

Report of experimental work on protective inoculation for anthrax and quarter ill / by William Robertson and John Penberthy.

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REPORT OF EXPERIMENTAL WORK

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ON

PROTECTIVE INOCULATION

FOR

ANTHRAX AND QUARTER ILL.

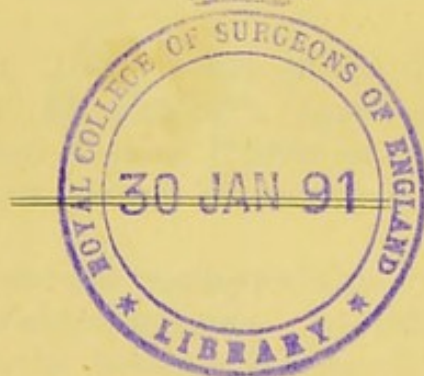
By WILLIAM ROBERTSON,

PRINCIPAL OF,

AND

JOHN PENBERTHY,

PROFESSOR OF THERAPEUTICS AT THE ROYAL VETERINARY COLLEGE.



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REPORT OF EXPERIMENTAL WORK
ON
PROTECTIVE INOCULATION FOR ANTHRAX
AND QUARTER ILL.

IN obedience to instructions received from the Veterinary Committee of the Society, we have been engaged in experimental inquiries into Protective Inoculation for Anthrax and Quarter Ill, and, acting on the suggestion of the Committee, our efforts have been principally directed to providing means of protective inoculation against Quarter Ill, and with the view of testing the statements regarding the efficacy of Pasteur's preventive for Anthrax.

The commencement of this work was, we regret to say, much delayed by the tedious process of obtaining a licence from the Government to carry out such part as was deemed purely experimental; four months having elapsed between the dates of application for and receipt of the official permission.

In further detailing what has been done, we will relate first what pertains to Anthrax—or Splenic fever—and to the employment as a preventive for the disease of the Pasteurian prepared "vaccine."

On October 13th, 1886, some tubes of Anthrax vaccine were obtained from M. Pasteur's agent in Paris. On the same day the specified dose of "premier vaccin" was injected into the subcutaneous tissue of two young bullocks at the College.

Up to the 24th there was no appreciable systemic disturbance. The temperature remained normal. At this date each animal was inoculated with the prescribed dose of "deuxième vaccin." As little disturbance followed this latter part of the operation as was noticeable after the former. (It should be mentioned that two inoculations, with an interval of ten days between each, are deemed necessary for the provision of protection.) This vaccine, microscopically examined and cultivated, was found to contain the anthrax bacillus. At the time of the last protective inoculation a small quantity of "deuxième vaccin" was injected

into a rabbit and guinea-pig. On the following day both these small creatures were found very unwell. Sixty-four hours after inoculation the guinea-pig was found dead, and 108 hours after inoculation the rabbit. The blood of both contained anthrax bacilli in considerable numbers. A yellow mouse, inoculated with a drop of blood from the dead guinea-pig, died in 36 hours.

Cultivation with blood from these several sources verify the statement that in Pasteur's "deuxième vaccin" there are bacilli capable of killing the rabbit, guinea-pig, and mouse, but which in due time, after inoculation with "premier vaccin," have no deleterious effect on young bovines.

We now experienced some difficulty in obtaining unattenuated virus from a naturally contracted case of anthrax. It was not till November 29th that we received from Dartford an affected spleen, about the condition of which for experiment there might be some doubt. Its virulence was, however, proved by inoculation of a rabbit, which died 21 hours after, its blood swarming with the anthrax bacilli. Cultivation yielded a fine growth in 24 hours, some of which injected into another rabbit induced death in 32 hours.

On December 10th, some viscera were obtained from another

REGISTER OF TEMPERATURES OF TWO CALVES INOCULATED WITH VIRULENT ANTHRAX MATTER, DECEMBER 10TH, 1886.

Dates.	"Vaccinated."	Not "Vaccinated."		"Vaccinated."	Not "Vaccinated."
1886.	Fahr.	Fahr.	1886.	Fahr.	Fahr.
Dec. 10, at 9.30 P.M.	101.6	102.7	Dec. 18, at 5.5 P.M.	102.1	103.1
" 11, " 9.30 A.M.	105.1	103	" 19, " 1.0 A.M.	102.3	101.5
" 11, " 3.50 P.M.	106.8	106	" 19, " 9.15 A.M.	103.1	101.1
" 12, " 12.50 A.M.	105.8	107.3	" 19, " 11.30 P.M.	102.2	102.2
" 12, " 10.40 A.M.	102.2	106.4	" 20, " 9.35 A.M.	100.6	101
" 12, " 3.10 P.M.	102.2	106.1	" 20, " 5.20 P.M.	102	100.5
" 13, " 9.55 A.M.	102.6	105	" 21, " 9.30 A.M.	101.2	102
" 13, " 4.5 P.M.	103.3	105.2	" 21, " 4.5 P.M.	102.2	103
" 14, " 12.5 A.M.	103.2	103.8	" 22, " 9.50 A.M.	100.6	102.3
" 14, " 9.30 A.M.	103.2	102.5	" 22, " 4.30 P.M.	102.8	104
" 14, " 4.5 P.M.	103.6	105.2	" 23, " 9.0 A.M.	102.2	102.5
" 15, " 12.5 A.M.	103.6	107.6	" 23, " 5.0 P.M.	101.4	101.4
" 15, " 9.30 A.M.	103.7	106.5	" 24, " 4.0 P.M.	102.0	103.4
" 15, " 5.35 P.M.	102.5	107.6	" 25, "	103.0	102
" 16, " 9.0 A.M.	101.8	103.3*	" 26, "	103.2	102.6
" 16, " 12.5 A.M.	Not taken.	106.7*	" 27, "	102.4	103.6
" 16, " 5.30 P.M.	102.5	105.7	" 28, "	103.8	101.4
" 17, " 12.40 A.M.	103.5	107	" 29, "	102	101.4
" 17, " 9.45 A.M.	102.5	105.2	" 30, "	102.4	102.4
" 17, " 3.45 P.M.	103	103.7	" 31, "	101.3	102.3
" 18, " 12.20 A.M.	102.8	103.6	Taken several days after this; no appreciable variation.		
" 18, " 10.0 A.M.	102.4	103.1			

fatal case of anthrax in a bullock. Microscopic examination of spleen pulp showed anthrax bacilli in large numbers. It was decided to use this as a test. About 30 drops of a mixture of spleen pulp and distilled water, were injected into the subcutaneous tissue of one of the oxen "protected" with Pasteur's vaccine, and of an ox which had not been so protected. There was no appreciable general indisposition in either case. The thermometer, however, showed a very high temperature in the unprotected animal, remaining with slight variation up to near 107 till the 16th. That of the "protected" animal, it will be seen, became elevated on the day following the injection to 106·8, after which it almost immediately receded to normal.

At the same time, and with the same material, a rabbit was inoculated in the subcutaneous tissue of the abdomen. Up to the night of the 13th, there was no evidence of systemic disturbance, but at 5 A.M. on the 14th, 85 hours after inoculation, it died. Its blood swarmed with anthrax bacilli.

On January 28th, 1887, an outbreak of anthrax of a most viru-

REGISTER of TEMPERATURES of CALF VACCINATED OCTOBER 13TH, 1886, and INOCULATED with VIRULENT ANTHRAX BLOOD, JANUARY 28TH, 1887.

Dates.	Fahr.	Dates.	Fahr.
1887.		1887.	
Jan. 29, at 9.45 A.M. ..	105·8	Jan. 30, at 11.45 P.M. ..	104·3
„ 29 „ 4.30 P.M. ..	106·7	„ 31 „ 9.45 A.M. ..	102·4
„ 29 „ 9.45 P.M. ..	105	„ 31 „ 11.55 P.M. ..	103·3
„ 30 „ 9.45 A.M. ..	103·6	Feb. 1 „ 10.10 A.M. ..	102·5
„ 30 „ 5. 0 P.M. ..	104·1		

lent type occurred at Chelmsford, in which 46 adult cattle died within a few hours. Being personally engaged in the investigation of this matter, we selected some material from a case most rapidly fatal. The same night we injected 30 drops of a mixture of spleen pulp and distilled water into the remaining ox, which had been "protected" with Pasteur's vaccine. Beyond the fact that the temperature rose on the following day to 106° F., and a little swelling at the point of inoculation, there was nothing worthy of remark till February 13th, when an abscess was noticed at the seat of previous swelling. This became very large—much pus escaping on its being opened. There was no bovine or other large susceptible animal at hand which could be used as a control. A rabbit, however, subjected to the same test showed much swelling at seat of inoculation, and died of typical anthrax in 78 hours.

The foregoing cannot be regarded as absolute and in itself

sufficient proof of the protective power of M. Pasteur's vaccine, though whatever demonstration there may exist, it is certainly favourable to it.

Taking into consideration the expressed desire of the Veterinary Committee of the Society, and the means at our disposal, it was deemed unadvisable to prosecute this matter farther.

The apparatus essential to the production of the "vaccine" by Pasteur's method is now in working order at the College, and can be utilized for the purpose at the Society's pleasure; but in view of the limited nature of outbreaks of the disease in Great Britain, it will in all probability be more economical to obtain the necessary protective material direct from M. Pasteur's laboratory in Paris, should such be required.

The disease technically known as "Symptomatic Anthrax," "Charbon Symptomatique," &c., has in different localities in this country received various appellations, as "Quarter Ill," "Black Quarter," "Black Leg," "Strike," "Felling," "Irons," "Puck," &c., most of which are in some way significant of its more manifest characteristics. Its distribution throughout Great Britain is general, and the national loss from its ravages considerable. On many farms the annual average has been computed at from 5 to 10 per cent. Essentially a malady of young stock (here, at least, particularly cattle), its effects are most marked in breeding and rearing districts.

Its more prominent features may be summarized as follows:— Its ordinary subjects are young cattle with ages ranging from six months to two years. Animals above and below this age seem to enjoy a large share of immunity. The attack is sudden, and the course usually rapid and fatal. Lameness and crepitating swelling of some parts of the limbs or trunk, the first manifestations, generally terminate in death in from seven to fifty hours. On removal of the skin covering the swelling, the underlying tissues are found very dark in colour, and on being cut into, much serosity exudes. The rapidity of its course, and the remarkable change in structure referred to, have caused Quarter Ill to be commonly mistaken for and confounded with "Anthrax" proper. Though contagious, and belonging to the same class, the essential features of the two diseases are absolutely distinctive. Both are induced only by the entrance of minute organisms (bacilli) into the healthy body.

The bacillus to which Quarter Ill is attributed is stouter than that of Anthrax, and rounded at its extremities. The anthrax bacillus requires the presence of oxygen for the manifestation of its vital phenomena, and when viewed under the microscope, shows no motion of its own. The Quarter-Ill bacillus, on the

contrary, appears to move freely in the field of the microscope, and does not require oxygen in its medium of support and development. Experiment goes to prove that this gas is highly deleterious to it, muscle-juice on being exposed to atmospheric air for some time becoming free from the organism.

While the bacillus of Anthrax shows a great selective affinity for the blood of its victim, it being usually found there in large numbers soon after death, and the blood most virulent, the blood from an animal dead of Quarter Ill is free of its bacilli, or contains them only in nearly unappreciable quantity, and is innocuous when inoculated in considerable amount. Examination and cultivation, however, demonstrate the constancy of the Quarter-Ill bacillus in affected muscle.

Klein* asserts that injection of the bacilli of Quarter Ill into the subcutaneous tissue of guinea-pigs, rabbits, sheep, and calves, *always* proves fatal. Our experiments appear to prove that this statement in general is not consistent, while inoculations into the rabbit show that animal to be in a special degree refractory to the effect of the bacilli. Injection of blood containing the anthrax bacilli into rabbits is in the large majority of instances fatal.

Though aware of certain positive statements regarding the possibility of producing the disease by infection or inoculation, as the fact was not generally appreciated, and as previously published experiments appear to have been carried out with virulent matter obtained direct from France, we deemed it expedient at the outset to direct our attention to this point.

In reply to advertisement, we received on the 3rd of November, 1886, the first reliable material (some fresh muscle-juice) from a naturally contracted case, and expeditiously despatched in bulk to us, by Mr. Clark, M.R.C.V.S., of Horncastle. Examination showed the characteristic bacilli.

At 3.45 P.M., one drop of muscle-juice was injected into the subcutaneous tissue of each of two guinea-pigs. Five drops from the same source were injected into the muscles of the haunch of a young bullock of about ten months.

At 6 A.M. on the 4th, one guinea-pig was ill, the parts about the seat of inoculation showing characteristic emphysematous swelling; at 4 P.M., the same conditions were manifest in the other guinea-pig. At 10 A.M. (4th), the bullock's health appeared much disturbed, temperature 105.7, the limb much swollen. A course typical of Quarter Ill terminated fatally in case of one guinea-pig 18½ hours after inoculation, of the other

* Klein's 'Micro-organisms and Disease,' 1885.

guinea-pig in 36 hours, and of the young bullock in 57½ hours. Post-mortem examination revealed positive evidence of the disease.

Microscopic inspection and sowings of both blood and muscle-juice from each of the above cases were made. In no instance could bacilli be detected in the blood, while in variable numbers they were always seen in muscle-juice. Cultivation of muscle-juice in veal broth never failed to yield growths of characteristic bacilli. Sowings of blood under same circumstances were invariably sterile.

With muscle-juice from another very malignant case reported to us on November 11th we inoculated two guinea-pigs, which died respectively 34 and 84 hours after inoculation. Soon after death of that which survived only 34 hours, two other guinea-pigs were inoculated with its blood. These animals seemed to suffer little or no inconvenience, and lived under our observation for two months.

Cultivations of serosity taken from the above young bullock 12 hours prior to death (from Quarter Ill) by puncturing with a glass pipette showed bacillar growth in 24 hours similar to that of muscle-juice of guinea-pigs inoculated at the same time. The spores were, however, very numerous. Inoculation of rabbits with this material did not produce the disease.

The point of inoculability of the disease, and its identity with that described by Continental writers, appear from the foregoing to be satisfactorily established.

Our investigations into methods for obtaining protection from the disease have been prosecuted on principles enunciated by three eminent French veterinary surgeons, MM. Arloing, Cornevin and Thomas, of the Lyons Veterinary School, in a work published in 1883. These *savans* describe measures, all of which they state to be effectual in inducing a state of immunity, by the introduction into the healthy animal of the virus in its natural state or in a state of attenuation.

Discarding the others as less practical, we have directed our experiments mainly to two plans apparently more easy of application in this country. From public report it would appear that in France "vaccination" for Quarter Ill is chiefly carried out (and this extensively) by introduction of dried muscle-juice into the subcutaneous tissue. The great advantages claimed for this method over most others are that the material ready for use may at any time be obtained by the veterinary surgeon, and that in its proper use there is not the slightest danger to the vaccinated animal. The "vaccin" is in France now regarded as an article of commerce, and having first ascertained by letters

from M. Arloing that M. Fromage of Paris was his agent for the sale of it, we obtained from this source a supply of material. Through the kindness of C. De Murrieta, Esq., of Wadhurst Park, Sussex, who, in August, 1886, wrote expressing his great interest in the matter, and generously placed some of his young stock at our disposal, we were enabled to commence testing the efficacy of Arloing's dried muscle-juice.

On August 21st, Professor Penberthy proceeded to Wadhurst Park, and carried out the first part of the process of protective inoculation by injecting into the subcutaneous tissue of the tails of six young beasts (carefully marked for distinction) a few drops of a solution in pure water of Arloing's prepared muscle-juice subjected to a temperature of 100° C. After an interval of ten days, Professor Robertson proceeded to Wadhurst Park and completed the process by injection in the same situation of the vaccine No. 2, which is dried muscle-juice subjected to a temperature of 85° C.

These animals were then, with six others unprotected, placed in a pasture traditionally notorious for the fatalities from Black Quarter occurring in it. Notwithstanding the assurances of the attendants acquainted with the place, that some, if not all, would be attacked, two months elapsed without the slightest appreciable change in the animals, save a little soreness at the seat of inoculation. Temperatures regularly taken and carefully registered by Mr. Chapman, agent at Wadhurst, showed little variation.

It was determined to test further the efficacy of this operation by introduction of muscle-juice from a naturally contracted case. The first opportunity occurred on November 30th, on receipt of some muscle forwarded by Major Percy, of Hodnet, and said by him to have been taken from a typical case. A considerable quantity (4 cc.) of expressed muscle-juice was injected into the thighs of each of three vaccinated and three unvaccinated calves. Save slight lameness of two of the animals (one vaccinated and one unvaccinated), they appeared to remain in perfect health, the thermometer indicating normal temperature for six following days.

On January 7th, 1887, Mr. Couchman, V.S., of Wadhurst, telegraphed that he had met with a marked case of Quarter Ill in a young bull still alive, and within two miles of Wadhurst Park. On the 8th, Mr. Penberthy proceeded by early train to the spot, recognized a clear case, obtained the necessary material from the affected muscles of the bull, which had died 18 hours previously, and injected 5 cc. into the thigh of each of the so far untested experimental animals, *i.e.* three vaccinated and

three unvaccinated. Strangely enough, with the exception of a considerable local swelling in one of the vaccinated calves at the seat of inoculation, and the elevation of its temperature on the following morning to 106.2° , there was no disturbance. The temperature of this animal dropped by degrees daily to normal.

On return to College, a guinea-pig was inoculated with one drop of muscle-juice, and three rabbits with three drops each. The rabbits suffered no ill-effects, while the guinea-pig died of Quarter Ill in $28\frac{1}{2}$ hours. The principal inference from this experiment, one item of which entailed four journeys into the middle of Sussex, appears to be evidence in direction of the view that circumstances may materially modify the infecting power of the virus, or the susceptibility of the animal. To the fact of a mild attack of the naturally contracted disease establishing protection we shall again refer. In absence of satisfactory elucidation of the reason we must, at least for the time, be content to accept it as an axiom that extrinsic and intrinsic conditions affecting the animal, and probably the virus, obtain to a greater extent in this than in many other contagious diseases. This bears out the result of clinical observation generally as to its erratic occurrence.

In order to give another trial to M. Arloing's "vaccine" powder, a fresh quantity was obtained from his agents in Paris, and on January 18th at the College, a heifer and steer, each about 10 months old, and a guinea-pig, were vaccinated in accordance with the directions with the powder. After the prescribed interval the process was completed. Careful observation revealed no disturbance from the operation.

On February 3rd, Mr. Godman of Woldingfold, Horsham, with whom we were previously in communication, wrote saying that one of his heifers was attacked with Quarter Ill, and that he wished the remainder of his young stock "protected." Accordingly, on the following morning, Mr. Penberthy proceeded to Woldingfold, and "vaccinated" twenty-three animals with Arloing's No. 1 vaccine, intending to go down and finish the process in due course. The case of the heifer was characteristic, and some muscle-juice containing the virus in considerable numbers was used to test the effect of the powder on the vaccinated steer and heifer at the College, and on a guinea-pig. In the case of the cattle a few drops were injected at 8.35 P.M. into the thigh of each, and one drop into the subcutaneous tissue of the guinea-pig. On the following morning the animals were noticeably ill. Symptoms of acute Quarter Ill developed; the guinea-pig died $24\frac{1}{2}$ hours, the steer $44\frac{1}{2}$ hours, and the

heifer 47 hours after inoculation. The following chart shows temperatures at:—

4th Feb. 1887.		Steer.			Heifer.
9 A.M.	104·3 Fahr.	105·6 Fahr.
2 P.M.	102·3	106·4
9·30 P.M.	101·5	102·2

Post-mortem examination revealed typical Quarter Ill. After this, with the concurrence of Mr. Godman, it was decided that the remainder of the process of vaccination by Arloing's powder method should not be carried out on the 23 calves.

At the same time that the heifer, steer, and guinea-pig were vaccinated with Arloing's powder, we inoculated another guinea-pig with muscle-juice dried at 32° C. prepared by ourselves. This little animal was subjected to precisely the same condition of inoculation of virulent material from Godman's case as the calves and a guinea-pig, and experienced no ill-effect, living apparently in perfect health for two months after.

This latter circumstance induced us to make further trials of material prepared on the principle of the last mentioned. Muscle-juice was dried and subjected to various temperatures. On February 14th, six guinea-pigs were vaccinated, three with powder produced at a temperature of 32° C., and three with some produced at 39° C. Forty-eight hours after inoculation one of the latter died, and 98 hours after one of those inoculated with 32° C. powder.

On February 25th and March 7th, three fresh guinea-pigs were vaccinated with Arloing's powder.

Up to the latter date the four surviving inoculated guinea-pigs appeared perfectly well. On the morning of the 8th of March one was found dead, which was inoculated with 32° C. material on February 14th,—one which had been inoculated and, as above, resisted virulent injection. One by one they were found dead, until by the 4th of April the whole of our experimental animals had succumbed. During the occurrence of this mortality there was great variation of atmospheric temperature, in addition to which we now have reason for supposing there was neglect on the part of the special attendant, who was discharged at this date. Post-mortem examination yielded no evidence of Quarter Ill.

These accidents were of course disastrous to this aspect of our work, which there had been reason for hoping might yield some practical result. We may, however, learn from it that three out of four guinea-pigs which had been inoculated with muscle-juice dried at 32° C., and two out of three inoculated with muscle-juice dried at 39° C., survived without manifesting

ill-effects. It is also remarkable that one guinea-pig inoculated with muscle-juice dried at 32° C. suffered no inconvenience from injection of virulent matter, which killed two calves and a guinea-pig "vaccinated" with Arloing's powder.

It should perhaps be mentioned here that the French experimenters first dry the muscle-juice at 32° C., and then submit it—mixed with water—for some hours to dry heat of 100° C., and 85° C., respectively for "first and second vaccines." The effect of the high temperature is probably the attenuation of the virus. It seems to us highly probable that the system of the calf would resist the injurious effects of a more potent virus than that in use in the form of M. Arloing's powder, and that a more potent virus would be more likely to make a protective impression on its subject. We therefore think it desirable that further investigation into this matter should be prosecuted.

Another phase of the disease is very interesting, and partly depending on it is the basis of another system of protective inoculation. The virus when introduced in moderate quantities (a fraction of which into the muscles would be fatal) into the blood stream of a susceptible animal, is incapable of inducing the affection. It has been asserted, by the writers before referred to, that introduction of fresh virus into the blood current of the living animal does not induce the disease, but that it actually confers on the animal so treated immunity for periods ranging from one to two years.

Experiment on this system was rendered practicable by the magnanimous offer of Major Algernon Heber Percy, of Hodnet Hall, Shropshire, who communicated to us an expression of his great interest in the work in hand, and volunteered to provide the necessary animals and attention for carrying out a series of trials on this special point at his farm adjoining Hodnet Hall. The offer was cordially accepted, and the necessary preliminary arrangements made. Commencement of operations depended on the occurrence of a typical case of Quarter Ill at or near Hodnet. The opportunity arose on November 11th, when, in response to a telegram from Major Percy, Mr. Penberthy proceeded to Hodnet the same night. On arrival it was found that the animal which was to provide the protective material had died at 11.45 A.M. Major Percy, with the kind assistance of his neighbours, Viscount Hill, Sir Vincent Corbett, Sir Thomas Meyrick, Mr. J. Tayleur, Mr. J. Bibby, and Rev. J. Hill, had collected early in November eight calves of different sizes, and of ages varying from 6 to 10 months. They had for some days been grazing together, and were now placed at our disposal for the experiment. It was decided that four of these animals should be protected by intravenous injection and placed with

the remaining four; the whole to be subject, save for inoculation, to precisely same conditions. To facilitate reference, the calves were marked and known as 1, 2, 3 and 4, and A, B, C and D.

On the morning of November 12th, a large number of agriculturists, veterinary surgeons and others had assembled to witness the operation. Examination of the dead calf revealed a clear case of Quarter Ill, apparently free from putrefactive change. Pieces of muscle from the most affected part were then taken and the juice obtained by squeezing through coarse linen. After diluting with water, the material for inoculation was ready. Calf No. 1 was then cast and secured with ropes, the jugular vein laid bare by dissection, and with the utmost precaution, to avoid contaminating the connective tissues, a few drops of the diluted muscle-juice were injected into the blood stream by means of a very fine hypodermic syringe. No. 2 was treated in the same way. In the case of Nos. 3 and 4, the material was introduced into the jugular vein by passing the hypodermic syringe point directly into it through the skin without previous dissection. Duly marked for identification, the eight calves were turned into a pasture and treated in every way alike, except that the temperatures of the inoculated were taken twice daily for eleven days. The register on page 14 shows that the heat-regulating functions were scarcely noticeably disturbed. The thermometry was undertaken by Major Percy, who personally superintended it, and Mr. Tomes, bailiff, who evinced the greatest care and interest throughout, states that up to the 24th all the calves appeared perfectly well.

It was previously arranged that at the expiration of seven days the virtue of this intravenous inoculation should be tested by injection into the muscles of virulent matter obtained from a naturally occurring case of Quarter Ill; the whole eight being subjected to the same treatment. To obtain the fresh virus for this purpose we put ourselves in communication with several stock-owners and veterinary surgeons. On receipt of a telegram advising us of a case in the practice of Mr. Brett, M.R.C.V.S. of Mansfield, on November 23rd, Mr. Penberthy proceeded thither, informing Major Percy that he expected to be able to apply the test at Hodnet Hall on the following day. On arrival at Mansfield the animal was still alive and suffering from Quarter Ill; but this being of a mild type and not likely to die immediately, we deemed it not quite satisfactory for so crucial a test. We determined to utilize this case for another purpose, and communicated our intention to Major Percy. By a happy coincidence preliminary signs of the disease were noticed in a heifer at a farm near Hodnet Hall, and being made aware of this fact by telegram, Mr.

REGISTER of TEMPERATURES of FOUR CALVES, INOCULATED with QUARTER ILL by INTRAVENOUS
INJECTION.

(INOCULATED NOVEMBER 12TH, 1886, 11.30 A.M.)

CALVES	Nov. 12th.	Nov. 13th.	Nov. 14th.	Nov. 15th.	Nov. 16th.	Nov. 17th.	Nov. 18th.	Nov. 19th.	Nov. 20th.	Nov. 21st.	Nov. 22nd.	Nov. 23rd.	Nov. 24th.	Nov. 25th.
No.	4.30 P.M.	9.30 A.M.	9.45 A.M.	9.30 A.M.	10.0 A.M.	11.0 A.M.	Noon P.M.	7.30 A.M.	7.30 A.M.	7.25 A.M.	7.30 A.M.	7.35 A.M.	7.25 A.M.	10.45 A.M.
1	102.2	103.2	102.6	101.4	103.0	103.0	103.4	103.0	104.6	102.2	102.2	104.6	103.8	103.8
2	102.0	102.4	103.6	102.0	103.0	103.0	103.2	103.0	102.4	103.2	103.4	102.8	103.2	103.4
3	103.0	102.6	102.4	102.2	103.0	102.4	103.4	103.0	103.4	102.4	103.6	102.2	102.2	102.8
4	102.4	101.8	103.4	101.4	102.2	102.0	101.6	102.2	102.4	103.4	103.4	102.4	102.6	103.0

Penberthy started from Nottingham for Hodnet immediately. On arrival it was announced that, in addition to the heifer referred to, one of the experimental animals (uninoculated) A. was noticed lame at 4.30 P.M.; at 9.30 P.M. we found it obviously dying of Quarter Ill. Next morning at 6.30, A. was dead and cold, and at 11.20 A.M. the heifer first seized died.

At 12 noon on the same day, in presence of a large number of gentlemen interested, the majority of whom had witnessed the inoculation on November 12th, Professor Penberthy injected, with all antiseptic precautions, into the subcutaneous tissue of the limbs of the remaining seven calves, a considerable quantity of virulent matter taken from the muscles of the still warm heifer. The seven were then placed and kept together in a meadow, under identical conditions. The table on page 16 is a register of the temperature taken twice daily.

It will be borne in mind that A. uninoculated had died of the disease naturally contracted while grazing with the others.

On the early morning of the 26th, C. was found so lame of the right hind limb that it could not walk. D. also was lame of the right hind limb.

At 9 A.M. of the 27th, C. died, and Major Percy writes: "C. was opened in presence of several friends, and was proved to be a bad case of 'striking'" (Quarter Ill), "spreading in all directions from point of inoculation."

On the 28th D. appeared very stiff in both hind limbs, mostly the left; he died at 10 A.M. on the 29th, and was opened in the presence of several farmers, and was also a clear case of "striking," spreading on both sides from the point of inoculation.

On the 28th a crepitating swelling was noticed on B.'s side at seat of inoculation; she appeared very dull and unwell. On the 29th a "lump" was found at the above-named spot, and she was very unwell. On November 30th the swelling was hard and much more defined. It gradually softened on the subsequent days, the calf returning to a state of health.

Of those inoculated intravenously on November 12th, *i.e.* Nos. 1, 2, 3 and 4, neither has appeared to suffer in the slightest degree. Of the four unprotected, one A. died of the disease taken naturally while grazing with the others; two, C. and D., died of the disease produced by the test inoculation on the 25th; and one, B., through contracting the disease from the test inoculation, survived in a manner we have frequently had occasion to notice. It was suggested to Major Percy that after perfect recovery B. should again be inoculated with virulent matter, with a view of ascertaining how far his mild attack had afforded him immunity, as it is usual for mild or resisted attacks

REGISTER OF TEMPERATURES OF SEVEN CALVES INOCULATED IN TISSUE.

(INOCULATED NOVEMBER 25TH, 1886, 12 NOON.)

Calves.	Nov. 25th.	26th.		27th.		28th.		29th.		30th.		Dec. 1st.		2nd.		3rd.	4th.	5th.	6th.	7th.	8th.
		A.M.	P.M.	noon.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.						
No.		3.45	3.35	12	5	7.44	3.40	7.35	5.45	10	3.15	4.35	5.45	7.25	4.40	7					
	Noon. Inoculated.	P.M.	P.M.		P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.					
1	{ Behind Left Fore Elbow. }	103.2	103.2 102.2	102.4	102.6	103.2	102.6	103	102.8	103.4	103	103.6	102.6	103.6	102	103					
2	{ Left Thigh. }	103	103.2 102.4	103	103.2	103.2	102.8	104	103	103.6	102.6	103.4	103	103.4	103.2	103.4					
3	{ Left Shoulder. }	102.2	102.2 103	102.6	102.2	103.2	102.8	103	102.4	103.8	102.8	102.8	102	103.2	102.4	103.2					
4	{ Right Hind Thigh. }	102.8	102.6 102.2	102	102.2	103	102	103.2	102.2	102.8	102.4	102.4	102.4	103	103	103					
A.																					
B.	{ Left Fore Shoulder. }	102.4	103.6 102.6	103.8	104.2	103.8	104.6	104.6	103.4	104	102	103.4	102.6	103.6	102.8	103.8	103.4	103.4	103.4	102.8	103.6
C.	{ Right Hind Thigh. }	102.8	105 104.4																		
D.	{ Right Hind Thigh. }	103	104.6 105.2	105.6	106	107.4	104.8	104.6													

Registration not continued.

Died of the naturally contracted disease on November 24th.

Died at 9 A.M. November 27th.

Died at 10 A.M. November 29th.

of such diseases to act in this fashion ; but it was decided that it could not be conveniently carried out.

However, to test this view, we utilized the mild case previously referred to as occurring near Nottingham. On December 29th, after perfect recovery of the bullock, sixty minims of muscle-juice taken fresh from a fatal case of Quarter Ill were injected into its thigh. Following this there was little general and no appreciable local disturbance. The temperature four hours after the operation rose to 104·2, in eighteen hours it became normal, and from this time the animal fattened rapidly.

Simultaneously with the bullock, two guinea-pigs were inoculated each with two drops of muscle-juice from the same source, and died within 25 hours, post-mortem examination revealing conditions characteristic of Quarter Ill.

In order to prove the practicability of the intravenous method of protective inoculation on December 22nd, Professor Penberthy, with aid of Mr. Tomes, the bailiff, inoculated 24 young cattle at Hodnet Hall. On this occasion, the injection was done in every case by piercing direct through the skin into the vein. The injected virus was taken from a heifer which had died some hours previously. The operation lasted about 1 hour 20 minutes. There was, Major Percy states, no sign of indisposition in the animals after the inoculation, and up to the date of writing this report there has been no case of Quarter Ill amongst these calves.

It may be interesting to add here the statement that each of the seven animals which died during the carrying out of the experiments at Hodnet Hall had been duly setoned and drenched.

The practical outcome of this work appears to us to be that it has made clear the fact of the communicability by inoculation of the disease known in England as Quarter Ill, and its identity with that spoken of by both Arloing, Cornevin and Thomas as "Charbon Symptomatique." Without further proof of its efficacy, we cannot recommend the use of Arloing's dried muscle-juice as a means of protecting cattle from the disease in Great Britain.

The simplicity of the method of inoculation described, as well as its general practicability, favours its adoption. Inasmuch as some of our experimental guinea-pigs, after being "vaccinated" with muscle-juice subjected to the effect of a lower temperature, resisted the influence of injected virulent matter, some of which killed cattle and other guinea-pigs vaccinated with Arloing's powder, further experiment in this direction should be made. In such case, and indeed in all, the result

is likely to be incomparably more valuable and trustworthy if experiment for protection be made with animals of the class for which practical protection is sought.

The Hodnet Hall experiments go very far to prove that the intravenous injection of considerable quantities of fresh virus is protective, and to a large extent practicable. The greatest drawback to its general adoption seems to be the necessity for fresh material with which to inoculate. This difficulty is not really as great as at first sight may appear. It at least has this in its favour, that it presupposes the existence of the disease on the estate, and does not encourage the chance of introducing fresh disease from without, as is probably the case with some systems of inoculation.

Resulting from the publication of a report of the experiments at Hodnet by Major Percy, several stock owners have applied to us to have their animals protected by the intravenous method. In view of the results already obtained, we strongly advise the further and fuller adoption of this plan.

In concluding our Report, we must again acknowledge our indebtedness to Mr. C. De Murrieta for placing animals and services at our disposal; to Major Percy, whose untiring interest in the whole matter and great solicitude for the proper observance of all details in connection with the Hodnet experiments have had no small share in the attainment of their success; to Messrs. Bennett, Pyatt, Kettle and Barron, veterinary surgeons, who, together with other gentlemen before referred to, have rendered material assistance.

After the above Report was written, one lot of 53 animals, belonging to Lord Egerton of Tatton, were inoculated, after the manner described in the Hodnet Hall experiments, with the virulent matter of Quarter Ill, taken from an animal of the herd which died on the spot after the arrival of Mr. Penberthy. Of this number, 4 animals died of Quarter Ill, and another subsequently, though not of the same disease.

Such an untoward result may, it is hoped, be guarded against by greater experience in the manipulatory work.—W. R.



