

From this date all pathological changes disappeared. The animal was killed on the 20th April, that is forty-eight days after inoculation.

*Post-mortem examination.*—Donkey foal, two and a half years old, condition very good. In the middle of the right jugular furrow an ulcer measuring  $7 \times 5$  cm. (bacillus necrosis?) was found. In the peritoneal cavity were a few drops of liquid. The intestinal tract was normal. Spiroptera tumours were present in the stomach. Both kidneys were of normal size, the capsule was easily detachable; in the right kidney was a small, egg-shaped infarct the size of a pea, passing through the cortex into the intermediate zone. In the left kidney was a white patch the size of a pin's head, and small streaks were noted in the cortex. The rest of the parenchyma and other urogenous organs were normal. The spleen measured  $32 \times 35 \times 17$  cm.; the pulp was distinctly granular and slightly protruding. In the liver were a few calcareous nodules, otherwise it was normal. All thoracic organs were normal. Cultures from the infarcts and microscopical examination was made, but no bacteria could be demonstrated.

*Diagnosis.*—Renal infarcts, probably as a sequel of *Nephritis specifica*.

*Histological examination.*—The tubules contained a few casts; the epithelial cells were slightly swollen, otherwise no pathological changes. In the infarcts described above, we found the tubules considerably enlarged, forming cavities and sinuses. They contained a small quantity of coagulated albumine, and in other places a few leucocytes. The epithelial cells were flat, cubical and compressed, probably as a sequel of the urine stasis. From the propria several small papillar elevations entered into the lumen of the tubule appearing like real septa. Probably we have here the beginning stages of an adenoma. The whole infarct was surrounded by an enlarged interstitial tissue which contained leucocytes in large numbers. The kidney parenchyma was nearly intact in the peripheral zone and only showed an irregularly distributed infiltration. Naturally the tubules were compressed, the glomeruli were normal and the whole process seemed to begin in the interstitial tissue. Connective tissue formation indicated the commencement of resolution. We have here a typical chronic interstitial nephritis, probably as a sequel of emboli, caused by the bacillus *Nephritis equi*. It is rather interesting to note that a similar adenomatous growth as described in horse 3746 was found. It is not my intention to explain the development of the adenoma; I only wish to point out that a certain kind of growth, especially adenoma, may be observed as a sequel of parenchymatous nephritis.

*Experiments with toxines.*—The general lesions found on the *post-mortem* examination showed that probably the action of a toxine is the cause of the heart paralysis, and therefore in the two following cases, not only the bacillus itself, but the bouillon with it was used for the injection.

*Horse 4236.*—Injected on the 8th April, 1909, intravenously with 5 c.c. of a four-days glycerine bouillon culture, strain "A", generation 16, and 10 c.c. of a three-day glycerine bouillon culture, strain "B", generation 9. (It was necessary to use this mixture, because the attenuation in the virulence of strain "A" was proved in a control experiment.) The temperature of the animal rose the next morning, and in the afternoon the animal laid in the stable, showing muscular tremors, ecchymoses of the conjunctiva, a pulse of 120, and in the urine a small quantity of albumine, leucocytes, a few bacteria and epithelial cells, were present. The specific gravity was 1.02. On the 10th April, 1909, the animal was found dead in the stable.

*Post-mortem examination.*—The kidneys showed early stages of alterations. There was a slight tympanitis. The muscles of the neck and head showed a

strong stasis of the blood, and the mucous membranes in the head were of a bluish colour. In the peritoneal cavity was a small quantity of liquid. The caecum contained some liquid; there was a slate discolouration of the mucous membranes, and the other parts of the intestinal tract were normal. The kidneys were embedded in a compact adipose tissue, the left capsule was easily detachable, was dark blue in colour, and there was injection of the *Stellulae verheinii*. The whole parenchyma was fibrous, and showed a few small greyish patches in the cortex. The right kidney showed similar lesions; in the cortex and in the intermediate zone, the blood vessels were very distinct. The super-renal glands had a typical haemorrhagic infiltration in the zona fasciculata. The spleen measured  $46 \times 48 \times 21$  cm. Sub-capsular haemorrhages were very frequent, the pulp was dark brownish and soft, and the follicles were indistinct. The liver showed patches of commencing decomposition. In the pleural cavity was a small quantity of liquid. Both lobes of the lungs were in inspirium, and showed on section hyperaemia and oedema. In the trachea, near the bifurcation, was a slight imbibition, and blood-stained foam. On the pericardium along the blood vessels, and near the auricles were a considerable number of ecchymoses, and in the slightly greyish myocardium were a few haemorrhages. In the left ventricle were well-formed blood coagula. The whole endocardium, and especially the muscular papillae, showed effusions, ecchymoses, and petechiae, as are generally found in horse-sickness. In several places the ecchymoses entered through the endocardium into the muscle, and there formed livid areas. In the right ventricle were a few blood coagula. The endocardium was slightly haemorrhagic, and the imbibition of the whole endocardium (with the exception of a few petechiae under the valves) was noted. The blood vessels at the base of the skull showed slight stasis, and there was a distinct filling of the pia vessels. The whole parenchyma of the brain was moist and oedematous. Microscopically, the kidney abscesses contained the bacillus nephritis in small numbers.

*Diagnosis*.—Acute toxæmia, due to the soluble toxine in the bouillon, and commencing nephritis.

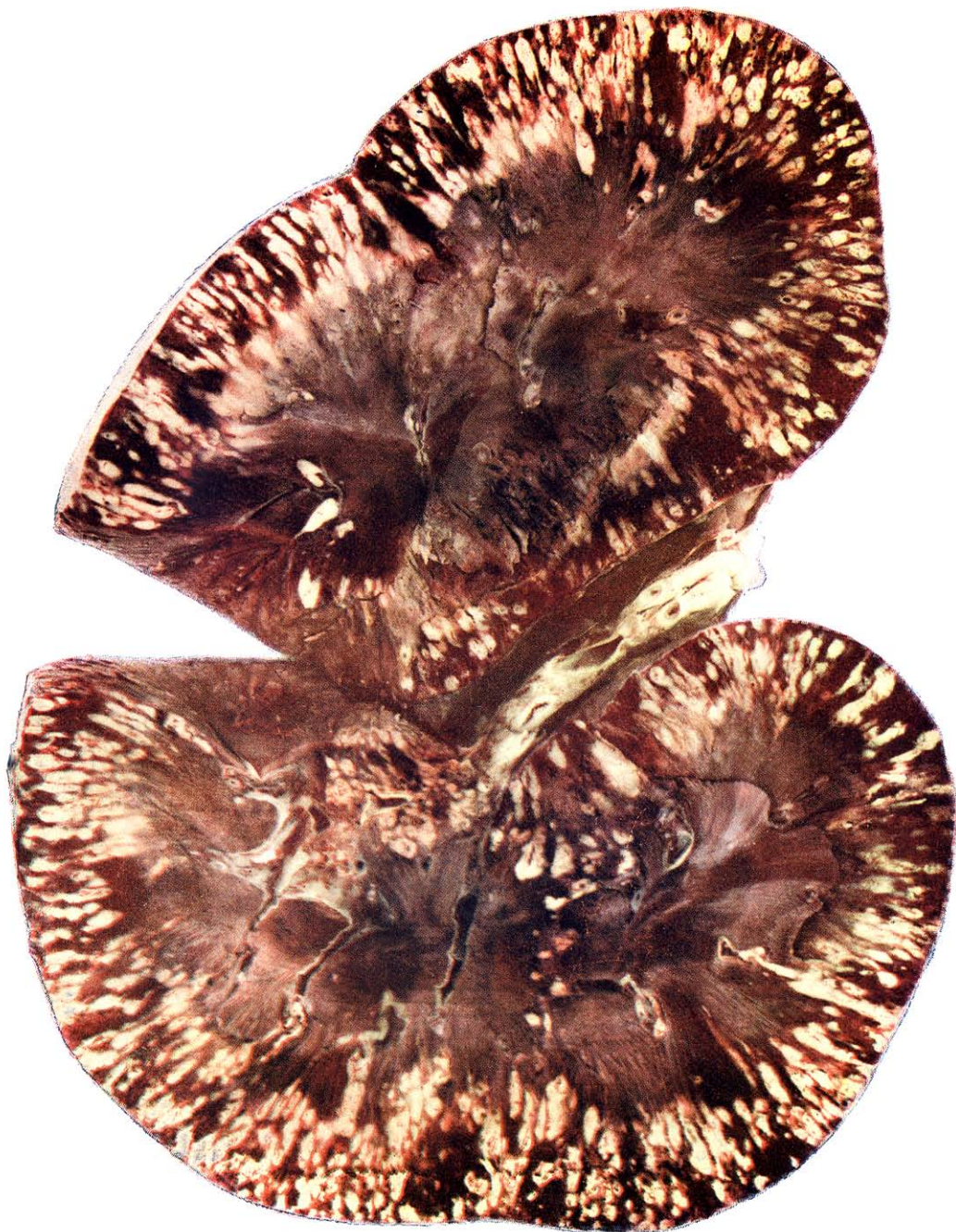
*Histologically*.—There was distinct congestion of the capillaries and other blood vessels. The desquamation of the epithelial cells in the tubuli recti was well marked, and confirmed the diagnosis.

*Horse 3929*.—Injected intravenously on the 19th March, 1909, with an emulsion of a six-days bouillon culture, strain "B", generation 15, shaken for twenty-four hours, and filtered through sterile blotting paper. About twenty-five minutes afterwards the toxic action started, and five hours later the animal died with constant trembling of the muscles of the body.

*Post-mortem examination* showed nothing specific with the exception of pulmonary oedema, ecchymoses on the epicardium and endocardium, and commencing *Glomerulo nephritis*.

*Histologically*.—All the blood vessels were distended; the glomeruli were partly blocked with erythrocytes. The epithelial lining of the tubes were slightly granular and irregular in form and staining reactions.

(4) *Intravascular injections*.—The anatomo-pathological lesions, the histological alterations and the results of the experiments, gave the proof that the bacillus nephritis was a micro-organism of specific pyogenous action, causing, when it reaches the kidneys by way of the blood stream, abscesses as described before. In order to give the proof that similar conditions in the kidneys could not be caused by a pathogenic toxine agent reaching them by the urinary tract, an injection was made into the urinary bladder. This question is of a



*Nephritis purulenta embolica*; left kidney of Horse 3944; natural size; photographed direct from specimen preserved in Kaiserling's solution No. 3.





Section of same kidney as Plate IX.



*Nephritis purulenta embolica*, produced experimentally ; left kidney of Mule 4013 ; natural size ; photographed direct from specimen preserved in Kaiserling's solution No. 3.





Section of same kidney as Plate XI.

certain importance, as recent literature on the subject of a purulent *Nephritis bovis* shows that this disease is also caused by a pyaemic infection. *Ernst* especially gave the proof histologically that the necrosis of the papillae is only of a secondary nature.

*Horse 3604*.—Received by means of a catheter on the 9th March, 1909, 80 c.c. of a crushed glycerine agar culture intravesically. (In pyelo-nephritis the particles of the agar irritate the mucous membrane of the urinary bladder and an increase of bacteria was always observed in the experiments carried on by *Ernst*.) A few days previously the urine was examined and proved to be normal. The temperature during the whole time the animal was under observation remained normal. On the 10th March the animal showed symptoms of slight irritation when passing urine, thought to arise from a typical *Tenesmus vesicae*. The urine was only passed in small quantities. The orifice of the ureter showed a slightly gelatinous and oedematous infiltration. The specific gravity of the urine was 1.035; it was slightly turbid, the reaction was alkaline, there was albumine, and after a few hours a heavy deposit formed. Microscopically, coagula, a few carbonates, triple phosphates, granular and fatty degeneration of the epithelial cells of the urinary bladder, leucocytes, and bacteria were found. In the stained smear a few bacilli mixed with streptococci and gram-positive bacilli were found. Nephritis bacillus could not be isolated in culture. On the 11th March, 1909, the local symptoms had nearly disappeared. The function of the urinary bladder had become normal and in the collective urine a few epithelial cells, flakes and mucus, and a few leucocytes were present. No nephritis bacillus could be found on culture. During the next few days, the deposit increased slightly and all the clinical symptoms pointed to a slight catarrhal cystitis. On the 17th March, 1909, the urine was normal and the kidneys appeared normal when examined per rectum.

(5) *Intra-ocular injection*.—That the nephritis bacillus is an exciting cause of suppuration, could be demonstrated in an illustrative way by the intra-ocular injection where all the stages of suppuration could be easily observed.

*Horse 4033*.—Injected on the 5th March, 1909, with  $\frac{1}{2}$  c.c. glycerine agar culture into the anterior chamber of the left eye. On 6th March a slight oedematous swelling of the eyelids and slight catarrhal conjunctivitis was noted. The cornea was slightly injected, greyish in colour, and sensitive. Slight injection of the episclerotic blood vessels was present. The general health was normal. On the 7th the swelling of the eyelids was pronounced, the catarrhal inflammation was very pronounced; no bacteria were found. Photophobia was much in evidence. On the 8th March the conjunctiva had the same appearance, the cornea was greyish in colour. Slightly protruding in the inferior quadrant of the cornea was a small abscess the size of a pin's head. The temperature was slightly higher. On the 9th March, both conjunctivae were dark bluish-red, the pericorneal blood vessels were injected, and the eye-ball was enlarged; the tensity was increased and a typical hypopion was noticeable. On the 10th March a commencing necrosis of the cornea and exudate of pus became evident. In the right eye, photophobia and slight greyish discolouration of the cornea were noticeable. On the 11th March an abscess formed in the left eye. In the right eye the formation of hypopion was pronounced. By means of cultures the injected bacteria could be found. The animal showed a slight depression and a high temperature on this date. On the 13th March there was an ulcer in the left eye-ball with granulated edges, the base consisted of the iris covered with fibrine. In the right eye the hypopion began to rupture. On the 18th March, both eye-balls:

showed the commencement of cicatrization. The swelling on the conjunctivae disappeared. In both eyes after twenty-five days only irregular rudiments of the eye-balls were found. The animal was in poor condition, and was therefore killed.

With the exception of the lesions in both orbits, only the two kidneys showed lesions. The capsule was easily detachable; in the cortex were streaks and patches and distinct injection of the glomeruli. All the other organs were normal.

Histologically, these patches proved to be the beginning of an interstitial nephritis with a chronic catarrh in the tubuli recti. The attenuation of the virulence was proved in an experiment where strain "B", generation 20, was injected intravenously and subcutaneously without causing any reaction. The urine did not show any signs of a nephritis during the forty-five days of observation.

All the experiments showed the pathogenity of the bacillus for equidae in a distinct manner. So far as we can ascertain from literature on the subject, this is the first time that a pyaemic nephritis has been produced experimentally in domesticated animals, and at the same time these lesions were proved to be due to a specific bacterium and not to a multiple infection. The result of the intravenous injections removes all doubts as to the specificity of the bacterium.

I may mention that, for example, in pyelo-nephritis bovis, the pseudodiphtheria bacillus injected in enormous quantities never produced either a similar disease or the real purulent nephritis. The experiments here proved also in an illustrative way that the bacillus *Nephritidis equi* is the real exciting cause of suppuration. Subcutaneous and intra-ocular injections with rather small quantities, produced (as long as the virulence of the bacillus had not diminished) a purulent process ending in cicatrization or encapsulation. Of two horses injected intravenously both died. Here the quantity of the material showed a certain influence because the animal injected with 10 c.c. lived six days and showed more lesions than the other injected with 25 c.c., and the mule injected with 15 c.c. of cultures only lived four days. The injection of 1 c.c. only produced a slight temperature reaction. The experiments on the donkey were carried out with a rather attenuated strain and therefore the lesions were only those of a chronic interstitial inflammation and their sequels. But it may also be a certain resistance of the donkey, which may be less susceptible than the horse and mule, in the same way as it is in some other diseases of the country. The examination of the urine of course indicated whether an infection occurred. In all cases where the animal died, the bacillus could be easily isolated from the organs, and its identity could be proved with that injected. Passage through animals had not the expected effect of increasing the virulence or keeping it at least at the same height. The intravesicular injection gave a negative result as could be expected from our knowledge concerning the pathological anatomy and histology of the disease. It can therefore be said that the natural infection also takes place through the blood stream. The two horses which were injected with liquid culture material showed but slight purulent processes, because toxic action on the heart caused death before metastasis could occur. The inoculation into the eyes was interesting, as not only the injected eye, but the other also, was affected in a similar way. From these experiments it is impossible to decide whether the conjunctiva of the right eye was due to a secretion formed by the bacillus in the left eye, or to a true pyaemic infection.

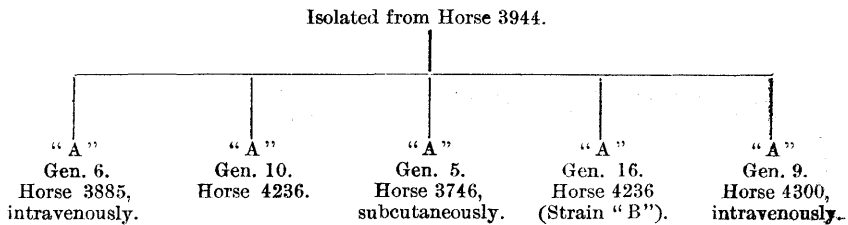


The lesions in the kidney and the other local lesions were not old abscesses, and here again it is difficult to say whether the lesions found in the organs were really caused by the nephritis bacillus, because horse-sickness and piroplasmiasis as common diseases of the country produces similar alterations in the kidneys. Clinically, throughout the experiments we could state that intravenous injections of culture material was always followed by fever, distinct depression, and loss of appetite. The slight discolouration of the conjunctival membranes was always significant, the pulse was generally weak and accelerated, the respiration was slightly hurried and shortly before death, spasmodic and of abdominal type. During the last few days, the back was slightly curved and irritation in the urogenous organs was always noted. By palpitation per rectum, the left kidney was always found enlarged, laying in a gelatinous tissue, and very painful. The urinary bladder always contained urine, otherwise the organs in the pelvis were normal. From a diagnostic point of view, the changes in the urine were important. Generally speaking, in all cases at the height of the reaction, typical oliguria ensued. The urine was dark, with the appearance of stout; in the early stages of the inflammation more or less clear, later on turbid, the consistence being sometimes viscid and gelatinous, and sometimes mucus and stringy. The mucous consistence was often so pronounced that by touching it with a glass rod, strings of varying length could be taken off, and the liquid would not pass through blotting paper. The specific gravity always varied within normal limits. The reaction was distinctly alkaline and often very pronounced, because under the influence of the fermentation which takes place in the kidney and in the urinary bladder, free ammonia had developed. If not indicated by its smell, its presence could be detected by the method of *Hess*. The albumine was always enormously increased, and the increase in amount corresponded with the stage of the inflammatory processes in the kidneys. Quantities over 2 per cent. were not rare; albumose, which always appears in the urine of the horse when a suppurative process takes place in the internal organs, was constantly found. Corresponding with the presence of haematuria, haemoglobine could be detected spectroscopically or by means of potassium hydro-oxide as a red flocculent deposit. Among the crystalline constituents of the urine, were carbonates, oxalates in small quantities, and triple phosphates in their typical forms. Organized elements as leucocytes, epithelial cells, renal casts, and a few red blood corpuscles were found in hanging drop preparations, and often the casts had a wax-like appearance pointing to a more advanced degeneration. It was noted that the bacteria were found in the urine six to ten hours after intravenous injection. Probably as the lesions in the kidneys progressed, the number of the micro-organisms eliminated by this organ increased. The nephritis bacillus probably acts in the same way as the *Staphylococcus*. It is stated in the paper of *Josef Koch* that, firstly through the primarily acting toxine, the blood vessels and epithelial cells in the kidney degenerate, and therefore gives the bacteria an opportunity to pass with the urine. A physiological elimination probably never takes place; it is a peculiarity of the bacillus itself that it can be found in the urine as it is found in other acute diseases, as typhoid fever, etc. The experiments proved that the typical *Nephritis purulenta disseminata* was produced in both kidneys. In three cases the metastasis in the lungs described in the original cases was also observed, and as proved by microscopical examination, these nodules were typical emboli, in the capillaries of the lung. Histologically, it was not difficult to decide that the nodules were not of glandular or parasitic nature, because in the first instance caryorrhesis never took place and calcification with eosinophile leucocytes was never observed.

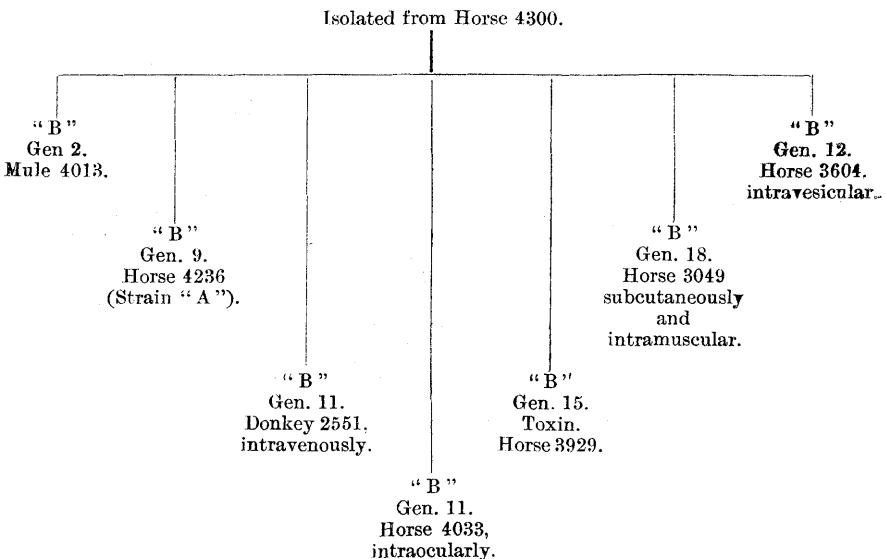
Several inoculations into guinea-pigs and the deviation of complement test always gave a negative result. Microscopical examination revealed merely an enormous infiltration of leucocytes in the alveoli, corresponding with the macroscopical lesions found and described in every standard work. I must say that I failed to test whether the development of the bacillus takes place in the blood, or if the blood is the carrier of the bacillus, metastasis resulting. The experiments show more or less that the last conjecture is probably the correct one. Whether the liver and spleen are also concerned in the elimination of the bacillus can be answered in the affirmative, because by cultural methods it was also isolated from these organs. In other cases where the virulence had diminished, these organs probably played to some extent the role of a destructor of the micro-organisms.

The following table shows the genealogy of the culture strains used in the experiments on horses. At the same time the attenuation of the virulency can be demonstrated in an illustrative way:—

STRAIN "A".



STRAIN "B".



*Immunity Reactions.*—In modern bacteriology, in addition to isolating the bacillus, the serological methods are taken into consideration for the diagnosis and differentiation between various strains. It was therefore advisable to carry out some experiments on these lines. The mucous consistence of the cultures did not permit the use of the agglutination test. Experiments with the precipitine reaction, using extract of bacilli in horse serum as described by *Pfeifer*, gave irregular results, and definite conclusions could not be obtained. Often in healthy animals, slight precipitine rings were observed after a short interval. Whether this reaction stands in relation to the presence of the bacillus in the body or whether non-specific anti-bodies cause the ring cannot be decided. The complement deviation test demonstrated in a few cases the specific anti-bodies. The test was carried out according to *Wasserman's* method. As haemolytic system, rabbit haemolysines for sheep-blood corpuscles (standard 0.00025 to 0.0001, for the experiment used in double quantity of its standard) were inactivated at 56° C. Fresh guinea-pig serum served as complement, and sheep-corpuscles washed from three to six times were used. As antigene I used the filtrate of a 48 to 72 hours glycerine agar culture, shaken in carbolized normal saline (0.5 per cent. in 0.85 per cent. Nor. Sal.). It was kept on ice in dark bottles. The deviation action of the three extracts used varied slightly, because often the emulsion used was of a different concentration than the other. The following table shows its action :—

Extract of the Bacillus.	Haemolysine (0.002).	Complement (1 : 10).	Sheep-blood Corpuscles (1 : 20).	Normal Saline.	Result.
1.0	1.0	1.0	1.0	1.0	Deviation.
0.75	1.0	1.0	1.0	1.25	Deviation.
0.5	1.0	1.0	1.0	1.5	Deviation.
0.4	1.0	1.0	1.0	1.6	Slight deviation.
0.3	1.0	1.0	1.0	1.7	Very slight deviation
0.2	1.0	1.0	1.0	1.8	Nearly complete haemolysis.
*0.1	1.0	1.0	1.0	1.9	Haemolysis.
0.05	1.0	1.0	1.0	2.0	Haemolysis.
0.001	1.0	1.0	1.0	1.0	Haemolysis.
0.005	1.0	1.0	1.0	1.0	Haemolysis.

\* Normal saline solution.

Accordingly the extract was used in the quantity of 1.0 c.c. diluted in 1:10. The complement was used in the smallest possible doses. It varied between 0.04 and 0.01 c.c. in the different tests, therefore it had to be tested previously in the morning for those tests which were carried out during the day. The immune serum was collected from the jugular vein and kept on ice without any preservative. It was inactivated at 56° C. to 62° C. in diminishing doses from 0.5 c.c. to 0.005 c.c. Higher doses could never be used. The horse serum contained generally non-specific bodies, which if not heated at 60° C. caused in the dose of 0.5 c.c. or less a non-specific deviation. For each dilution a control without extract was therefore necessary, and by this means errors could be avoided. A test carried out for horse 4300 was as follows :—



## HORSE 4300.

Serum, 13th February, 1910 (on ice without any preservative).

Test, 2nd March, 1910.

Immune Serum.	Extract, 1 : 10.	Complement, 3 : 100.	Haemoly-sine, 1 : 1200.	Sheep-blood Corpuscles 5 : 100.	Normal Saline Solution, 0·85 %.	Result.
0·5	1·0 (-0·1)	1·0 (-0·03)	1·0 (0·0002)	1·0 (0·057)	0·5	Complete deviation.
0·5	—	1·0	1·0	1·0	0·5	Haemolysis.
0·25	1·0	1·0	1·0	1·0	0·75	Slight deviation.
0·25	—	1·0	1·0	1·0	0·75	Haemolysis.
0·1	1·0	1·0	1·0	1·0	0·9	Very slight deviation
0·1	—	1·0	1·0	1·0	0·9	Haemolysis.
0·05	1·0	1·0	1·0	1·0	1·0	Haemolysis.
0·05	—	1·0	1·0	1·0	1·0	Haemolysis.
0·02	1·0	1·0	1·0	1·0	1·0	Haemolysis.
0·02	—	1·0	1·0	1·0	1·0	Haemolysis.
0·01	1·0	1·0	1·0	1·0	1·0	Haemolysis.
0·01	—	1·0	1·0	1·0	1·0	Haemolysis.
0·005	1·0	1·0	1·0	1·0	1·0	Haemolysis.
0·005	—	1·0	1·0	1·0	1·0	Haemolysis.

## Controls.

Immune Serum.	Extract, 1 : 10.	Complement, 3 : 100.	Haemoly-sine, 1 : 100.	Sheep-blood Corpuscles 5 : 100.	Normal Saline Solution, 0·85 %.	Result.
1·0	—	1·0	1·0	1·0	1·0	Haemolysis.
0·5	1·0	1·0	1·0	1·0	0·5	Haemolysis.
Normal Serum 1·0	1·0	1·0	1·0	1·0	—	Slight deviation.
Normal Serum 1·0	—	1·0	1·0	1·0	1·0	Slight deviation.
—	2·0	1·0	1·0	1·0	1·0	Haemolysis.
0·5	—	1·0	1·0	1·0	1·0	Haemolysis.
0·5	—	1·0	—	1·0	2·5	Complete deviation.
—	—	—	1·0	1·0	2·5	Complete deviation.
—	—	1·0	—	1·0	3·0	Complete deviation.
—	—	—	1·0	1·0	3·0	Complete deviation.
—	—	—	—	1·0	4·0	Complete deviation.

Serum extract and complement were kept for one hour at a temperature of 37° C., and then the components of the haemolytic system were added to the mixture. The serum, as shown in the table, contained specific anti-bodies, which were demonstrated by a deviation with 0·5 to 0·2 c.c. serum. In a similar way, complement deviating substances were demonstrated in the serum of horse 4236 and horse 4033. The serum of mule 4013 showed with 0·02 c.c.

a non-specific deviation, an observation which is made frequently in a number of sera of mules tested at the institute. The specific anti-bodies may perhaps have been present in small quantities, but in the dose used, it was impossible to demonstrate them. *Miessner* and *Trapp* stated in their paper that heating at 60° C. always destroys the non-specific anti-bodies. Our experience with a considerable amount of material proved that, especially in mules, this is often impossible. Several sera of mules were heated at 62° C. and 63° C., and with 0·01 c.c. serum always gave a non-specific deviation. In the glanders diagnosis only the agglutination test can help us out of this difficulty. It was interesting to note the presence of two anti-bodies in the serum of horse 4300, where in addition to the nephritis bacillus anti-body, immune bodies for the glanders bacillus were also found in a dilution of 0·01 c.c. The agglutination in the same horse was 1:800, and the autopsy proved the accuracy of the previous mallein cuti and serum reactions. This reaction will never have any importance from a diagnostic point of view, because, as stated before, it is impossible to keep a strain of bacteria virulent which could be used as material for the tests. The extracts deteriorate very rapidly, and the collection and preservation of serum in the field is very difficult. The examination of the urine from a diagnostic point of view is easier and quicker. I missed the opportunity of daily examining the serum, and thus was unable to find the date when the immune bodies appeared in the blood for the first time.

(b) *Guinea-pigs.*

The following transmission experiments were carried out:—

*Case No. 1.*—Three guinea-pigs, Nos. 30, 31, and 32, were injected as follows: No. 30, injected intraperitoneally with 5 c.c. on the 7th March, 1902; No. 31, injected intraperitoneally with 5 c.c. on the same date. No. 32, injected intravenously with 2 c.c. on the same date. No. 30 died on the 8th March, and showed an accurate peritonitis of a fibrinous haemorrhagic character. The exudate was very stringy. No bacteria were present, and all organs were rather rich in blood but sterile. Cultures made from the exudate developed a few small colonies on glycerine agar. The peritoneal exudate of guinea-pig No. 30 was injected intraperitoneally into guinea-pig No. 33; this latter animal was dead the next morning, and showed lesions similar to those of guinea-pig No. 30. Cultures were positive. A few other animals were also injected intraperitoneally, but all with negative results. In the first twenty-four hours they showed symptoms of sickness, but these passed over.

*Case No. 2.*—Two guinea-pigs were injected intraperitoneally with a glycerine agar culture, second generation; one died from a similar peritonitis to that described above. Positive cultures were obtained from the exudate.

*Case No. 3.*—Culture material from strain "A", isolated from the animal. Experiment No. 1: Of four guinea-pigs injected intraperitoneally on the 19th January, 1909, with 5 c.c. glycerine agar culture, generation five, No. 1, weight 470 grams, died on the 20th January, 1909. *Post-mortem* examination showed a purulent, fibrinous peritonitis, distinct haemorrhagic infiltration of the serosa of the intestines, a few ecchymoses on the epicardium, pulmonary oedema, and a slight enlargement of the spleen. Microscopically, the bacilli were present in the peritoneal exudate, lungs, blood stream and liver, and cultures from these were positive. Guinea-pig No. 2, weight 670 grams—at the end of the experiment 720 grams—was kept till the 14th May, but with negative results. Guinea-pig No. 3, weight 210 grams—died on the 21st January; the *post-mortem* appearance resembled that of guinea-pig No. 1. Microscopically,

by means of culture, bacilli were found in the peritoneal exudate, lungs, spleen, and kidney. Guinea-pig No. 4, weight 425 grams—at the end of the experiment 625 grams—was killed on the 14th May; result negative.

*Experiment No. 2.*—Two guinea-pigs were injected on the 20th January intraperitoneally, with the peritoneal exudate of guinea-pig No. 1. The animals were sick for a few days but recovered. They were killed on the 3rd June, and found quite healthy.

*Experiment No. 3.*—Two other guinea-pigs received on the 20th February, 1910, 10 c.c. of a glycerine bouillon culture, intraperitoneally. No. 1, with a weight of 395 grams, showed no symptoms. No. 2, with a weight of 230 grams, died two days afterwards, and showed the same peritonitis as observed in the other cases.

*Experiment No. 4.*—Eight guinea-pigs were injected on the 12th February, 1909, intraperitoneally with 5 c.c. of glycerine agar culture. None of the animals died, but two were killed, and on *post-mortem* examination no pathological lesions were noted.

*Experiment No. 5.*—Two guinea-pigs were injected intravenously with 1 c.c. glycerine bouillon culture, generation eleven; the animals were refractory.

*Experiment No. 6.*—Five guinea-pigs were injected intraperitoneally on the 4th February, 1909, with 2 c.c. of a glycerine agar culture, strain "B", generation two; the following day one guinea-pig, weighing 250 grams, died of a peritonitis. The other animals showed no reaction.

*Experiment No. 7.*—Five guinea-pigs were injected with 2 c.c. of glycerine bouillon culture, strain "B", generation two, all with negative results.

*Experiment No. 8.*—Four guinea-pigs received on the 19th January, 1909, 2 c.c. of glycerine agar culture, subcutaneously, strain "A", generation five. At the seat of the injection a slight swelling formed which disappeared two days later.

These experiments show that only under certain conditions can guinea-pigs be infected. The quantity of virulent material and the age of the animals are important factors. The lesions were always the same after intraperitoneal injection, namely, purulent serofibrinosa, peritonitis with a mucous and stringy exudate, and haemorrhagic exudation on the serous membranes. Of thirty-eight animals injected, only four died after peritoneal injections. These were all young and rather small animals. The cultures of the first generation were probably more pathogenic for guinea-pigs than those of later generations which never caused any reactions. Passage through horses seems to restore the virulency for guinea-pigs. It is remarkable that older and larger guinea-pigs which were injected with most virulent material (which in small intravenous injections caused a fatal disease in horses) never developed an infection. We can therefore state that only under certain conditions is the nephritis bacillus pathogenic for guinea-pigs. In all these animals, the bacillus was also an exciting cause of suppuration.

### (c) Rabbits.

Experiments on rabbits showed similar lesions to those described in guinea-pigs.

*Experiment No. 1.*—Two rabbits were injected intraperitoneally on the 19th January, 1909, with 5 c.c. glycerine agar culture, strain "A", generation five. Rabbit No. 1, weight 1255 grams, died on the 20th September. A small quantity of a stringy exudate was found in the peritoneal cavity. The intestines and mesentery were thinly covered with fibrine. The peritoneum, capsule of the kidneys, and the diaphragm were slightly injected. Under the capsule of the spleen were two small abscesses. On the epicardium were



ecchymoses; microscopically, bacilli in the peritoneal exudate were very frequent, and cultures made from the exudate were positive. The pleura, lungs, blood, liver, spleen, and kidney were normal. Rabbit No. 2, weight 1100 grams, showed no symptoms. It was used later in a trypanosoma experiment, and when killed on the 23rd June showed no lesions of an old peritonitis.

*Experiment No. 2.*—Four rabbits were injected on the 19th January, 1909, intravenously with 2 c.c. glycerine agar culture, strain "A", generation four. None of these animals showed any symptoms.

*Experiment No. 3.*—Two rabbits were injected on the 19th January, 1909, with 5 c.c. culture subcutaneously in the dorsal region. The next days there were hot, slightly fluctuating swellings, which disappeared four days later. In one case only, an abscess the size of an egg was formed, in which the bacilli could be found.

*Experiment No. 4.*—Three rabbits were injected intraperitoneally on the 12th February, 1909, with 10 c.c. glycerine agar culture, strain "B". None of these animals showed any symptoms.

*Experiment No. 5.*—Four rabbits were injected intraperitoneally on the 4th February, 1909, with 5 c.c. glycerine agar culture, and with 1 c.c. intravenously. These animals were sick for a few days, not feeding well. After this time all symptoms disappeared; the rabbits were killed and the *post-mortem* examination was negative. This experiment shows that out of fifteen rabbits, fourteen were refractory. It is therefore difficult, or probably in certain cases impossible, to successfully infect rabbits. By intraperitoneal injection of culture material, a peritonitis of similar character as described for guinea-pigs may be produced. The age of the animals and the virulence of the culture probably had no influence. The nephritis bacillus was only pathogenic for rabbits in the first generation.

(e) *Rats and mice.*

Several rats were injected subcutaneously and intraperitoneally with negative results. Mice were also injected and fed with bread soaked in a glycerine bouillon culture. All experiments were negative. When killed, twenty-four to thirty-six hours later, in a few cases, the bacilli could be isolated from the intestines.

(f) *Pigeons and fowls.*

Intraperitoneal and intramuscular injections of culture material in large doses to birds gave no results. A slight infiltration was observed during the first few days, but disappeared later.

(g) *Dogs.*

The fact is well known that dogs have a certain predisposition to kidney diseases, and therefore experiments in this direction were carried out. Subcutaneous, intraperitoneal, and in two cases intravenous, injections gave negative results. In one case I tried to reduce the resistance by intravenous injection of ordinary water; twelve hours later a large dose of the nephritis bacillus was inoculated, but no result could be obtained.

(h) *Ruminants.*

Comparative experiments on these animals were considered necessary. Bull 569 was injected on the 19th February, 1909, intrajugularly with 10 c.c. of a thirty-six hour glycerine agar culture, strain "B", generation three. The animal did not show any symptoms whatever. The urine was tested every day, but never showed pathological changes. No bacilli were present. The several

urine tests in the first twelve hours were all negative. The animal died in an East Coast fever experiment on the 26th June, but did not show any lesions of an old nephritis.

*Goat 1350.*—Injected intravenously on the 3rd March, 1909, with 10 c.c. of glycerine agar culture, strain "B", generation eleven, intravenously. No symptoms appeared.

*Goat 1611.*—Was injected subcutaneously on the 3rd March, 1909, with 10 c.c. glycerine agar culture. The temperature curve at the end of twenty-two days showed a few rises in the evening, but when the animal was killed only two small white cicatrized patches, like infarcts, were found in the left kidney. All the other organs were normal.

*Histological examination.*—In one of the capillaries was a fine thrombosis with a typical regenerative reaction of the interstitial tissue, causing an increased formation of connective tissue. A few interlobular arteries along the edge of the infarcts were slightly dilated and filled with red blood corpuscles. Bacteria, or signs of a purulent process, could not be detected, so that the nature of these infarcts and the genesis is still doubtful.

*Sheep 1756.*—Was injected on the 8th December, 1909, intravenously with 5 c.c. glycerine bouillon culture, strain "A", generation ten. No old nephritis lesions were found when it died later in a trypanosoma experiment.

*Sheep 1880.*—Was injected intravenously on the 19th March, 1909, with 10 c.c. glycerine bouillon culture, strain "B", generation ten. It was killed on the 20th April and found to be healthy.

*Sheep 1234.*—Was injected subcutaneously with 20 c.c. glycerine bouillon culture. For a few days it showed a distinct infiltration at the place of injection. The swelling disappeared at the end of the first week, without having formed an abscess.

So far the experiments on the ruminants with virulent material showed the impossibility of transmitting or producing a purulent infection in these animals. Summarizing, we can state that the pathogenicity of the bacillus *Nephritis equi* is only pronounced for horses. All the other animals which were tested gave negative results.

With regard to the place in the classification occupied by this organism, for which I have proposed the name "*Bacillus Nephritidis equi*" (*Transvaal*), I wish to point out that it has great similarities to the group of bacteria called "Coryne bacteria". Morphologically and in cultures, it resembles the bacillus diphtheria or of pseudo-diphtheria, which are represented in this group. The formation of mucus, the fermentation of carbo-hydrates, and the gram-negative staining reaction, distinguish it from this bacillus. It may be a variety of this group, but the presence of similar bacteria in the kidney of cattle and pigs, which are morphologically like the *Bacillus Nephritidis equi*, warrant us in grouping them together. All these bacteria probably belong to a family which have a special tendency to act on the kidney and to be an exciting cause of suppuration.

#### *Experiments with the toxine secreted by the bacteria.*

All *post-mortem* examinations showed lesions similar to those found in acute intoxication, and the inoculation of liquid material proved this in a distinct manner. Concerning the question whether uraemia is the cause of all the symptoms and lesions, we can state that in several cases from the pathological anatomical point of view, the conditions for a retention of the urine in the body had not yet taken place at the time of death, and also

uraemic processes are but rarely met with in horses. Only in a few experiments were they therefore carried out to prove that a real toxine was present in the filtrate of liquid cultures. Three to five days glycerine bouillon cultures, or cultures in Bouillon "Martin", were used for this purpose.

Without having been sterilized they were filtered through *Chamberland* candles, the filtrate was tested as to its sterility, and used *in toto* or concentrated *in vacuo* at 37° C. Sterilization was omitted in order to avoid the endotoxines being mixed with the filtrate. A few experiments on horses were carried out, which all had the same result, and I am therefore only bringing forward the two following instances:—

*Horse 4309.*—Was injected subcutaneously with 10 c.c. filtrate. It showed no symptoms. It was injected later intrajugularly with 10 c.c. of a concentrated bouillon, and six weeks later with 10 c.c. intravenously of the concentrated filtrate, and it died within five hours. The *post-mortem* examination only showed lesions in the heart and a pulmonary oedema.

*Horse 4319.*—Injected intravenously at 4 p.m. on the 19th February, 1909, with 30 c.c. of filtrate. At 5 o'clock the animal was found lying in the stable and showed symptoms of pain, respiration more or less hurried, the eyes staring, the pupils slightly distended. At 5.30 p.m. the same symptoms were present, also profuse diarrhoea, pulse 84, respirations 32, and trembling of the muscles. These symptoms were similar to those observed after the injection of arecolin. At 6 p.m. paralysis of the anus, pulse 120, weak and jerky, respirations 48, were noticed. At 6.30 the condition was the same. At 7 p.m. foam collected around the nostrils. At 8.30 the horse was standing, profusely sweating, pulse 64, respiration 30. The following morning the animal had lost its appetite, and frequently laid down. The temperature was slightly increased, but returned to normal the next morning. The toxic action therefore gave rise to only nervous affections, and the symptoms showed the tendency of the body to eliminate these toxines. Similar reactions were observed in horses 3063 and 4309.

One guinea-pig received 5 c.c. filtrate subcutaneously, and showed symptoms for a short time. Several animals injected intraperitoneally with concentrated filtrates showed a rapid loss of condition, depression, and erection of hair. Control animals which were injected with the same sterile bouillon remained healthy and did not show any loss of condition. An active endotoxine did not seem to be isolated by the destruction of the bacilli, which could be demonstrated in two cases where glycerine agar cultures were ground in a mortar, shaken, and afterwards injected subcutaneously without causing any results. The experiments showed that by filtration a soluble toxine may be obtained from bouillon cultures. The toxine is found in fairly small quantities, therefore large doses were necessary for the production of symptoms and death of the animal. In guinea-pigs, the loss of condition was remarkable. The toxine affected the central nervous system and the vasomotor centres. As a sequel of this, an alteration of the tonus, haemorrhagic exudations, endothelial swellings and degeneration of the parenchymatous organs can be found.

#### *Pathogenesis.*

Generally all cases of purulent nephritis in both kidneys have a pyaemic origin, and are secondary to purulent alterations in other parts of the body. The channel of entrance of the infection has only been studied in a few special diseases (strangles, infectious pneumonia, etc.), and therefore we know but little of the flora of other processes which may give rise to metastatic lesions in the kidneys. That purulent processes, such as endocarditis, pneumonia,



and abscesses in the hoof, may produce metastasis in the kidney is well known, but it is rather doubtful whether an infection through the intestinal tract offers a possible explanation in our cases. All experimental research on these lines failed to demonstrate the *Bacillus nephritidis equi*, and we have therefore to look for other possible channels of entrance. In one case, necrosis of the ligamentum nuchae was observed, but systematic investigation of all organs did not prove that the infection in the kidney originated in this wound. That purulent nephritis has a pyaemic origin was proved experimentally. Where the bacillus enters into the body, and whether the blood stream only acts as a carrier, causing in this way metastasis, could not be proved definitely.

The experiments to produce the disease by means of urogenous infections gave negative results. In *post-mortem* examinations the organs of the urinary system, other than the kidneys, never showed any lesions, and therefore infection by way of the urinary tract may be definitely excluded. It has been explained that positive precipitine reactions were often observed in normal horses. It was therefore thought that the bacillus *Nephritidis equi* might be a saphrophyte like the other pseudo-diphtheria bacilli, which under certain conditions becomes pathogenic. In the literature on this subject, *Ernst* records the presence of diphtheria bacilli in and on the bodies of cattle; *Bongert* reports on a case of strangles where he could isolate from the pus, not only *Streptococcus equi*, but also a bacillus which showed resemblances to the diphtheria bacillus. In our cases the presence of the bacillus in the healthy animal was never proved bacteriologically. All these bacteria grow with difficulty and are not easily differentiated on culture plates. Negative results in this direction therefore did not exclude the possibility of their existence in the body. The absence of the bacillus in a larger proportion of purulent lesions examined does not prove that the bacillus is uncommon. We may conclude that the experiments gave the proof of a pyaemic infection, but the channel of entrance of the infection remains unknown.

The bacillus can be compared with two others isolated from the kidneys, namely, "*Bacillus polymorphus*", described by *Grixomi*, and "*Bacillus polymorphus suis*", described by *Degen*. Both show polymorphism, no gram-staining, they form small colonies on agar, they were very adherent, viscid, mucus, and produced a flocculent deposit in the water of condensation. In contradistinction to the bacillus *Nephritidis equi* they grew on potato, showed no liquifaction of the gelatine, no coagulation in milk, and no fermentation of carbohydrates. The *Grixomi* bacillus is only pathogenic for mice (and man?). The *Polymorphus suis* is pathogenic for rabbits, and produced metastatic purulent lesions as a sequel of intravenous injection. It is also less resistant, and therefore the sub-cultures must be made every third day. Guinea-pigs infected with bacillus *Polymorphus suis* in large doses also died of a purulent peritonitis and corresponded in this respect with the bacillus *Nephritidis equi*.

### Conclusions.

The following conclusions may be drawn:—

1. Occasionally in the Transvaal a purulent equine nephritis was observed, caused by a specific bacillus, "*Nephritidis equi*" (*Transvaal*). The infection is of pyaemic nature, as proved by the lesions and experimental research. The three animals affected naturally with bacillus nephritidis succumbed.

2. *Clinical symptoms*.—Fever and depression are present, and examination of the urine shows distinct albuminuria, presence of casts (hyaline, granular,

leucocytes, and epithelial cells). Elimination of the bacilli is always observed. The formation of triple phosphates can constantly be proved by microscopical examination of the urine.

3. *Pathological anatomy.*—The autopsy regularly showed a typical pyaemia and toxæmia, with well-developed *Nephritis purulenta disseminata embolica* and metastasis in the lungs. There are never any signs of an ascending infection in the urinary tract.

*Histology.*—The symptoms of abscess formation as sequels of the lesions in the blood vessels of the kidney or the lungs are present. The infiltration degeneration and necrosis may be of considerable extent, so that the enormously enlarged kidneys are almost totally destroyed by purulent infiltrations. When the renal lesions resolve, as was observed in two cases, a fibrinous interstitial nephritis with commencing formation of an adenoma occurred.

4. The bacillus can be easily isolated from the abscesses in the kidneys and the lungs and from the urine.

*Morphology.*—The bacterium varies in length and shape and shows typical polymorphism. It is rather difficult to stain with the different aniline dyes, and does not take the gram stain. It is non-motile and shows no resistant forms.

*Biology.*—Cultures show slight liquifaction of gelatine. On glycerine agar and serum, small very mucous colonies form. Milk culture is very characteristic with its formation of stringy mucus and coagulation, which takes place after three or four days as a sequel of the acid formation. It shows no growth on potato and no characteristics on other media. The fermentation of different carbohydrates and the formation of acids and mucus are constantly observed. The optimum temperature of growth in air is 37° F. The vitality is low, and therefore experimental research is often interrupted. By filtration of four to five days' cultures, a sterile filtrate may be obtained, which shows in rather small quantities very remarkable toxic action. The distribution of the micro-organism outside the body and the channel of entrance into the body are unknown.

5. In the serum of horses suffering from *Nephritis purulenta specifica* amboceptors were demonstrated by means of the deviation of complement. The method has no diagnostic value because non-specific anti-bodies often interfere with the reaction.

6. By intravenous injection of fully virulent cultures, which were washed off in normal saline, horses and mules could be infected and showed *post-mortem* the typical lesions in kidneys and lungs. Subcutaneous injections in large quantities produced only local suppuration, and in one case small lesions in the kidney. Cultures introduced in the urinary bladder gave negative results. By means of intra-ocular injection, the positive chemiotatic character was proved through the formation of a hypopyon. The injection of liquid cultures, containing the toxine, caused the death of the animal in a very short time (twelve to twenty-four hours).

7. Cattle, sheep, goats, dogs, rats, mice, and birds injected by different methods are refractory.

8. Guinea-pigs and rabbits often die as a sequel of intraperitoneal injection of culture material in large doses, the mucous purulent peritonitis being probably only caused by mechanical irritation.

9. For the first time, experimentally by inoculation of a specific bacillus, the proof of the pyaemic origin of a purulent descending nephritis could be given.

10. Morphologically and culturally, the bacillus shows similar characters to those of the bacillus *Polymorphus suis* and the bacillus *Polymorphus (Grixomi)*, and it therefore probably belongs to the group of the Coryne bacteria (*Lehm* and *Neum*).

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## DESCRIPTION OF PLATES.

- Plate No. VII* .. Bacillus *Nephritidis equi*.  
 (a) Glycerin-agar-culture, twenty-four hours, old, diluted carbol-fuchsin (1 : 950).  
 (b) Serum-culture, forty-eight hours old, diluted carbol-fuchsin (1 : 950).
- Plate No. VIII* .. Growth of the bacillus *Nephritidis equi*.  
 (a) Stab-culture in alkalic gelatine, eight days old, at 23°.  
 (b) Stroke-culture on glycerin-agar. Strain "A" (gen. 6), twenty-four hours old, at 37°.  
 (c) Stroke-culture on glycerin-agar isolated from mule 4013 (gen. 1), twenty-four hours old, at 37°.
- Plate No. IX* .. *Nephritis purulenta embolica*, left kidney of horse 3944.  
 (Taken by three-colour process.) Natural size.  
 Specimen preserved in Kaiserling's solution No. 3.
- Plate No. X* .. Section of the same kidney.
- Plate No. XI* .. *Nephritis purulenta embolica* produced experimentally, left kidney of mule 4013 (natural size).  
 Specimen preserved in Kaiserling's solution No. 3.
- Plate No. XII* .. Section of the same kidney.