.*

No. of experiment.	Weight of rabbit.	Dilution of sulphuric or hydrochloric acid in normal saline solution.	Amount given	Symptoms produced.	Results.	Remarks.
2. Sulphuric acid.	1,420	2½ per cent.	1½ c.c. into vein of ear.	Difficulty of respiration for a minute or so; no other symptom.	Remained well.	
3. Ditto .	1,350	1½ per cent.	2 ditto .	*Nil* . .	Ditto.	
4. Hydrochloric acid.	1,390	5 per cent.	1½ ditto .	*Nil* . .	Ditto.	
5. Ditto .	1,410	5 per cent.	2 ditto	*Nil* . .	Ditto.	
6. Ditto .	1,550	1½ per cent.	2 ditto .	*Nil* . .	Ditto.	

This table proves that the results of the intravenous injections of quinine recorded in Table VII were due to the quinine, and not to the acidity of the dilution; because, when quinine bihydrochloride is dissolved in normal saline solution in the proprotion of 1 gr. to 1 c.c. the acidity as represented by the amount of combined acid is equal to 1·3 per cent. H_2SO_4, or 1 per cent. H. Cl.

It is evident that H_2SO_4 is more poisonous for rabbits when given intravenously than H. Cl. 1½ c.c. of a 5 per cent. H_2SO_4 killed a 1,450 grammes rabbit within 1 minute, whereas 1½ c.c. and 2 c.c. of a 5 per cent. H. Cl. produced no symptoms in rabbits weighing 1,390 and 1,410 grammes respectively. See experiments 1 and 4 and 5.

Table IX.

Experiments on Rabbits to prove whether a non-lethal dose of Quinine given one day would establish a tolerance to a lethal dose given the next day, or at a later period.

No. of experiment.	Weight of rabbit.	Salt of quinine used.	Amount given and dates.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
	grms.		grs.	c.c.			
1 . .	1,840	Bihydrochloride	24th August . 7	7	No apparent symptoms.	Not ill after 1st dose.	
		Ditto .	25th August . 12	12	Very quickened breathing after 15 minutes.	Died ¾ hours after 2nd dose.	*P. M.* immediately after death. Heart continued to beat several minutes after death. Blood apparently normal, no clots in heart or large vessels. Traces of hæmolysis on centrifuging a specimen of blood in 1½ per cent. citrate of soda in normal saline solution.

Table IX—*contd.*

Experiments on Rabbits to prove whether a non-lethal dose of Quinine given one day would establish a tolerance to a lethal dose given the next day, or at a later period—*contd.*

No. of experiment.	Weight of rabbit	Salt of quinine used.	Amount given and dates.	Quantity of normal saline in which dissolved.	Symptoms produced.	Result.	Remarks.
			grs.	c.c.			
2 . .	1,870	Bihydrochloride	24th August . 9	9	Respiration quickened after one hour; apparently well after 2 hours.	Well 2 hours after 1st dose.	
		Ditto .	25th August . 12	12	Rapid breathing after 10 minutes.	Died 1½ hours after 2nd dose.	*P. M.* immediately after death. Same condition found as in No. 1.
3 . .	1,650	Ditto .	11th August . 6	6	Excited after 30 minutes. Seemed well after 1 hour.	Well 1 hour after 1st dose.	
		Ditto .	26th August . 10	10	Rapid respiration after 40 minutes; very ill after 2 hours; seemed dying after 5 hours; began to improve after 6 hours.	Recovered from the effects of 2nd dose.	Blood normal before 10 grs. quinine given, but slight hæmolysis 5 hours after. No hæmolysis 3 days afterwards. Severe sloughing at seat of injection.
4. Control	1,880	Ditto .	25th August . 12	12	Difficulty in breathing after 40 minutes. This difficulty increased until death.	Died after 4 hours.	*P. M.* immediately after death. No clots in heart or large vessels. Blood apparently normal, clotted quickly and serum slightly tinged red. Slight hæmolysis on centrifuging a specimen in 1½ per cent. citrate of soda in normal saline solution.

The experiments in this table furnish no evidence of any tolerance having been established.

CHAPTER IV.

Remarks preliminary to a Detailed Examination of the Experiments recorded in the next chapter.

Before dealing with the experiments in which tetanus spores and quinine were used, it may perhaps be well to recapitulate a few of the leading points already mentioned.

When tetanus spores are carefully prepared and freed from any traces of toxine or contaminations of any kind, they may be injected into susceptible animals without producing tetanus. This is an old and well known experiment, and never fails when properly carried out. Its success depends on the fact that the phagocytes can pick up and digest tetanus spores in the absence of any irritant or other material likely to distract them ; but when virulent spores mixed with toxine, other bacteria, or other spores, sterile powdered charcoal, sterile sand, quinine, lactic acid, or anything which keeps away phagocytes, or occupies their attention, are injected hypodermically into susceptible animals such as guinea pigs, or monkeys, tetanus is the result.

These are facts which justify the statement that tetanus spores alone do not produce tetanus. Before spores can give rise to tetanus they must germinate and grow into bacilli, and these bacilli must elaborate an extra-cellular toxine which is responsible for the disease and all its symptoms.

Now the question arises, when tetanus spores are injected hypodermically, are they in all cases picked up and destroyed by the phagocytes ? or are some of them stowed away locally, to remain dormant until some irritant or some other depressing influence produces favourable conditions for their liberation and growth into toxine producing bacilli ? Would a similar locking-up of tetanus spores take place when wounds or abrasions are infected with spore containing material ?

The experiments recorded in this paper in addition to proving the influence of quinine in favouring tetanus infections, will also throw some light on these obscure points.

In some of these experiments tetanus cultures were used, but in most of them washed spores, with and without quinine were used, the object being to prove whether quinine helped in any way in the production of tetanus, and if so, what was the nature of the assistance, and how it was brought about.

To save unnecessary repetition and at the same time to explain what actually takes place when quinine and tetanus spores are injected into different parts of the body of an animal, it may perhaps be well briefly to follow up each injection separately.

1. *Quinine.*—When quinine is injected hypodermically into guinea pigs, it soon becomes absorbed, but immediately on injection and before absorption has taken place, the tissues at the site of injection are severely damaged. This local destruction of tissues is so complete that in most cases a slough forms, which involves the skin and comes to the surface, leaving an open sore within a few days from the time the quinine was given. The acidity of the solution may to a certain extent account for this local action, but it happens also when "Quinoforme Lacroix," a patent French preparation absolutely neutral in reaction, is given.

The effect of a localised necrotic subcutaneous focus would be to establish a suitable anærobic medium for the growth of tetanus spores should they by any chance become lodged there. At the same time, one of the immediate effects of a large dose of quinine would be to produce a more or less paralysing effect on the phagocytes generally, and a negative chemiotaxis on the phagocytes locally, conditions which would still further the growth of any tetanus spores which happened to get lodged at the spot.

2. "*Washed tetanus spores.*"—Vaillard and Vincent[15] have proved that when "washed tetanus spores" are injected hypodermically into guinea pigs, they are picked up and digested by the phagocytes which crowd to the seat of injection, provided there is nothing introduced with the spores to destroy tissue, produce a negative chemiotaxis, or in any way to divert the phagocytes from efficiently performing their function of bactericidal agents.

It may happen even with active phagocytes that some of the spores escape destruction, and become stowed away locally, to grow and produce infection at some future time when conditions become favourable. It may also happen that some of the phagocytes require a considerable time to kill off and digest the toughest of the spores, and that phagocytes in this condition on re-entering the circulation may get stranded or lodged in a suitable site where the spores can grow and give rise to tetanus. The subcutaneous or intramuscular injections of quinine would produce such foci provided spore-laden phagocytes become lodged there.

On the assumption that this is what happens when quinine and tetanus spores are injected into separate parts of the body, one would expect more infections in susceptible animals when the quinine is injected on the day before, or at the same time as the spores, and fewer infections when the spores are injected a day or more before the quinine. Also one would expect never to fail in producing tetanus when solutions of quinine or other irritants such as lactic acid are injected mixed with the spores.

On the other hand, one would expect to fail in producing tetanus when non-irritants (which become rapidly absorbed), such as morphia or sterile normal saline solution, are injected mixed with washed tetanus spores. As a matter of fact the accuracy of these conjectures is verified by experimental tests as will be seen by

referring to the numerous experiments detailed in the succeeding part of this paper.

In order to bring facts to bear upon what has been briefly outlined in the foregoing remarks, it will be necessary to take up and deal *seriatim* with the experiments upon which these conjectures and explanations are based.

In the first and second series of experiments the virulence of the strain of tetanus used had become attenuated, and the subsequent experiments were carried out with another strain, which possessed a higher degree of virulence, but even this strain was not very virulent.

Care was taken to use only absolutely pure cultures. The purity of every culture used was controlled by examining stained specimens under the microscope, and planting out fresh cultures. In every case in which "washed spores" were used, a control cultivation was made to prove that the spores were alive and pure. The "washed spores" were used immediately after their preparation, in order to avoid any subsequent chance of growth or contamination taking place, as either of these mishaps would have vitiated the experiments. This necessitated the preparation of a large number of tetanus cultivations, so as always to have a culture at a suitable stage of growth when required.

The "washed spores" were prepared as follows: Ten c.c. anærobic broth cultures which had been grown for a month at a temperature of 37° C. were used; 2 c.c. were pipetted off from the bottom of a culture and transferred to a sterile test tube, and then heated in a water bath at a temperature of 75° C. for 5 minutes. Before heating, a specimen was stained and examined to test for purity, and also a cultivation made so as to be available when required. Another cultivation was made after heating tc prove that the spores were alive, and of course this culture could also be utilised when it had sufficiently grown. When broth cultures of tetanus have been allowed to incubate at a temperature of 37° C. for a month, the spores are well formed and resistant, and by that time they have sunk down to the bottom of the tube, so that, provided the tube has not been shaken, it is possible practically to remove nearly all the spores by pipetting off the fluid from the bottom part of the cultivation.

When 2 c.c. are pipetted off from a 10 c.c. culture for heating purposes, and when the same quantity of this heated fluid containing spores is used for a series of experiments, each anima! experimented on receives practically the same amount of spores; this is what was done in all cases in which washed spores were used. Guinea pigs received 0·25 c. c. and in the case of monkeys $\frac{1}{2}$ c. c. was given.

The strictest aseptic precautions were observed in the preparation and administration of quinine, spores, tetanus cultures, or other materials used, and in no instance were control experiments omitted.

The animals experimented upon were in most cases full grown, and in all cases healthy, they were kept isolated in separate cages, and under the observation

of trustworthy attendants night and day. At night they were visited every two hours by a man on duty, and during the daytime they were under my own observation, but there was always an attendant on duty to note down anything that happened and bring it to my notice.

The details of these experiments are plainly given in the Tables in which they are recorded, so that only a few remarks are necessary to draw attention to each series separately.

CHAPTER V.

A Series of Experiments recorded in Tables I to XIX.

THE 1ST SERIES OF EXPERIMENTS.

In this series four guinea pigs were inoculated with "washed tetanus spores" under the skin of the hind leg, and next half a grain of quinine was injected hypodermically on the front of the chest, and repeated for three days. On the fourth day one animal developed tetanus, and died three days later. The other three animals remained well. Two control guinea pigs inoculated with "washed tetanus spores" only, remained well. Another control inoculated with 0·5 c.c. of the tetanus culture from which the spores were prepared developed tetanus on the fourth day, and died next day.

It will be seen from this last experiment that the tetauns culture used was not very virulent, otherwise it would not have required four days for 0·5 c.c. to produce tetanus in a guinea pig.

The results of the experiments recorded in this table show that when quinine is injected hypodermically into guinea pigs it produces conditions favourable for infection with "washed tetanus spores," even when the spores are of low virulence.

Table I.

Experiments on Guinea Pigs to demonstrate the effects of Quinine in producing favourable conditions for infection with "Washed Tetanus Spores," when Spores are given one day before Quinine.

No. of experiment.	Date of injection of spores.	Date of injection of quinine.	Amount of quinine injected.	Result.	REMARKS.
			gr.		
1 . .	1st March 1910, right *hind leg.*	2nd March 1910.	1	Tetanus, 5th March.	Symptoms local at outset, then became general. An anærobic culture made from the tissues at the site of injection of spores grew tetanus bacilli.
		3rd March 1910.	½ on front of *chest.*	Died, 8th March.	
		4th March 1910.	½		
2 . .	Ditto . .	Ditto .	Ditto .	Remained well.	
3 . .	Ditto . .	Ditto .	Ditto .	Ditto.	
4 . .	Ditto . .	Ditto .	Ditto .	Ditto.	
5 and 6 . (Control of spores.)	Ditto . .	..	..	Both controls remained well.	
7 (Control of the culture from which spores were prepared.)	0·5 c.c. tetanus culture, 1st March.			Tetanus, 4th March. Died, 5th March.	Symptoms local at first, then became general.

The culture of tetanus used for this series of experiments was of low virulence, as evidenced by the fact that three days elapsed before ½ c.c. produced tetanus in a guinea pig. (See No. 7 control experiment.) This series shows 25 per cent. of infections when spores were given one day before quinine; the spores were injected in the hind leg, and quinine on front of the chest.

THE 2ND SERIES OF EXPERIMENTS.

A series of experiments in which quinine injections were commenced one day before spores were given.

Three guinea pigs were each given half a grain of quinine hypodermically on the front of the chest, and next day "washed tetanus spores" hypodermically in the hind leg. The quinine injections in ½ grain doses were continued for a week.

On the 10th day from the time the spores were given one animal developed tetanus in the leg into which the spores had been injected, and next day the tetanus symptoms had extended to the side, and the disease became chronic, and lasted for a month, when recovery took place.

The other two animals remained well, as also did the control guinea pig which received spores only.

The control which received 0·5 c. c. of the culture from which the spores were prepared contracted tetanus on the second day, and died three days later.

The results of the experiments recorded in this table show that when quinine is injected hypodermically into guinea pigs it produces conditions favourable for infection with "washed tetanus spores."

Table II.

Experiments on Guinea Pigs to demonstrate the effects of Quinine in producing favourable conditions for infection with "Washed Tetanus Spores" when Quinine is commenced one day before Spores are given.

No. of experiment.	Date of injection of spores.	Date of injection of quinine.	Amount of quinine injected.	Result.	REMARKS.
1 . .	4th March 1910, right *hind leg.*	3rd to 10th March.	½ gr. daily on front of *chest.*	Tetanus, 10th March. Severe and localised to right leg and right side; became chronic, and after a month recovered.	Disease localised to the side on which spores were injected.
2 . .	Ditto .	Ditto .	Ditto .	Remained well.	
3 . .	Ditto .	Ditto .	Ditto .	Ditto.	
4 (Control of spores).	Ditto .	..	..	Ditto.	
5 (Control of the culture from which spores were prepared.)	0·5 c.c. tetanus culture, 4th March.	..	..	Tetanus, 5th March. Died, 8th March.	Symptoms local at first, then became general.

This series shows 33·3 per cent. of infections when quinine was commenced one day before spores were given: the spores were injected in the hind leg, and quinine on front of the chest.

THE 3RD SERIES OF EXPERIMENTS.

This series is a repetition of the 2nd series, but a more virulent strain of tetanus culture was used for the preparation of the "washed spores."

Four guinea pigs were given ½ gr. quinine hypodermically on front of the chest, and next day "washed tetanus spores" hypodermically in the right hind leg. The quinine treatment was continued daily in ½ gr. doses in one animal for three days, and in the other three for five days. No. 1 which had quinine for three days developed tetanus on the third day after spores were given and died on the fifth day. No. 2 developed tetanus on the fifth day after spores were given, and died on the thirteenth day. Nos. 3 and 4 developed tetanus on the sixth day after spores were given, and both these animals passed through a severe attack of local tetanus, which involved the right leg and right side of the body for three weeks, when they eventually recovered.

Two control guinea pigs inoculated with "washed spores" only, remained well, and the control culture guinea pig developed tetanus after 24 hours and died next day.

In the No. 2 guinea pig of this series, local tetanus developed on the fifth day after spores were given, and the disease remained localised to the leg inoculated with spores for nine days, after which acute generalised tetanus set in, and the animal died two days later. Immediately after death, anærobic cultivations in broth tubes were made from seat of injection of spores (right hind leg); seat of injection of quinine (chest); spleen; liver; lungs; kidneys; heart's blood. When these cultivations had been incubated for a month at 37°C. they were opened and examined with the following results :—

> A rich growth of tetanus bacilli with well formed spores from the seat of injection of quinine on the chest, and also from the seat of injection of spores in the leg. A few micrococci in the cultivation from the lungs, and the remaining four cultivations from the liver, spleen, kidneys, and heart's blood were sterile.

After this result it was deemed advisable to test further whether tetanus germs can be conveyed from a remote site of infection such as the leg, to another part of the body such as the chest, where quinine has been injected. The results of the experiments detailed in Tables VIII, IX and XII prove that this is what happens in probably every case, and that a hypodermic injection of quinine establishes a very favourable local site for the growth of tetanus germs should any ever reach the spot. A phagocyte carrying a few tetanus spores which happened to get stranded in such a site would prove the starting point of a fresh focus of infection.

Is this what happened in No. 2 guinea pig of this series when it had chronic and localised tetanus for nine days, and then acute and fatal symptoms suddenly set in? In 1904, Vincent,[16] when experimenting with quinine and tetanus infections,

also obtained results which favour the view that quinine injections produce foci favourable for the growth of tetanus bacili, and that infection may be conveyed from the original site of the disease to these foci.

Table III.

Experiments on Guinea Pigs to demonstrate the effects of Quinine in producing favourable conditions for infection with "Washed Tetanus Spores" when Quinine is given one day before Spores.

No. of experiment.	Date of injection of spores.	Date of injection of quinine.	Amount of quinine injected.	Result.	Remarks.
1.	7th April 1910, right *hind leg.*	6th to 9th April.	½ gr. daily on *front chest.*	Tetanus, 10th April. Died, 12th April.	Symptoms local at outset, then became general.
*2	Ditto .	Ditto .	Ditto .	Tetanus, 12th April. Died, 23rd April.	Symptoms local at first, advanced slowly, then became severe and general the day before death. *See footnote*.*
3.	Ditto .	Ditto .	Ditto .	Tetanus, 13th April. Severe, but eventually recovered after three weeks.	Symptoms localized to right leg and right side of body.
4.	Ditto .	Ditto .	Ditto .	Same as experiment No. 3.	Ditto ditto.
5 and 6 (Controls of spores.)	Ditto .	...	...	Both controls remained well.	
7 (Control of culture used for preparation of spores.)	0·25 c.c. of culture on 7th April.	...	...	Tetanus, 8th April. Severe. Died, 9th April.	Symptoms local at outset, but soon became general.

This Table shows 100 per cent. of infections when quinine is commenced one day before spores are given.

* *Experiment No. 2.*

As the disease in this guinea pig assumed a somewhat chronic and localised character for nine days at the outset, and then became acute and general, anærobic cultivations in broth were made from the following tissues and localities immediately after death, to prove whether infection had been transferred further than the seat of injection of spores :—Seat of injection of quinine ; seat of injection of spores ; spleen ; liver ; lungs ; kidney ; heart's blood.

Result.—After incubating at 37°C. for a month, there was a rich growth of tetanus bacilli from the seat of injection of spores and also from the seat of injection of quinine. All other tubes remained sterile, with the exception of the one from the lungs which grew micrococci only.

The skin at the site of the quinine injections was unbroken at the time of death, but there was subcutaneous evidence of the escharotic action of the quinine.

In this case infection was conveyed from the seat of injection of spores in the hind leg, to the seat of injection of quinine on front of the chest.

The 4th series of experiments.

A repetition of the experiments recorded in Tables II and III. Quinine was given hypodermically on the front of the chest to four guinea pigs one day before

"washed tetanus spores" were given hypodermically in the hind leg, and two out of the four animals treated in this manner developed tetanus; one on the fifth day after the spores were given, the other on the sixth day.

The one which developed tetanus on the sixth day died three days later. Anærobic cultivations made from the site of spores, and the site of quinine grew tetanus bacilli; but those from the liver and spleen gave negative results.

The other guinea pig remained in a state of chronic tetanus for five weeks, and eventually recovered. The control guinea pig which received "washed spores" only, remained well, and the control culture animal developed tetanus on the second day, and died on the fourth day.

The result of this series of experiments confirms those recorded in Tables II and III, but the percentage of infections are 50 per cent. as compared with 100 per cent. in Table III and 33·3 per cent. in Table II.

Table IV.

Experiments on Guinea Pigs to prove the influence of Quinine in producing favourable conditions for Tetanus infection when Quinine is commenced one day before "Washed Tetanus Spores" are given.

No. of experiment.	Date of injection of spores.	Date of injection of quinine.	Amount of quinine injected.	Result.	Remarks.
1	27th August 1910, right *hind leg.*	26th to 28th August.	½ gr. daily for two days on front of *chest.*	Tetanus, 3rd September. Died, 6th September.	Disease local at first, then became general. Tetanus bacilli grew in anærobic cultivations made from site of injection of spores, and from site of injection of quinine, but not from the liver, or spleen.
2	Ditto .	Ditto .	Ditto .	Tetanus, 2nd September; remained chronic for five weeks, then recovered.	Disease localised to the right leg, and right side of body; very severe for a week, when daily improvement set in.
3	Ditto .	Ditto .	Ditto .	Remained well.	
4	Ditto .	Ditto .	Ditto .	Ditto.	
5 (Control of spores.)	Ditto .	*Nil.*	*Nil.*	Ditto.	
6 (Control of culture from which spores were prepared.)	½ c.c. culture, right *hind leg.*	*Nil.*	*Nil.*	Tetanus, 28th August. Died, 30th August.	Local at outset, then became general.

This Table shows 50 per cent. of infections when quinine is given one day before spores; the quinine was injected on front of the chest, and spores in the hind leg.

THE 5TH SERIES OF EXPERIMENTS.

The experiments in this series illustrate the effects of quinine in producing favourable conditions for infection with "washed tetanus" (which are by themselves non-infective) when injected into guinea pigs under varying conditions.

Six guinea pigs of approximately the same age and size were selected, and divided into three lots of two each, so as to contrast the effects of quinine when given :—

1. One day before "washed tetanus spores" were given.
2. Same day and time as "washed tetanus spores" were given.
3. One day after "washed tetanus spores" were given.

The spores were injected hypodermically into the left hind leg and only one dose given, and the quinine hypodermically on the front of the chest in $\frac{1}{2}$ grain doses for four days.

In the first lot tetanus set in on the third day from the time the spores were given, and in the other two lots on the fourth day.

In all these six animals the disease ran a chronic course, and was confined to the left hind leg (seat of injection of spores), and left side of the body : and for over a fortnight they were bent to the infected side, and the infected leg stuck out stiff when they crawled along on being taken out of their cages. After a month they all eventually recovered. In the two animals which got quinine one day before spores, the disease was more severe than in the others, and as I have already mentioned it set in one day earlier ; so that in these two experiments the quinine had some influence in preparing more favourable conditions for spores to develop, than in the other two lots in which it was given in one lot same day as spores, and in the other the day after.

Two control guinea pigs were given spores only, and both remained well. Another control guinea pig was given $\frac{1}{4}$ c.c. of the tetanus culture from which the spores were prepared, and tetanus set in on the second day, and it died next day.

The results of this series of experiments in addition to proving the favouring influence of quinine in producing tetanus when "washed spores" are given, also prove that an earlier infection is likely to take place when the quinine is given some time before the spores.

Table V.

Experiments to contrast the effects of Quinine on Guinea Pigs when given

(*a*) One day before "Washed Tetanus Spores" were given.
(*b*) Same day as "Washed Tetanus Spores" were given.
(*c*) One day after "Washed Tetanus Spores" were given.

No. of experiment.	Date and seat of injection of spores.	Date and seat of injection of quinine.	Amount of quinine injected.	Result.	Remarks.
1	2nd July 1910, left *hind leg.*	1st to 4th July on *front of chest.*	½ gr. daily for 4 days.	Tetanus, 5th July; severe and confined to left side. Recovered after a month.	In both these experiments quinine was commenced one day before spores, and tetanus set in on the third day.
2	Ditto .	Ditto .	Ditto .	Same as experiment No. 1.	
3	Ditto .	2nd to 6th July, on front of chest.	Ditto .	Tetanus. 6th July; confined to left side, not severe. Recovered after a month.	In both these experiments quinine was commenced same day as spores, and tetanus set in on the fourth day.
4	Ditto .	Ditto .	Ditto .	Same as experiment No. 3.	
5	Ditto .	3rd to 6th July, on front of chest.	Ditto .	Tetanus, 6th July; severe but confined to left side. Recovered after a month.	In both these experiments quinine was commenced one day after spores, and tetanus set in on the fourth day.
6	Ditto .	Ditto	Ditto .	Same as experiment No. 5, but disease less severe.	
7 and 8 (Controls of spores used.)	Ditto .	*Nil.*	*Nil.*	Remained well .	Both controls remained well.
9 (Control of culture from which spores were prepared.)	0·5 c.c. tetanus culture, 2nd July.	*Nil.*	*Nil.*	Tetanus, 3rd July; severe and acute. Died 4th July.	Symptoms local at outset but rapidly became general.

In experiments 1 and 2 in which quinine was commenced one day before spores were given, tetanus set in one day earlier than in the other four experiments, in two of which quinine was given the same day as spores, and in the other two the day after spores.

The 6th series of experiments.

In this series, two guinea pigs were inoculated with "washed tetanus spores," and immediately afterwards exposed to a temperature of about 38°F. in a cold chamber.

In both cases the spores were injected into the muscles of the right hind leg, and one of them had at the same time ½ gr. quinine, given subcutaneously on the front of the chest. The one which got spores only, developed tetanus on the third day; and the one which got ½ gr. quinine in addition to spores developed tetanus on the second day, so that the quinine probably hastened the onset of tetanus by one day.

As soon as tetanus symptoms set in, both were taken out of the cold chamber and kept at room temperature, when the disease became chronic, and remained localised to the right leg (seat of spores) and side, and was severe for three weeks, when both began to improve, and eventually recovered.

The control guinea pig inoculated with spores only, and kept at room temperature remained well; and another control inoculated with $\frac{1}{4}$ c.c. of the tetanus culture from which the spores were prepared developed tetanus on the second day, and died on the third day.

It is evident from these two experiments that cold has an influence in producing conditions favourable for infection with "washed tetanus spores," and that this influence is greater when quinine is given in addition. One would infer from these experiments that it is possible for a latent infection to be stirred into activity by cold or other depressing influences.

Table VI.

Experiments to test the influence of Cold on Guinea Pigs in the production of Tetanus after the injection of "Washed Tetanus Spores."

No. of experiment.	Date of injection of spores.	Time of exposure to cold.	Whether quinine was given, and if so the amount.	Result.	Remarks.
1	9th April 1910, right *hind leg.*	9th to 12th April, kept in cold chamber at about 38° F.	No quinine .	Tetanus, 12th April, three days after spores.	Severe and confined to right leg and right side of body. Lasted for three weeks, when it gradually cleared away.
2	Ditto .	9th to 11th April kept in cold chamber at about 38° F.	$\frac{1}{2}$ gr. only, on front of chest, 9th April.	Tetanus, 11th April, two days after spores.	Ditto ditto.
3 (Control of spores.)	Ditto .	Not exposed to cold.	No quinine .	Remained well.	
4 (Control of tetanus culture from which spores were prepared.)	0·25 c.c. tetanus culture, 9th April.	Ditto .	Ditto .	Tetanus, 11th April. Died, 12th April.	Symptoms local at outset, then became general.

In No. 1 experiment, cold diminished the animal's powers of resistance to such an extent that "washed tetanus spores" were able to grow and produce infection; and in No. 2 experiment the $\frac{1}{2}$ gr. of quinine hastened the influence of cold by one day.

The 7th series of experiments.

This series was primarily intended to demonstrate the favouring influence of quinine in producing tetanus in guinea pigs, when given hypodermically on front of the chest, and for two days before washed tetanus spores were given hypodermically in the hind leg. Owing to the accidental contamination of the culture from which the spores were prepared the series proves nothing so far as quinine is concerned, as the two control animals contracted tetanus the same time as the four experimental ones.

The control anærobic cultivation made from the washed spores, in addition to growing tetanus bacilli, grew another spore-bearing bacillus resembling malignant œdema; and a similar bacillus (in addition to tetanus bacilli) was isolated from one of the controls and also from one of the experimental animals after death.

The results of this series are given as an illustration of the necessity of using *pure* "*washed spores*," and the importance of never omitting control experiments. The series also serves another purpose, *viz.*, as an example of the fact that "washed tetanus spores" mixed with other spores produce tetanus, when pure "washed tetanus spores" would fail to do so.

Table VII.

The results of this series of experiments on Guinea Pigs are given as an illustration of the importance of using pure cultures only when experimenting with "Washed Tetanus Spores," and the necessity for control experiments. The culture used had got accidentally contaminated with another spore-forming bacillus.

No. of experiment.	Date of injection of spores.	Date of injection of quinine.	Quantity of quinine injected.	Results.	Remarks.
1	25th February 1910.	23rd to 25th February.	½ gr. daily for two days.	Tetanus, 26th February. Died, 27th February.	This series proves nothing so far as quinine is concerned, owing to the fact that the two controls contracted tetanus. In all seven experiments there was local swelling at the seat of injection of spores, and bacilli resembling malignant œdema present in stained specimens. Both these and tetanus bacilli were isolated by cultivations from two cases (one control and one experiment).
2	Ditto .	Ditto .	Ditto .	Ditto ditto.	
3	Ditto .	Ditto .	Ditto .	Ditto ditto.	
4	Ditto .	Ditto .	Ditto .	Ditto ditto.	
5 and 6 (Controls to spores.)	25th February 1910.	*Nil.*	*Nil.*	Tetanus, 26th February. Died, 27th February.	Both controls contracted tetanus, which rendered the series of experiments useless.
7 (Control of culture from which spores were prepared.)	½ c.c. tetanus culture, 25th February.	*Nil.*	*Nil.*	Ditto ditto .	Symptoms very acute, and disease proved rapidly fatal.

A cultivation made from the spores used for this series after heating for five minutes at a temperature of 75°C. grew a mixture of tetanus and other spore-forming bacilli. The series proves that tetanus spores mixed with other spores produce tetanus.

The 8th series of experiments.

The object of this series of experiments was to follow up the information, gained from experiment 2, Table III (3rd series), in which tetanus bacilli were isolated from the site of a quinine injection on front of the chest following the inoculation of "washed tetanus spores" in the hind leg.

A tetanus culture was used for this series, and not "washed spores," as it was necessary to produce tetanus without fail, in order to demonstrate whether tetanus bacilli can be conveyed from the seat of infection on a distant part of the body, such as the leg, to another part where quinine had been injected, such as the front of the chest.

Seven guinea pigs were inoculated subcutaneously in the right hind leg with $\frac{1}{4}$ c.c. of a pure tetanus culture, and on the second day when local tetanus was well marked in all, six of them were given $\frac{1}{2}$ grain quinine subcutaneously on the front of the chest. Soon after the quinine was given the disease became severe and general, and they all died from generalised tetanus two days afterwards.

The control animal which received the same amount of tetanus culture, but no quinine, also died on the same day, but later on in the evening.

Immediately after death, anærobic cultivations were made from the subcutaneous tissues at the sites of injection of tetanus cultures and quinine respectively; and after incubating at a temperature 37° C. for a week, a good growth of tetanus bacilli, showing well formed spores was present in each of the tubes from the seat of injection of the tetanus culture in the leg, and in five out of six from the seat of injection of quinine on front of the chest.

Every precaution was taken to sterilise the surface of the skin before cutting into it to remove a small piece of subcutaneous tissue for inseminating the broth tubes, and separate sterilised instruments were used for the chest and leg, so there was no possiblity of carrying over contaminations from one site to the other when making the cultivations.

In five out of six experiments, infection was conveyed from the original site of infection in the leg to the seat of quinine injection on the front of chest.

When these results are interpreted, it means that quinine given hypodermically in the early stages of a tetanus infection starts a fresh focus of infection, and a focus from which tetanus bacilli (which have not been injected into it) can be isolated.

The question arises how did infection get conveyed from the leg to the chest in these cases? One can only surmise that early in a tetanus infection, the efficiency of the phagocytes is severely taxed, and that some of them re-enter the circulation carrying tetanus spores, and before they have been able to deal successfully with these spores they get stranded at the site where the quinine had been

injected, where they find dead tissue under anærobic conditions, the most suitable conditions for the germination of tetanus spores.

A spore infected phagocyte entering the circulation would not be likely to get stranded in healthy living tissue, and if it did, it would not find conditions suitable for the spores to develop, and its phagocytic action would not be interfered with.

As evidence in favour of this might be adduced the fact that after death in cases of tetanus the bacilli are not found in anærobic cultivations made from the internal organs or heart's blood. (See Tables IX, X, XII, and XIV.)

Table VIII.

Experiments on Guinea Pigs to prove whether hypodermic injections of Quinine in cases of Tetanus infection produce local conditions suitable for the growth of Tetanus bacilli.

No. of experiment.	Date and seat of injection of tetanus *culture*.	Date and seat of injection of quinine.	Result.	Result of anærobic cultivations made after death from		Remarks.
				Seat of injection of tetanus culture.	Seat of injection of quinine.	
1	25th August 1910, right *hind leg.*	27th August 1910, ½ gr., *chest.*	Tetanus, 27th August. Died, 29th August.	Growth of tetanus bacilli.	Growth of tetanus bacilli.	Tetanus local at first; then became severe and general after quinine was given. Infection was conveyed from the hind leg to seat of quinine injection on front of chest.
2	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.
3	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.
4	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.
5	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.
6	Ditto .	Ditto .	Ditto .	Ditto .	No growth of tetanus bacilli.	Symptoms same as experiments Nos. 1 to 5.
7 (Control of tetanus culture used.)	Ditto .	*Nil.*	Ditto .	Ditto .	..	Tetanus local at outset; then became general.

In all these experiments the tetanus bacillus was recovered from the seat of injection of culture (hind leg) and in out of five of the six cases in which quinine was given, tetanus bacilli were also isolated from the seat of injection of the quinine (chest). The failure to isolate tetanus bacilli from the seat of quinine injection in No. 6 experiment was very probably due to the fact that a hot glass rod was accidentally used to push down into a broth tube the small piece of subcutaneous tissue used to inseminate it. The tetanus bacilli isolated from the seat of injection of quinine were virulent for guinea pigs.

THE 9TH SERIES OF EXPERIMENTS.

The object of this series of experiments was to prove whether quinine when given in tetanus infections, in addition to producing a local site to which the infection becomes transferred, would also give rise to conditions in which infection would be found in the internal organs.

Three guinea pigs were taken, and each was injected subcutaneously in the hind leg with a tetanus culture, and at the same time each was given 1 grain of quinine subcutaneously on the front of the chest. On the next day local tetanus appeared in all three animals, and death took place two days later.

Immediately after death, anærobic cultivations were made from the seat of injections of spores, seat of injection of quinine, and from the liver, and the spleen. A growth of tetanus bacilli took place in all the cultivations made from site of spores, and site of quinine, but not from the liver and the spleen.

The control guinea pig which received $\frac{1}{4}$ c.c. tetanus culture only, developed tetanus, and died about the same time as the others. Anærobic cultivations made from the site of injection of quinine and site of injection of tetanus culture grew tetanus bacilli, but the liver and the spleen gave negative results.

This series of experiments in addition to confirming the results of the anærobic cultivations recorded in Table VIII, also proves that quinine has no influence in transferring the infection of tetanus from a localised site to the internal organs, although this influence is marked in transferring infection from a localised site to the site of injection of quinine.

Table IX.

Experiments on Guinea Pigs, to prove whether Quinine, in addition to producing favourable local conditions for the growth of the Tetanus bacillus, has any influence in producing conditions in which the bacillus may be found in the internal organs.

No. of experiment.	Date and seat of injection of tetanus *culture.*	Date and seat of injection of quinine.	Result.	RESULT OF ANÆROBIC CULTIVATIONS MADE FROM				REMARKS.
				Seat of injection of tetanus culture.	Seat of quinine injection.	Liver.	Spleen.	
1	30th August 1910, right *hind leg.*	30th August 1910, 1 gr. on front of *chest.*	Tetanus, 31st August. Died, 2nd September.	Growth of tetanus bacilli.	Growth of tetanus bacilli.	Negative	Negative	Tetanus bacilli were cultivated from seat of injection of culture, and from seat of injection of quinine, but not from the liver or the spleen.
2	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	
3	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	
4 Control of tetanus culture used.	Ditto .	*Nil.*	Ditto .	Ditto .	..	Ditto .	Ditto .	Tetanus bacilli were cultivated from seat of injection of culture, but not from the liver or the spleen.

This series of experiments proves that the influence of quinine in producing local favouring conditions for the transference of tetanus bacilli from the seat of infection to the seat of quinine injection, does not extend to producing conditions in which the bacillus is found in the internal organs. The tetanus bacilli isolated from the seat of injection of quinine in this series of experiments were virulent for guinea pigs.

THE 10TH SERIES OF EXPERIMENTS.

The object of this series of experiments was to demonstrate what effects (if any) quinine, morphia, lactic acid, and normal saline solution have in preparing suitable local foci for the development of "washed tetanus spores," when either of these materials are given subcutaneously, and followed 30 minutes later by the injection of "washed tetanus spores" into the same site.

Six guinea pigs were taken and treated as follows :—

1. Two received $\frac{1}{2}$ gr. quinine disolved in $\frac{1}{2}$ c.c. normal saline solution.
2. Two others received $\frac{1}{20}$ grain morphia in $\frac{1}{2}$ c.c. normal saline solution.
3. One received $\frac{1}{2}$ c.c. of a weak dilution of lactic acid (dilution made with normal saline solution).
4. One control guinea pig received $\frac{1}{2}$ c.c. normal saline solution.

The injections to these six guinea pigs were given subcutaneously in the right hind leg. When 30 minutes had elapsed to allow for absorption to have taken place, each received into the site of the previous injection $\frac{1}{4}$ c.c. of "washed tetanus spores," and with the following results :—

The two animals which received quinine developed acute tetanus on the third day, and died next day.

The animal which received lactic acid developed acute tetanus on the second day, and died next day.

The two animals which received morphia, and the one which received normal saline solution remained well.

These experiments show that quinine and lactic acid, when injected hypodermically produce sites suitable for the development of tetanus spores should they ever reach these sites ; and that morphia and non-irritating solutions of normal saline do not have this effect.

On the death of the three animals which received quinine and lactic acid, anærobic cultivations were made from the subcutaneous tissues at the site of injections in the leg, and also from the liver and the spleen, with the result that tetanus bacilli were isolated from the leg in all three cases, but not from the liver and spleen—a further proof that infection is not found in the internal healthy organs in cases of acute tetanus.

Table X.

Experiments on Guinea Pigs to prove the influence of Quinine and Lactic Acid in producing Tetanus, as contrasted with morphia and normal saline solution, when "Washed Tetanus Spores" are used; the spores being injected into the same site as the quinine, lactic acid, morphia, or normal saline solution, but 30 minutes later.

No. of experiment.	Date and seat of injection of quinine, or lactic acid, or morphia, or normal saline solution.	Date and seat of injection of *spores*.	Result.	Result of anærobic cultivations made after death from			Remarks.
				Seat of inoculation.	Liver.	Spleen.	
1	29th August 1910, ½ gr. quinine, right *hind leg*.	29th August 1910, into same site as quinine, but 30 minutes later.	Tetanus, 1st September. Died, 2nd September.	Growth of tetanus bacilli.	Negative	Negative	Quinine, and lactic acid, when injected hypodermically, produce sites favourable for the development of "washed tetanus spores"; but normal saline solution and morphia do not produce any local favourable influence. The quinine solution and lactic acid did not produce tetanus by dissolving the tetanus spores, and liberating the toxine which they contained. The 30 minutes' time allowed for absorption and the growth of tetanus bacilli from the seat of injection would go against this view. In experiments 1, 2, and 3, although the disease was acute and rapid, the infection remained local, and the internal organs were free from tetanus bacilli.
2	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	
3	29th August 1910, 1 c.c. weak lactic acid, right *hind leg*.	29th August 1910, into same site as lactic acid, but 30 minutes later.	Tetanus, 31st August. Died, 1st September.	Ditto .	Ditto .	Ditto .	
4	29th August 1910, $\frac{1}{75}$ gr. morphia, right *hind leg*.	29th August 1910, into same site as morphia, but 30 minutes later.	Remained well.				
5	Ditto .	Ditto .	Ditto.				
6 (Control of the pores used.)	29th August 1910, 1 c.c. normal, saline solution, right *hind leg*.	29th August 1910, into same site as normal saline soultion, but 30 minutes later.	Ditto.				

Quinine and lactic acid, when injected hypodermically, prepare sites favourable for the development of tetanus spores, but morphia and normal saline solution do not produce this effect.

The 11th series of experiments.

This series of experiments was devised with the object of proving why it is that tetanus so rarely follows hypodermic injections other than those of quinine, due allowance being made for those exceptional cases in which tetanus germs were inoculated along with the injected material.

Morphia is perhaps more often given as a hypodermic than any other drug, and yet it is the rarest exception possible ever to hear of tetanus following its use. The same may be said with regard to bacterial vaccines, sera, and such remedies, which are always given hypodermically, and yet one seldom, if ever, hears of a case of tetanus following their use, although they are now given all over the world as a routine measure in the treatment of numerous human diseases, and doubtless by many who have hazy notions as to what asepticism really means.

The two circumstances above all others which make quinine a more dangerous weapon in the production of tetanus when given hypodermically are—

(1) The local destruction of tissue at the seat of injection.

(2) The possible paralysing effect on phagocytic activity when large doses are given.

The local destruction of tissue follows as a matter of course in all cases in which quinine is given hypodermically ; and the paralysing effect on phagocytes is a possibility when the drug is pushed and the patient's resistance lowered by the effects of malaria, a high temperature, or other influences.

In continuation of the line of reasoning suggested by the results of the experiments detailed in Table X, and with the object of testing experimentally the effects of " washed tetanus spores " in the production of tetanus when mixed with quinine and morphia respectively, and injected hypodermically, the following experiments were carried out. Eight healthy guinea pigs of approximately the same age and size were selected. Three were given $\frac{1}{2}$ grain quinine dissolved in $\frac{1}{2}$ c.c. normal saline solution, and mixed with $\frac{1}{4}$ c.c. " washed tetanus spores." Three others were given $\frac{1}{20}$ grain morphia in $\frac{1}{2}$ c.c. normal saline solution, and mixed with $\frac{1}{4}$ c.c. " washed tetanus spores."

One was given $\frac{1}{4}$ c.c. " washed tetanus spores " only, and the eighth guinea pig was given $\frac{1}{4}$ c.c. of the tetanus culture from which the spores were prepared.

The injections were in all cases given hypodermically in the hind leg, and with the following results :—

(1) The three animals which received the spores mixed with quinine all developed tetanus ; one on the second day, one on the third day, and the remaining one on the fourth day ; and death took place on the fourth, ninth, and tenth days respectively after receiving the injection.

After death anærobic cultivations were made from the sites of injection of quinine mixed with spores, and tetanus bacilli were isolated in all three cases.

(2) The three animals which received morphia mixed with spores remained well, and not the slightest trace of local tetanus showed itself at any time.

(3) The control animal which received " washed spores" only remained well.

(4) The other control which received $\frac{1}{4}$ c.c. of the culture from which the spores were prepared, contracted tetanus on the second day and died next day.

It is evident from the results of this series of experiments that the chances of contracting tetanus when tetanus spores are given hypodermically in a quinine

solution are much greater than when the spores are given with a hypodermic injection of morphia.

Table XI.

Experiments to test the effect of "Washed Tetanus Spores" when mixed with Morphia or Quinine and injected into Guinea Pigs.

No. of Experiment.	Date of injection of mixture of morphia, or quinine and "washed tetanus spores."	Seat of injection.	Result.	Remarks.
1	16th September 1910, $\frac{1}{20}$ gr. morphia mixed with washed tetanus spores.	Right hind leg .	Remained well.	
2	Ditto ditto .	Ditto .	Ditto	
3	Ditto ditto .	Ditto .	Ditto.	
4	16th September 1910, $\frac{1}{2}$ gr. quinine mixed with washed tetanus spores.	Ditto .	Tetanus, 18th September. Died, 20th September.	An anærobic cultivation made from seat of injection of quinine mixed with spores gave a growth of tetanus bacilli.
5	Ditto ditto .	Ditto .	Tetanus, 20th September. Died, 26th September	Ditto ditto.
6	Ditto ditto .	Ditto .	Tetanus, 19th September. Died, 26th September.	Ditto ditto.
7 (Control of spores used.)	16th September, "washed tetanus spores" only.	Ditto .	Remained well.	
8 (Control of culture from which spores were prepared.)	16th September, $\frac{1}{4}$ c.c. tetanus culture only.	Ditto .	Tetanus, 17th September. Died, 18th September.	Severe and rapid; tetanus local at outset; then became general.

This series of experiments demonstrates that tetanus spores when mixed with quinine and injected into guinea pigs produce tetanus, but when mixed with morphia do not produce tetanus.

The 12th series of experiments.

As all the foregoing experiments with quinine and tetanus spores had been carried out on guinea pigs, it was deemed advisable to control a few of them by similar experiments on monkeys.

The common brown monkey found in the hills in the neighbourhood of Kasauli was used. The animals were full grown weighing about 12 to 14 lbs., in good condition, and had been in captivity for over five months.

This series included five monkeys treated as follows:—

No. 1 Monkey.—Quinine was commenced at the same time that spores were given.

On September 2nd, $\frac{1}{2}$ c.c. "washed tetanus spores" were given hypodermically in the right hind leg, and three grains quinine hypodermically on front of chest. The quinine was again repeated on the 3rd and 8th September, and in 4-grain doses.

Tetanus developed on September 19th, but the animal was ill for two days before this. Lockjaw was present on the 19th September but not severe, and the animal was able to eat and drink.

On the 21st, there was difficulty of swallowing and the jaws could only be pressed open a little and with difficulty.

The symptoms advanced slowly and become generalised. On the 26th the animal was killed with chloroform.

Immediately after death, anærobic cultivations were made from the subcutaneous tissues taken from seat of injection of spores, seat of injection of quinine, and also from the liver, the spleen, and the heart's blood.

3 c.c. of the heart's blood were also injected subcutaneously into a guinea pig.

A growth of tetanus bacilli was obtained from the seat of injection of spores, and seat of injection of quinine, but not from the liver, the spleen, or the heart's blood.

The guinea pig inoculated with 3 c.c. of the heart's blood remained well for a week, when wasting set in, and it died on the 14th day, but showed no symptoms of tetanus at any time. An anærobic cultivation made from the site of injection of this monkey's blood into the guinea pig failed to grow tetanus bacilli.

No. 2 Monkey.—Quinine was commenced one day after spores were given.

On September 2nd, ½ c.c. "washed tetanus spores" was given hypodermically in the right hind leg.

On September 3rd, 4th and 8th, 4 grains quinine were given hypodermically on front of the chest.

Tetanus developed on September 17th, and on that date there was slight stiffness of the muscles of the neck and the jaws. On the 19th, lockjaw was well marked, and there was great difficulty of swallowing, and the animal died next day from acute generalised tetanus.

Immediately after death, anærobic cultivations were made from subcutaneous tissue taken from the seat of injection of spores, and from the seat of injection of quinine, and also from the liver and spleen.

A growth of tetanus bacilli was obtained from the seat of injection of spores, and seat of injection of quinine, but not from the liver or the spleen.

No. 3 Monkey.—Quinine was commenced one day before spores were given.

On September 2nd, 3 grains quinine were given hypodermically on the front of the chest and next day ½ c.c. "washed tetanus spores" were given hypodermically in the right hind leg, and at the same time 4 grains quinine on front of chest. On September 8th it received another 4 grains quinine on front of chest. Tetanus developed on September 15th, slight at first, but more marked on the 16th when lockjaw was present.

On the 17th generalised tetanus was present, and the animal lay in its cage bent forward (emprosthotonos) with shallow breathing, muscles of arms, legs,

chest, and abdomen rigid, and jaws firmly clenched. It remained in this condition until the 21st when it died.

Immediately after death anærobic cultivations were made from subcutaneous tissue taken from the seat of injection of spores, and seat of injection of quinine, and also from the liver and the spleen.

A growth of tetanus bacilli was obtained from the seat of injection of spores, and seat of injection of quinine, but not from the liver or the spleen.

No. 4 Monkey.—(Control of "washed spores" used.)

This monkey was given ½ c.c., "washed tetanus spores" hypodermically in the right hind leg on September 2nd. The spores were from the same strain and culture of tetanus as those used for the other three monkeys, and prepared at the same time, and in the same way. No quinine was given. This animal remained well.

No. 5 Monkey.—Control of the culture of tetanus from which the "washed spores" were prepared.

On September 2nd, 1 c.c. of tetanus culture was given hypodermically in the right hind leg.

Tetanus developed on September 4th, and death took place the next day.

The disease commenced with contraction of the masseter muscles (lockjaw), difficulty of swallowing, and rapidly advanced to generalised tetanus, and before death the animal was bent back (opisthotonos).

The rapid course of the disease was no doubt due to the amount of toxine contained in 1 c.c. of the culture used. The tetanic symptoms were not any more marked in the inoculated hind leg than in the other leg.

Immediately after death anærobic cultivations were made from the subcutaneous tissue taken from the seat of injection of the tetanus culture, and also from the liver and the spleen.

A growth of tetanus bacilli was obtained from the seat of injection of culture, but not from the liver or the spleen.

It will be seen that the results of the experiments on these three monkeys which contracted tetanus after inoculation of "washed tetanus spores" in the hind leg, and hypodermic injections of quinine on front of the chest, harmonise with similar experiments on guinea pigs. The incubation period was more prolonged in the case of the monkeys, and when symptoms set in they resembled those found in the cases of tetanus in the human subject, lockjaw being an early and prominent symptom, and local tetanus at the site of inoculation being absent (as an early symptom at any rate) in all three cases, whereas it is always the first symptom in guinea pigs, and when not lethal, is often the only symptom.

In these cases the infecting micro-organism was recovered from the seat of injection of quinine and also from the seat of injection of spores, but not from

the liver or the spleen in any case, nor from the blood in the case of No. 1 experiment.

The tetanus bacillus isolated from the seat of injection of quinine in these three monkeys was virulent, and killed guinea pigs in small doses ($\frac{1}{2}$ c.c. of a 14 days' growth killed guinea pigs in two days; less would have sufficed).

Table XII.

Experiment on *Monkeys* with "Washed Tetanus Spores" and Quinine.

No of experiment.	Date and seat of injection of ½ c.c. spores.	Date and seat of injection of quinine.	Result.	Result of anærobic cultivations made from				Remarks.
				Seat of injection of spores.	Seat of injection of quinine.	Lever.	Spleen.	
1	2nd September 1910, right *hind leg.*	2nd September 1910, 3 grs., *chest.* 3rd September, 4 grs., *chest.* 8th September, 4 grs., *chest.*	Tetanus, 19th September. Died, 26th September.	Growth of tetanus bacilli.	Growth of tetanus bacilli.	*Nil.*	*Nil.*	Quinine commenced same day as spores. Lockjaw, an early symptom, followed by stiffness of neck and shoulder muscles, and difficulty in swallowing.
2	2nd September 1910, right *hind leg.*	3rd September 1910, 4 grs., *chest.* 4th September, 4 grs., *chest.* 8th September, 4 grs., *chest.*	Tetanus, 17th September. Died, 20th September.	Growth of tetanus bacilli.	Growth of tetanus bacilli.	*Nil.*	*Nil.*	Quinine commenced one day after spores. Lockjaw, an early symptom, and soon after tetanus became general and very acute.
3	3rd September 1910, right *hind leg.*	2nd September 1910, 3 grs., *chest.* 3rd September, 4 grs., *chest.* 8th September, 4 grs., *chest.*	Tetanus, 15th September. Died, 21st September.	Growth of tetanus bacilli.	Growth of tetanus bacilli.	*Nil.*	*Nil.*	Quinine commenced one day before spores. Lockjaw, an early sympton, then slow general tetanus set in, finishing up with emprosthotonos.
4 (Control of spores used.)	2nd September 1910, right *hind leg.*	..	Remained well.					
5 (Control of tetanus culture used to prepare spores.)	2nd September 1910, 1 c.c. tetanus culture, right *hind leg.*	..	Tetanus, 4th September. Died, 5th September.	Growth of tetanus bacilli.	..	*Nil.*	*Nil.*	Lockjaw, an early and prominent symptom. Opisthotonos marked before death.

In No. 1 experiment, immediately after death, a guinea pig was injected subcutaneously with 3 c.c. of blood taken from the heart, but the animal did not contract tetanus. An anærobic cultivation made from ½ c.c. of the heart's blood transferred to a broth tube also gave negative results.

It is evident from the results of the experiments recorded in this table that quinine when given hypodermically to monkeys produces conditions in which pure "washed tetanus spores" become infective, and capable of producing fatal tetanus. It will be observed that lockjaw was an early and prominent symptom in all four cases, and that opisthotonos was present in one case, and emprosthotonos in another.

The 13th series of experiments.

The possibility of tetanus spores remaining alive and localised at the seat of a former injury is of the utmost importance in relation to the hypodermic administration of quinine. It will be noticed that in Tables I, II, III, IV, V, VI, X, XI,

and XII, that there is direct experimental proof to the effect that pure tetanus spores, free from toxine and other irritants, fail to give tetanus to guinea pigs and monkeys when injected hypodermically ; but in a large percentage of these animals when quinine is given hypodermically, and in a part of the body far removed from the spores, tetanus is the result, although the quinine, syringe, and fluid in which the quinine was dissolved were beyond suspicion, and contained not a trace of tetanus infection.

The object of this series of experiments was to demonstrate whether tetanus bacilli can be cultivated from the site of the hypodermic injection of pure "washed tetanus spores" into guinea pigs, after various periods had elapsed since the spores were injected.

Cultivations were made from eight guinea pigs. Most of these animals had been used as controls in several other experiments in which quinine had been given in addition to spores. None of them had shown any signs of tetanus and all were healthy and full grown. The time which elapsed between the date of injection of pure spores, and the date on which cultivations were made from the site into which the spores had been injected, varied from five weeks to seven months ; so that ample time had elapsed to allow of the spores producing infection, or being disposed of by phagocytosis, or stowed away locally in the tissues at the seat of injection, and alive and capable of producing tetanus should conditions become favourable.

The animals were killed with chloroform. The skin at the site of injection of spores (right hind leg in all cases) was rendered aseptic, cut into, and a small portion of the subcutaneous tissue and adjacent muscles removed, and placed in broth tubes. The tubes were then rendered anærobic, and incubated at a temperature of 37°C., for a week at least. In all these eight experiments true tetanus bacilli were isolated, and proved virulent by giving tetanus to other guinea pigs.

It is evident from these results that tetanus spores can remain localised in the tissues for months. We know from experiments carried out years ago by Metchnikoff, Vincent and others, that when washed tetanus spores are injected hypodermically into animals the phagocytes pick them up and kill them off, and that the spores can be demonstrated inside phagocytes taken from the site soon after injection. Some of the spores must escape being efficiently disposed of by the phagocytes, otherwise it would be impossible to obtain evidence of their presence in the tissues months afterwards. No doubt most of them are destroyed by the phagocytes, and those which escape get fixed in a safe place in the tissues, and remain there without giving rise to any symptoms.

When a person contracts tetanus through a wound, it means that tetanus spores have entered the wound, and found suitable conditions in which to germinate and give rise to the disease.

On the other hand, should the local conditions be unfavourable for the tetanus spores to germinate, and should they escape phagocytosis, what is to prevent them remaining stowed away and locked up in the tissue locally for months after the wound has healed ? A condition of this kind would correspond to what is found in guinea pigs, when washed tetanus spores are injected locally, except that in a tetanus-infected wound, the chances are more in favour of the spores finding suitable conditions for germination on account of other wound-infecting organisms accompanying them. There is still another condition necessary when a wound is infected with tetanus spores, *viz.*, anærobic conditions. I have no doubt that in many tetanus-infected wounds, owing to the absence of anærobic conditions, tetanus fails to set in, and that the spores which escaped phagocytosis get hidden away in the tissues locally.

Table XIII.

Experiments to test whether Tetanus Spores can remain alive and localised at the site of a subcutaneous injection in Guinea Pigs.

No. of experiment.	Date and site of injection of spores.	Date on which the animal was chloroformed and tissue taken from site of injection for making cultivations.	Result of anærobic cultivations in broth.	Remarks.
1	30th August 1910, hind leg .	30th September 1910	Growth of tetanus bacilli .	The tetanus bacilli isolated from this series of experiments were virulent for guinea pigs.
2	27th August 1910, hind leg .	Ditto .	Ditto.	
3	Ditto ditto.	Ditto .	Ditto.	
4	Ditto ditto.	Ditto .	Ditto.	
5	7th April 1910, hind leg .	3rd October 1910 .	Ditto.	
6	9th April 1910, hind leg .	Ditto .	Ditto.	
7	30th August 1910, hind leg .	Ditto .	Ditto.	
8	itto ditto.	Ditto .	Ditto.	

The results of this series of experiments prove the possibility of tetanus spores remaining latent and localised in the tissues for months.

The 14th series of experiments.

In the experiments given, advantage was taken, on the death of a monkey and two guinea pigs from acute tetanus, to prepare anærobic cultivations from blood taken from the heart, and at the same time to inoculate three other guinea pigs with blood also taken from the heart of these three cases of acute tetanus.

The blood was taken immediately after death and before it had had time to clot.

No. 1 received 1 c.c. from a tetanus guinea pig.

No. 2 received 2 c.c., from another tetanus guinea pig.

No. 3 received 3 c.c., from a tetanus monkey.

The anærobic blood cultivations gave negative results in all three cases; no tetanus bacilli appeared in any of them.

Nos. 1 and 2 guinea pigs remained well.

No. 3 guinea pig inoculated with 3 c.c., of monkey's blood remained well for a week, when rapid wasting set in, and it died on the 12th day. It showed no symptoms of tetanus at any time. An anærobic cultivation made from the site of injection of blood failed to grow tetanus bacilli.

In these three cases of acute tetanus the blood furnished no evidence of containing tetanus infection.

Table XIV.

Experiments to prove whether the blood in case of acute Tetanus contains infection at the time of death.

No. of experiment.	Source from which the blood was taken.	Result of animal experiment with blood.	Result of anærobic cultivation made from blood.	Remarks.
1	A guinea pig which had died from severe and well marked experimental tetanus; blood was taken from the heart immediately after death.	1 c.c. injected subcutaneously in the hind leg of a guinea pig failed to give the animal tetanus.	½ c.c. transferred to a broth tube, and incubated anærobically at a temperature of 37°C for 10 days gave negative results.	The two guinea pigs and the monkey from which blood was taken for these experiments were typical severe cases of experimental tetanus, and in each of them a cultivation made after death from the site of inoculation gave a growth of tetanus bacilli. It is evident from these results that the blood in a case of tetanus does not contain any living tetanus spores, or tetanus bacilli at the time of death.
2	Ditto .	2 c.c. injected subcutaneously in the hind leg of a guinea pig failed to give the animal tetanus.	Ditto.	
3	A monkey which had died from severe and well marked experimental tetanus; blood was taken from the heart immediately after death.	3 c.c. injected subcutaneously in the hind leg of a guinea pig failed to give the animal tetanus.	Ditto.	

The results of this series of experiments show that the blood in cases of acute tetanus does not contain infection at the time of death.

The 15th series of experiments.

Considering the fact that tetanus is the most widely distributed of all pathogenic micro-organisms, and that it has been isolated from the intestinal canal of horses, sheep, cattle, and other animals, it is only reasonable that one would expect to find a similar infection of the intestinal tract of men. The fact that it is present in the intestinal tract of man and has been isolated from human excreta

is mentioned in most text books on bacteriology, but I cannot find any information about the percentage of cases in which it is present, or whether tetanus bacilli from this source have ever been proved virulent, and capable of giving tetanus to susceptible animals. With a view to gaining some information on these two points the series of experiments detailed in Table XV were carried out.

Ten samples of fæces were obtained haphazard from 10 separate sources and from healthy individuals.

In each case an emulsion was made by adding about 2 grammes to 15 c.c. of sterile normal saline solution. After thoroughly mixing, ½ c.c. was filled into a glass pipette and heated in a water bath to a temperature of 80° C. for 5 minutes. After heating, the contents of the capsule were transferred to an ordinary broth tube, which was made anærobic, and incubated at a temperature of 37°C. for a week. At the end of this time tetanus bacilli when present were easily distinguished by staining specimens.

In no case was this sufficient to obtain a pure growth, as many other spore bearing anærobic bacilli found in the intestinal contents of man survive heating for 5 minutes at a temperature of 80°C., and as a matter of fact, in every instance there was evidence that such bacilli were present when the tubes were examined. In all ten cases pseudo-tetanus bacilli were found, and in four out of the ten cases true tetanus bacilli were also found.

The pseudo-tetanus bacillus found in these cases might, on a casual glance and without experience, be mistaken for true tetanus bacilli; but on a close examination there is a marked difference. In the true tetanus bacillus when spores have formed they are round, and at the very end of a thin straight rod. In the psuedo-tetanus bacillus when spores have formed they are slightly oval, not quite at the end of the bacillus, which is shorter and stouter than the true tetanus bacillus, and the end opposite to the spore is often pointed. When the culture is a few days old, the rod part of the pseudo-tetanus bacillus disappears, and spores only are found; whereas in the true tetanus bacillus, the spores remain on the ends of the rods for weeks. Finally the pseudo-tetanus bacillus also grows anærobically, and is non-pathogenic for animals.

Bacilli answering in every detail to the description of true tetanus bacilli were isolated in four out of the ten cases in which an attempt was made.

The virulence of these four cultures was tested on guinea pigs, and with the following results: Two of the cultures were virulent; 1 c.c. of a 14 days' growth produced marked tetanus in guinea pigs in 20 hours and death in 42 hours. One culture was only slightly virulent as it required 48 hours for 1 c.c. of a 14 days' culture to produce local tetanus in a guinea pig. The fourth culture was non-virulent—1 c.c. of a 14 days' growth failed to give local tetanus to a guinea pig.

The very fact that tetanus spores are to be found in the intestinal tracts of a

large percentage of individuals opens up the possibility of so-called idiopathic tetanus occurring, provided the spores are virulent and get lodged in a focus of dead tissue, and under anærobic condition. Granted that it is possible for tetanus spores to enter the circulation from the intestinal tract where the mucous membrance is injured, ulcerated, or in any way damaged to such an extent as to be incapable of keeping back infection, what is to prevent such spores getting stranded in an injury somewhere else in the body where there is dead anærobic tissue ? The hypodermic or intramuscular injection of quinine would prepare a suitable focus in a case of this kind, and so would injuries of various sorts which are constantly occurring. It seems to me that this is a reasonable possibility of infection in cases of so-called idiopathic tetanus, and in many other injuries including quinine injections, but to say more on the subject at present would savour of conclusions arrived at in the absence of proof.

Table XV.

Experiments proving the presence of Tetanus germs in normal human fæces.

No. of experiment.	Preparation of material used.	Cultivation used.	Result.	Remarks.
1	Emulsion of normal fæces in normal saline solution and heated 5 minutes at 80°C.	Anærobic broth culture.	Growth of tetanus bacilli	In the four specimens which contained true tetanus bacilli, there were also other spore-forming bacilli, including the pseudo-tetanus bacilli described by Tavel and Zimmerman, but these were easily distinguished from the true form. Four out of the six negative specimens contained pseudo-tetanus bacilli, and other spore-forming germs; numerous spore-forming bacilli with no resemblance to either the true or pseudo tetanus bacillus were found in the remaining two specimens. In experiments Nos. *1, 6 and 9* the tetanus bacilli isolated *were virulent for guinea pigs in small doses.* In experiment No. 2 the tetanus bacilli isolated were non-virulent for guinea pigs in a dose of 1 c.c. of a 14 days' culture.
2	Ditto .	Ditto .	Ditto.	
3	Ditto .	Ditto .	Negative.	
4	Ditto .	Ditto .	Ditto.	
5	Ditto .	Ditto .	Ditto.	
6	Ditto .	Ditto .	Growth of tetanus bacilli.	
7	Ditto .	Ditto .	Negative.	
8	Ditto .	Ditto .	Ditto.	
9	Ditto .	Ditto .	Growth of tetanus bacilli.	
10	Ditto .	Ditto .	Negative.	

The experiments in this table prove that a large percentage of normal healthy persons harbour virluent tetanus bacilli in their intestinal tracts (4 out of 10, or 40 per cent.).

The 16th series of experiments.

This series of experiments was primarily intended to serve as a control of the tetanus bacilli isolated from the site of quinine injections in guinea pigs and monkeys in the experiments recorded in Tables VIII, IX and XII in which quinine was given hypodermically on front of the chest, and "washed tetanus spores" or tetanus cultures in the hind leg.

On referring to the above-mentioned tables it will be seen that in every instance except one, in which cultivations were made from the site of quinine injections in the circumstances mentioned, tetanus bacilli were recovered. Vincent[17] in 1904 recorded similar results in some cases of experimental tetanus in guinea pigs in which quinine had been given hypodermically. It is therefore obvious from the results recorded in this paper, and from those recorded by Vincent, that the transference of tetanus micro-organisms from the original site of infection to the site of quinine injection is one of the results to be expected when quinine is given hypodermically in tetanus infections; it is also a possible result to be expected in latent spore infections. We know that tetanus spores are to be found in the intestinal tracts of a large percentage of mankind, and in some animals; would a similar transference of infection take place from the intestinal tract in some of these cases when quinine is given hypodermically?

In one of the experiments recorded in this series a transference of infection from the intestinal tract is the only reasonable explanation which can be given to account for the bacilli isolated from the site of quinine injections.

Four healthy guinea pigs were each given ¾ grain quinine hypodermically on the front of the chest, and after an interval of two days the dose was repeated. Two days after the second dose the animals were killed with chloroform, and anærobic cultivations made from the site of the quinine injections. A pure growth of tetanus bacilli was obtained from one of these cases, but the other three gave negative results. The tetanus bacilli isolated from this one case were extremely virulent for guinea pigs; 0·1 c.c. of a six days' growth was sufficient to produce tetanus in a guinea pig after 12 hours, and death after 24 hours. None of the strains used for the experiments recorded in this paper was anything like so virulent as this.

The question is, what was the source of infection in this case?

Before the animal was chloroformed it showed no symptoms of tetanus; but this does not exclude the possibility of tetanus setting in later if it had been allowed to live longer. The fact of tetanus bacilli being absent from the site of quinine injections in the other three experiments would exclude the quinine as a source of infection, as all four guinea pigs were inoculated from the same quinine tabloid and at the same time, and with the same syringe. On each occasion on which quinine was injected a 5-grain tabloid was dissolved in 5 c.c. of sterile normal saline solution, and from this solution 3 c.c. were filled into a 3 c.c. syringe, and from this syringe each guinea pig received ¾ c.c.

In these circumstances the only reasonable source of infection in this case was the animal's own intestinal tract. It is to be regretted that cultivations were not made from the intestinal tracts of these four animals; on this account it was decided to repeat the series, and include intestinal cultivations in addition to cultivations from the seat of quinine injections. (See Table XVII.)

Table XVI.

Experiments on Guinea Pigs to determine whether Tetanus bacilli can be cultivated from the site of hypodermic injections of Quinine in cases where no Tetanus spores or Tetanus cultures had been given.

No. of experiment.	Dates on which quinine was given hypodermically.	Site of injection of quinine.	Date on which anærobic cultivations were made from site of quinine injection.	Result.	Remarks.
1	6th October 1910, ¾ gr. 8th October 1910, ¾ gr.	Front of chest .	10th October 1910.	Negative .	The cultivations from Nos. 1, 2 and 3 experiments were examined on several occasions up to the 14th day, and in none of them were there any bacteria resembling true tetanus bacilli.
2	Ditto .	Ditto .	Ditto .	Ditto.	
3	Ditto .	Ditto .	Ditto .	Ditto	
4	Ditto .	Ditto .	Ditto .	Growth of tetanus bacilli.	In the cultivation from No. 4 experiment, a pure growth of tetanus bacilli was obtained on the third day. This culture was very virulent, as evidenced by the fact that 0·1 c.c. of a 6 days' growth was sufficient to produce tetanus in a guinea pig in 12 hours and death in 24 hours.

The 17th series of experiments.

In the experiments on guinea pigs and monkeys recorded in Tables VIII, IX and XII, tetanus bacilli were isolated from the site of hypodermic injections of quinine given on front of the chest, when tetanus cultures, or "washed tetanus spores" had been injected in the hind leg.

The only reasonable conclusion to be arrived at in order to explain the presence of tetanus bacilli at the site of quinine injections in the experiments referred to is, that they had been transferred from the site of infection in the leg to the site of quinine injections on the chest; and with the object of controlling this conclusion the following experiments were carried out.

Four healthy full grown guinea pigs were each given a hypodermic injection of ¾ grain quinine on front of the chest, and after an interval of two days the dose was repeated. Two days after the second dose the animals were killed with chloroform and anærobic cultivations made from the subcutaneous tissue at the site of quinine injections on front of the chest, and also from the intestinal contents.

No evidence was obtained of the presence of tetanus infection in any of these cultivations.

The results of this series of experiments prove that the conclusion arrived at to explain the presence of tetanus bacilli isolated from the site of quinine injection in the experiments recorded in Tables VIII, IX and XII is probably correct.

Table XVII.

Experiments on Guinea Pigs to determine whether Tetanus bacilli can be cultivated from the site of hypodermic injections of Quinine, or from the intestinal tract in cases where no Tetanus spores, and no Tetanus cultures had been given.

No. of experiment.	Dates on which quinine was given hypodermically.	Site of injection of quinine.	Dates on which anærobic cultivations were made from		Result of anærobic cultivations made from		Remarks.
			Site of quinine injection.	Intestinal tract.	Site of quinine injection.	Intestinal tract.	
1	15th and 17th October 1910, ¾ gr. each time.	Front of chest.	19th October 1910.	19th October 1910.	Tetanus bacilli negative.	Tetanus bacilli negative.	
2	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.	
3	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.	
4	Ditto .	Ditto .	Ditto .	Ditto .	Ditto .	Ditto.	

The 18th series of experiments.

The result of No. 4 experiment in Table XVI rendered it necessary to prove whether guinea pigs harbour tetanus infection in their intestinal tract. Ten healthy guinea pigs were killed with chloroform, and a portion of the contents of the rectum and large intestine removed and emulsified in sterile broth, 1 c.c. from each emulsion was filled into glass pipettes, and heated in a water bath for 5 minutes at a temperature of 80°C., and used to inseminate 10 broth tubes. When these tubes had been incubated for 10 days at a temperature of 37°C., and under anærobic conditions, tetanus bacilli were found in one case, but not in the other nine. In three of the ten cases examined pseudo-tetanus bacilli were found; and other spore-forming bacilli were present in all. The tetanus bacilli found in the one case were probably non-virulent, as 1 c.c. of a 10 days' culture failed to give a guinea pig tetanus.

It is evident from the result of this series of experiments that only about 10 per cent. of guinea pigs harbour tetanus spores in their intestinal tract; and that these spores are not in all cases virulent. This possibly accounts for the fact that in numerous experiments on these animals with hypodermic injections of quinine only, none of them contract tetanus.

On the other hand the result of No. 4 experiment in Table XVI would point to the possibility of virulent spores being present in the intestinal tract of guinea pigs in exceptional cases.

Table XVIII.

Experiments to determine whether normal healthy Guinea Pigs harbour Tetanus spores in their intestinal tract.

No. of experiment.	Preparation of material used.	Cultivation used.	Result.	Remarks.
1	Emulsion in normal saline solution from portions of the rectum and the cæcum. Emulsion was heated for 5 minutes at a temperature of 80°C.	Anærobic broth culture	Negative.	
2	Ditto .	Ditto .	Growth of tetanus bacilli.	Non-virulent for guinea pigs in a dose of 1 c.c. of a 10 days' culture.
3	Ditto . .	Ditto .	Negative.	
4	Ditto . .	Ditto .	Ditto.	
5	Ditto . .	Ditto .	Ditto.	
6	Ditto . .	Ditto .	Ditto.	
7	Ditto . .	Ditto .	Ditto.	
8	Ditto . .	Ditto .	Ditto.	
9	Ditto . .	Ditto .	Ditto.	
10	Ditto . .	Ditto .	Ditto.	

The 19th series of experiments.

The object of this series of experiments was to test whether antitetanic serum can be trusted to protect against a certain lethal dose of a virulent tetanus culture, followed next day by a hypodermic injection of quinine. The tetanus culture used as a test was extremely virulent: $\frac{1}{50}$ c. c. was sufficient to kill a full grown guinea pig in 24 hours. It contained well formed bacilli with spores and toxine.

When a culture of this kind is injected hypodermically into a guinea pig the toxine is soon absorbed, and the spores proceed to germinate into fresh bacilli, which in their turn elaborate more toxine, and so the disease advances unchecked; but when antitetanic serum has been given before the tetanus culture, the toxine is neutralised, and a passive immunity is conferred which prevents the spores from developing, and leaves them an easy prey to the phagocytes. Two guinea pigs were each given 3 c.c. of tetanus antitoxin hypodermically, on the front of the chest, and after 3 hours a dose of a virulent tetanus culture in the hind leg. Next day they were given $\frac{3}{4}$ grain quinine hypodermically in the side. Both remained well. A control guinea pig which received half the amount of the same tetanus culture

used for the two experimental animals contracted tetanus after 12 hours, and died after 24 hours.

The fact that both animals survived the further test of a hypodermic injection of quinine would point to the spores being effectually dealt with at the site of injection.

The result of this series of experiments is direct experimental proof that anti-tetanic serum is a trustworthy prophylactic against tetanus when hypodermic injections of quinine are given.

Table XIX.

Experiments on Guinea Pigs to determine whether the hypodermic injection of Tetanus antitoxin can protect against the hypodermic injection of a virulent Tetanus culture.

No. of experiment.	Site of injection of antitoxin.	Site of injection of tetanus culture.	Result.	Remarks.
1	3 c.c. front of chest	Hind leg	Remained well	The culture used in these experiments was extremely virulent. Nos. 1 and 2 received 0·1 c.c., and No. 3 (control) 0·05 c.c., *i.e.*, half the quantity given to Nos. 1 and 2.
2	Ditto	Ditto	Ditto	24 hours after the antitoxin tetanus culture were given, Nos. 1 and 2 received ¾ gr. quinine hypodermically, but this had no effect in producing tetanus.
3 (Control of tetanus culture used.)	Ditto	Ditto	Tetanus after 12 hours. Died after 24 hours.	

The results of this series of experiments prove that tetanus antitoxin is a trustworthy prophylactic against tetanus when it is necessary to give quinine hypodermically.

CHAPTER VI.

Antitetanic Serum as a prophylactic in cases of Malaria in which it is necessary to give Quinine hypodermically.

Although cases of tetanus are, comparatively speaking, of rare occurrence after hypodermic injections of quinine, it is a terrible calamity when they do occur. To avoid with certainty what the late Professor Maclean [18] described as "something revolting in a death brought about directly or indirectly by a remedy intended to cure," the only reliable safeguard against tetanus when quinine must be injected hypodermically is a dose of tetanus antitoxin. (See Table XIX.)

In countries where severe forms of malaria occur, and in those cases where quinine cannot be tolerated by the stomach, or for other reasons, it may be sometimes necessary to give it hypodermically. When such a necessity arises, and especially in those localities of tropical countries where tetanus frequently occurs, it would be advisable to give a dose of antitetanic serum immediately before or immediately after giving quinine hypodermically. A dose of from 10 to 15 c.c. given hypodermically confers a passive immunity to tetanus for two or three weeks, and by that time the patient would probably not require any further injections of quinine.

It is well known that the principal use of antitetanic serum is not so much curative as preventive in its action; as a prophylactic it is most reliable. An injection of serum into the loose subcutaneous tissues of the side of the abdomen would not cause any pain or inconvenience to the patient, and this amount, for the time being, would render several daily injections of quinine safe as far as tetanus is concerned.

Those who have had an extensive experience in treating malaria in tropical climates assert that there are cases in which it is possible to save the patient by hypodermic injections when it would be impossible to do so by the ordinary administration of quinine. It is in such cases, and not as a routine measure in those who can tolerate quinine by the stomach, that hypodermic injections are justifiable. Given with the precautions which a dose of antitetanic serum would ensure, there would be no risk of tetanus ensuing, and the patient would only have to contend with the local reaction caused by the quinine. The passive immunity to tetanus which is so quickly conferred by antitetanic serum would prevent the local effects of quinine from acting as a focus for the growth of tetanus spores, and it would also prevent any latent spore infection from giving rise to tetanus under the combined favouring influences of quinine and an attack of malaria.

CHAPTER VII.

Conclusions.

1. Rabbits and guinea pigs are susceptible to the action of quinine when given hypodermically, or by the stomach. When a large dose is given to these animals it produces muscular spasms, convulsions, and death by asphyxia. When given hypodermically a certain lethal dose for a guinea pig is 1 grain per 150 grammes of body weight, and for a rabbit 6 grains per kilogramme of body weight; but in some cases less would suffice. When given by the stomach a certain lethal dose for a guinea pig is 1¼ grains per 150 grammes of body weight, and for a rabbit 15 grains per kilogramme; but in some cases less would prove fatal.

Rabbits are very susceptible to small doses of quinine given intravenously. In these animals ¾ grain per kilogramme of rabbit is a certain lethal dose, and kills in most cases within one minute, asphyxia being the immediate cause of death.

The fatal results of intravenous injections of quinine are not due to the acidity of the solutions, as evidenced by the fact that rabbits can withstand the intravenous injection of dilutions of sulphuric or hydrochloric acid of much greater acidity than the solutions of quinine which rapidly prove fatal.

¼ grain per kilogramme of body weight is a non-lethal dose of quinine for a rabbit when given intravenously.

2. When quinine is injected hypodermically, or into the muscles, it has a well marked destructive action on the tissues at the site of injection; and in addition to producing these foci of dead tissue which would serve as suitable anærobic media for the growth of tetanus spores should they by any chance become lodged there, it also gives rise to conditions favourable for infection with "washed tetanus spores" injected into other sites.

3. When quinine is given hypodermically to tetanus infected animals, tetanus germs are transferred from the original site of the tetanus infection to the site where the quinine has been injected. The experiments on this point confirm those carried out by Vincent in 1904.

4. Pure "washed tetanus spores" given hypodermically to guinea pigs and monkeys do not produce tetanus; but when quinine is injected hypodermically into a different part of the body, either the day before, the same day, or the day after spores are given, a large percentage of these animals contract tetanus.

5. Pure "washed tetanus spores" when mixed with quinine, or weak lactic acid, and injected hypodermically into guinea pigs invariably produce tetanus, but when given mixed with morphia the animals remain well. Quinine and lactic acid when injected hypodermically produce sites favourable for the development of tetanus spores; but morphia, and normal saline solution do not produce this effect.

6. Pure "washed tetanus spores" when injected hypodermically into guinea pigs remain latent at the site of injection for months, as evidenced by the fact that

virulent tetanus bacilli may be recovered from these sites after a period of seven months, and possibly after a much longer period. The importance of this fact in its relation to the hypodermic injection of quinine is evident.

7. Tetanus bacilli or tetanus spores are not found in the blood and internal organs in cases of acute tetanus, although they are invariably found at the original site of infection, and at the site of quinine injections given during the disease ; they are also found at the site of quinine injections when quinine has been the means of bringing about an infection with " washed tetanus spores " injected into a different part of the body.

8. Tetanus infection was present in the intestinal tract of healthy human subjects in four cases out of ten examined. In three of these cases the tetanus bacilli isolated were virulent for guinea pigs.

9. Cold has an influence in producing tetanus in guinea pigs when " washed tetanus spores " are given hypodermically : and this influence is increased by the hypodermic injection of quinine.

10. No evidence has been obtained of the presence of tetanus infection in any of the solutions of quinine used in the experiments recorded in this paper.

11. Some strains of tetanus spores are extremely resistant, and may remain alive and retain their virulence on a rusty nib for as long as 18 years, when the nib is placed in a test tube capped with rubber, and kept in a cupboard at room temperature.

12. Tetanus antitoxine is an efficient prophylactic against tetanus when it is necessary to give quinine hypodermically.

References.

1. Fournier Pescay, quoted by Burnet in " Campaign against Microbes," 1909.
2. Baron Larrey, quoted by Burnet in " Campaign against Microbes," 1909.
3. Seidillot, quoted by Etienne Burnet in " Campaign against Microbes," 1909.
4. Vincent, Annales de l'Institut Pasteur, December 1904.
5. Maclean, Diseases of Tropical Climates, page 69.
6. Mansop, Tropical Diseases, 4th edition, page 122.
7. Vincent, Annales de l'Institut Pasteur, December 1904.
8. Theobald Smith,—Journal of the American Medical Association, March 20th, 1908.
9. Roseneau,—A Practical Guide to Sterilisation, 1903.
10. Meyer & Ransome, Proceedings of the Royal Society, Volume 72, No. 477 for 1903.
11. Henri & Cenrodeannu,. Compt. Rendu, May 4th, 1907.
12. Burnet, Campaign against Microbes, 1909.
13. Metchnikoff on Immunity.
14. McFarland's Bacteriology, 4th edition, 1904.
15. Vaillard & Vincent, Annales de l'Institut Pasteur, 25th January 1891.
16. Vincent, Annales de l'Institut Pasteur, December 1904.
17. Vincent, Annales de l'Institut Pasteur, December 1904.
18. Maclean, Diseases of Tropical Climates, page 69.

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OF THE

GOVERNMENT OF INDIA

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ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA

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SUPERINTENDENT GOVERNMENT PRINTING, INDIA
1911

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