**The veterinary bacteriological laboratories : Issued in commemoration of the opening of the new laboratories at Onderstepoort, Pretoria, October, 1908.**

# THE VETERINARY BACTERIOLOGICAL LABORATORIES OF THE TRANSVAAL.

THE RIGHT HON. GENERAL LOUIS BOTHA, P.C.,
*Minister of Agriculture.*

F. B. SMITH, ESQ.,
*Director of Agriculture.*

DR. ARNOLD THEILER, C.M.G.,
*Government Veterinary Bacteriologist.*

Transvaal Department of Agriculture.

THE

# VETERINARY BACTERIOLOGICAL LABORATORIES.

*ISSUED IN COMMEMORATION OF THE OPENING OF THE NEW LABORATORIES AT ONDERSTEPOORT, PRETORIA, OCTOBER*, 1908.

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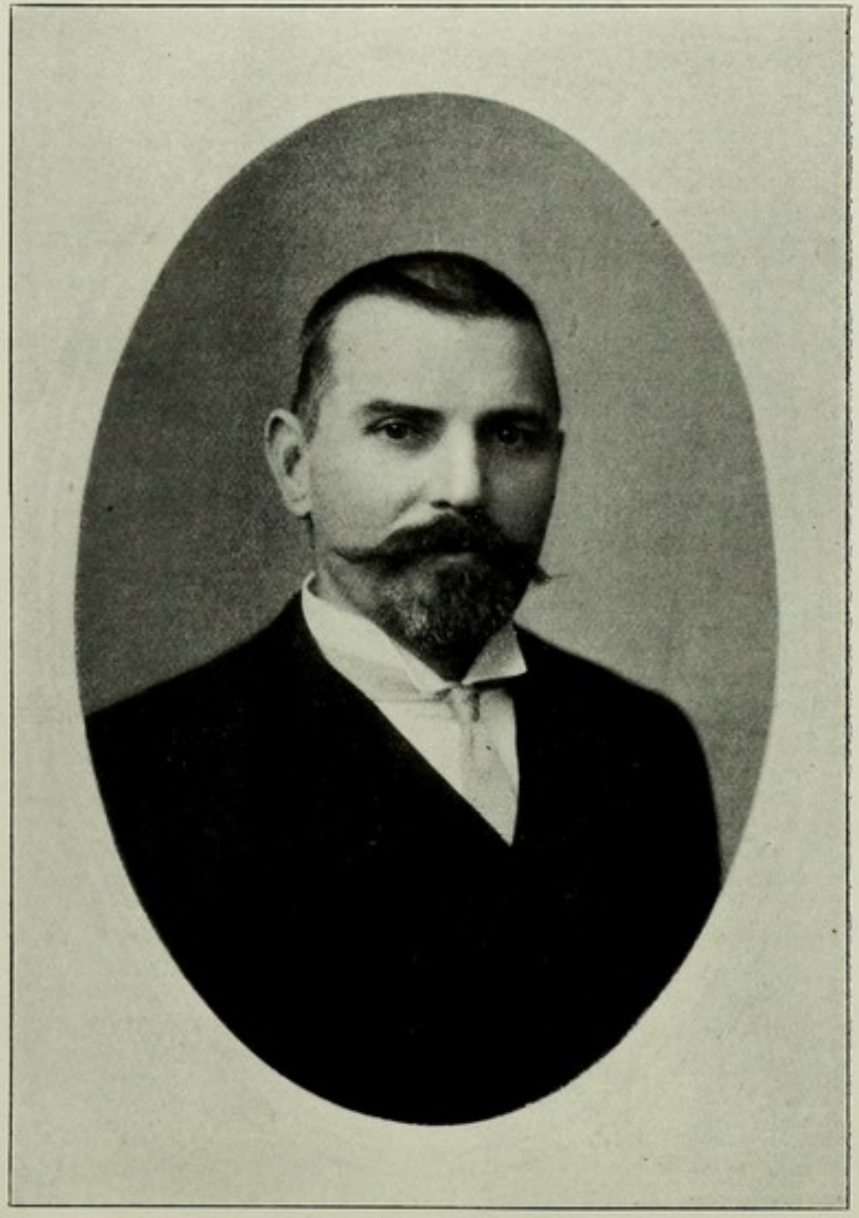
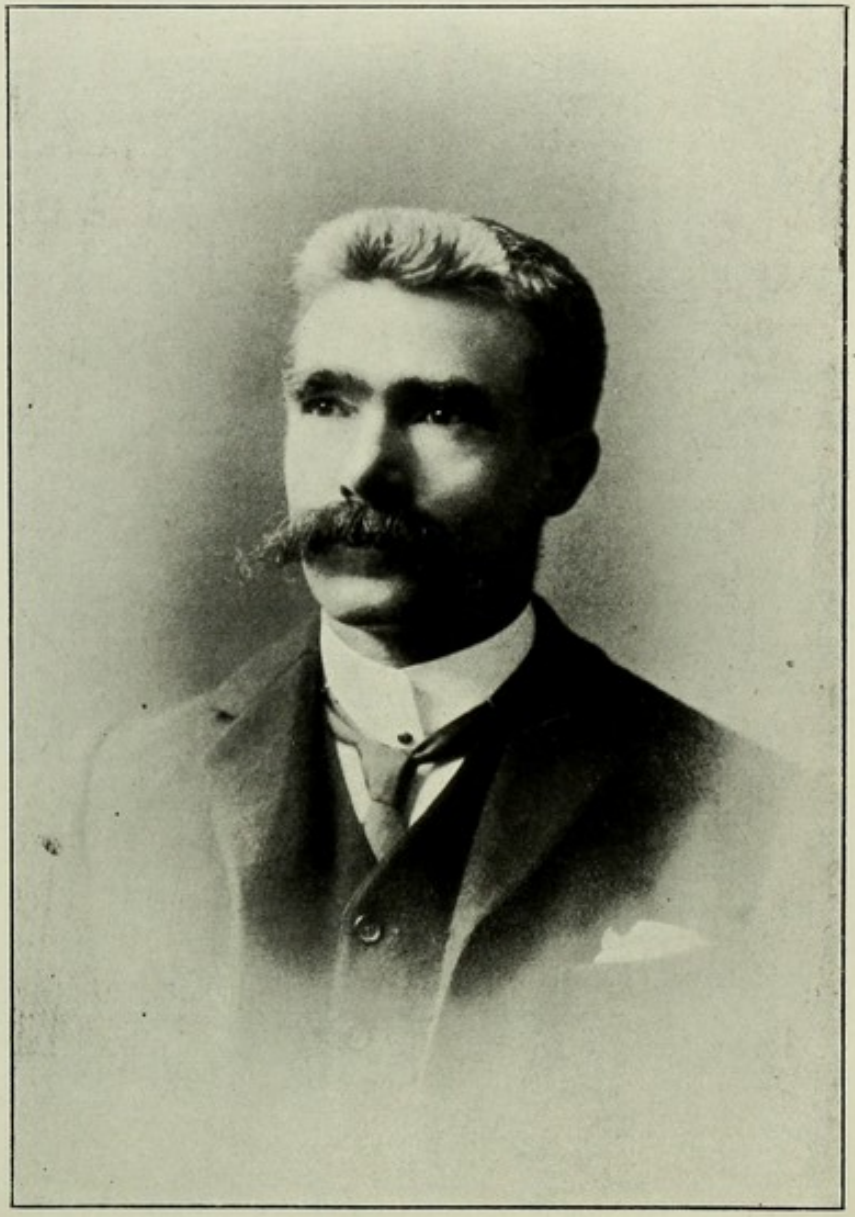


Dr. Arnold Theiler, C.M.G., *Government Veterinary Bacteriologist.*



C. E. Gray, Esq., *Principal Veterinary Surgeon.*

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# TRANSVAAL DEPARTMENT OF AGRICULTURE.

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*Director of Agriculture :*—F. B. Smith, Esq.

The Veterinary Bacteriological Division.

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Lewis Henry Gough, Phil. Dr., Basle.

*Pathologist :—*

Karl Friedrich Meyer, Schweiz. Tierarz. Staats-diploma ; Dr. Med. Vet. University, Zurich.

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*Farrier :* S. B. Tees.
*Storekeeper :* J. B. Gildea.
*Caretaker :* F. W. Pfister.
*Yard Foreman :* R. J. Varley.
*Engineer :* S. Solomon.
*Assistant Engineer :* W. R. Palmer.

*The Staff of the Veterinary Division.*

THE VETERINARY DIVISION (for Contagious Diseases).

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C. E. GRAY, M.R.C.V.S.

*Assistant Principal Veterinary Surgeon :—*

J. M. CHRISTY, M.R.C.V.S.

*District Government Veterinary Surgeons :—*

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G. MAY, M.R.C.V.S., Government Veterinary Surgeon for Waterberg.
J. I. EDGAR, M.R.C.V.S., Government Veterinary Surgeon for Zoutpansberg.
S. I. JOHNSTON, M.R.C.V.S., Government Veterinary Surgeon for Wakkerstroom.
H. M. WEBB, M.R.C.V.S., Government Veterinary Surgeon for Lydenburg.
W. G. EVANS, M.R.C.V.S., Government Veterinary Surgeon for Lichtenburg and Marico.
J. DONALDSON, M.R.C.V.S., Government Veterinary Surgeon for Ermelo and Carolina.
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J. M. TATE, M.R.C.V.S., Government Veterinary Surgeon for Heidelberg and Standerton.
J. G. BUSH, M.R.C.V.S., Government Veterinary Surgeon for Krugersdorp.
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F. LINDSAY, M.R.C.V.S., Government Veterinary Surgeon for Middelburg.
G. W. LEE, M.R.C.V.S., Government Veterinary Surgeon for Rustenburg.
T. H. DALE, M.R.C.V.S., Government Veterinary Surgeon for Potchefstroom, Wolmaransstad, and Bloemhof.
F. J. DUNNING, M.R.C.V.S., Relieving Officer.
D. T. MITCHELL, M.R.C.V.S., Relieving Officer.
P. CONACHER, M.R.C.V.S., Seconded for Service with Portuguese East Africa Administration.

# History of the Laboratories.

THE Veterinary Research Laboratories of the Transvaal Department of Agriculture comprise a large block of buildings on the farm Onderstepoort, situated eight miles to the north of Pretoria.

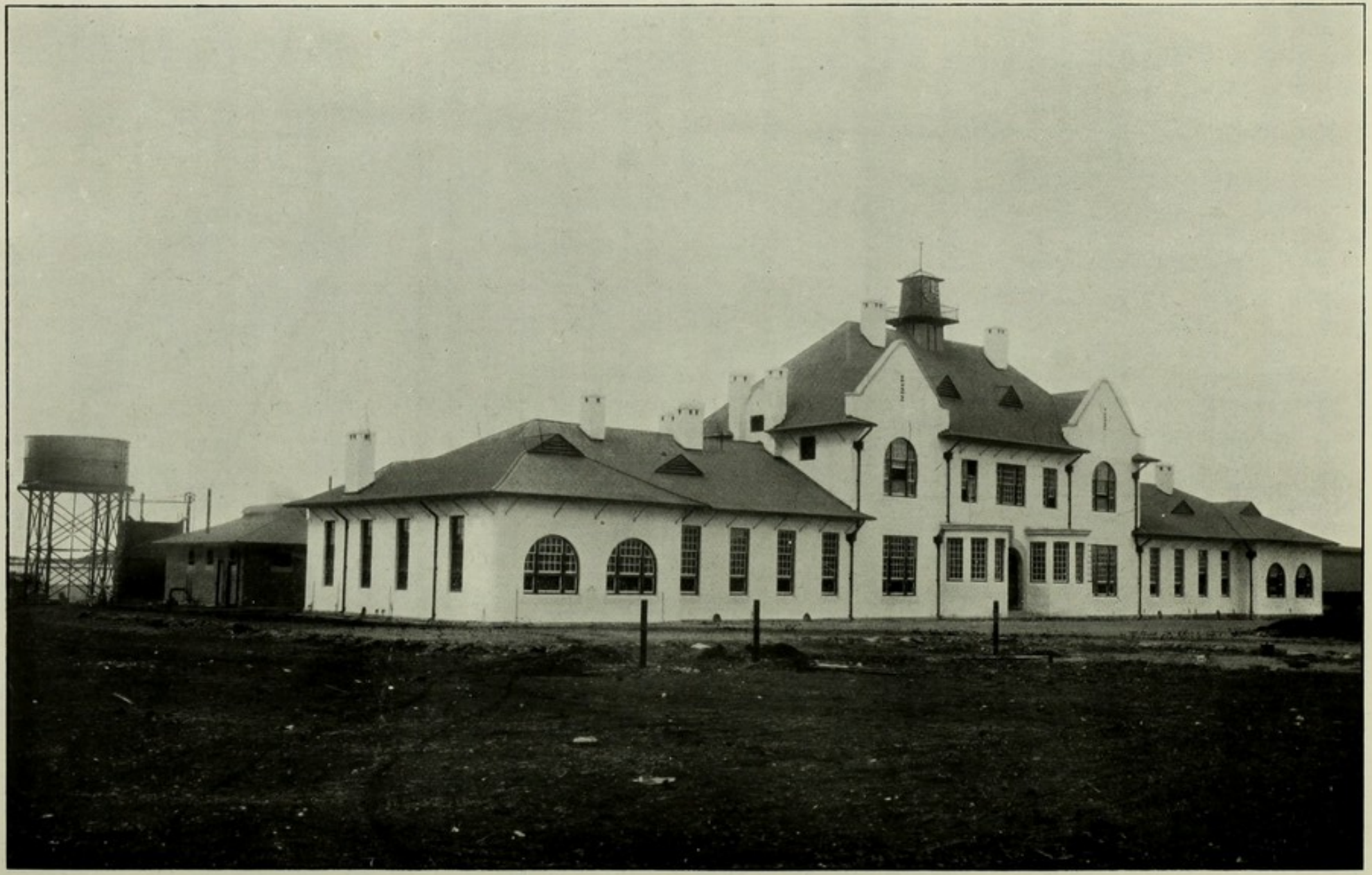
The site was chosen first, for its central position, being in the vicinity of Pretoria, the capital of the Transvaal; the laboratory is in close touch with the headquarters of the Department of Agriculture and the Government of the Colony. It is also well served by the railway, Pretoria being a junction for the eastern line to Delagoa Bay, the northern line to Pietersburg, and the western line to Rustenburg, and on the south by the line via Germiston, it is connected up with the south-eastern line to Natal, the south-western line via Johannesburg to Potchefstroom, and the Cape border, and by the direct southern line to Orange River Colony border at the Vaal River.

A railway siding at the laboratory makes it possible for animals from infected areas to be sent through in quarantine with the least possible delay, and a good service of local trains runs between the siding and Pretoria. The laboratory is also connected by telephone with the Pretoria Central Exchange, and in this way is in direct telephone communication with Johannesburg and the whole of the Rand, as well as the towns of Pietersburg, Klerksdorp, Potchefstroom, and Zeerust.

A site to the south of Pretoria would in some ways have been preferable had other considerations been as easily satisfied, but climatic conditions had to be considered, and the high veld would not have offered the same facilities of studying the progress of the diseases of the low veld as the situation that was decided upon. Being practically on the verge of the bushveld, it has a climate perceptibly warmer than Pretoria, but at the same time perfectly healthy for human beings; it is consequently not only geographically but also climatically centrally situated.

A further consideration in the selection of a site was the necessity of there being attached to the laboratory a farm of sufficient area to afford grazing ground for the live stock in addition to some arable land on which to raise crops for feeding the stabled animals under experiment, and so it would not have been convenient for the laboratory to have been situated in the town itself.

The portion of the farm Onderstepoort that was purchased meets all these requirements. In area it is 512 acres, and is now divided up into paddocks. Sixty acres are under cultivation, irrigated from a furrow taken from the Aapies River, which flows along the whole of the eastern



*A Front View of the Laboratory.*

country, and accordingly a small sum of money was voted to fit up a temporary laboratory on the town lands of Pretoria.

At that time there was at Daspoort (a suburb of Pretoria) a wood and iron shed which had been erected as an outcome of a resolution of the Inter-Colonial Rinderpest Congress held at Vryburg in the year 1896. Delegates from the various States and Colonies of South Africa met at this congress to discuss the position with regard to rinderpest, and the best measures to be taken to combat the disease in future. One of the resolutions passed was to the effect that the exportation of hides should be permitted only if they were first subjected to proper disinfection, and it was for this purpose that the building in question had been erected. It had, however, never been used, and the suggestion was now made to utilise it with such alterations and additions as might be necessary as a veterinary research laboratory. The scheme received the hearty support of the landdrost, Mr. C. E. Schutte, and eventually a new three-room building of wood and iron, lined with brick, was erected for a laboratory, the existing building being turned into a stable, and after a few small additions made with material and fittings taken from the temporary Rinderpest Field Station, the buildings were ready for occupation by the middle of 1898; the equipment, however, was sadly deficient as for the present the best had to be made of such as was available from the temporary Rinderpest Laboratory which was now no longer needed.

The first undertaking in the new laboratory was the preparation of calf vaccine lymph. A serious outbreak of small-pox had occurred in the Transvaal, and the necessity of providing a large supply of lymph for vaccination of Kaffirs prevented much useful research work being undertaken, and the war which broke out in the later part of the year 1899 temporarily stopped further work.

After the occupation of Pretoria by the British troops in 1900 the laboratory premises were made use of by the military authorities as a stable for the horses of the Transvaal Constabulary, and it was not until the following year when rinderpest had again broken out in Basutoland and the Orange River Colony that thoughts were again turned to a veterinary laboratory.

Though the outbreak of rinderpest had not occurred in the Transvaal, it was feared that due to the movement of troops in the field, there was very little doubt that it would soon spread into this Colony, and on the recommendation of Dr. George Turner, at that time Medical Officer of Health for the Transvaal, it was decided to start a rinderpest station at Daspoort, so that the laboratory could be utilised in connection with the preparation of serum. Dr. Turner took charge of this station, at which considerable additions had to be made. A yard 200 ft. square enclosed by a galvanised iron fence was made, in which the cattle could be tethered to poles, and on two sides open sheds were erected, an

incinerator for the disposal of the carcases was built, and outside the yard a brick building to serve as further laboratory accommodation.

From this time on as necessity arose small additions were made, first a building for the preparation of vaccine lymph, then some quarters for the staff engaged in the rinderpest work, later some stables and more quarters; but always the additions were of a temporary nature and chiefly constructed from such old wood and iron as was obtainable from buildings pulled down during the war, so by the end of the year 1905 the station had already grown to some size, though it was merely a heterogeneous collection of old wood and iron buildings; most of them of a very unsuitable nature for scientific work.

But an important change had in the meantime taken place in the administrative control of the laboratory. Until the conclusion of the war in the year 1902, both the laboratory and the rinderpest station were a subordinate branch of the Public Health Department, but with the establishment of a Department of Agriculture under the direction of Mr. F. B. Smith, they were both transferred to his Department, and the laboratory which had hitherto received but half-hearted support as a merely temporary institution, now became organised as the Veterinary Bacteriological Division of the Department of Agriculture, and from this time onward a steady policy of progress has been pursued.

The preparation of rinderpest serum was continued until in 1903 the country was considered free from the disease; it was then thought advisable to close down the serum depot, as the keeping of infected animals at a place where the facilities for segregation were by no means perfect created a quite unnecessary risk of starting a new outbreak in the immediate neighbourhood of the laboratory.

Meanwhile at the close of the war, importations of stock on a large scale had begun, not only from neighbouring Colonies and States in South Africa, but also from oversea; and due to these unrestricted importations, both during the war and after, many diseases before not known in the Transvaal, were introduced, and amongst them a new disease of cattle, which at one time seemed as if it would rival rinderpest in destruction and sweep off the last head of cattle that rinderpest and the war had left. The history of the introduction of East Coast fever, and the methods taken to combat it are now a matter of history, but the Transvaal farmer has need to congratulate the Department of Agriculture on its foresight in establishing a laboratory which was ready at once to take up research into the nature of the disease and so arrive at the best means of attacking it and preventing its spread.

From the first inception of the laboratory horse-sickness, which is so peculiarly a disease of South Africa, and which annually takes so heavy a toll of the horses and mules of this country, was naturally studied, but at first the facilities were scanty and money lacking. As, however, with a

BACTERIOLOGICAL LABORATORY
ADMINISTRATION BLOCK
AT ONDERSTE POORT

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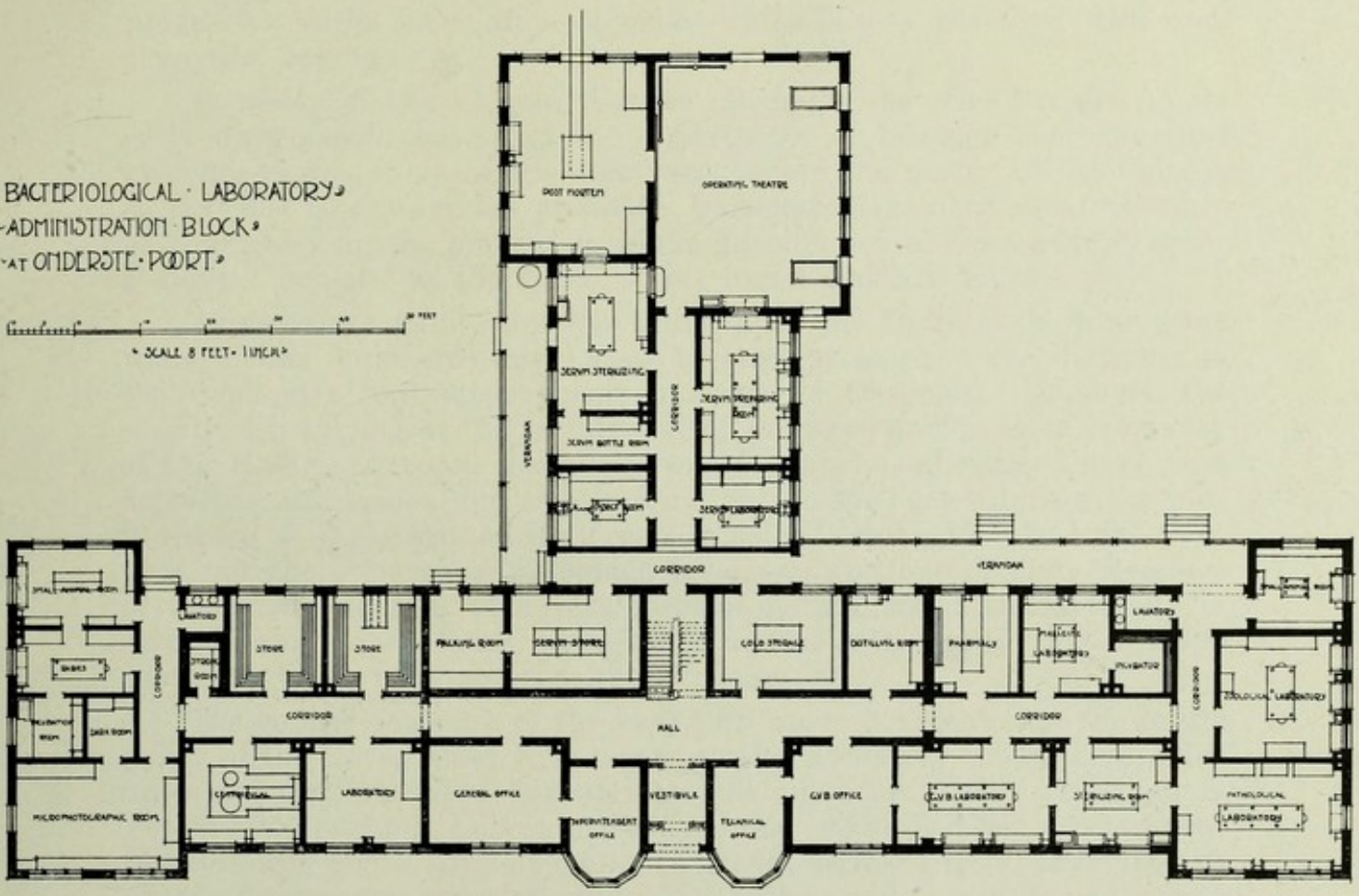
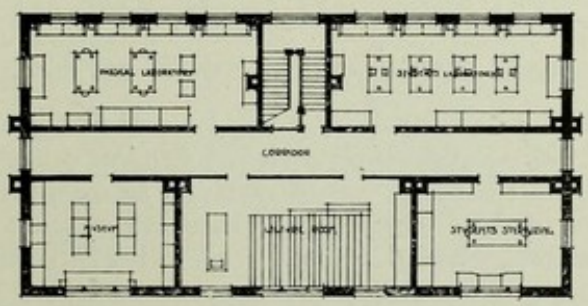



GROUND PLAN




1ST FLOOR PLAN

ARCHITECT

C. MURRAY
CHIEF ENGINEER
P.W.D. TRANSVAAL

more progressive policy the laboratory increased in size and funds were more liberally supplied, more attention was paid to this disease, and it was possible to enter upon a systematic course of experiments, which eventually culminated in the introduction in the autumn of 1905 of the preventive serum inoculation of mules which is now practised with such successful results.

It was a matter of good fortune no doubt that the Division in its early days should have had the opportunity of bringing such practical evidence of the value of scientific work before the people of the Colony, so that the laboratory has gradually become to be looked upon not only as a necessity for the prevention of the introduction of diseases from other countries, but also as the farmer's best friend and adviser.

As more and more work was thrust upon the Division the time came when it was impossible any longer to attempt to meet the demands in the temporary and inconvenient quarters of Daspoort. Moreover, the situation, low lying at the farther end of the town and in close proximity of the Kaffir and coolie locations, was extremely unhealthy; used as a depositing site before, and as a burying ground for horses during, the war, it seemed as if the ground itself were infected. From 1902 to 1906 every year saw the occurrence of typhoid fever amongst one or more members of the staff, and that of a very virulent nature, which ended fatally in several cases; it became, therefore, a matter of necessity that a move should be made to a more healthy situation.

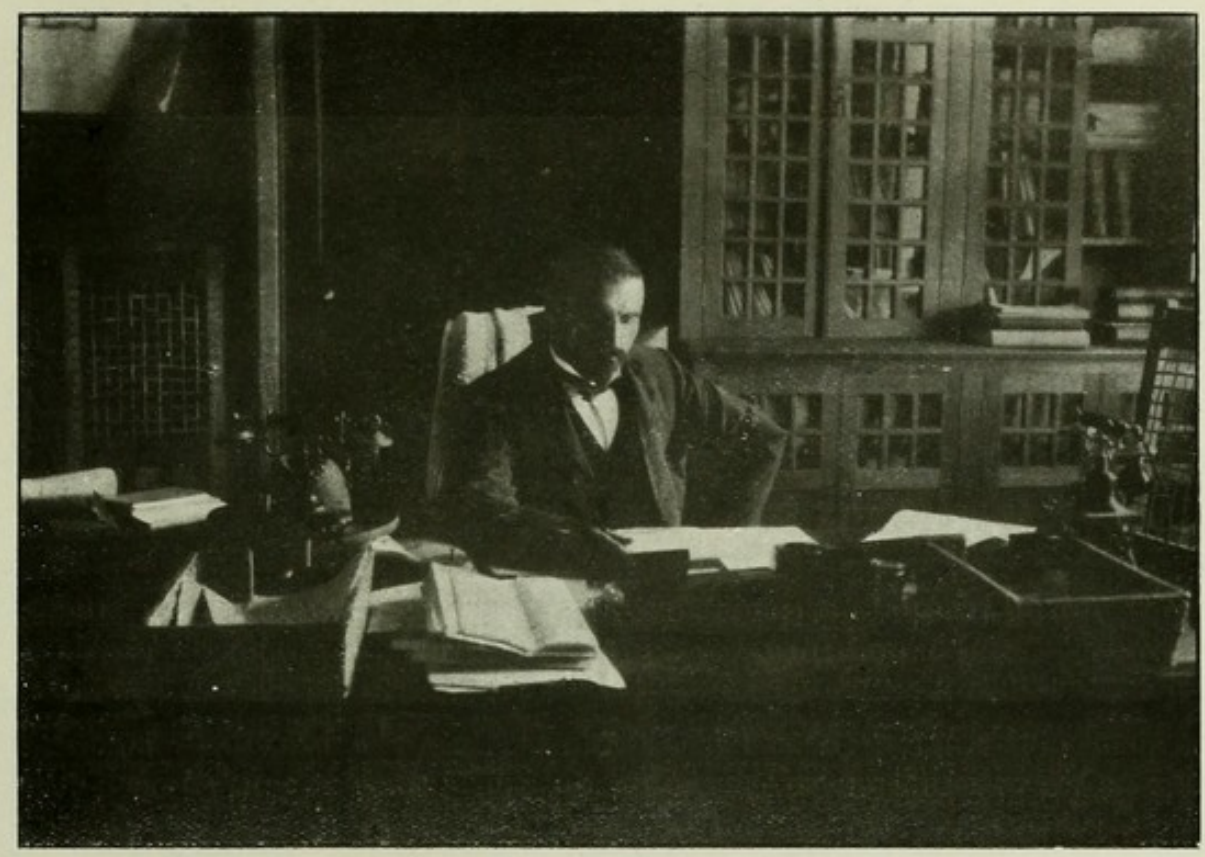
The enteric outbreak of the year 1906 made it urgent that the future policy of the Government with regard to the laboratory should be decided upon at once; whether appropriate buildings should be erected and a Veterinary Research Laboratory should be established as a permanent institution in South Africa, or a move should be made to other temporary quarters on a new site. Happily the former policy was decided upon without hesitation, and South Africa is now able to take a place amongst the older countries of Europe as a leader in veterinary scientific research.

## Publications of Work done in the Laboratories.

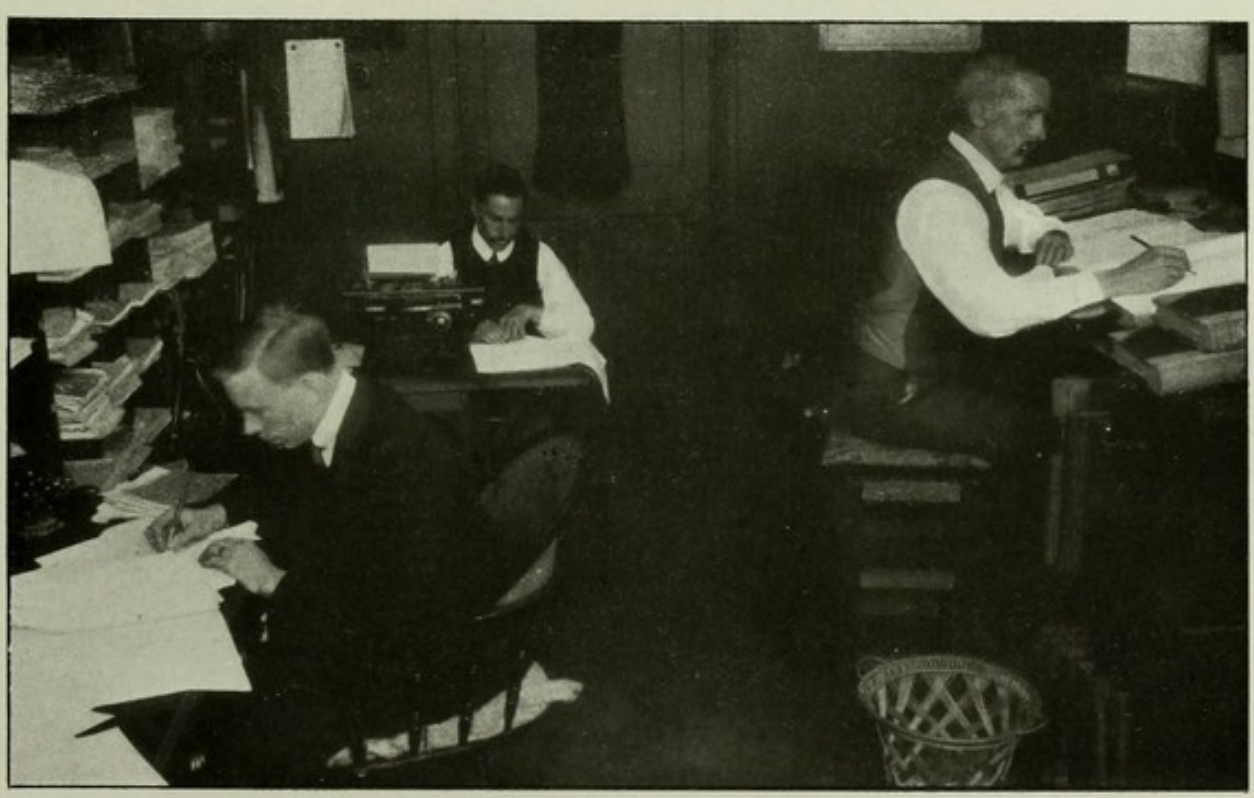
By Dr. Arnold Theiler, C.M.G.

1. Das Wiedererscheinen der Rinderpest und die Erfolge der Schutzimpfung in Sued Afrika. (Published in Monatshefte fuer Praktische Tierheilkunde, Band. 13.)
2. The Danger of the Simultaneous Immunisation with Serum and Virulent Blood for Rinderpest in Cattle not Immune against Redwater. (Annual Report, 1903–04.)
3. Horse-sickness (Results from Former Experiments and Serum Treatment applied to Horse-sickness). (Annual Report, 1903–04.)
4. Notes on Haemolysis. (Annual Report, 1903–04.)
5. Horse-sickness Experiments. (Annual Report, 1904–05.)
6. Further Experiments with Immunisation of Mules against Horse sickness. (Annual Report, 1905–06.)
7. Transmission of Horse-sickness into Dogs. (Annual Report, 1905–06.)

8. The Immunity in Horse-sickness. (Annual Report, 1905–06.)
9. Horse-sickness. The results of Inoculation in Practice during 1905–06. (Annual Report, same year.)
10. Horse-sickness. The Results of Inoculation in Practice during 1906–07. (Annual Report, same year.)
11. Horse-sickness. The Results of Inoculation in Practice during 1907–08. (Annual Report, same year.)
12. Further Notes on Immunity in Horse-sickness. (Annual Report, 1906–07.)
13. The Immunisation of Mules with Inadequate and Adequate Serum and Virus, and the Immunity obtained therefrom. (Annual Report, 1906–07.)
14. The Inoculation of Mules with Polyvalent Virus and Serum. (Annual Report, 1906–07.)
15. The Inoculation of Mules with Polyvalent Virus. (Annual Report, 1907–08.)
16. On the Variability of a Certain Strain of Horse-sickness Virus. (Annual Report, 1907–08.)
17. Fever Reactions Simulating Horse-sickness. (Annual Report, 1907–08.)
18. The Rhodesian Tick Fever. (Transvaal Agricultural Journal, 1903.)
19. The Rhodesian Tick Fever. (Report of the South African Association for the Advancement of Science, 1904.)
20. East Coast Fever. (Journal of the Royal Army Medical Corps, 1904, and in the Annual Report, 1903–04, also partially in Fortschritte der Veterinaer-Hygiene, 1903.)
21. Further Transmission Experiments with East Coast Fever. (Annual Report, 1906–07.)
22. The Influence of Cold on Ticks and Piroplasma Parvum. (Annual Report, 1907–08.)
23. Equine Malaria. Journal of Comparative Pathology and Therapeutics, 1902.)
24. Equine Malaria and its Sequelae. (As above.)
25. Notes on Piroplasmosis of the Horse, Mule, and Donkey. (Annual Report, 1903–04, and Zeitschrift fuer Thiermedicin, 1904.)
26. Further Notes on Piroplasmosis of the Horse, Mule, and Donkey. (Annual Report, 1904–05, and Journal of Comparative Pathology and Therapeutics, 1905.)
27. Piroplasma Equi as a Complication of Horse-sickness. (Annual Report, 1904–05.)
28. Inoculation against Equine Piroplasmosis. (Annual Report, 1905–06.)
29. Transmission of Equine Piroplasmosis by Ticks in South Africa. (Annual Report, 1905–06, and Journal of Comparative Pathology and Therapeutics, 1906.)
30. Piroplasmosis in Horses due to Hyperimmunisation. (Annual Report, 1905–06.)
31. Continuation of Experiments for Inoculation against Equine Piroplasmosis. (Annual Report, 1906–07.)
32. Further Inoculation Experiments against Biliary Fever of Equines. (Annual Report, 1907–08.)
33. The Piroplasma Bigeminum of the Immune Ox. (Annual Report, 1903–04, and Journal of the Royal Army Medical Corps, 1904.)
34. Piroplasma Mutans (Nova Species) of South African Cattle. (Annual Report, 1905–06, and Journal of Comparative Pathology and Therapeutics, 1906.)
35. Further Notes on Piroplasma Mutans. (Annual Report, 1906–07, and Journal of Comparative Pathology and Therapeutics, 1907.)
36. The Immunity of Cattle Inoculated against Piroplasma Mutans. (Annual Report, 1907–08.)
37. Experiments with English and South African Redwater. (Annual Report, 1906–07.)
38. Immunity in Tropical and Sub-tropical Diseases. (Commemoration Publication, 1909.)



*The Office of the Government Veterinary Bacteriologist.*



*The Technical Records Office.*

39. Trypanosomiasis in Camels. (Annual Report, 1904–05.)
40. A New Trypanosoma. (Journal of Comparative Pathology and Therapeutics, 1903.)
41. Spirillosis of Cattle. (Journal of Comparative Pathology and Therapeutics, 1904.)
42. Transmission and Inoculability of Spirillosis Thieleri (Laveran.) (Proceedings of the Royal Society, 1905.)
43. Transmission and Inoculability of Spirillosis in Cattle. (Annual Report, 1904–05.)
44. Notes on the Immunity of the Piroplasmosis of the Dog. (Annual Report, 1903–04, and Centralblatt fuer Bakteriologie, 1904.)
45. Heartwater in Cattle. (Annual Report, 1903–04.)
46. Immunisation against Heartwater. (Annual Report, 1904–05.)
47. Blue-Tongue in Sheep. (Annual Report, 1904–05.)
48. The Inoculation of Sheep against Blue-Tongue, and the Results in Practice. Annual Report, 1906–07.)

By Dr. A. Theiler and Stewart Stockman, M.R.C.V.S. (late Principal Veterinary Surgeon, Transvaal, 1902 to 1903).

1. Experiments to Show how long an Area which was at one time Infected (with East Coast Fever) will remain Infected. (Annual Report, 1903–04, and Journal of Comparative Pathology and Therapeutics, 1904.)
2. Inoculation Experiments according to the Methods of Professor Koch. (As above.)
3. Dipping Experiments. (As above.)
4. Possible Influence of the Different Seasons on the Outbreak of East Coast Fever. (As above.)
5. Further Experiments to note how long an Area will remain Infected with East Coast Fever. (Annual Report, 1904–05, and Journal of Comparative Pathology and Therapeutics, 1905.)
6. Do Salted Cattle contain Piroplasma Parvum in their Blood ? (As above.)
7. Experiments with Serum against East Coast Fever. (Annual Report, 1905–06, and Journal of Tropical and Veterinary Science, 1906.)

By Sydney Dodd, M.R.C.V.S. (late Assistant Government Veterinary Bacteriologist, 1906–07).

1. A Disease of a Pig due to a Spirochaete. (Journal of Comparative Pathology and Therapeutics, 1906.)
2. A Preliminary Note on the Identity of the Spirochaete found in the Horse, Ox, and Sheep. (Journal of Comparative Pathology and Therapeutics, December, 1906.)

By James Walker, M.R.C.V.S., Assistant Government Veterinary Bacteriologist (appointed July, 1908).

1. The Diagnosis of Bacillary Piroplasmosis of Bovines in the Transvaal. (Commemoration Publication, 1909.)

By Dr. Walter Frei, Assistant Government Veterinary Bacteriologist (appointed November, 1906).

1. Viscosity of Blood. (Transvaal Medical Journal, April, 1908.)
2. Surface Tension of Serum. (Transvaal Medical Journal, August, 1908.)
3. Physical Chemistry in Veterinary Science. (South African Association for the Advancement of Science, Grahamstown, 1908.)
4. Remarks on some Experiments with Snake Poison. (Royal Society of South Africa Proceedings, 1909.)

5. Vergleigende Physikalische Chemische Blut und Serum Untersuchungen, mit besonderer Berucksichtigung der Pferdesterbe. (Zeitsch. fur Infektionskrankheiten, 1909.)
6. Physikalische Chemische Untersuchungen uber Piroplasmosis. (Zeitsch. fur Infektionskrankheiten, 1909.)
7. Physical Chemical Investigations into South African Diseases. (Annual Report Government Veterinary Bacteriologist, 1907-08.)
8. Haemolysis in Practical Veterinary Science. (Commemoration Publication, 1909.)

By Dr. Lewis Henry Gough, Zoologist (appointed July, 1908).
1. On a Coenurus in the Duiker. (Proceedings Royal Society of South Africa.)
2. The Anatomy of Stilisia Centripunctata. (Commemoration Publication, 1909.)

By Dr. Karl Friedrich Meyer, Pathologist (appointed October, 1908).
Notes on the Pathological Anatomy of Pleuro-pneumonia. (Commemoration Publication, 1909.)

## Description of the Laboratory Buildings.

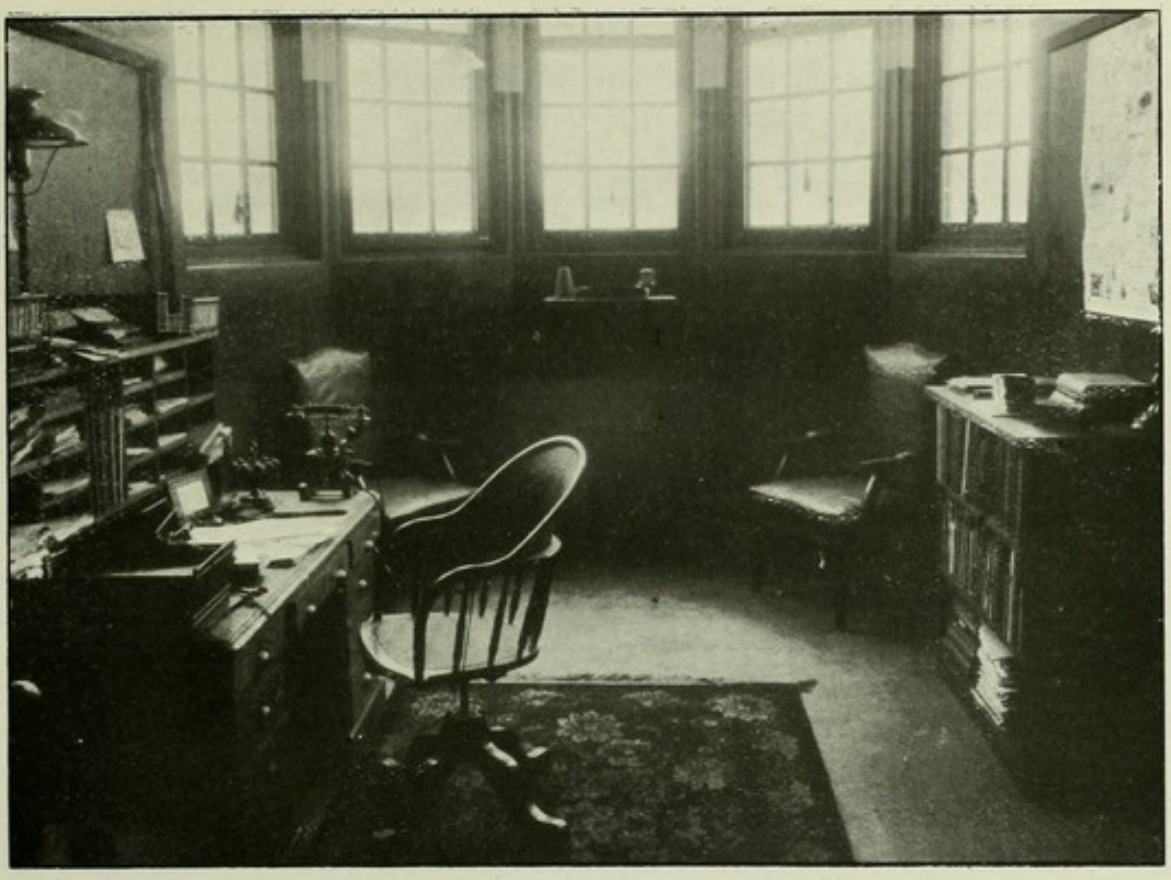
The laboratory building, which is in the Colonial Dutch style of architecture, is of brick, faced with cement. It is 303 ft. in length × 51 ft. wide and from the centre a wing runs back another 80 ft., making the whole a T-shaped building.

A second storey extends for 77 ft. over the central part, and this is surmounted by a clock turret, from where a magnificient view of the surrounding country is obtained.

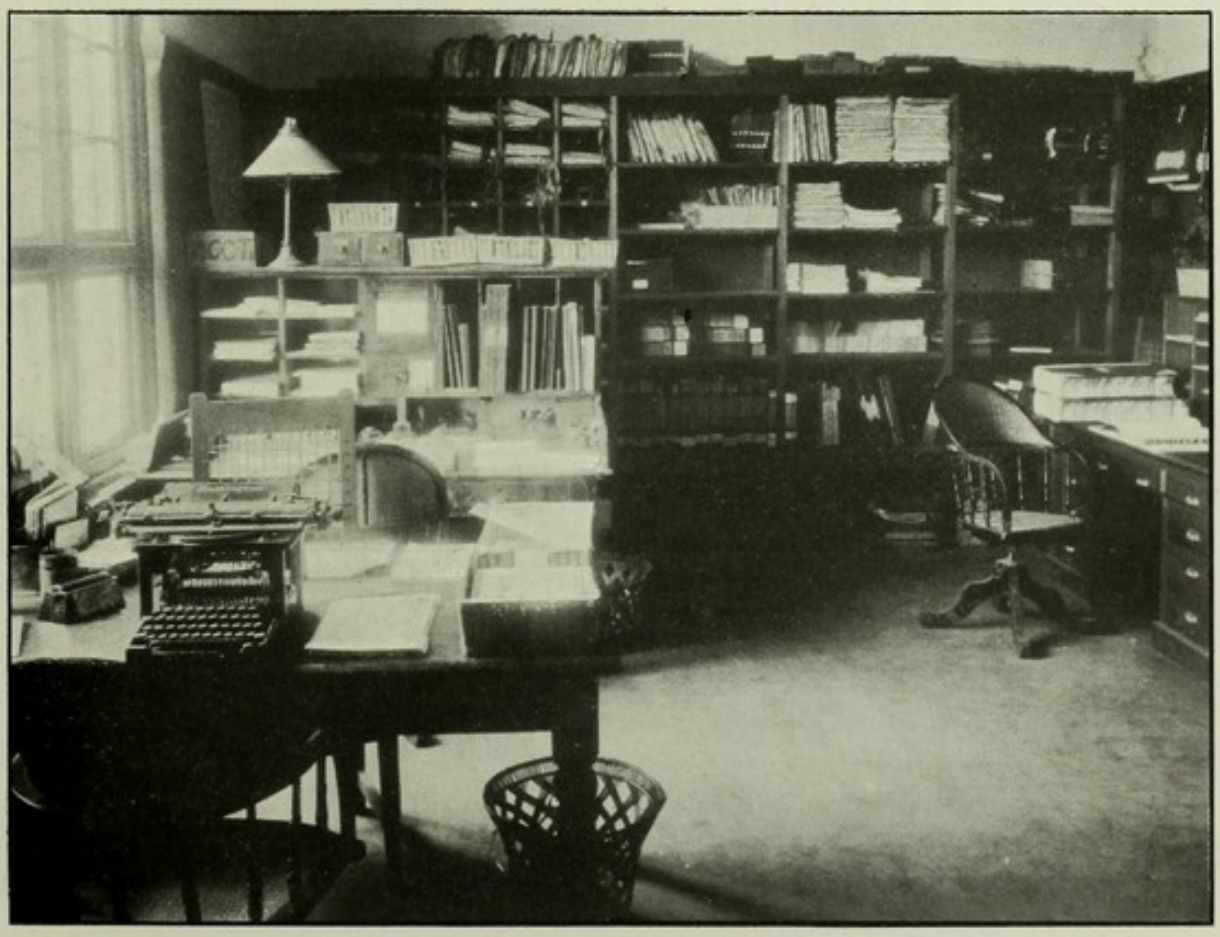
The building lies east and west with the main entrance on the south side. A corridor runs from end to end, and beneath the floor of the corridor, with branches into all the rooms, is a conduit covered with removable granolithic slabs; in this conduit run the pipes for hot and cold water, steam, gas, and waste.

The rooms on the ground floor are 13 ft. high, and the walls are distempered above and painted with a chocolate coloured dado below. There are steel ceilings throughout the building. The floors, except in the offices, are of granolithic and are finished off at the sides of the rooms rising in a curve to meet the skirting so as to avoid corners that might collect dust, and the laboratories have been designed throughout with a view to absolute cleanliness and freedom from dust. The windows in all the laboratories are 2 ft. 6 in. from the ground and on a level with the slate slab tables used for microscope work, and the lower part of the windows are fitted with panes of plate glass 36 in. × 2 ft. so that there shall be no obstruction to the light on the tables. The whole building has been constructed to be perfectly insect proof, wire screens being fitted to all the windows and doors.

The several laboratories are fitted on a uniform plan as far as such will conform to the particular requirements of each, and hot and cold



*The Superintendent's Office.*



*The General Office.*

water, gas, steam, and electric light are laid on throughout the building. Down the centre of each laboratory runs a teak table 3 ft. 3 in. high, having a row of drawers on each side and semi-circular sinks fitted to the ends; gas nozzles are provided on each side at suitable distances down the whole length of the tables; they are let into hollows scooped out of the tables so as to be flush with the table tops. Hot and cold water taps are provided at all the sinks.

In front of the windows are tables for microscope work made of slate slabs 2 ft. wide, fitted on iron brackets fixed in the wall; between the windows and at each end of these tables are small sinks.

Fume closets are provided in all the laboratories 6 ft. long × 2 ft. wide, tapering to the top, which is connected by a flue with the chimney; glass doors sliding upwards open the closets to a height of 2 ft. 4 in., and in each hot and cold water, gas and electric power are laid on. A sink is also provided, and the floor of the closets are covered with sheet lead. These fume closets are of great value in a hot climate as a place for keeping the gas stoves as well as for carrying on the smaller sterilizing operations. Brackets 2 ft. 6 in. long × 11 in. wide, each fitted below with two plate glass shelves 6 in. wide are fixed to the walls over all the microscope tables; the brackets are used for carrying the larger bottles of disinfectants, and the shelves for reagents.

Small incubators, heated by gas, are in those laboratories where they are required. Vacuum pumps as well as small centrifugal machines driven by electric motors are also provided where necessary, and there are large cupboards with sliding glass doors in all laboratories.

The sterilizing rooms, which are three in number, in addition to tables similar to those provided in the laboratories, have, in each, large slate tables fitted against the wall, which is lined above them to a height of 18 in. with glazed white tiles. These tables are for sterilizing operations and are in two sections—one 2 ft. 8 in. high for smaller apparatus, and one 2 ft. high for carrying the large autoclaves, which are heated by steam from the central supply. Beneath the former a smaller slab is fixed to be used as a shelf, and over the whole is a hood of sheet iron connected at the top with the chimney to carry away the hot air. There are also two large sinks with draining racks, bottle racks, and other necessary accessories; hot and cold water, gas, and electric light are provided as in the laboratories as well as glass-blowing apparatus and vacuum pumps in each.

A general idea of the arrangement of the laboratory can best be obtained from the plan, from which it will be seen that on entering at the door on the south side and passing through a small vestibule, the main corridor runs right and left. This corridor taking a turn at each end leads to two doors at the back of the building, that on the

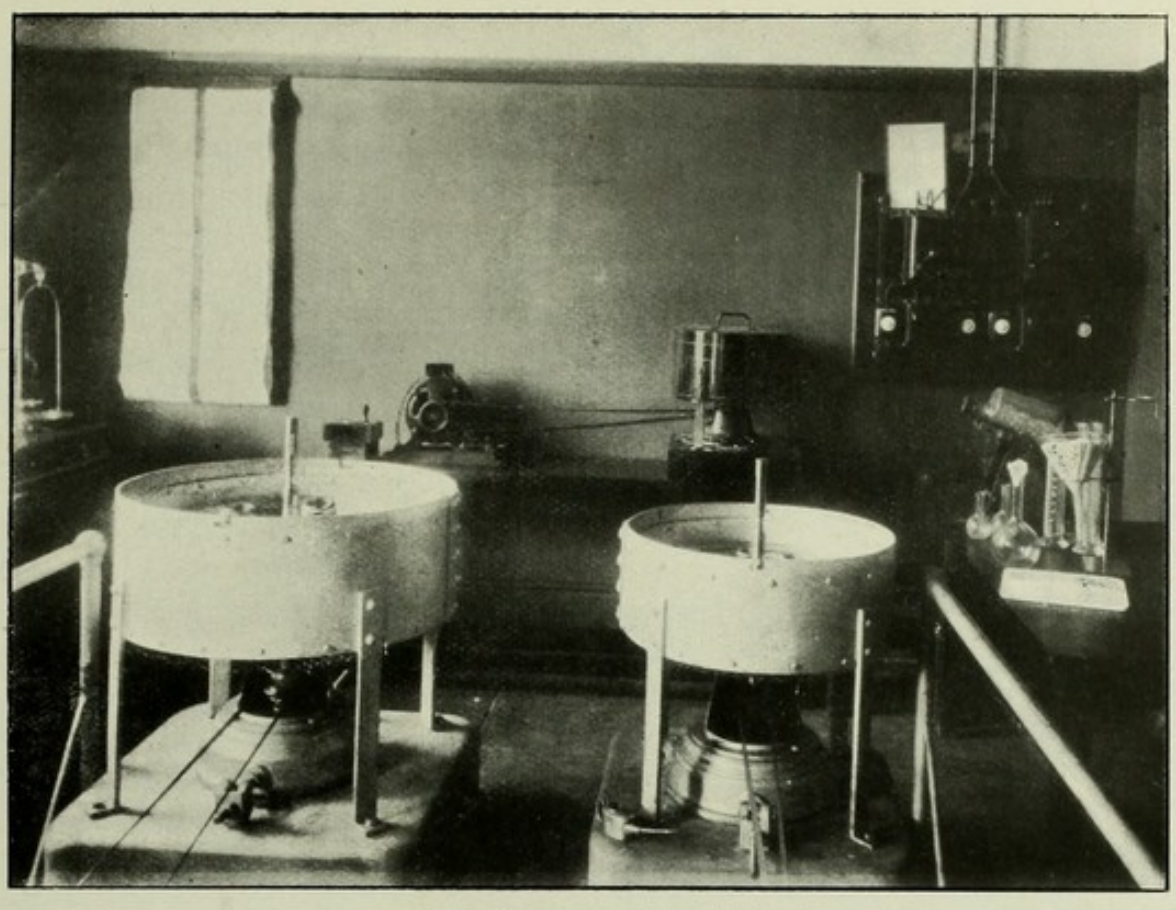
east side opening out into the quadrangle yard, and that on the west side into the yard in which the power house is situated. Facing the front door is the staircase which leads to the upper floor, and going direct from the door and passing the staircase a passage leads through a glass panelled door to the back wing of the building. Into these two corridors all the rooms on the ground floor open, excepting the serum store and the packing room, which open on the back. The four front rooms in the centre of the building are the offices, the two to the right are, first the office in which all the technical records are kept, and next to it, with a connecting door, that of the Government Veterinary Bacteriologist; those to the left are first the office of the Superintendent, under whose control is the organisation and all the business management of the station, and next, opening out of it, the general clerical office.

Adjoining the office of the Government Veterinary Bacteriologist and connected by a door is his private laboratory, which opens out at the farther end also into a sterilizing room. This laboratory is at present chiefly used in connection with the study of Protozoa and their relation to diseases of live stock.

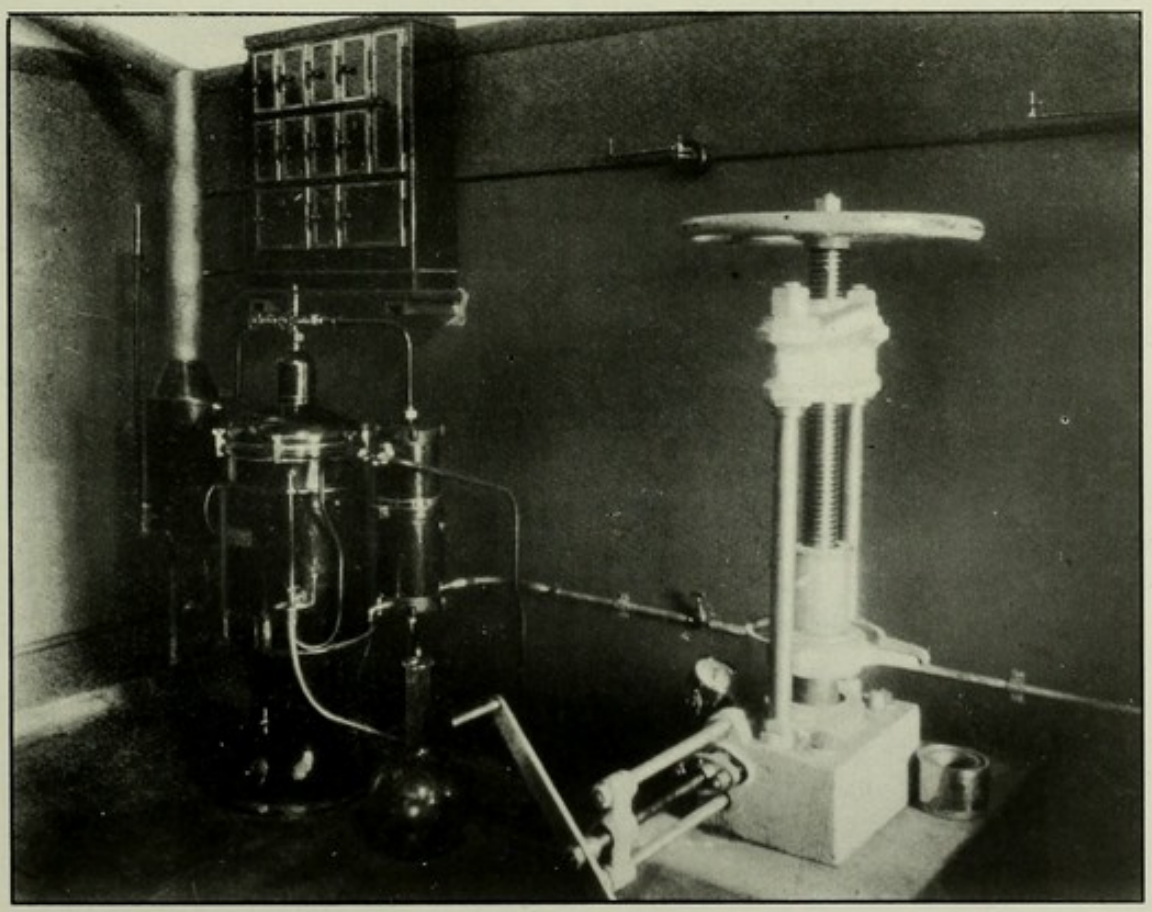
The last room on this side of the building is the Pathological Laboratory, which also opens by another door into the same sterilizing room. This laboratory is 25 ft. long × 16 ft. wide, and lighted by two large windows facing the south, and one facing east, and is very completely fitted up for histological and bacteriological research. Here also the examination of blood and other smears is carried on, as well as of numbers of pathological specimens that daily come in from the country. The extent of this branch of the work will be realised when it is stated that during the past year 1,362 specimens were examined, and the number is on a steady increase. The principal object of this laboratory is the study of histological pathology of tropical diseases of the country, and more especially of horse-sickness, East Coast fever, and the various piroplasms.

Next to the Pathological Laboratory, with two windows looking on to the east, is the Zoological Laboratory. Though not directly connected it is conveniently situated for using the same sterilizing room mentioned before. This room is 18 ft. × 18 ft. 6 in., and is now chiefly devoted to the study of intestinal parasites, and more especially to those found in sheep, a matter of most vital importance to the sheep farmer of this Colony. Here also the breeding of mosquitos is carried on and experiments on their relation to the transmission of disease.

Passing from the corridor through the door at the back of the building, one comes out on to a wide verandah which faces the north and runs half the length of the building. This was designed to protect from the sun's rays the windows of the rooms facing the north, and so to preserve a more equable temperature in the laboratory. On the left of the door is



*The Centrifugal Room.*



*The Still Room.*

a small lavatory, and on the right, fitted with shelves for the cages, is the room in which small animals under experiment are kept; this room has a table in the middle and is fitted with slate slabs and a sink by the window.

Returning through the door into the laboratory building, the first room on the north side is a room used exclusively for bacteriological work, and mainly for the preparation of mallein, tuberculin, pleuro-pneumonia cultures, and quarter-evil vaccine, and connected with it by a double door is an incubation room 8 ft. square and 8 ft. high insulated with asbestos and heated by a gas stove which is automatically regulated by the Roux system. Next to this room is a small room fitted as a pharmacy, which is also used as a chemical and drug store; against the walls on each side are large cupboards with sliding glass doors, and a table with a sink is in the centre.

Adjoining is another small room used for making distilled water, for which purpose a large Murrle distilling apparatus, fed by steam from the main supply, has been provided, and is so arranged that it can also be used with gas; connected with it is a drying oven, the temperature of which can be raised if necessary by super-heating the steam by means of a gas jet. A Buchner press, mounted on a concrete stand is placed in this room, and there is a small fume closet containing the serum coagulating apparatus.

Adjoining is the cold storage room, provided with double doors, the walls and ceiling of which are completely insulated with asbestos and silicate cotton. In the centre is a large ice chest in which compartments are provided for keeping pathological specimens. The walls are lined with shelves filled chiefly with bottles of horse-sickness virus.

Returning again to the other side of the building next to the general office is a room at present used as a workroom for the lay assistants; adjoining this is the room set aside for the large centrifugal machines; these, two in number, are by Lautenschlager, of Berlin; the larger, driven by a 5 horse-power electric motor and making 3,000 revolutions a minute, is provided with four receptacles each of a capacity of one litre; the smaller, driven by a $2\frac{1}{2}$ horse-power motor, makes 4,000 revolutions per minute, and is capable of centrifugalising one litre of liquid. A smaller machine, driven by $\frac{1}{2}$ horse-power motor, is fixed against the wall, and the room is fitted up in other respects as a laboratory. Opposite the last two rooms are two storerooms for laboratory apparatus and glassware, and on the same side is a strongroom for the records.

The corner room on the front of the building is the room used for photography, in size similar to the Pathological Laboratory, situated on the opposite corner of the building, with two large windows facing the south and a window on the west. A working bench extends the length of the room under the windows, with a sink at each end, and the walls are fitted with glass panelled cupboards. In this room there is a Zeiss

micro-photographic apparatus and appliances for ultra microscope work. The illumination for these is provided by a thirty ampere arc lamp, or by means of a heliostat placed on a brick pedestal outside the window. Heavy curtains are hung at the windows so that the room can be completely darkened when required. A well fitted up dark room adjoins and opens out of this room.

The three next rooms, all facing west, are used for the preparation of rabies vaccine, according to the Pasteur method. The centre room is a small laboratory. An inner room opens out from it which is fitted with double doors and windows, and here the temperature is regulated by a similar stove to the one in the incubation room. This room is exclusively used for drying the Rabies cords. On the other side of the laboratory is a room for keeping the small animals and fitted similarly to the small animal room on the eastern side of the building.

In the back wing the rooms devoted to the preparation of horse-sickness serum and blue-tongue vaccine are situated, and the last two rooms to the north are the operating theatre and the post-mortem room. Passing from the main corridor through the glass panelled door mentioned before, on right and left are doors opening out on the verandah facing the quadrangle yard, and on to a verandah running north and south on the west side of the back wing; in front is a corridor with a door at the end opening into the operating theatre. There are two rooms on the right—first, a small laboratory used for testing the serum for its haemolitic effect and sterility; second, the serum preparation room, a large room with two windows facing east and a hatch opening into the operating theatre, so that the bottles filled after tapping can be expeditiously passed through from the latter. In this room the preparation of horse-sickness serum for the immunisation of mules is carried on. The laboratory annually sends out more than 1,000 litres of this serum to all parts of South Africa. Blue-tongue vaccine is also prepared here, and of this in the past year nearly 200,000 doses were issued.

Facing this room on the left of the corridor is a sterilizing room which is also provided with a hatch opening into the operating theatre, to facilitate the easy passing through of sterilized bottles; and there is a second hatch opening into the post-mortem room, which adjoins the operating theatre. Next to the sterilizing room, and connected with it by a door, is a small room fitted with shelves for sterilized bottles, and opposite the Serum Laboratory is the tick and insect room, where ticks used in connection with the experiments in the transmission of the various protozoa and diseases of the country are bred and kept during their moulting stages.

To the left of the door opening on to the west verandah is a door leading into the serum storeroom, where the serum, blue-tongue vaccine, and other preparations, after having been bottled, sealed, and labelled are

*The Serum Store.*

*The Small Animals Room.*

kept ready to be sent out as required ; adjoining and connected by a door is the packing room, which also opens by a door into the yard. In this room the bottles of serum and other preparations are packed in boxes for despatch by train into the country districts.

The operating theatre and the post-mortem room are situated at the end of the back wing and are connected by sliding doors; the former is 38 ft. × 28 ft., and the latter 29 ft. × 21 ft. The flooring of both rooms is asphalt, and they are well lighted by windows placed high up on the walls.

The post-mortem room has in addition two large windows on the west, beneath which are slate slabs similar to those provided in the laboratories for microscope tables. Tram lines are laid on the floor which, passing through the double doors on the north side, circle all round the back of the stables and branch off to the destructor. Carcases are thus easily taken from the stables on a truck and brought into the post-mortem room, and, after autopsy is completed, can be quickly removed to the destructor. The truck is so arranged that the four sides fall down to form a table on which the post-mortem examination is made, and they can afterwards be replaced in position for the removal of the carcase. There is a desk for entering in the register at the time the record of each post-mortem, and all conveniences for the curing and preserving of pathological specimens are provided.

The operating theatre, in addition to windows on the north and east side, has a large skylight in the roof with windows on all four sides ; there is therefore an excellent light in all parts of the hall. Tethering rails are fixed around the walls, and there are two padded boxes for retaining horses under operation, and, in addition to every necessary convenience, a small centrifugal machine with haematocrytes is fixed on the wall, which facilitates the measuring of the blood volume of the living animal on the spot. Double doors open out on the north side towards the stables, and there are two doors on the east side for bringing horses into the boxes, and a small door at the south-east corner leading into the quadrangle yard.

On the first floor there are six rooms ; the centre room on the south side is a lecture room and library, arranged with benches in tiers facing a table fitted with a sink and provided with water and gas so that laboratory demonstrations can be made ; behind are the blackboards. In this room there is a large projection lantern illuminated by a seven ampere arc lamp, which is fitted with a projection microscope, and is arranged for direct projection of diapositives by transmitted light or for projection of solid objects by reflected light.

On the north side there are only two rooms, each 15 ft. × 32 ft. One is fitted up as a students' laboratory with all the necessary facilities for bacteriological work and having four working tables arranged down the

centre fitted with sinks, hot and cold water, and gas ; they are also provided with small cupboards and drawers below, and are modelled after the pattern of the students' tables at the bacteriological laboratory at Berne. The other room is devoted to physical chemical research, and has also complete laboratory equipment and it is proposed will be used later for teaching physiology to students.

Opposite the students' laboratory is a sterilizing room, fitted up similarly to those in the other parts of the building.

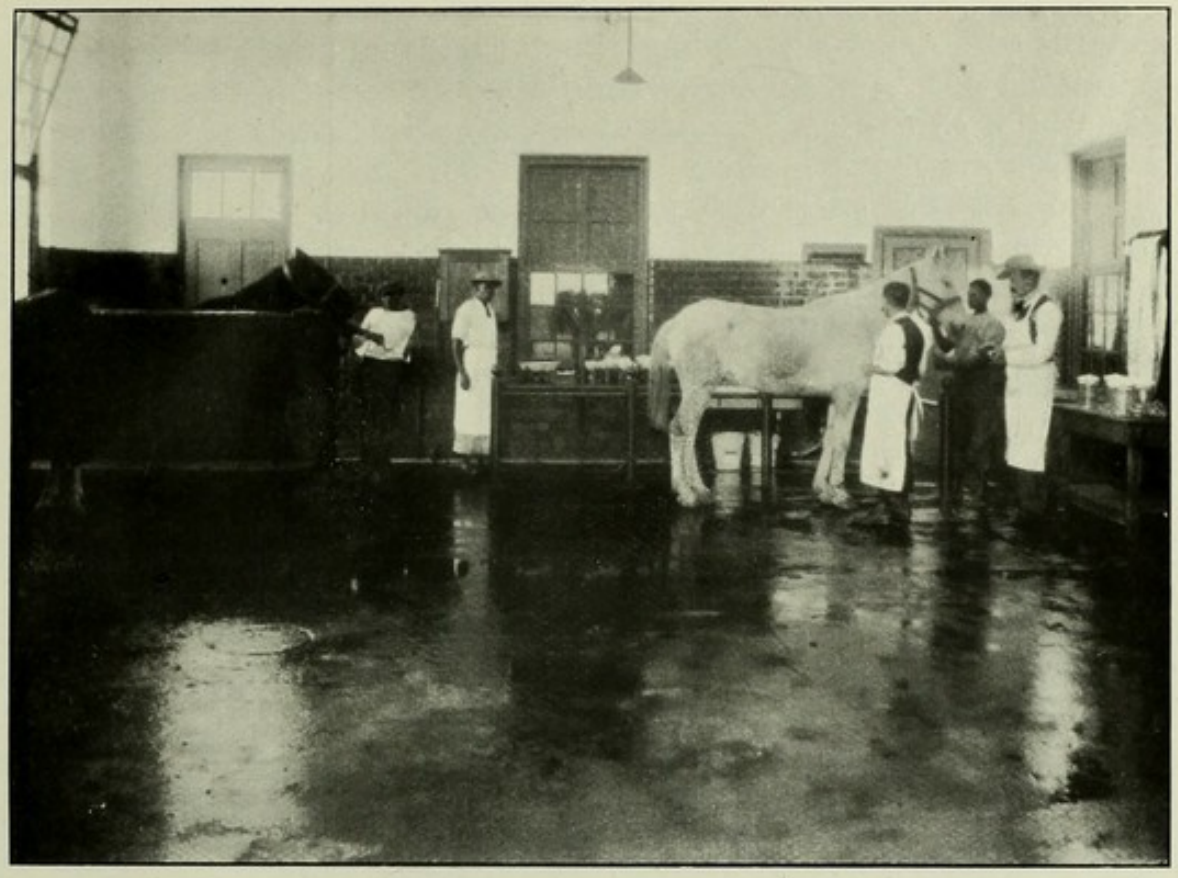
The remaining room on this floor is the pathological anatomical museum. Here already there is a fine collection of specimens, and the large glazed cases on the walls are filled with a very representative collection, amongst which are some interesting specimens of tropical diseases. In the centre of the room is a chest fitted with drawers for specimens, and under the window is a table so that work connected with the museum can be conveniently carried on.

All the principal rooms are connected with a telephone installation provided for communication within the building, a matter of great convenience in a large laboratory where a numerous staff is employed.

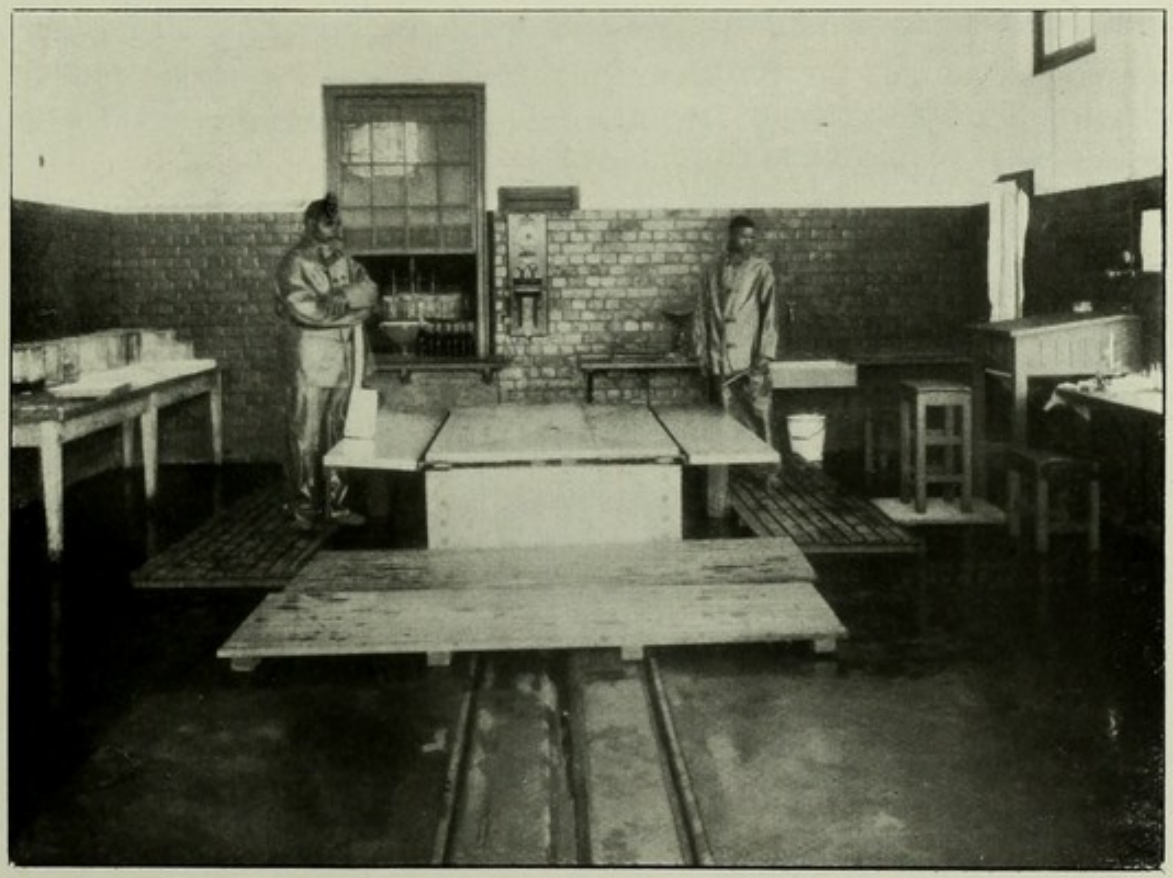
To the west of the laboratory, completely detached, is a separate building designed for the preparation of calf vaccine lymph. This building complete in itself has four rooms ; a large room well lighted by two windows facing the south and fitted with a table is for the vaccinating operations ; a door leads from here into a stable with three loose boxes for the calves, to which also there is an outer door for attendance. In front, opening on to a verandah, as well as connected by a door with the operating room, is the laboratory for preparing the vaccine and filling the tubes ; there is also a small room at the back, opening into the stable, used as a storeroom for forage. The preparation of vaccine lymph is an important branch of the laboratory work, over three-quarters of a million tubes having been sent out into all parts of South Africa during the last two years, and the work is now carried out under the most modern hygienic conditions.

The situations of the other buildings are seen on the block plan.

The stables are all built of brick and are lofty and well ventilated ; the mangers are of iron and the stall partitions are made with iron pillars and iron frames covered with boarding ; the floors are paved with blue bricks set in cement, and are drained by open channels, which pass through a pipe into an open gulley that runs the whole way along the outside of the stables and discharges through traps at intervals into the main drainage system. The windows are placed high up and are covered, as also are all ventilators, with wire gauze to keep out insects, a matter of some importance for the prevention of natural infection of horse-sickness.



*The Operation Theatre.*



*The Post-Mortem Hall.*

The stables used for the animals under experiment are situated at the back and form, with the east and north wings of the laboratory, a quadrangle. In these stables, accommodation is provided for 100 horses and mules, 50 cattle, and 100 sheep and goats. There are twelve loose boxes as well as six isolation boxes, which latter are arranged for slinging horses if required. Doors open into the quadrangle yard, and there are also doors at the back, and passages for feeding with doors at each end run between each stable. In this way in the hottest of weather there is abundance of ventilation, and all cleaning and feeding operations are carried out through the doors at the back, the doors opening into the quadrangle yard being only used for bringing out animals in connection with the laboratory work. A tram line runs the whole length of the stables at the back to facilitate feeding and cleaning, and dead animals are removed to the post-mortem room by this means.

Behind the stables are the forage stores and feed mixing room, with chaff-cutter and corn-grinding machine, the buggy house, harness and tool sheds, a carpenter's shop, a saddler's shop, and the wagon and implement shed. There is also a farrier's shop and a large platform scale.

The dog kennels are in a building which contains 20 pens, with a passage down the middle of the building; each opens into a yard paved with granolithic and enclosed by iron railings on dwarf walls.

The piggeries are built on the same plan, but the yards are enclosed by walls 3 ft. high, and are fitted with cast iron feeding troughs and gates.

There is also a segregation stable capable of holding 30 horses, to receive horses and mules when first admitted to the station, in which they can be malleined, and a room is fitted up at one end of the stable for facilitating this.

An isolation stable more especially for suspected cases of glanders is apart from the other buildings, and has twelve loose boxes with a feeding passage running down the centre, but the latter has no communication with boxes except by a sliding trap door through which the food can be passed; a tap supplies water to a trough in each box operated by a cock in the passage.

There is also a house for breeding small animals, rabbits, and guinea pigs; this is fitted with removable partitions so that a number of small boxes or larger runs can be arranged as desired, and the whole can be taken down and thoroughly cleansed and disinfected when necessary. The design for this house was copied from the plans of a house for the same purpose in the Berne Laboratory.

There are eight wood and iron stables, 50 ft. long × 15 ft. wide, opening into yards 50 ft. × 50 ft., fenced with corrugated iron 8 ft. high. These are used for horses kept for serum and for animals not under experiment and reserve animals. Water is laid on to troughs in the yards.

The cremator is situated at a little distance from the other buildings and was supplied by Messrs. Manlove, Alliott & Co., of Nottingham. It consists of two separate destructors placed end to end and working through one chimney, and is capable of cremating five or six horses a day.

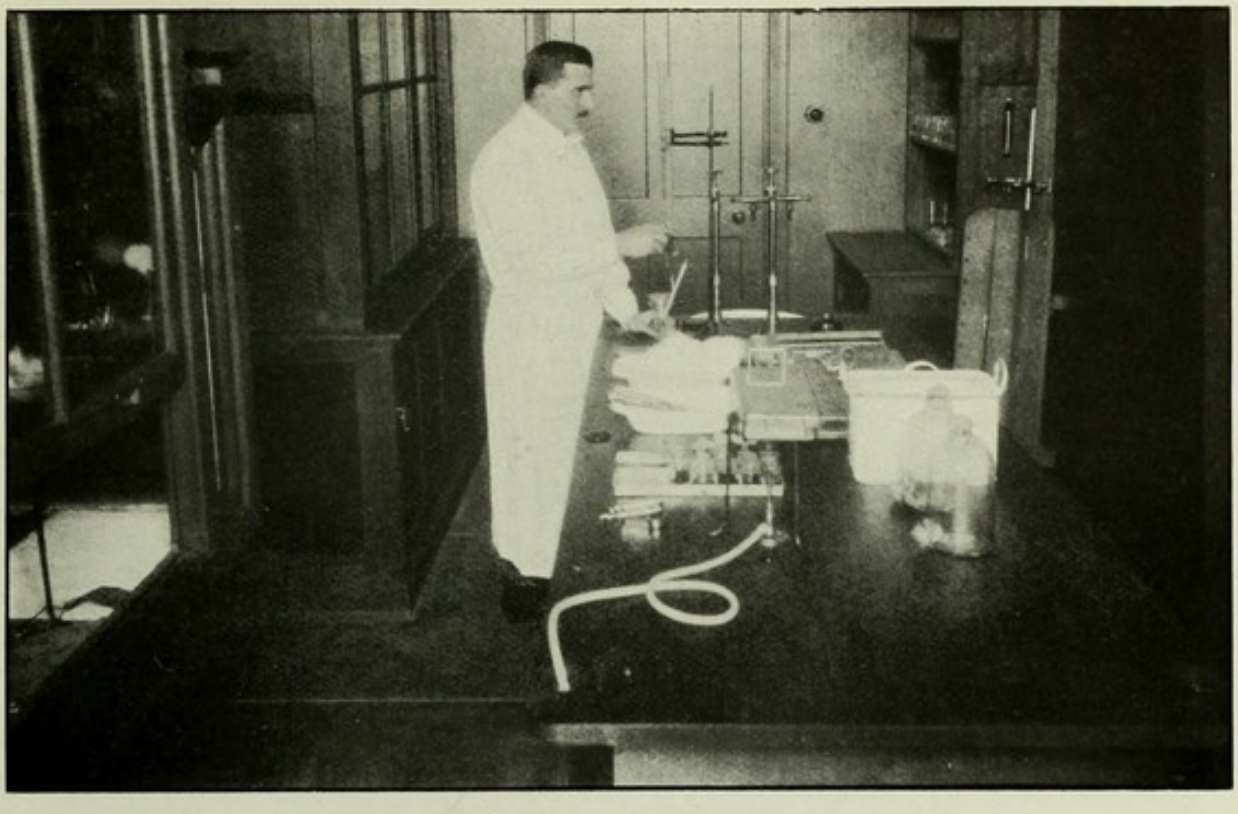
The electric installation, the work on which was carried out under the direction of Mr. F. Stephens, the Electrical Engineer to the Public Works Department, consists of an engine and dynamo supplying current at 250 volts pressure to about 200 lamps throughout the laboratory building and to a number of points whence current can be conveniently drawn for working small motors for various purposes. Electricity is also supplied for driving the centrifugal machines by means of two motors, one of 5 horse-power, and another of $2\frac{1}{2}$ horse-power, the motors being on raised platforms to economise space. The system of distribution for power purposes is kept entirely separate from the lighting circuits. Current up to 40 amperes is also available for running the large arc lamp used for photo-micrography and ultra microscope work, and for the projection lantern in the lecture room.

A pump driven by a small electric motor is provided for filling a tank in the roof. This pump is automatically stopped when the tank is full by a float operating an electrical device, so that the pump requires no attention when once started.

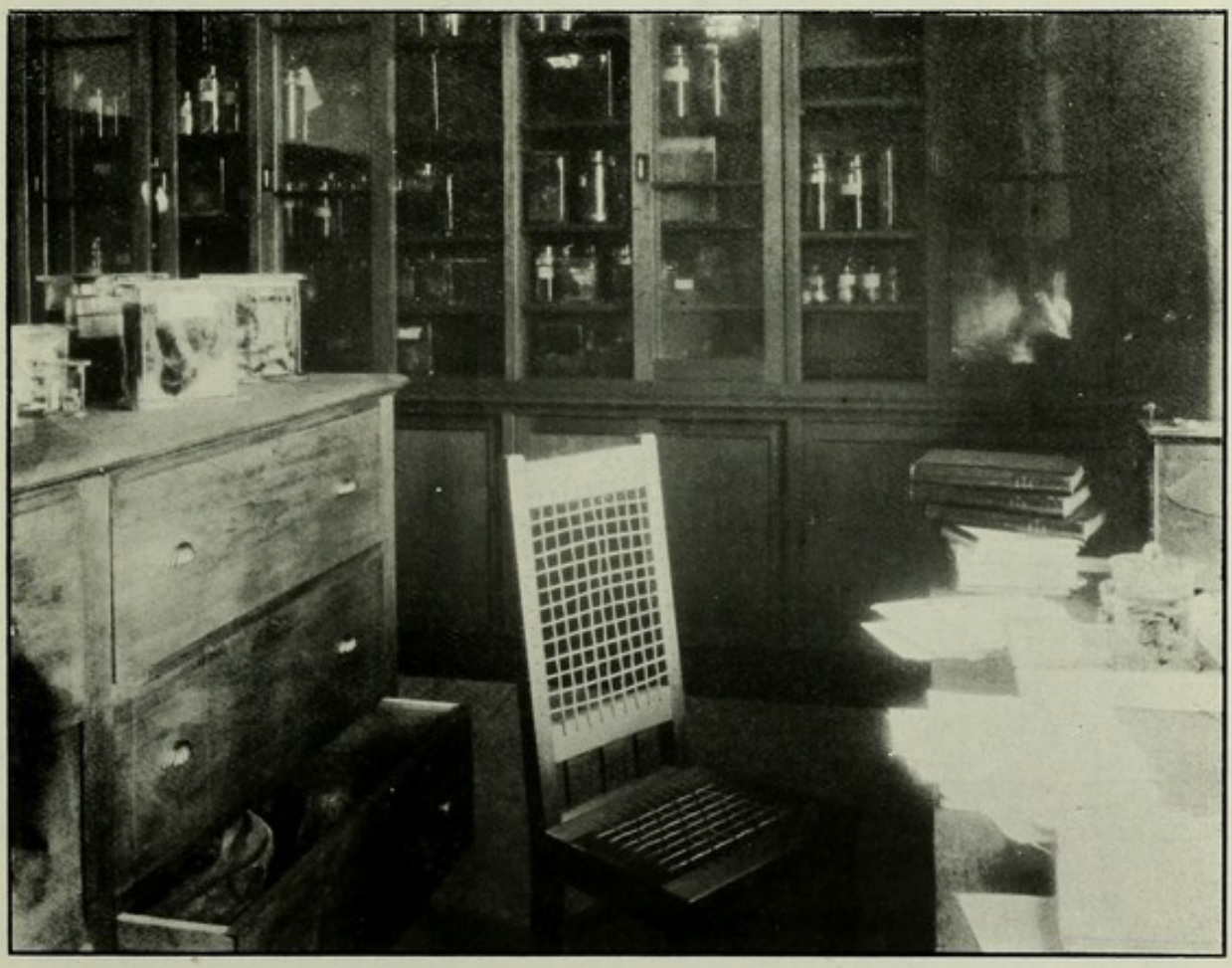
The electric light plant is located in a detached building on the north-west side of the laboratory. It consists of a steam-driven high speed, direct-coupled Crompton-Belliss set, running at 400 revolutions per minute, and capable of developing 80 amperes at 250 volts pressure. The current from the dynamo is taken direct to a main switchboard located in the engine house, whence the cables radiate to the main building and to the pump house. Several spare circuits are provided on the board for future extensions. The boiler is of the Robey semi-portable type. It is fixed under the same roof as the engine, but in a separate room, and is capable of supplying all the steam required in connection with the supply of electricity, and for sterilising purposes, as well as for making distilled water.

The auxiliary plant in connection with the boiler consists of a feed-water heater worked by exhaust steam from the engine and a hot water cylinder for the supply of hot water for all purposes throughout the laboratories. This water is also heated by means of exhaust steam, use being thus made of heat which would otherwise be wasted. These heaters are situated in a chamber below the level of the boiler house floor.

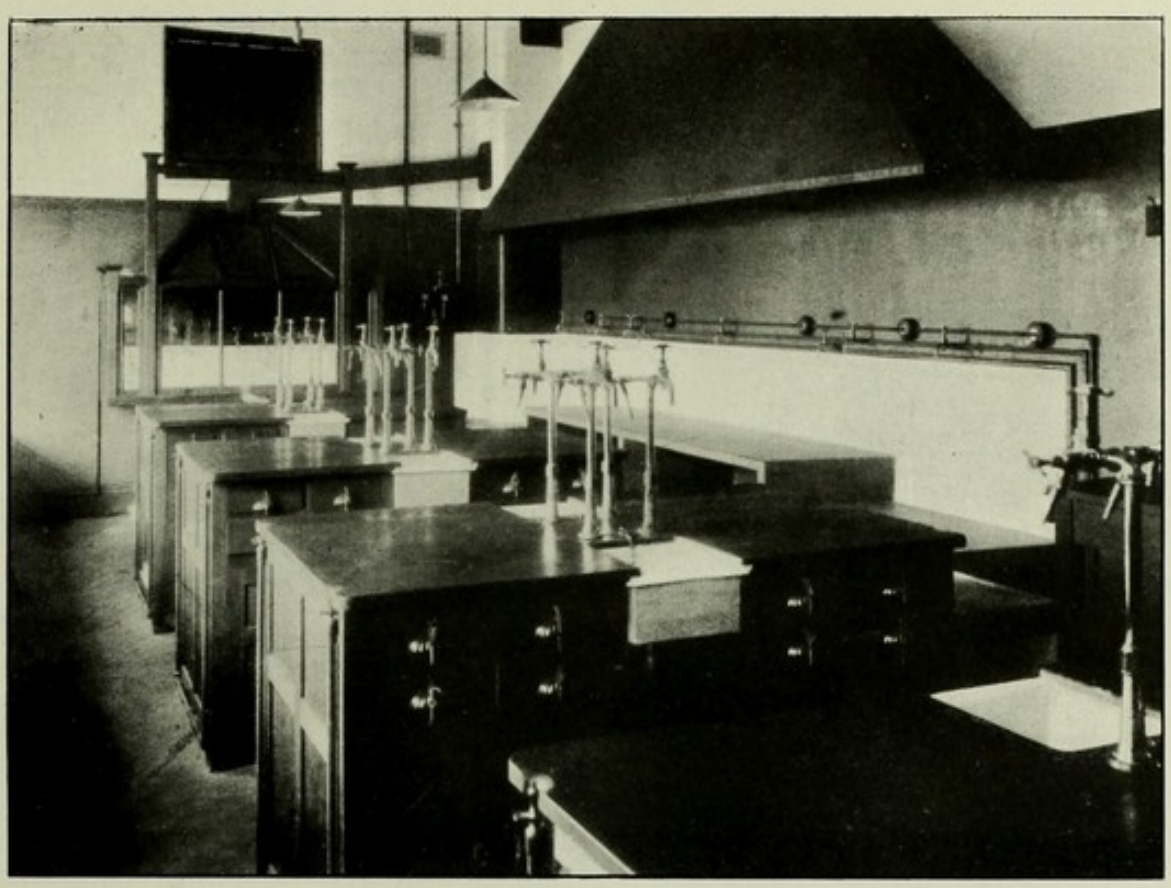
Installed in a recess in the boiler house is the retort for making gas from petroleum, a Mansfield generator being used. As the gas is produced it flows from the generator to the gasometer about thirty yards away ; this gasometer has a capacity of 1,000 cubic ft. and holds sufficient for about two days' supply. Gas is of course largely used in the



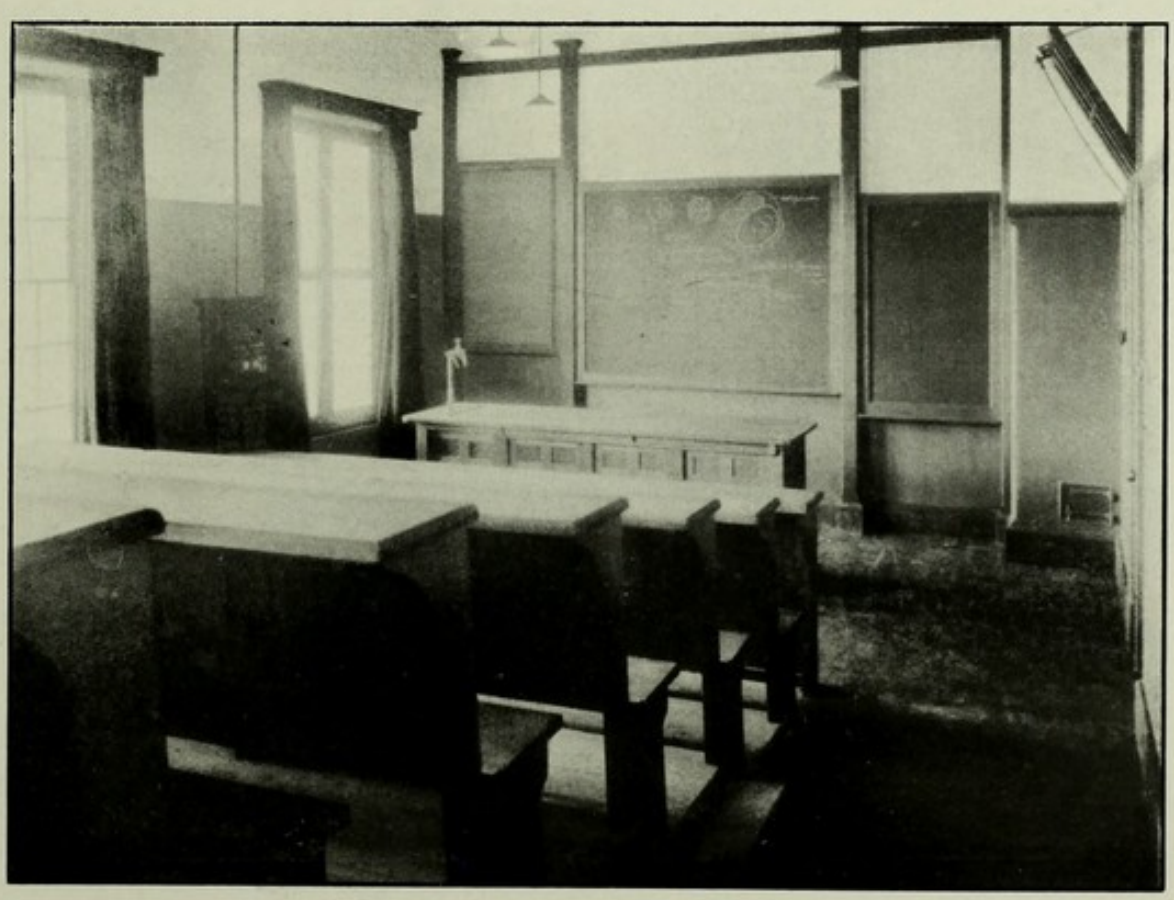
*The Rabies Laboratory.*



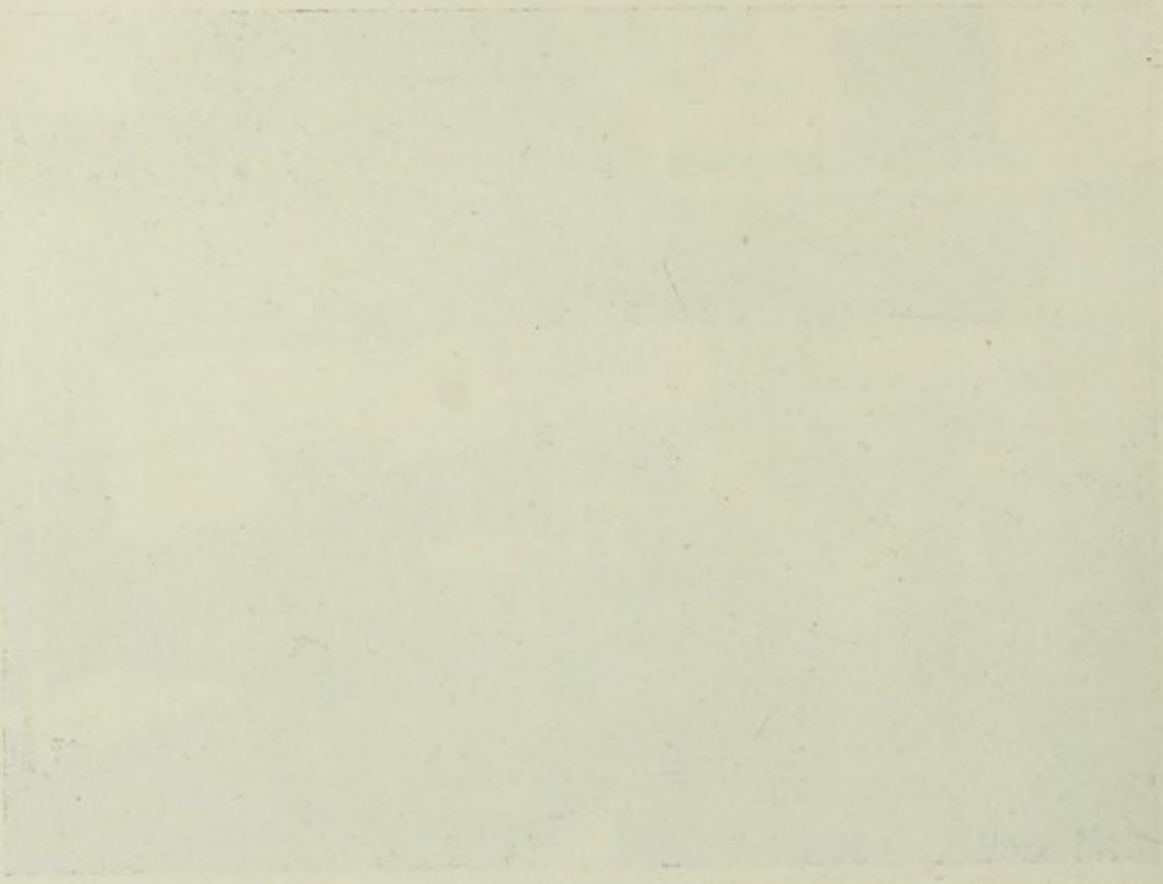
*The Pathological-Anatomical Museum.*



*The Students' Laboratory.*



*The Lecture Hall.*

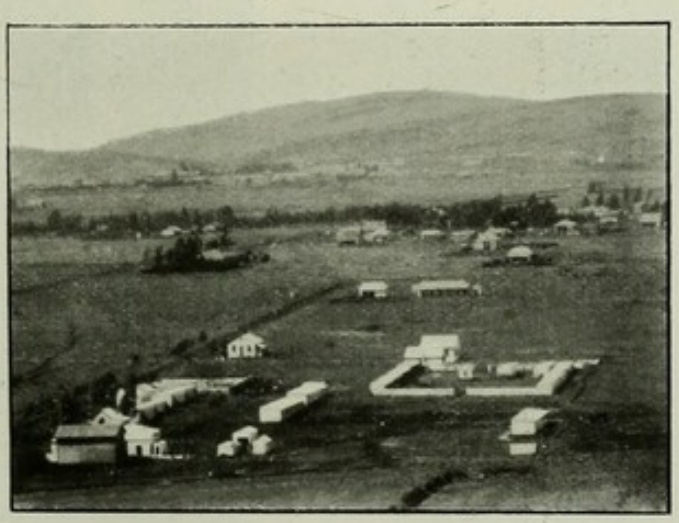


laboratories, and fittings for incandescent and ordinary gas lamps are provided through the building, so that it can be used also as an auxiliary to the electric light in the event of light being required at any time when the electric plant may not be running.

An ample supply of water for all purposes in the stables and laboratories is drawn from a borehole situated about 200 yards to the east of the main building. A Rees Roturbo high efficiency pump is installed in the pump house. This is driven by a 5 horse-power vertical motor direct coupled to the pump spindle, and is capable of delivering about 3,000 gallons per hour when running at a speed of 2,500 revolutions per minute. The water pumped from the borehole is delivered to a tank situated near the gasometer, this tank being raised to a height of 40 ft. above ground level in order to produce sufficient pressure for the water to reach the taps in the upstairs rooms of the laboratory. The tank has a capacity of 30,000 gallons which suffices for a two days' supply. A system of mains distributes the water to the various laboratories, stables, and the dwelling-houses where the staff reside.

The whole of the above-mentioned plant is under the charge of an electrician resident on the spot, an assistant being employed to run the engine for lighting purposes at night.

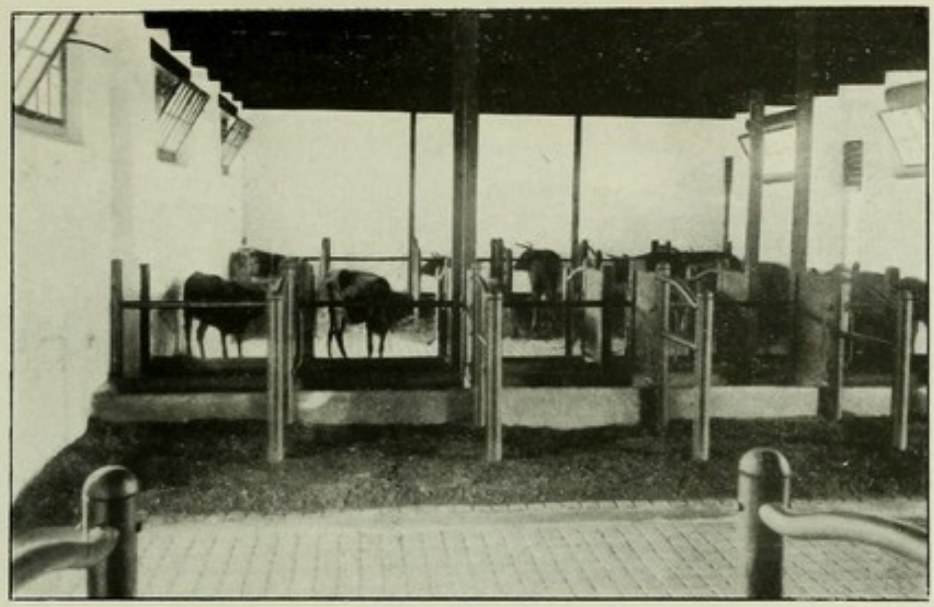
In every way most complete both in buildings and all accessories and fitted up with the best and most modern equipment, the new Laboratories have a wide field of work before them in research into the many and hitherto little studied tropical and sub-tropical diseases of live stock of South Africa.



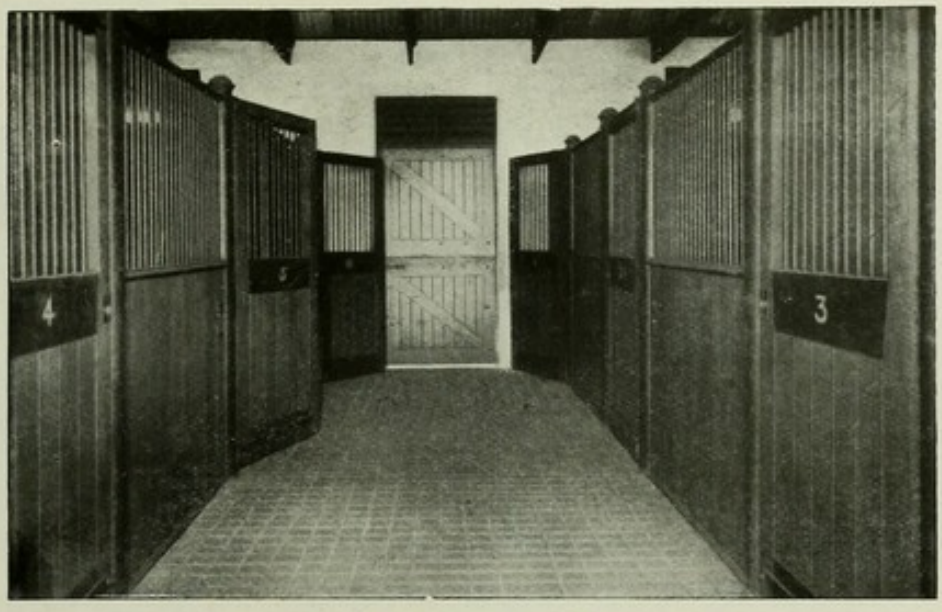
*The Old Laboratories at Daspoort.*

*The Back of the Laboratory from the Quadrangle Yard.*

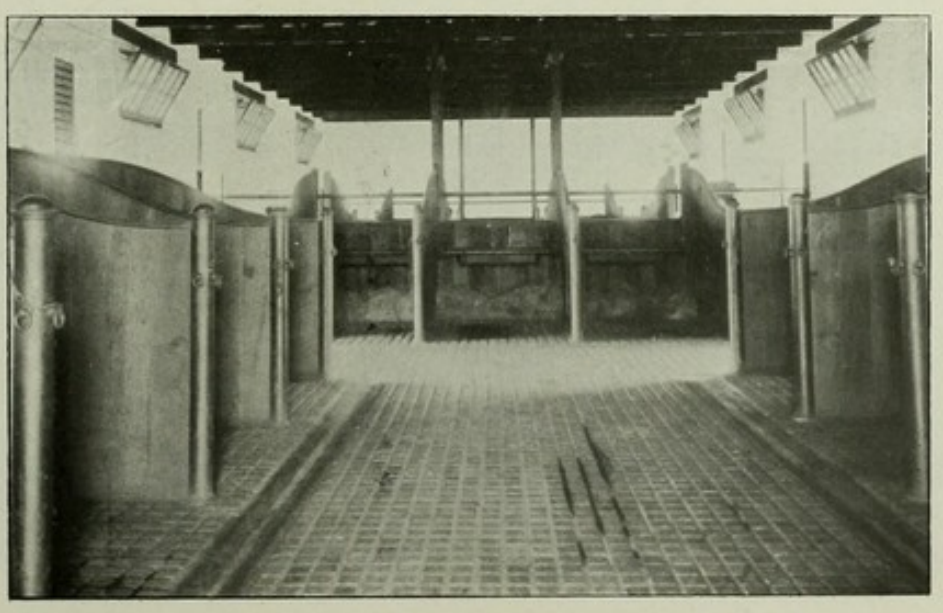
*The Quadrangle Yard.*



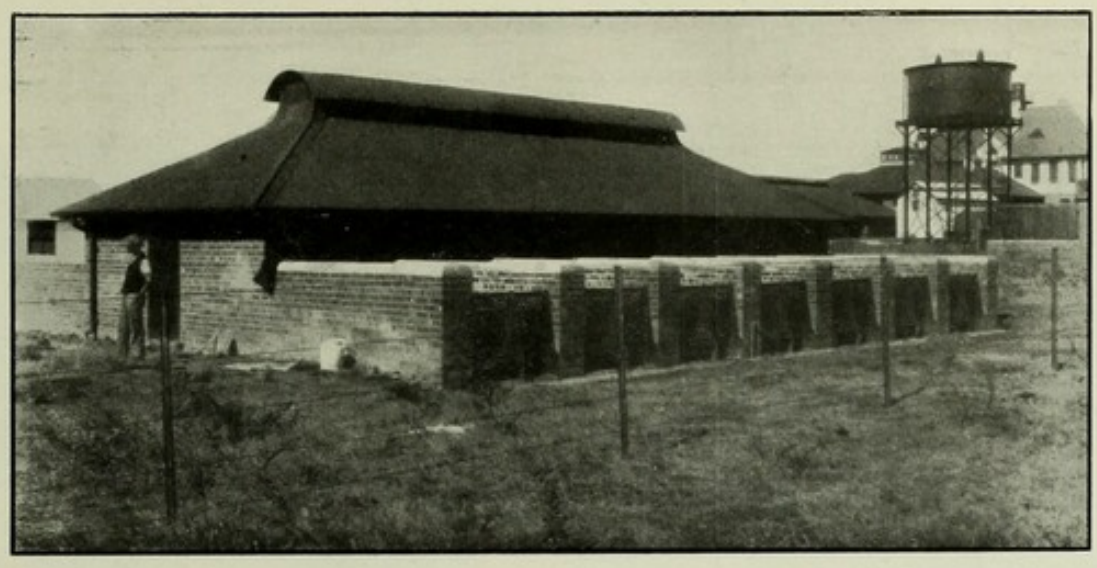
*A Cattle Byre.*



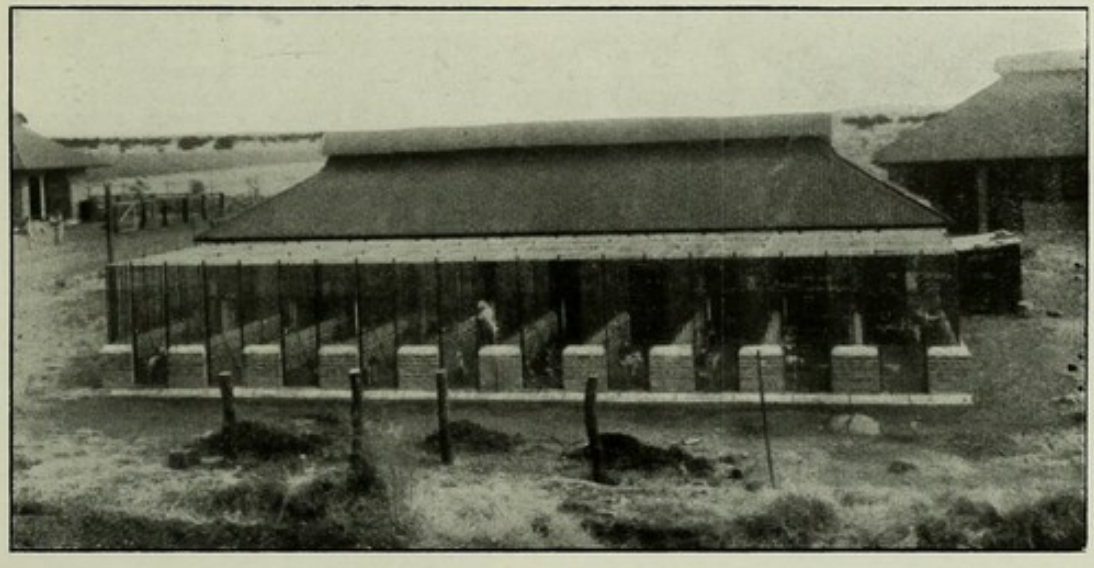

*Some Loose Boxes.*



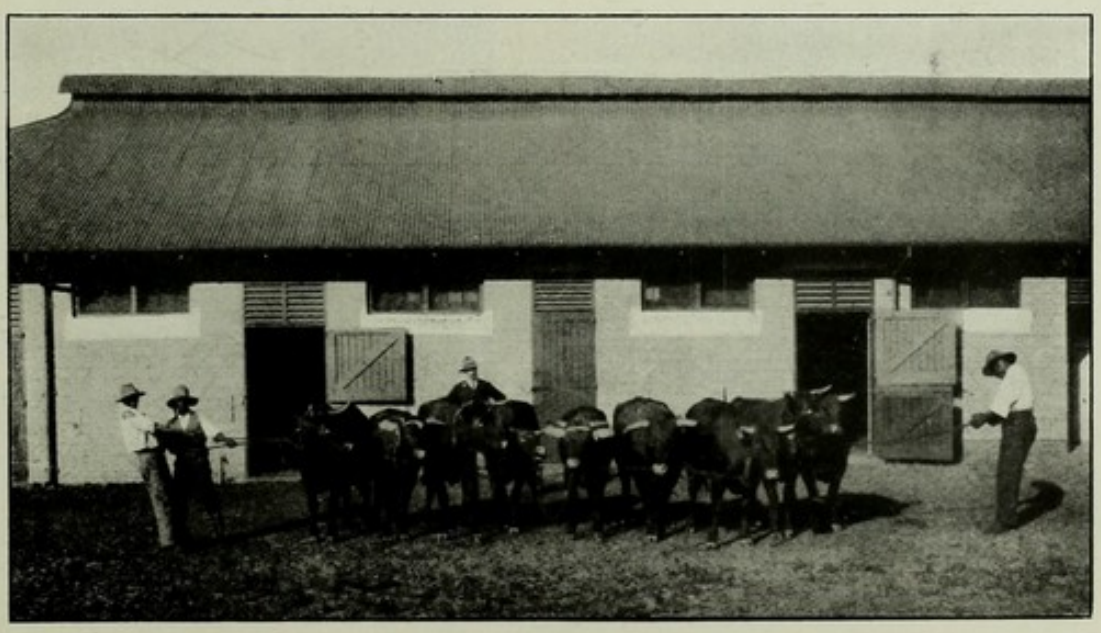
*One of the Stables.*



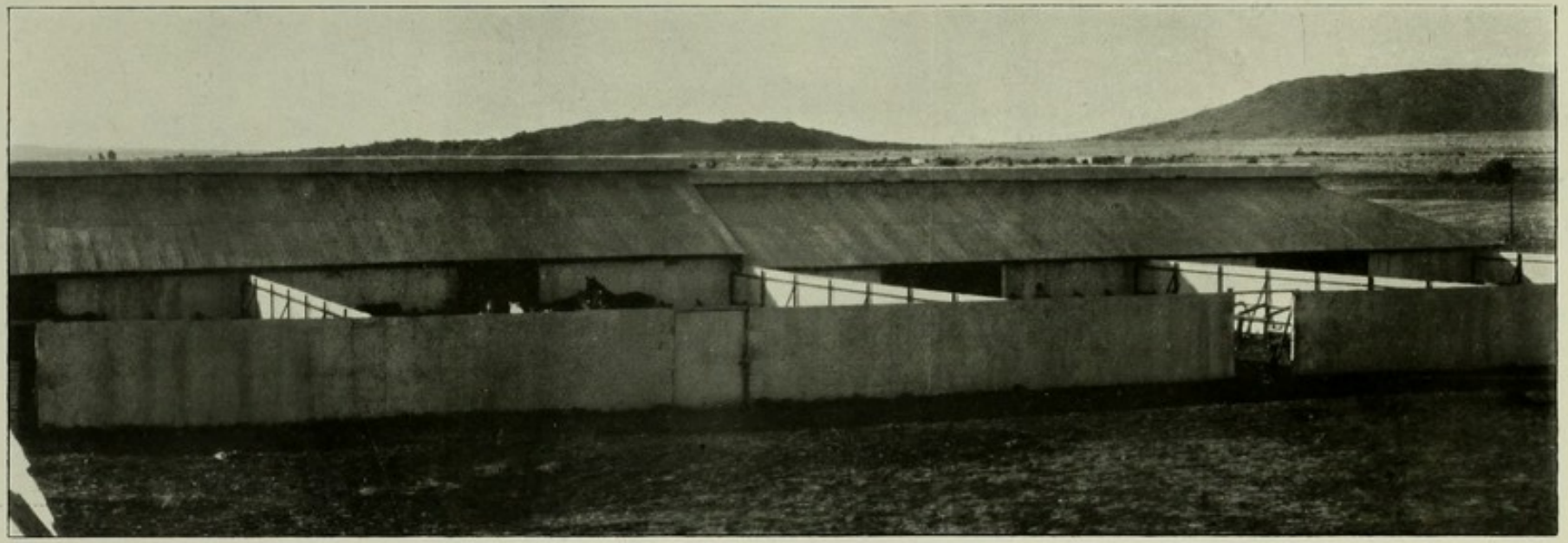
*The Piggeries.*



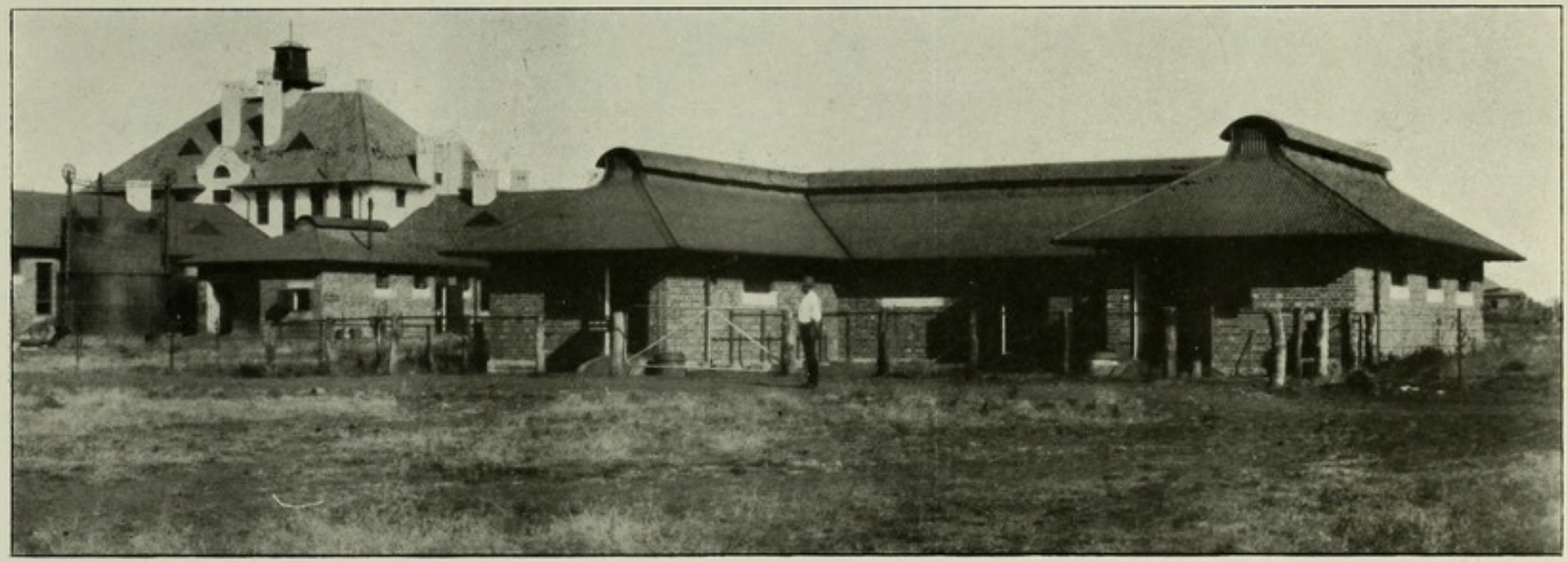
*The Dog Kennels.*



*In the Quadrangle Yard. Some Sussex Cattle imported for Experiment,*



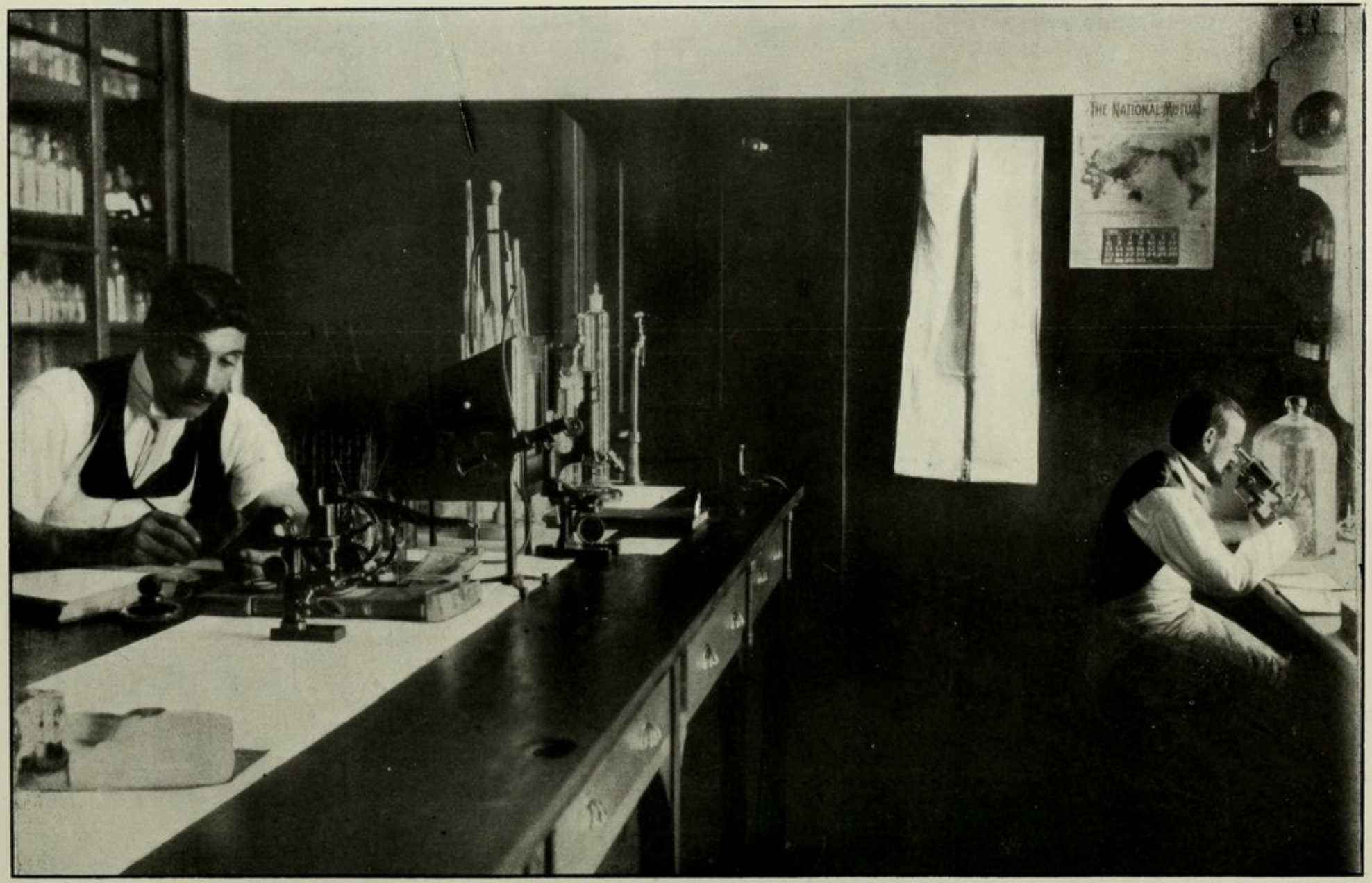
*The Horse and Cattle Kraals.*



*The Receiving Stable.*

# Immunity in Tropical and Sub-tropical Diseases.

By Dr. ARNOLD THEILER, C.M.G.




*The Laboratory of the Government Veterinary Bacteriologist.*

# Immunity in Tropical and Sub-tropical Diseases.

THE term "tropical" or "sub-tropical" disease is applied in this paper to such maladies of stock as are principally encountered in Africa, and more particularly in South Africa.

Those of any economic importance are, so far without exception, either due to protozoa (*Piroplasmoses*, *Trypanosomiases*, *Spirochaetoses theileria*) or to a specific group of ultravisible organisms (*Chlamydozoa*) which, in the same way as the protozoa, are dependent for their propagation on a host in the form of a tick or an insect. (Heartwater, horse-sickness, blue-tongue.)

In South Africa other infectious diseases also exist, such as anthrax, quarter-evil, glanders, strangles, etc., caused by specific bacteria, and not so many years ago epizooties such as pleuro-pneumonia, foot and mouth disease, and rinderpest have played considerable havoc amongst stock. These latter two are also due to ultravisible organisms, but are spread by direct contact with the sick animals and their morbid products. These and the mentioned diseases caused by bacteria do not require hosts as propagators, and in this fact lies the important difference between those designated as "tropical" or "sub-tropical." They are also encountered in colder regions and have been known for a considerable length of time, whereas the tropical ones are more or less new to science.

Ever since I have undertaken the study of the diseases of South Africa, I have laid particular stress on the question of immunity. A possible application of any immunity for the purpose of protecting the greatest number of animals exposed to infection must bear immediate results, and for this reason, wherever possible, it forms the best weapon under present conditions for successfully combatting these maladies.

It is my object in this paper to compile the facts known concerning the immunity in tropical diseases and to compare them with those known in other diseases, selecting for examples such as are known or have been observed in this sub-continent.

Immunity is spoken of as general or specific. In the first instance is usually meant the absolute insusceptibility of a certain animal or a class of animals to a disease. When we apply this to some of the tropical diseases, it would, for instance, express that cattle and sheep are immune against horse-sickness; cattle, sheep, and dogs are immune against the piroplasmosis of horses; sheep and dogs immune against the piroplasmosis of the ox; cattle immune against blue-tongue of sheep; horses immune against heartwater, etc.

The reverse of immunity is susceptibility, and in speaking generally about certain diseases having no influence on particular animals, we most frequently use the term non-susceptibility ; thus indicating that the word immunity has a more specific sense. It is more especially applied in such instances where animals or certain classes of animals which are usually susceptible to a certain disease have, by the process of recovering from that disease, acquired such new qualities as to render them insusceptible against the further attacks of the same disease.

For the purpose of this article, the term will be used in its restricted sense ; for instance, a horse which has recovered from horse-sickness or from piroplasmosis will be called immune against these diseases. For the present the question will be left open as to the nature of such immunity ; this will follow as a corollary from the deliberations.

## Complete Immunity.

An immunity is complete when the cause of the disease has no influence on the recovered animal under any conditions whatsoever ; that is to say whether the test is applied naturally in the form of an exposure, or artificially by introducing the specific germs, and irrespective of the quantity, manner of injection, and lapse of time succeeding the recovery. We know of one disease where this has been observed to be the case, viz., in rinderpest. In South Africa two epidemics of this zoonose have been observed—the first during the years 1896–97, and the other during 1901–03. Animals which recovered in the first outbreak were refractory to the second one, and it is admitted that in rinderpest immunity lasts for life.

After the fact was established that the serum of immune animals had protective properties, use was made of this fact and immune oxen were hyperimmunised to a great extent by injecting several litres of virulent blood. In no instance under my observation did such an injection lead to a breakdown of immunity. We may thus safely accept that immunity in rinderpest acquired by recovery cannot be broken, neither naturally nor artificially.

## Temporary Immunity.

The immunity of pleuro-pneumonia in cattle has been made use of in South Africa probably ever since that disease has been introduced. It has been noted that an ox which has recovered from the disease is immune. The expression pleuro-pneumonia indicates that the pathological lesions are found on the lungs and the pleura. Although the primary seat is in the lungs, yet the disease is by no means specific for this organ. It is rather specific for the lymphatic system, starting in the interstitial tissue of the lung and involving the lung parenchym proper secondarily. Therefore typical lesions can after artificial introduction of virus develop

in any region of the body; they correspond with those of the interstitium of the lungs. Recovery from these lesions are succeeded by immunity, that is to say, a subsequent inoculation within a reasonable short time no longer produces the typical lesions, neither does an animal so treated contract lung-sickness within that time. When at a later period, a year or more, the same animal is again injected, a reaction can take place, or when exposed to natural infection, it may contract pleuro-pneumonia.

We thus stand here before the fact that an acquired immunity diminishes in the course of time. This, of course, varies with the animal, and in some occurs sooner than in others. Immunity acquired by recovery from the disease naturally acquired lasts, according to Nocard and Leclainche,* three to four years.

### Immunity to Different Quantities of Virus.

Quarter-evil in cattle is due to the introduction of a specific bacterium into the subcutaneous tissue of young cattle. Recovery from the lesions thus produced is succeeded by immunity. This immunity can be brought about in various ways; one of them is the introduction of the virus in small quantities, and here the observation is made that the immunity stands in direct relation to the quantity of virus injected. Thus, if the dose of virus in increased, the immunity previously obtained does not protect, and breakdowns occur (Kitt). This fact is generally observed in diseases caused by bacteria; an immunity obtained through the introduction of the minimum lethal doses will protect against this dose, or perhaps a small multiple of it, but not so against a larger one.

### Immunity to Different Virulency.

Another contingency which occurs in experiments with bacteria is the different virulency of one and the same bacterium. Artificial immunity against anthrax, for instance, is obtained by a double injection of a first and second vaccine, differing in virulency. This differentiation in virulency can be so exalted that the immunity thus obtained can be broken by such a virus. This exaltation can be obtained by passing the virus through a series of susceptible animals of the same or of different species. It is noted and generally accepted that the more virulent the virus, the more solid becomes the resulting immunity.

### The Immune Animal as a Reservoir of Virus.

An important fact to be considered in connection with the immunity obtained, either naturally or by inoculation in the above-mentioned diseases, with the exception of pleuro-pneumonia, is the observation that these animals no longer propagate the disease. The principle holds also

* Nocard and Leclainche, "Les Maladies Microbiennes les Animaux," 1903.

for pleuro-pneumonia, inasmuch as a completely recovered animal is harmless; only such animals in which the sequesters have been found in the lungs carry the contagion with them as long as this sequester has access to a bronchus.

## Immune Serum.

The recovery from an infection is accompanied with the acquisition of certain qualities of the blood, more particularly of its serum; the most prominent one of which is the preventive effect such a serum exerts on the virus to which it corresponds, when injected previously, simultaneously, or subsequently to the virus or when mixed with the virus, either totally or partially prohibiting the effect of such virus. In addition to this, in certain cases direct influences are noted in vitro on the virus known as precipitation, agglutination and cytolysis (Bacteriolysis). These qualities become specially pronounced in hyperimmune animals, which process is carried out by subcutaneous or intrajugular injections of large amounts of virus. *In rinderpest* the injection of an appropriate dose of serum some hours previous to virus prevents the latter from developing; when injected simultaneously, a reaction ensues which, in the majority of cases, ends with recovery. The same can be noticed when the serum is injected not too long after the virus; the action of the virus is completely destroyed when it is mixed in vitro and then injected. *In pleuro-pneumonia*, according to Nocard, the inoculation of an immune serum (40 c.c.) protects against a subsequent inoculation of virus (1 c.c.). In a mixture of serum and virus in equal quantities injected subcutaneously, the virus does not develop. *In quarter-evil*, according to Kitt, an immune serum (40 c.c.) injected some days previously to virus protects against this virus. In a mixture of serum and virus injected subcutaneously, the virus becomes inert. The serum has agglutinating properties. *In anthrax* an immune serum protects against a subsequent virus injection. A simultaneous inoculation of serum and virus produces active immunity (Sobernheim).

The résumé concerning immunity in non-tropical diseases may be classified as follows:—

(1) Immunity may be complete, both regarding quantity of virus and length of time it lasts.
(2) Immunity lasts only for a limited time.
(3) Immunity varies with the quantity of injected virus.
(4) Immunity varies with the virulency of the virus used.
(5) Immunity renders an animal unfit for the propagation of the contagion.
(6) Immunity and the process of hyperimmunisation gives the serum preventive qualities.

# I.—DISEASES DUE TO ULTRAVISIBLE VIRA.

## *A.*—Horse-sickness, Blue-tongue, Heartwater.

Horse-sickness is due to a filtrable ultravisible organism, which in vitro preserves its virulency for several years. Blood of a sick animal acts as a virus. A horse or a mule which has recovered from an attack of this disease is known to have acquired immunity (salted); it is generally understood that immune animals do suffer from relapses (aanmanings), from which as a rule they recover.

The following notes will demonstrate the nature of the immunity acquired, and the cause of the relapses. It will be advisable to record the observation in chronological order, as this will best explain the development of our knowledge concerning the immunity in this disease.

Immunity in Mules.*—The method of immunising mules consists in the simultaneous injection of virus (2 c.c.) and serum (average dose, 300 c.c.). The serum is obtained from horses or mules which have recovered and which have been hyperimmunised. This process consists in the intrajugular transfusion of blood from the sick into the immune animal, averaging in amounts to 10 litres, transfused in two to four operations in intervals of 6–18–24 hours. About three to four weeks after infusion, the serum of the infused animal is fit for use. The virus with which the experiments were started was collected from a horse in Pretoria, which had contracted the disease spontaneously. As nearly all our initial experiments were carried out with this virus, and later it became necessary to distinguish it from vira collected from other animals, it was called the "Ordinary Virus." Once the fact had become established that through the simultaneous injection of virus and a corresponding dose of serum immunity could be obtained, it had to be decided whether this immunity was complete, both concerning quantity of virus and time it lasts. The former could naturally easily be settled, whereas the latter has not yet found a definite solution.

The best proof for immunity was the process of hyperimmunisation. The statistics at our disposal show that up to January, 1907,† the total number of 295 mules were tested with the Ordinary Virus. Amongst this number were 41 mules which were hyperimmunised twice, 13 mules three times, 4 mules four times, and 2 mules five times. It must be remembered here that the infusion for one hyperimmunisation averages 8 to 10 litres. The observation made was, that in *no* instance did the infused animal show any reaction typical for horse-sickness; indeed usually not even a rise of temperature due to the huge infusion of sick febrile blood was noticed. The animals used for hyperimmunisation had

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* Annual Report, Gov. Vet. Bac., Transvaal, 1903–04.

† Annual Report, Gov. Vet. Bac., Transvaal, 1907–08.

passed through immunisation within eight months previous to the infusion; those hyperimmunised a second or third time were done so after an interval of three months to a year.* The longest period between immunisation and hyperimmunisation was two years without breakdowns. The longest period between immunisation and test with the same virus was six years.

CONCLUSION.—*The immunity obtained in mules by the simultaneous injection of serum and Ordinary Virus is complete concerning quantity of Ordinary Virus with which it is tested; concerning time, it is so at least for six years.*

IMMUNITY IN HORSES.—No method of immunising horses has as yet been introduced into practice. The horses referred to here have recovered in experiments carried out for the purpose of finding such a method. With a few exceptions the immunity obtained was due to the injection of serum and virus in various combinations, but principally by simultaneous injection. As this article deals with the immunity, the details of immunisation does not enter within its scope. All the recovered horses were used for hyperimmunisation in a similar way as indicated in connection with the mules. Thus were hyperimmunised or otherwise tested with Ordinary Virus 104 horses, amongst which were 10 hyperimmunised twice. The amount infused for hyperimmunisation averages 8 to 10 litres. Five horses were hyperimmunised three times. The intervals between hyperimmunisation was usually short, averaging two to three months. The longest interval between two hyperimmunisations was two years. The observation was, that out of this number no horses showed a typical reaction due to this test of hyperimmunisation.

NOTE.—There was one horse which reacted with symptoms of dikkop. At that time this occurrence was not understood. Soon after the test injection, the horse was grazed at Onderstepoort during the horse-sickness season, and a new infection must have been contracted naturally, a fact which will be explained later.

CONCLUSION.—*The immunity of horses obtained through the recovery from an injection of Ordinary Virus is complete concerning quantity of Ordinary Virus. Concerning time it is for at least two years.*

The virus used for hyperimmunisation and test did not correspond in generation to that used for immunisation. The vira used principally for immunisation were the fifth and the thirty-seventh generations, counting every subsequent animal through which the ordinary strain had passed as a generation. These animals were indiscriminately taken amongst horses and mules, and did not follow each other with any regularity. Only after the sixty-fifth generation the virus was continued through horses exclusively. The greatest interval between the generation

* Annual Report, G.V.B., Transvaal, 1906–07—Immunisation of Mules with Ordinary Virus.

used for immunisation and hyperimmunisation in mules was forty-three generations ; in horses, seventy generations.

It is usually observed in other diseases that the passage of virus from animal to animal of the same species increases the virulency, and it may be expected that such virulency can break the immunity obtained by a lower generation. However, the virus of the Ordinary strain of a high generation, and even the hyperimmunisation with such had no effect on the animal immunised with a lower generation.

Conclusion.—*The immunity of mules and horses obtained by the recovery from the injection of Ordinary Virus protected completely against the infusion of large quantities of virus of a higher generation.*

(The reservation should be made here, that in the passage of virus through the different generations, mules and horses were used, and it is possible that this may account for the stability of the virus.)

Experiences in Practice.—*A.*—Tzaneen Virus.—Once it had been settled that the immunity in mules could not be broken by huge quantities of virus, it was expected that such an immunity would stand natural exposure, and accordingly the method was introduced into practice.

During the horse-sickness season, 1905–06,* a total number of 3,235 mules had been inoculated, and were exposed to natural infection. At the end of the season, of this number 21 were reported to have died of horse-sickness, and 45 had shown symptoms of relapses (aanmanings). There was but little doubt that death was due to horse-sickness. Post-mortem reports in several instances were obtained from the District Veterinary Surgeon. At the same time some horses which had recovered from experiments, and had also been hyperimmunised, were exposed to be tested. One of these died in Tzaneen, the Government estate in the Letaba Low Veld, a notorious country for horse-sickness. Blood from the dead animal was obtained, and named " Tzaneen Virus." It was then used in the following experiments. A number of mules, totalling 139, all immune against Ordinary Virus, were tested with Tzaneen Virus, with the result that 12 showed typical horse-sickness fever reactions, 4 with lesions of dikkop, and 1 died of horse-sickness. A number of horses, totalling 17, all immune against Ordinary Virus were tested with Tzaneen Virus, with the result that 5 showed reactions and recovered, 3 had reactions with symptoms of dikkop, 1 died from the pulmonary and 1 from the dikkop form of horse-sickness. There were only 7 animals out of the 17 which did not react. Some of the tests were made within a few months after recovery from Ordinary Virus, and on animals (horses) which had been previously hyperimmunised once, twice, and three times with Ordinary Virus.

* Annual Report, G.V.B., Transvaal, 1906–07.

Conclusion.—*The immunity obtained by the Ordinary Virus did not protect all the mules and horses against the test with Tzaneen Virus, even when this test was carried out soon after immunisation, and even in horses which had been hyperimmunised.*

*B.*—Bulawayo Virus and Ordinary Immunity.

This virus was obtained from a mule which had been immunised in Bulawayo during the season 1905–06 with Ordinary Virus. When exposed, it showed a relapse, from which it recovered.

A number of mules, totalling 36, all immunised with Ordinary Virus, were tested with this Bulawayo Virus, with the result that 7 mules showed fever reactions typical for horse-sickness, 6 animals showed the symptoms of dikkop and recovered, 1 mule had a doubtful reaction, and 6 died of horse-sickness. Of 9 horses immune to Ordinary Virus, which were tested with the Bulawayo Virus, 3 showed reactions with dikkop, and 4 died of horse-sickness. There were 2 animals out of the 9 which did not react. Some of these tests were made within a few weeks after immunisation.

Conclusion.—*The immunity obtained by the Ordinary Virus did not protect all the mules and horses against the test with Bulawayo Virus, even when this test was carried out soon after immunisation.*

*C.*—Immunity from Tzaneen Virus and Tests with Ordinary Virus.

Shortly after it had been noticed that Tzaneen Virus could break the immunity of the Ordinary Virus, it was concluded that the former would be stronger or, better, more virulent, and therefore probably better suitable for the practice. It was at the same time ascertained that the serum of animals hyperimmunised with the Ordinary Virus could advantageously be used with the Tzaneen Virus, the mortality from inoculation averaging about the same percentage.

In the first half of the season 1906–07, the Tzaneen Virus was introduced, but in the latter half withdrawn, as it was found to have changed spontaneously in virulency. Subsequent to this, for the purpose of ascertaining the immunity obtained from the Tzaneen Virus, tests were made.

(1) *Tests with Ordinary Virus.*—A number of mules, immune against Tzaneen Virus, totalling 59, were tested with Ordinary Virus a comparatively short time after immunisation. The result was that 47 animals showed fever reactions more or less typical for horse-sickness, 2 animals showed the symptoms of dikkop and recovered, and 5 animals died of horse-sickness. There were tested 19 horses immune to Tzaneen Virus, with Ordinary Virus, of which 7 showed fever reactions typical for horse-sickness, 5 had reactions with symptoms of dikkop, 2 died of horse-sickness, and 5 had no reactions.

Conclusion.—*The immunity obtained by the Tzaneen Virus did not protect all the mules and horses against the Ordinary Virus.*

(2) *Tests with Bulawayo Virus.*—There were tested 13 mules immune to Tzaneen Virus, with Bulawayo Virus, also a comparatively short time after immunisation, with the result that 8 showed horse-sickness reactions and 1 died of horse-sickness. Of 4 horses immune to Tzaneen Virus and tested with Bulawayo Virus, 2 showed horse-sickness reactions, and 1 a reaction with the symptoms of dikkop.

Conclusion.—*The immunity obtained by the Tzaneen Virus did not protect all the mules and horses against the Bulawayo Virus.*

*D.*—Immunity from Bulawayo Virus and Tests with Ordinary Virus and Tzaneen Virus.

(*a*) Three mules immune against Bulawayo Virus and tested with Ordinary Virus all showed reactions; one immune horse reacted to the virus.

(*b*) Of 5 mules immune against Bulawayo Virus and tested with Tzaneen Virus, 3 gave reactions. Of 2 horses, 1 died from the test.

Conclusion.—*The immunity obtained from the Bulawayo Virus did not protect all the mules and horses against a subsequent test of either Ordinary or Tzaneen Virus.*

*E.*—Tests of Mules immune to Tzaneen Virus with Tzaneen Virus of a Higher Generation.

The experience of a rather large number of animals had shown that the immunity obtained by the Ordinary Virus could not be broken by the same virus, by whatever quantity of virus and whatever generation, and after even a lapse of two years. This proved not to be the case with the Tzaneen Virus; it is particularly typically pronounced in horses. Of 81 horses which were immunised with the Tzaneen Virus of a lower generation, and within a comparatively short time tested with the same strain of a higher generation, 16 showed reaction typical for horse-sickness, 6 showed reactions with dikkop, 9 showed reactions and died of pulmonary form, and 3 showed reactions and died of dikkop. A similar observation was made with horses injected twice with the Tzaneen strain of two different generations, first of a lower one and later with a higher one. When tested with the virus still of a higher generation, breakdowns of immunity were noted. Of 40 horses thus treated, 4 showed simple horse-sickness reactions, 3 showed reactions of dikkop, 9 died of pulmonary form, and 3 died of dikkop. The same observation was repeated to a smaller extent even in horses which had received three subsequent injections of Tzaneen Virus of different succeeding generations, as the following statistic shows: Of 15 horses thus treated, 3 showed simple reactions, and 2 died of pulmonary horse-sickness.

Conclusion.—*The immunity obtained from Tzaneen Virus of a lower generation did not completely protect against the test of a higher generation; even the immunity obtained from a Virus two or three generations below that of the test was not complete.*

Immune Animals do not Act as Reservoirs.

It has experimentally been proved that the blood of an animal suffering from horse-sickness is virulent during the febrile reaction and only for a limited time afterwards. This applies of course only to tests made by inoculation with blood from recovered animals, and these have in all instances proved to be negative if carried out almost at any time after recovery up to a number of years, but the reservation must be made here that under natural conditions, that is to say, through the host, a transmission might occur from immune to susceptible animals, a contingency which so far has not any support by analogy with another disease. We are therefore entitled to conclude for the present that the immune animal does not act as a reservoir.

Serum of Recovered Animals.—In speaking of serum and virus in horse-sickness, one has in the first instance to consider whether they are adequate to each other, in other words, whether the serum used corresponds to the same strain of virus with which the animal is injected.

The serum of recovered animals has but little preventive value, but acquires this when injected or infused in short or longer intervals as already described.

The observations refer to a virus which in the dose of 1 c.c. subcutaneously injected is invariably fatal, and may be summarised as as follows :—*

(1) Adequate serum and virus mixture of equal quantities and injected in multiples of the minimum dose, *subcutaneously or intrajugularly*, is, as a rule, harmless, but no immunity results. (2) Inadequate serum and virus mixtures of equal quantities, mixed and injected in multiples of the minimum dose are usually harmless. (3) The inoculation of adequate serum previous to the subcutaneous injection of virus, generally speaking, prevents the development of the disease; the same is sometimes observed when virus is injected intrajugularly; no immunity results. It is also sometimes noticed with inadequate serum and virus. (4) The simultaneous inoculation of adequate serum and virus does not prevent development of the disease in mules, and when the doses of serum are properly adjusted, 98 per cent. of animals recover. Under similar conditions recoveries in horses are less (about 60 per cent.); when the dose of serum is increased all development may be stopped. This refers also to some inadequate sera. The recovered animals are immune. (5) Adequate virus injected previously to serum

* Annual Report, G.V.B., Transvaal, 1904–05.

is generally not influenced by the latter ; the longer the interval between the two, the surer a reaction will result. This applies also to inadequate virus.

RÉSUMÉ.—Our present knowledge concerning immunity in horse-sickness can be résuméd as follows :—

(1) Recovery from an attack of horse-sickness causes immunity ; this immunity is complete to a virus adequate to the one which caused the disease (Ordinary strain).

(2) Immunity may vary according to the virulency of a strain which can be influenced by passage from animal to animal. (This applies to the Tzaneen strain.)

(3) Immunity obtained by one strain of virus does not protect all the animals against a different strain of virus.

(4) Serum of immune animals, hyperimmunised by either strain, has protective qualities against virus of the same and of different strains.

(5) Recovered animals do not retain the infection in their blood.

## BLUE-TONGUE IN SHEEP.

This disease is due to a filtrable ultravisible micro-organism, which in vitro preserves its virulency for several years. Blood of a sick animal acts as virus. A sheep which has recovered from an attack of blue-tongue is considered immune ; there are no definite data at our disposal to show how long the natural acquired immunity lasts, but it is generally admitted that " salted " sheep contract the disease in a lesser degree.

IMMUNISATION.—There exists two methods of immunisation, viz., the first introduced by Spruell,* consisting of an injection of adequate serum and virus in appropriate quantities either simultaneously or mixed at the time of injection ; the second one is the vaccination introduced by the writer of an attenuated virus obtained by passing it through a number of generations.

Sheep which recover as a result of either inoculation are immune.

The following data refer principally to my own observations, where not otherwise noted :—Mortality from vaccination is almost nil. The sheep show visible symptoms of blue-tongue in a slight degree ; the intensity varies in various flocks and under adverse conditions (rain, for instance).

CHARACTER OF IMMUNITY.—The following note explains the nature of the immunity :—

A sheep which recovered from a 20 c.c. injection of virus reacted to a subsequent injection of 30 c.c. virulent blood injected 33 days later

* " Cape Colony Agricultural Journal,"

of the same strain, and again an injection of 100 c.c. another 33 days later caused a typical reaction. A lamb which had contracted blue-tongue and recovered from an injection of virulent blood showed typical lesions of blue-tongue when injected 71 days later with 60 c.c. virulent blood of the same strain. A number of sheep injected with increasing doses of virulent blood for the purpose of hyperimmunisation generally show fever reactions after the first and second injection. In later injections these reactions are no longer noticed. The fact is noteworthy that when the hyperimmunisation is carried out soon after immunisation no deaths result from this increasing amount of blood. The reaction becomes the more pronounced the later the injection of virus is carried out after immunisation.

Of 40 sheep tested with 20 c.c. virus four and seven months after vaccination, severe fever reactions were noticed in 15 sheep and slights reactions in 14 sheep; in some of these typical symptoms of the disease were noticed, and 1 actually died as a result of it.

Conclusion.—*Immunity against blue-tongue conferred by virulent or attenuated virus is not complete. It may be broken with a larger quantity of the same strain of virus, and this breakdown is more certain to occur the longer the period which elapses between immunisation and test.*

*The breakdowns of immunity in the majority of cases do not end with death.*

Experience in Practice.—The vaccination of sheep was introduced into practice in 1907, and during the time when blue-tongue was rampant.

The mortality within the first fourteen days after inoculation may principally be accounted for by natural infection. In the 5,875 sheep, of which records were kept, the disease stopped after this period; the mortality amounted to 7 animals, equal to 0·4 per cent. Of 16,218 susceptible non-treated sheep running under the same conditions as above, 1,817 died of blue-tongue, or 11 per cent.

Conclusion.—*The vaccination of sheep during an epidemic of blue-tongue stops the disease after fourteen days, and the sheep remain immune for the rest of the season.*

The post-mortem examinations of seven sheep mentioned in the foregoing notes out of the vaccinated number could not be controlled; thus some doubt as to correct diagnosis does exist. However, in the same season amongst the sheep belonging to the laboratory exposed on the notorious farm Onderstepoort, some breakdowns were noted, of which the particulars are as follows:—

One sheep contracted spontaneous blue-tongue ten months, a second one eight months, a third one fourteen months, and a fourth one fifteen months after immunisation.

In the year 1907–08 about 200,000 sheep were vaccinated, the season was a good one; the only breakdown which occurred was in the Orange River Colony affecting a ewe.

In the year 1908–09 about 85,000 were inoculated, and about 80 breakdowns were noted in three different farms of three different districts (Lydenburg, Ermelo, Piet Retief).

Conclusion.—*A certain number of breakdowns under natural conditions are observed, even soon after vaccination; these breakdowns vary in their extent according to certain localities.*

The Blood of Immune Animals.—A number of sheep immunised six to eighteen months ago were tapped, and the blood was injected in the doses of 5 c.c. into susceptible sheep. In no instance were the lesions of blue-tongue noted.

When immediately afterwards tested on their immunity, with the minimum doses of virus, these injected sheep proved to be susceptible to blue-tongue.

Conclusion.—*Immune animals do not retain the infection in the blood.*

The same reservation as in horse-sickness has to be made here.

Serum of Immune Animals.—Serum of hyperimmunised sheep was tested on its preventive value, with the following results:—

(1) Simultaneous subcutaneous injections of serum and virus, with the excess of the former, prevented all development of disease; intrajugularly injection was followed by reaction and death.

(2) Serum injected twenty-four hours previous to virus prevented the development of the disease.

Conclusion.—*The serum has preventive qualities.*

Résumé.

(1) Immunity in blue-tongue of sheep is never complete; it can be broken either by virus of the same strain if applied in larger doses or by a virus of a different strain.

(2) The serum has preventive qualities, and immune animals do not act as reservoir for virus.

## Heartwater.

Heartwater is a specific disease of cattle, sheep, and goats, due to a filtrable micro-organism present in the blood, which preserves its virulency for not longer than forty-eight hours. Fresh blood of sick animals must, therefore, be used as virus. Any animal belonging to the genera mentioned above acquires immunity through the recovery from the disease.

There does not yet exist any practical method of immunisation, and the observations made on the immunity refer to animals recovered from a disease transmitted to them by injection of virulent blood.

Character of Immunity.—Oxen, sheep, and goats which have recovered from an attack of heartwater can be injected with large quantities of virulent blood adequate to the virus which produced immunity.

Five cattle were hyperimmunised to the extent of 1,000 to 3,000 c.c. virulent blood in one operation, and eight sheep from 50 c.c. to 400 c.c. in one injection were hyperimmunised in this way.

This hyperimmunisation was carried out in various intervals. No breakdowns occurred. The longest interval between two hyperimmunisations was in cattle, seventeen months, and in sheep, eighteen months.

Conclusion.—*Immunity against heartwater when tests were carried out with the adequate virus was complete concerning the quantity of virus, and, concerning time, at least for eighteen months.*

Experience with Inadequate Virus.—Eight sheep, all immune and hyperimmunised to a particular strain of virus called the Sjamboks Kraal Virus, which were injected with virus of a different strain obtained from Komatipoort, showed the typical heartwater reaction, from which 1 died. Spreull* injected 3 sheep and 2 goats previously immunised by virulent blood injection and then exposed to natural infection with 5 c.c. blood of a sick animal into the jugular vein; 1 sheep sickened and recovered, 1 goat sickened and recovered, and 1 died.

To judge from Spreull's statement, the virus must have been inadequate to the one with which immunity was produced. The experiment of the same man proved that immunity obtained by the inoculation of a virulent blood did not protect against natural infection (tick infection). These facts are probably also due to a virus of a different strain.

Conclusion.—*The immunity obtained from one particular strain of virus can be broken by an inadequate strain. (Strain of a different locality.)*

Experience in Practice.—This is only limited and refers specially to a lot of cattle which were the recoveries from the experiments and had been hyperimmunised to various extents; they were exposed as controls in connection with an experiment of a different nature two years after immunisation.

There were altogether exposed eight animals immune against heartwater. In no case could with certainty a breakdown due to the tick infection be noticed, although the control animals contracted this disease. Dixon,† in his experiments with goats which had been immunised by

* "Cape Agricultural Journal," Vol. XXIV, No. 4, 1904.

† "Cape Agricultural Journal," Vol. XV, No. 12, 1899.

subcutaneous inoculation of virulent blood, found that they were not protected when exposed to natural infection. His results after five months' exposure were : Death from heartwater in 30 per cent. of Boer goats ; 50 per cent. of Cape sheep ; 40 per cent of Angora goats ; 40 per cent. of merino sheep, and lambs 25 per cent.

CONCLUSION.—*Immunity acquired by inoculation and increased by hyperimmunisation protected eight cattle against natural infection of heartwater. The immunity conferred on sheep and goats by inoculation of virulent blood broke to a great percentage.*

BLOOD OF IMMUNE ANIMALS.—The blood of an ox which had recovered from an attack of heartwater was injected into two susceptible sheep without any result.* The same results were obtained by Lounsbury†, who injected blood of recovered goats into susceptible goats. Further, his experiments with ticks reared on goats which recovered from heartwater demonstrated that these were unable to transmit the disease.

CONCLUSION.—*Animals which have recovered from an attack of heartwater do not retain the infection in the blood.*

PREVENTIVE VALUE OF SERUM.—The serum of an immune animal which has been hyperimmunised has acquired preventive qualities which may be summarised as follows :—(1) Mixture of serum and virus, with the excess of the former, injected subcutaneously, did not cause the disease. (2) Serum injected subcutaneously in excess and virus into the jugular vein or subcutaneously prevented a reaction. (3) Serum injected twenty-four hours previously to the virus prevented a reaction. (4) Serum injected twenty-four hours after virus did not prevent development of reaction.

CONCLUSION.—*The serum of hyperimmunised animals have preventive qualities.*

RÉSUMÉ.

(1) Immunity in heartwater is not complete ; it can be broken by virus of a different strain.

(2) Immune cattle seem to have a better protection than immune goats of sheep against virus of different localities.

(3) Serum of hyperimmunised animals has protective properties.

(4) Immune animals do not act as reservoirs.

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## II.—PIROPLASMOSES.

Redwater of cattle (bovine piroplasmosis). Biliary fever of equines (equine piroplasmosis). Biliary fever of dogs (canine piroplasmosis). Infection due to *Piroplasma mutans*.

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* "Cape Agricultural Journal," Vol. XXI, No. 4.

† Annual Report, Government Entomologist, 1901.

## Redwater or Texas Fever.

This disease is due to the introduction of *Piroplasma bigeminum* into a susceptible beast naturally by the blue ticks, artificially by inoculation of blood containing this parasite. The blood kept in vitro retains its infectivity only for a limited number of days (fourteen days). Animals which have recovered from an attack of this disease are known to be immune. An immune Africander ox injected with various amounts of blood from cattle actually suffering from redwater, in intervals and varying in quantity to the total extent of 8 litres, showed no breakdowns of immunity.

In connection with the immunisation of oxen against rinderpest, country-bred oxen were injected with large doses of blood, which undoubtedly contained *Piroplasma bigeminum*—no accurate records were kept about the reaction, but judging from results, no breakdowns occurred.

English Cattle.—Of 10 English heifers, which were all immunised against redwater and had shown *Piroplasma bigeminum* in the blood, 6 showed reactions and rare *Piroplasma bigeminum* when injected with blood containing this parasite. Of 6 English heifers recovered from an attack of redwater brought on by injection of blood, when exposed to natural infection, 2 died. Smith and Kilborne* also, in their experiments came to the conclusion that one attack of Texas fever does not completely protect against a succeeding exposure to new infection.

Conclusion.—*The immunity conferred by an injection of virulent blood can be broken by inoculation, which is, as a rule, not accompanied by severe symptoms or death. Immunity conferred by injection of virulent blood does not protect completely against natural infection. The immunity thus conveyed varies in the various breeds of animals; it is better and almost complete in South African born and bred cattle; it is not so good in imported cattle.*

The Blood of Immune Animals.—Animals recovered from redwater retain the infection in the blood and such blood remains infective when injected into susceptible animals. The progeny of the blue ticks feeding on such immune animals likewise transmit the disease. The blood of an immune animal exposed to tick infection remains so during the whole life of such an animal and probably so in an animal not exposed to tick infection. There is an observation recorded from America,† where the blood of such an immune animal proved to be infective after twelve years. It has been observed that the injection of immune blood causes in the majority of animals only a slight reaction during which *Piroplasma bigeminum* is found in the blood, and this fact has been made use of as a preventive inoculation. The immunity obtained in this way protects the greatest number of animals against heavy infection and deaths from redwater when exposed to redwater veld. (*Vide* foregoing conclusion.)

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* U.S.A. Bureau Animal Industry, 8th and 9th Annual Reports.

† Schroeder and Cotton, 22nd Annual Report, Bureau of Animal Industry, 1905.

Observation in Practice.—The history of the importation of cattle from various Texas fever countries into the Transvaal after the conclusion of the late war gives us a demonstration of the immunity under the condition of natural exposure. Importations from Madagascar numbered 10,000 oxen. These oxen are immune to redwater, as experience and experimental proof have shown. The general experience was that these animals did not contract redwater, and ever since cattle were imported from that country this fact is generally admitted. Contrary to this, an observation of mine shows that freshly imported Madagascar cattle can suffer from redwater. Eight Madagascar oxen brought from Natal, where they had been running for a short time, were brought to the laboratory and directly after arrival two succumbed to acute redwater.

Texas cattle were also imported to the number of 10,000. Speaking generally, these cattle proved to be immune to the South African redwater, but also here were exceptions to the general rule. Some cattle which had been running in Natal were brought to the Transvaal to be used in connection with the East Coast fever experiment, and four died within eight days after arrival from acute redwater. They undoubtedly must have contracted the disease previous to departure. From Queensland 500 head were imported. In this case the experience amounts to experimental value. The 500 head were inoculated with the blood of an immune South African ox, with the result that some of the cattle, few in number, contracted acute redwater and died.

Speaking from general observations, all cattle born and bred on the veld of the Transvaal must have gone through an attack of redwater and, therefore, must be immune. The immunity is a generally accepted fact. Yet during this summer outbreaks of redwater in all parts of the country were very numerous, and in many cases in cattle which were born and bred on the farm, or had been there at least for some years. In some instances the redwater took the form of a real epizootic.

It must be stated here that the climatical condition of the current year was exceptionally favourable for the breeding of ticks.

Conclusion.—*Observations in practice prove that immunity against redwater naturally acquired gives a great protection against subsequent exposure, but it is by no means complete. The breakdowns may be either due to infection with a different strain or to the over infection by means of ticks.*

Redwater noticed as Complication of Other Diseases.—When East Coast fever was first observed, it was noticed that in sick animals in conjunction with small piroplasms, the typical *Piroplasma bigeminum* was found. When later the small piroplasms were recognised as a species of their own—*Piroplasma parvum*—the fact of the simultaneous presence of both piroplasms in redwater immune animals found its interpretation in accepting that, under the influence of the acute East Coast fever, the *Piroplasma*

*bigeminum* undergoes a further multiplication process and leads to the appearance of redwater complicating the former disease. Statistics for the year 1903–04 show that of blood smears sent to the laboratory for diagnostic purposes :—

311 were pure East Coast fever infections ; and 23 complicated with redwater.

Statistics for the year 1904–05 show :—

334 pure East Coast fever infections ; and 16 complicated with redwater.

Statistics for the year 1905–06 show :—

152 pure East Coast fever infections ; and 5 complicated with redwater.

Statistics for the year 1906–07 show :—

133 pure East Coast fever infections ; and 5 complicated with redwater.

Statistics for the year 1907–08 show :—

239 pure East Coast fever infections ; and 6 complicated with redwater.

Conclusion.—*Immunity against redwater can be broken when the immune animal is suffering from some other febrile disease.* (It is possible that the cases of breakdowns noted by me in Texan and Madagascar cattle, shortly after a railway journey, were due to this.)

## Equine Piroplasmosis.

This disease is due to the introduction of *Piroplasma equi* into susceptible equines, either naturally by the bite of the red tick (*Rhipicephalus evertsi*) or artificially by inoculation of blood containing this parasite. The blood retains its infectivity for a limited time, about three weeks in our experiments. Animals which recover from the natural or artificially contracted disease may show more than one reaction.* The recovery means immunity. Horses born and bred in the Transvaal veld become immune by the natural tick infection. Foals suffer but little from the disease.

Blood of Immune Animals.—Experiments undertaken to this effect have demonstrated that the blood of immune horses is infective for horses, mules, and donkeys, and vice versa. The blood of a zebra caught in the bushveld proved to be infective for horses.

An immune animal retains the infection probably for a lifetime when exposed to ticks, and for a considerable time when not exposed.

In our experiments for the purpose of hyperimmunisation in horse-sickness by transfusion of blood from sick to healthy horses, we noted

*Annual Report, G.V.B., Transvaal, 1903–04.

equine piroplasms in 38 cases out of 402 horses treated, and in 3 mules out of 282, due to the infused blood, which kept the infection in a latent form.

A donkey foal kept for eighteen months out of the infection proved to be virulent in the inoculation of 100 mules. It has been observed that, similar to redwater, the injection of blood of immune foals is not so virulent and that the majority of injected animals recover. This has been made use of as a method of immunisation (blood of donkey foals).

In this way were treated with horse foal blood : 34 horses, 27 donkeys, and 135 mules. Three mules died of piroplasmosis.

With donkey foal blood were injected : 80 horses, 81 mules, 15 donkeys. There were no deaths.

Conclusion.—*The blood of an equine which has recovered from an attack of piroplasmosis remains infective.*

Test of Immunity by Injection of Blood.—A horse* which had recovered from the disease in July, 1904, was tested by the injection of 2,500 c.c. blood of a horse which at that time was suffering from piroplasmosis, and containing numerous piroplasms. No reaction was noticed in this horse.

Of 35 horses immunised against piroplasmosis by injection of immune blood, 3 horses died of piroplasmosis when hyperimmunised against horse-sickness.

Conclusion.—*Immunity against Piroplasma equi may be broken through infusion of large quantity of immune blood. (Horse-sickness virus.) (Probably different strain.)*

Equine Piroplasmosis as Complication of Other Diseases.—In our experiments with horse-sickness, we frequently met animals suffering from biliary fever whilst undergoing a horse-sickness reaction. All possibilities of an artificial infection simultaneously with the injection of serum or virus had to be excluded, since virus and serum were of old standing. Similarly to what has been described in redwater is the case here, a breakdown of immunity due to a concurrent fever.

During the year 1905–06 these breakdowns were noticed during the immunisation of 3,195 mules ; 26 showed piroplasmosis complicated with horse-sickness (0·8 per cent.), of which number 11 died (0·3 per cent.).

Of 402 horses immunised against horse-sickness and passing through a horse-sickness reaction, 12 showed complications with *Piroplasma equi.*

Conclusion.—*Immunity against equine piroplasmosis can be broken when the immune animal is suffering from some other febrile disease.*

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*Annual Report, G.V.B., Transvaal, 1904-05, page 104.

## PIROPLASMA MUTANS INFECTION.

A disease of cattle, due to the presence of *Piroplasma mutans* either naturally contracted by ticks or artificially by the inoculation of blood containing this parasite has, as a rule, a chronic course, microscopicaliy pronounced as an anemia. Recovery is usual, and recovered animals are known to be immune.

The Blood of Immune Animals.—Blood of an animal which has shown the presence of *Piroplasma mutans* at one time, either due to artificial or natural infection, is infective for susceptible cattle when inoculated.

The experiments never failed when animals were used of a country known to be free of *Piroplasma mutans* infection and the blood used for inoculation was derived from an animal in whose blood *Piroplasma mutans* have been traced ; whilst animals immune against redwater as a rule do not show the presence of *Piroplasma bigeminum* in microscopical examinations ; this is the rule with *Piroplasma mutans*, which for a long time can be traced microscopically.

Immunity under Influence of Intercurrent Fever.—The number of these parasites undergo variations and, similar to redwater, the existence of another febrile disease can evoke an increase of parasites.

Two head of cattle used in a heartwater experiment developed a typical heartwater reaction, due to the virus injection, and during this reaction *Piroplasma mutans* increased considerably. A similar observation was made in an ox which contracted heartwater naturally. Usually the presence of *Piroplasma mutans* is accompanied with that of *Piroplasma bigeminum*, which can easily be understood, since every animal born on the Transvaal veld is immune against redwater. We succeeded, however, in finding an animal which was infected with *Piroplasma mutans* alone, and, in the course of the various experiments, twenty-six animals were injected with blood of recovered animals and all showed pure *Piroplasma mutans* reactions.

Conclusion.—*The blood of an immune animal which recovered from mutans infection remains infective. Similar to Piroplasma bigeminum and equi, intercurrent fevers can cause an increase of Piroplasma mutans, which can be considered as a decrease in the existing immunity.*

### Piroplasmosis of the Dog.

Canine piroplasmosis is due to the infection with *Piroplasma canis*, introduced into susceptible dogs either naturally by means of ticks (*Haemaphysalis leachi*) or artificially with blood which contains the parasite. The disease has a relapsing character.

The final recovery renders a dog immune. This immunity in an experiment of ours by inoculation with virulent blood of one and the same strain could not be broken.

The Blood of Immune Dogs is infective and, contrary to the other piroplasmosis, such blood is as virulent both for young and old animals as is that of sick animals. The blood of an immune dog retained its infectivity in our experiments during one year.

Robertson* quotes an instance of thirteen months and another of two full years. Robertson was able to infect this latter dog again by injecting virulent blood.

Conclusion.—*Immunity against canine piroplasmosis is present as long as the dog retains the infection in the blood; when this wears off then it again becomes susceptible. Under natural conditions, this will hardly be noticed, as the presence of ticks is the permanent cause of re-infection.*

Serum of Immune Animals.—The knowledge concerning the serum of hyperimmunised animals can be summarised as follows† :—

(1) The mixture of serum and virulent blood injected into susceptible dogs was harmless; no immunity followed.

(2) Injection of serum twenty-four hours before virus injection prevented development of disease; no immunity followed.

(3) Injection of serum twenty-four hours after virus prevented development of disease; no immunity followed.

(4) The serum was active in the way indicated both against a virus of a different animal (*Heterologous*), as against the virus obtained from the serum supplying dog (*Homologous*).

Conclusion.—*The serum of a dog hyperimmunised with virulent blood has preventive qualities.*

Immunity in Inoculable Piroplasmosis.—Recovery from a piroplasmosis causes immunity. This immunity is not complete and can be broken by virus of a different strain. The immune animals retain the infection in the blood for a considerable length of time, and during this time intercurrent maladies can break the immunity. The degree of immunity varies somewhat in the various breeds of animals (redwater); it seems to last as long as the blood remains infective (canine piroplasmosis). The serum of hyperimmune dogs has protective properties. (Not tested in redwater and equine piroplasmosis.)

## III.—SPIROCHAETOSIS IN CATTLE.

Spirillum theileri causes in horses, cattle, sheep, and goats, a febrile reaction of short duration, sometimes of a relapsing character and the microscopical lesions of anemia. All animals suffering from a pure infection recover. The infection is naturally transmitted by the progeny of infected blue ticks, or artificially by the inoculation of blood containing the parasites.

* Jour. Comp. Path. and Therap., Vol. XIX, Part 2.
† Annual Report, G.V.B., Transvaal, 1903–04.

The presence of *Spirillum theileri* had been seen in animals suffering from other infections as, for instance, *Piroplasma mutans*, *Piroplasma bigeminum*, *Trypanosome theileri*. In the former two cases it is sometimes difficult to say which would be the primary infection, especially when the fact is considered that *Spirillum theileri* remains in the blood of immune animals. The appearance of the spirillum, together with trypanosomes, must be considered as a breakdown of immunity of spirillosis.

CONCLUSION.—*The immunity obtained through the recovery from a Spirochaetosis infection is not complete; the blood remains infective and intercurrent fevers cause an increase of the parasites.*

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## IV.—EAST COAST FEVER (*Theileria parva*).

East Coast fever is a disease in cattle due to the presence of *Theileria parva*, a parasite of the red corpuscles, which can so far only be introduced into the system in the natural way by means of ticks. Thus the disease is not inoculable, and all attempts to transmit it with blood by infusion or injection of juice of internal organs have failed. In one of our experiments, a young ox was infused during fifteen minutes with East Coast fever blood; there was no reaction due to this. The disease leads to a mortality of 95 per cent. The recovered animals are known to be immune. A number of eight oxen which had recovered in August, 1902, at Komatipoort from East Coast fever, were exposed three and a half years later at Sjamboks Kraal to natural infection; none of these animals contracted the disease.

CONCLUSION.—*The immunity is complete concerning natural infection and concerning time.*

THE BLOOD OF IMMUNE OXEN.—Immune animals do not retain the infection in the blood. This can be observed in practice where immune cattle have survived the outbreak of East Coast fever, where subsequently young, or new, fresh imported stock were running together on tick-infected farms without ever showing an infection. Oxen which recovered in the year 1902 from East Coast fever have been running since then with susceptible cattle in tick-infected areas without transmitting the disease to the susceptible ones.

Experiments* to prove this were also undertaken by feeding brown tick nymphae on oxen which had two years previously recovered from East Coast fever, and placing the moulted adults on susceptible cattle. In no instance did such ticks transmit the disease, hence it can safely be concluded that the blood of East Coast fever immune cattle harbours no longer the parasite of East Coast fever.

CONCLUSION.—*The blood of an ox recovered from East Coast fever does not retain its infectivity.*

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* Annual Report, G.V.B., Transvaal, 1903–04, pages 93 and 94.

Serum of Immune Animals.—Professor Koch had stated that the serum of cattle which were hyperimmunised with the blood of cattle suffering from East Coast fever acted, when injected into sick oxen, directly on the parasite, causing them to disappear; unfortunately such serum had also haemolytic effect, so that it could not be applied with safety. Having overcome the difficulty of haemolysis in horses by infusion of the virulent blood into the jugular vein, this method was also used in connection with East Coast fever and some oxen were hyperimmunised to the extent of a minimum of 20 and a maximum of 40 litres.

This serum was not haemolytic. It had no lytic action on the parasite, and when applied on an infected herd it had no preventive action.

Conclusion.—*Serum of East Coast fever immune oxen hyperimmunised by infusion of sick blood has no preventive action whatsoever.*

Résumé.

The immunity in East Coast fever is complete concerning natural infection and time.

The immune animals do not retain the infection in the blood.

The serum has no preventive properties.

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## V.—TRYPANOSOMIASES.

There exist many different species of these parasites in Africa, which from our point of view have not yet been studied. A certain number of records are registered in the literature, which will give us an idea as to the existence of immunity or otherwise. I shall only refer to these trypanosomiases.

The specific trypanosomiasis of cattle in South Africa is due to the presence of *Trypanosoma theileri*. Generally speaking, this parasite has but little pathogenic action; we meet it occasionally in bloodsmears sent to us from cattle reported as suffering from gall-sickness, invariably from the bushveld. This trypanosomiasis is only inoculable into cattle. Recovery from such an attack seems to create immunity.

The inoculation of large quantities of blood containing the trypanosomes into animals which had recovered from trypanosome infection gave in the majority of cases negative results, and in the positive results there were only a small number of parasites.

Conclusion.—*Immunity caused by the recovery from a Trypanosoma theileri infection can be broken by subsequent inoculation.*

Of more importance are the trypanosomes of *Trypanosoma evansi*, the type to which belong, in addition to the one mentioned, *Trypanosoma brucei* of Zululand, of Togo in West Africa, and the *Trypanosoma*

*sudanense*. Morphologically speaking, these various parasites could not with absolute certainty be separated from each other, although for epidemiological and geographical reasons a difference between *Trypanosoma evansi* and *brucei* suggested itself. French scientists introduced a method of differentiation, based on the observation, that animals which recovered from a trypanosome infection are immune against that particular strain of virus. The duality of *Trypanosoma evansi* and *brucei* was decided in the following way:*

Laveran and Mesnil made use of two goats, which had recovered from mal de caderas (*Trypanosoma equinum*) whose blood had proved to be sterile by tests on susceptible animals. They were then injected with *Trypanosoma brucei*, developed the disease and recovered; thus proving that recovery from mal de caderas does not cause immunity against nagana.

One of the recovered goats was re-injected with the *Trypanosoma brucei*, and since no development followed it had to be concluded that immunity was established. The goats were now injected with the trypanosome of surra; they both developed the disease and the presence of parasites could be demonstrated by sub-inoculation into mice.

Conclusion.—(1) *Recovery of goats from nagana gave immunity against the strain by which the immunity was caused.*

(2) *Recovery from mal de caderas did not give immunity against nagana or surra.*

(3) *Recovery from nagana did not cause immunity against surra.*

French scientists worked with surra of various origins, viz., a strain obtained from Mauritius, another from India, and a third one from Nha-trang, China, and based on the immunity re-action declared the trypanosomes found in the disease "mbori" of North Africa to be identical with *Trypanosoma evansi*.

Vallee and Panisset† inoculated two head ot cattle with surra of Mauritius. After the course of a year they no longer showed trypanosomes in their blood, and a subsequent inoculation with the same virus proved their immunity. They then were twice in succession inoculated with the trypanosome of mbori and proved refractory. Later they were inoculated with surra from India and again proved refractory.

Laveran experimented in the reverse order. A male goat which had recovered from mbori, and whose blood had proved to be sterile was first tested with the same virus and proved to be refractory. Subsequently it was inoculated with surra of Mauritius and no infection took place. From this, Laveran supported the views of Vallee and Panisset

* Compt. Rend. des Seances de l'Acad. de Sciencè, t. CXXXVI, p. 1529; A. Laveran et F. Mesnil.

† Comptes Rendus, 27/3/05.

of the identity of the trypanosome of Mauritius and mbori, but is inclined to consider trypanosome of mbori as a variety of *Trypanosoma evansi.* Similar experiments were undertaken by Laveran and Mesnil* to prove that the trypanosomiasis of Nha-trang in Annam was identical with surra, after it was proved that they did not differ either in morphology or in their pathological action. A male goat which was immune against both mbori and surra of Mauritius, and whose blood inoculated into susceptible animals had proved to be sterile, was inoculated with surra from Nha-trang. It contracted the disease and died.

A goat which had recovered from an infection of a Mauritius strain of surra and had proved to be immune to the test with the virus, and after sub-inoculations into smaller animals had proved that the blood had become sterile, was inoculated with virus of Nha-trang. The animal contracted the disease and died. Here the immunity of surra and mbori did not protect against a third strain of surra virus of Nha-trang. Laveran and Mesnil's interpretation of these facts is of a close relationship of the two strains, but of different variety; but they do not consider the Nha-trang strain to be an entity of its own.

Conclusion.

(1) *Cattle which recovered from surra of Mauritius proved immune to the test when reinjected with the same virus.*
(2) *Goats which recovered from mbori proved immune to the test when injected with the same virus.*
(3) *Cattle which were immune to surra of Mauritius proved to be immune to mbori and to surra of India.*
(4) *Goats which were immune to mbori proved to be immune against surra of Mauritius.*
(5) *Goats immune to surra of Mauritius and mbori, or to surra of Mauritius alone, proved not immune to surra of Nha-trang.*
(6) *The blood of animals whose immunity was tested proved to be sterile.*

Although there are pathogenic differences described by Laveran,† *Trypanosoma sudanense,* which morphologically cannot be distinguished from *Trypanosoma evansi*, had to be considered as a specie of its own, owing to the interpretation of the immunity test. Laveran used a goat which had recovered from an infection with *Trypanosoma sudanense.* After the blood had become sterile and had proved to be immune against a subsequent test it was injected with *Trypanosoma evansi* of the mbori disease with positive results.

In the reverse experiment, a goat recovered from mbori, and after the blood had become sterile proved to be immune against this strain, was successfully infected with *Trypanosoma sudanense.* One virus did

* Recherches Experimentales sur la Trypanosomiase des Chevaux de l'Annam.
† Tryp. du Haut Niger Ann. de l'Ins. Past. t. 21, No. 5.

not protect against the other, hence the conclusion of the duality of the two trypanosomes.

Conclusion.

(1) *Recovery of a goat from Trypanosoma sudanense gave immunity against this strain.*
(2) *The blood of the immune animal was sterile.*
(3) *The immunity did not protect against mbori strain, nor did mbori immunity protect against Sudanense strain.*

Morphologically identical with the *Trypanosoma brucei* of Zululand were also found to be two strains of trypanosomes found in Togo. Their identity or otherwise formed the subject of investigations by Lavern and Mesnil.*

They experimented with a Nagana from Togo obtained from Prof. Schilling, which they injected into a goat (this goat had recovered from an infection due to the Zululand strain and its blood proved to be sterile) together with a control; they both contracted the disease and died respectively on the 34th and 35th days. Here the immunity of the Zululand Nagana did not protect against Schilling's Togo strain; hence the conclusion that this latter one is not Nagana.

Conclusion.

(1) *Immunity of Trypanosoma brucei of Nagana strain of Zululand did not protect against Schilling's Nagana strain of Togo.*
(2) *The blood of the goat which had recovered from a Nagana infection proved to be sterile.*

Observations recorded by Koch† concerning a Togo strain of *Trypanosoma brucei* are of utmost interest to us.

Two horses, a mare and a stallion, were imported from the Hinterland of Togo to Berlin. En route they had to pass a tsetse belt. They contracted a trypanosomiasis (called the Togo-Martini strain of Nagana). The stallion died about four months after infection, and its blood proved to be highly virulent for any experimental animals which were injected. The mare remained in good condition; microscopical examination did not reveal the presence of trypanosomes, which were only demonstrated after large doses of blood had been injected into dogs. Contrary to the experience with the stallion, the mare's trypanosomes were but slightly virulent for smaller animals and these recovered to a great extent. Finally the mare, which remained over a year in perfect health, was inoculated with the trypanosome originating from the stallion, and now she developed an acute tsetse disease from which she died. Koch interpretes this to a different virulency of the two strains, although the horses had become infected in all likelihood at the same time and at the same place.

* Comptes Rendus, 25/6/06.
† Deutsche Med., Woch., 1904.

Martini, who carried out these experiments, was able to increase the virulency of the mare's strain to a great extent. Dogs, injected with virus of the first generation, only died after 100 days; but dogs injected with virus from later generations died in 10–15 days. It was also noticed that dogs which had recovered from an infection with the earlier generations died from the infection when injected with a later generation. Here no immunity was observed.

Conclusion.

(1) *The two strains of the stallion and the mare had for morphological and other reasons to be considered identical.*
(2) *They were of different virulency.*
(3) *The virulency could by passage be so increased that the immunity obtained from one virus, low in the scale of generations, did not protect against the same virus higher in the scale.*

Koch* has made some further observations which are useful from our point of view: Two head of cattle which had been inoculated with an attenuated strain of trypanosomes, apparently recovered, inasmuch as in their blood microscopically trypanosomes could no longer be seen. They were tested, together with some control animals with a virulent strain of trypanosomes. The controls showed the disease in the usual way; the two vaccinated animals showed trypanosomes only for a few days and remained healthy. Apparently by the first inoculation they acquired some immunity against the second strain. One of these animals remained for about six years under observation and was tested during this time on its immunity, again with positive results. After six years the blood of this animal which on microscopical examination proved to be sterile was tested on dogs, when the presence of trypanosomes was promptly proved. Thus in this instance we have a proof that cattle, although resisting to repeated inoculation of virulent blood and therefore apparently immune, can retain the trypanosome in the blood stream.

Bruce,† who studied the Nagana in Zululand in order to trace the connection between game and Nagana, inoculated the blood of game into dogs, which were kept outside of the infected area (on the Lebombo mountains). Thus the blood of 8 buffaloes proved to be infective once; of 13 wildebeeste three times; of 4 koedoes three times; and once the blood of a bushbuck and a hyena. This fact demonstrates the connection between game, fly, and disease in stock.

Conclusion.

(1) *An animal proved to be immune against the inoculation of Nagana, but its blood proved to be virulent for susceptible cattle.*
(2) *Game, which are considered immune against Nagana, may carry the infection in their blood.*

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* Loco cit.
† Further Report on the Tsetse Fly Disease or Nagana of Zululand.

The differentiation between the *Trypanosoma pecaudi*, the cause of a North African disease called baleri, and the *Trypanosoma dimorphon*, which in its smaller not free flagellated forms resemble each other to a certain extent, was also demonstrated by the immunity test.

In Laveran's experiments,* a sheep after it had recovered from a *Trypanosoma pecaudi* infection, and its blood was proved to be sterile, was tested with its own strain of virus, which in the meantime had passed some guinea pigs. It was found to be refractory to this injection. It was then inoculated with *Trypanosoma dimorphon* and developed the infection in typical time, thus proving that the immunity obtained from the former infection did not protect against the latter one.

The long flagellated forms of *Trypanosoma pecaudi* resemble *Trypanosoma evansi*. It was therefore possible that the original animal in which *Trypanosoma pecaudi* was first found contained a mixed infection of two different trypanosomes, a flagellated and a non-flagellated one, and the former might be *Trypanosoma evansi* of mbori disease. Laveran inoculated a goat, immune against mbori (*Trypanosoma evansi*) with *Trypanosoma pecaudi*; the goat developed the infection and the two forms of *Trypanosoma pecaudi* appeared again, thus pointing to the true species of *Trypanosoma pecaudi*.

Conclusion.

(1) *Recovery from Trypanosoma pecaudi gave immunity which protected against this particular virus, and the blood of recovered animals was sterile.*

(2) *Immunity thus obtained did not protect against an infection with Trypanosoma dimorphon.*

(3) *Immunity obtained through recovery from Trypanosoma evansi (mbori) infection did not protect against Trypanosoma pecaudi.*

*Trypanosoma dimorphon* was first described as a horse disease by Dutton and Todd. Experiments proved that all stock can be infected with it, and subsequently it has also been found in various species of domesticated animals. The disease runs usually a chronic course, and several animals have been observed to recover.

Concerning immunity, some observations have been made by Martin† in a goat and a sheep. These animals had recovered from a natural attack, and their blood injected into dogs proved to be sterile; when reinoculated, they again showed the infection.

Conclusion.—*Recovery from an attack of Trypanosoma dimorphon did not produce immunity. The blood of the recovered animal proved to be sterile.*

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* Comptes Rendus, 1907.

† Les Tryp. de la Guinea francaise : Ann. de l'Inst. Past., t. XXI, No. 5.

Somewhat related to *Trypanosoma dimorphon*, but more uniform in size, is the *Trypanosoma congolense*; according to the immunity test it must be differentiated from the former. Two goats which recovered from an injection were used in Laveran's experiments.* Sub-inoculation into smaller animals proved the sterility of the recovered goats' blood. The animals were reinjected with the same strain; again they both showed a slight infection. Further inoculation however did not cause any more infection; the animals had acquired immunity. The two recovered goats were now inoculated with *Trypanosoma dimorphon*. Both animals contracted a typical infection from which one died and the other was still alive on the date of publication (November, 1908).

Conclusion.—*Recovery from a first attack of Trypanosoma congolense did not convey a complete immunity. A second infection with the same strain proved successful. Subsequent recovery increased this immunity. The blood of immune animals was sterile. Immunity against Trypanosoma congolense did not protect against Trypanosoma dimorphon.*

Serum Experiments.—The experiments of Laveran,† Mesnil,‡ Martin,§ Kleine,|| and Mollers,|| and of others with trypanosomes can be résuméd as follows† :—

(1) Serum of animals suffering or recovered from an advanced trypanosomiasis has distinct preventive qualities. When homologous serum and virus were mixed before injection no infection took place; independently injected the results varied. In experiments of heterologous mixtures of serum and virus no preventive actions were noticed.

(2) The serum of an animal suffering from advanced trypanosomiasis may be active against the trypanosomes of the same blood from which the serum was derived (Kleine and Moller).

(3) Animals which supply a serum with preventive properties may retain the infection in the blood, and the serum of such blood has no preventive action on the corresponding trypanosomes, even if such trypanosomes have passed through a succession of animals (Mesnil and Brimont).

Summary.

Animals may recover from trypanosomiasis and then prove to be immune against the strain with which they have been infected; it is an exception that the same strain again causes an infection, but the different strains may cause reinfection.

Animals may be immune and their blood remain infective or they may be immune and the blood becomes sterile.

Serum of infected or recovered animals has slight protective properties.

* Contrib. à l'Etude de Tryp. congolense: Ann. de l'Inst. Past., t. XXII, Nov., 1908.
† Comptes Rendus, t. CXLII, p. 1482.
‡ Ann. de l'Inst. Past., t. X.
§ Zeitsch fur Hyg. t. L., 4/4/05.
|| Zeitsch fur Hyg. t. LII., 1906.

## COMPLETE SUMMARY OF CONCLUSIONS.

### I.

Two different types of immunity may be distinguished :—

(1) Immunity whereby the recovered animal no longer acts as a reservoir of virus. *Immunitas sterilisans* (East Coast fever and the tropical diseases due to ultravisible organisms ; trypanosomiasis partially.)

(2) Immunity whereby the recovered animal acts as a reservoir for virus. This immunity may cease by intercurrent fevers ; breakdowns. *Immunitas non sterilisans.* (All inoculable piroplasmoses and spirochaetoses ; trypanosomiasis partially.)

### II.

In speaking of immunity of a recovered animal, distinction must be made between immunity generally without reference to any particular strain of virus and immunity in particular with reference to definite strains of virus. (For instance, immunity against horse-sickness can be generally spoken of, or in particular the immunity obtained from the Ordinary strain, or Tzaneen, or any other.)

We can then conclude as follows : With the exception of East Coast fever in South Africa no other tropical disease produces complete immunity. The cause of the other diseases is not uniform ; there exist many different strains or varieties of the species of organisms which all form the cause of the disease.

Concerning a particular strain, the following may be concluded :

(1) An immunity against a particular strain may be complete. (Ordinary virus of horse-sickness ; some trypanosomiases ; heartwater.)

(2) An immunity against a particular strain may be broken

- (*a*) by the injection of large doses of the same strain of virus (blue-tongue) ; or
- (*b*) after a certain time has elapsed (blue-tongue) ; or
- (*c*) by a more virulent virus of the same strain (Tzaneen virus in horse-sickness) ; or
- (*d*) by a different strain of virus (horse-sickness, heartwater, redwater, and surra).

(3) Immunity can vary with the species and with the breed of animals susceptible to the disease (redwater, heartwater).

### III.

The facts noted in South Africa concerning variations of immunity may be explained as follows :—

East Coast fever is a freshly imported disease, and the outbreak throughout South Africa may all be due to one and the same source, whereas, for instance, horse-sickness, blue-tongue, etc., are diseases established since ages, and are localised ; the micro-organisms have undergone and are still undergoing certain variations, the result of which is the different immunity.

IV.

From a practical point of view, immunity against a tropical disease can only be spoken of as sufficient when it protects against the majority of various strains of the organisms forming the cause of the disease found in such region, for which it is intended to make use of for inoculation purposes.

V.

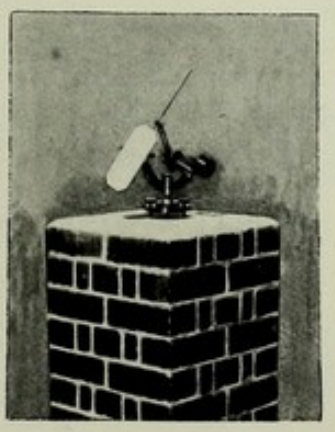
The immunity test for identifying two morphologically similar organisms can only allow of a definite conclusion when the test is positive, but not so when it is negative.

VI.

The serum of animals which have recovered from a disease and have been hyperimmunised to a certain extent acquires preventive properties. East Coast fever is an exception, but the injection of blood from an animal suffering from East Coast fever into susceptible ones does not communicate the disease, and this may explain the fact. The preventive action of a serum is principally pronounced against a homologous strain, either of the same animal (virus derived from the same immune animal which supplied the serum ; in some trypanosomiasis and in canine piroplasmosis) or of different animals and of different strain (horse-sickness) ; it may be deficient against heterologous strains (strains of different origin ; some trypanosomiases, surra, and horse-sickness) or even against a homologous strain derived from the animal which supplied the serum (some trypanosomiases).

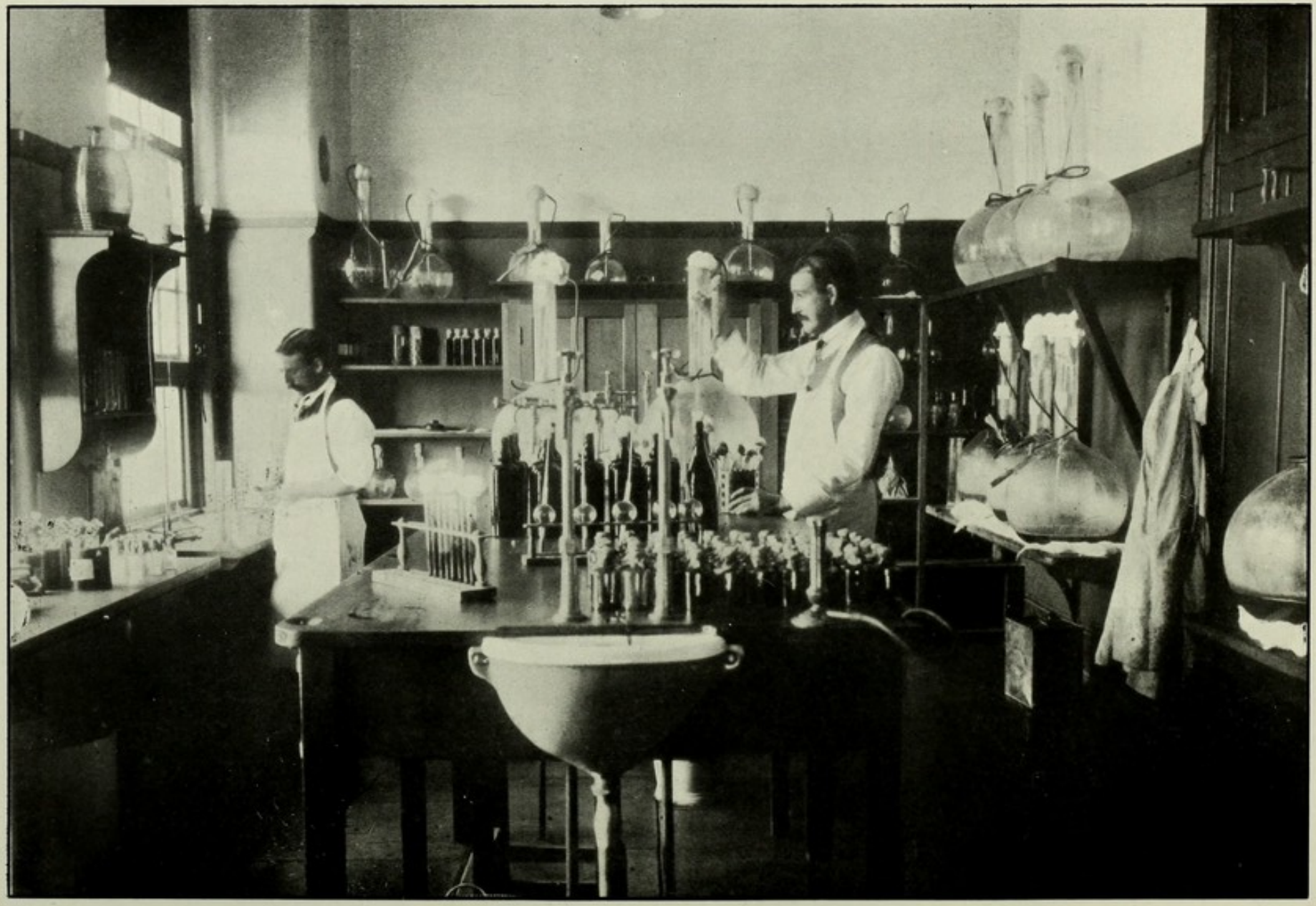
VII.

Concerning the immunity itself, there are no essential differences between that caused by bacteria and that caused by protozoa. The fact that an apparently recovered animal acts as a reservoir has its analogy in pleuro-pneumonia of cattle, although the two cases are not quite identical. In the latter, morbid lesions are still present, in which the infective cause is retained ; in the former, the cause remains in an apparently healthy animal.

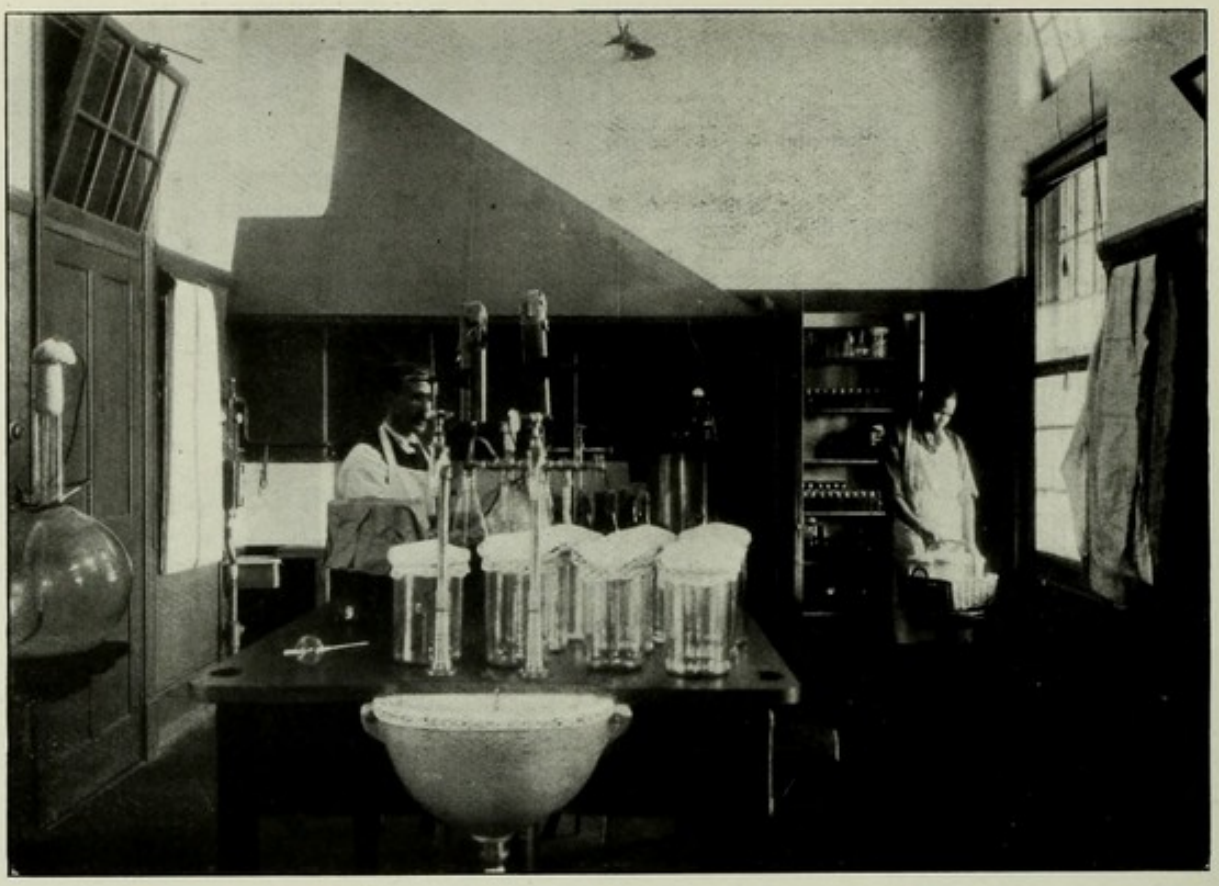


# The Diagnosis of Bacillary Piroplasmosis of Bovines in the Transvaal.

By JAMES WALKER, M.R.C.V.S.



*The Serum Room.*



*Sterilizing Room—Serum.*

*Sterilizing Room—Bacteriology.*

# The Diagnosis of Bacillary Piroplasmosis of Bovines in the Transvaal.

THE term "piroplasmosis" is applied to diseases caused by endoglobular parasites belonging to the group protozoa and called piroplasmata. Of the bovine piroplasmoses known in the Transvaal, two interest us more particularly, viz., the two caused by bacilliform piroplasms and which hitherto have been known under the name *Piroplasma parvum* and *Piroplasma mutans*; both from a diagnostic point of view are interesting; the former from an economic point of view is of extraordinary importance, and at one time threatened to destroy the cattle of South Africa. These two microscopical organisms are morphologically inseparable; this forms the reason for a difficulty in the microscopical diagnosis. An early decision with regard to a diagnosis of East Coast fever is always a matter of urgency, consequently the practical utility of observations which would allow of a differential diagnosis would be very considerable.

*Piroplasma parvum* is the cause of the disease popularly known as East Coast fever. A specific name for the disease caused by *Piroplasma mutans* does not exist, but the term "gall-sickness" probably includes that disease as well. The main characteristic of *Piroplasma parvum* is its non-inocubility which separates it absolutely from *Piroplasma mutans*, which is inoculable even with small quantities of blood.

At the Veterinary Conference held in Budapest * in 1905, the question of piroplasmosis received attention. Dschunkowsky stated the disease described first by him under the name Tropical Piroplasmosis, and caused by small endoglobular parasites in the shape of rings, rods, and flagellated forms, in Transcaucasia was inoculable; this statement reversed his former one, and by this differentiated it from the disease East Coast fever. Bitter communicated that he had observed a non-inoculable piroplasm in Egypt, likewise due to small piroplasms, and Ducloux, of Tunis, reported his experience of a similar parasite as the cause of a disease in that country. In view of these new discoveries the piroplasmata at that time were grouped as follows, viz. :—

> "TYPE.—*Piroplasma beginminum*, *Piroplasma bovis* (Babes) found in European Haemoglobinuria of cattle. *Piroplasma bigeminum* (Smith and Kilborne) of Texas fever."
>
> "TYPE.—*Piroplasma parvum* (*a*) Inoculable piroplasmoses, tropical piroplasmosis of Transcaucasia, *Piroplasma annulatum* (Dschunkowsky); (*b*) Piroplasmoses (not inoculable) *Piroplasma parvum* (Theiler) of East Coast fever, piroplasmosis of the North African disease (Bitter and Ducloux)."

* Proceedings of the 8th International Veterinary Congress, Budapest, Sept., 1905.

Since then new inoculable piroplasmata of the type *Piroplasma parvum* had been described by Theiler and called *Piroplasma mutans* * and by Miyajima and Shibayama in Japan,† which led Bettencourt to suggest the following classification, viz. :—

1*st Group.*—Piroplasmoses produced by parasites presenting in one of their evolution phases the form of a pear and disposing themselves in pairs in the same corpuscle.

*Babesia bovis* (Babes).

*Babesia bigeminum* (Smith and Kilborne).

Geographical distribution, Europe, America, Africa.

2*nd Group.*—Piroplasmosis produced by parasites presenting always at one phase of their evolution the form bacillary—*Piroplasmoses bacilliformes.*

(*a*) *Piroplasmoses bacilliformes, inoculable.*

*Babesia annulata* (*Piroplasma annulatum*) Dschunkowsky.

*Babesia mutans* (*Piroplasma mutans*) Theiler.

*Babesia bacilliforms* found amongst the cattle of Japan (Miyajima and Shibayama).

*Geographical distribution.*—Transcaucasia, Japan, South Africa, to which may be added the following, viz. :—Piroplasmosis, described by Stephens and Christopher, in Madras ; Shein in Annam ;‡ Bettencourt in Portugal ;§ Boeret on the Gold Coast of Africa ;‖ Does in the Dutch Indies ;¶ Martini in Peycheles, China ;** Lichtenheld in German East Africa ;†† Balfour in the Soudan ;‡‡ Broden and Rodhain, Congo (Stanley Pool).§§

(*b*) *Piroplasmoses bacilliformes, non-inoculable.*

*Babesia para*‖‖ (*Piroplasma parvum*) Theiler.

Babesia of Tunis and Egypt.

*Geographical distribution.*—South Africa (Theiler), Egypt (Bitter), Tunis (Ducloux).

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* Theiler : Piroplasma Mutans.—Annual Report Transvaal Department of Agriculture, 1905–06 ; Report of the Government Veterinary Bacteriologist, Transvaal Department of Agriculture, 1906–07.

† Bacilliformis : Zietsch. j. Hyg., t. LIV, Sept., 1906. Miyajima and Shibayama, pp. 189–200.

‡ Shein in Annam Ann. Institut Pasteur, t. XXI, 25/8/07, pp. 656, 657, 669.

§ Bettencourt, extract Archives Institute Royal of Bacteriology, t. I, Fax II, Lisboa, 1907.

‖ Boeret, Gold Coast, Bulletin Soc. Path. Exot t. I, No. 4, 1908, p. 234.

¶ Does, Dutch Indies. Mededeelingen uit het Geneeskundig Lab. te Weltevreden, 2de Serie, B. No. 4, p. 185, 1905.

** Martini in Peycheles, China, Arch f. Sch. u. Trop. Hygene, t. XI, 1907, pp. 507–511 ; 718–719, August and November, 1907.

†† Lichtenheld in German East Africa, Zietschrift f. Hygene u. Infektious Krankheiten, bl. 61, 1908, heft 2, p. 261.

‡‡ Balfour in Sudan, 3rd Report Wellcome Res. Lab. at the Gordon Memorial College, Khartoum.

§§ Broden and Rodhain, Congo, Stanley Pool. Bulletin Soc. Path. Exot t. II, seance 10/3/09, pp. 12–124.

‖‖ Theiler : Babesia Parva, Piroplasma Parvum.—Annual Report of the Transvaal Department of Agriculture, 1903–04 ; Annual Report of the Transvaal Department of Agriculture, 1904–05 ; Annual Report of the Transvaal Department of Agriculture, 1905–06 ; Report of the Government Veterinary Bacteriologist, Transvaal Department of Agriculture, 1906–07.

Bettencourt suggests creating a new genus which he proposes to call "Theileria," including all the parasites which present the bacillary form and which divide themselves in originating forms of a cross. In this manner the name Babesia would be reserved solely for parasites of the type *Babesia bigemina*.

From our present knowledge of *Piroplasma parvum*, principally its non-inocubility and the presence of certain intracellular bodies found in the internal organs of an affected animal which will be referred to later, and from the fact that an immune animal does not retain the infection in the blood, the genus—Theileria—has to be completely separated from the rest of the piroplasms and to be considered as a genus of its own. For the same reason it follows that the suggestion of Bettencourt to include the bacillary forms in one genus cannot be accepted, and the inoculable bacillary piroplasms either represent a genus of their own or should belong to the group of Babesia.

The following grouping may represent the present knowledge :—

*Inoculable* (Babesioses).

I.

(*a*) Babesia type, B. Bigeminum.

II.

(*b*) Babesia bacilliformes, type *Babesia mutans*.
Babesia of Madras (Christopher and Stephens).
,, Annam (Shein).
,, Gold Coast of Africa (Boeret).
,, Dutch Indies (Does).
,, Peycheles, China (Martini).
,, German East Africa (Lichtenheld).
,, Soudan (Balfour).
,, Congo (Stanley Pool) (A. Broden and J. Rodhain.

III.

*Non-Inoculable.*

(*a*) Theileria. Type : *Theileria parva*.

East Coast fever is a new disease for South Africa. In the year 1897, Professor Koch when investigating diseases in German East Africa described an endoglobular micro-organism of bacillary form, and which at that time was regarded by him to be young forms in the development of *Piroplasma bigeminum*, the cause of redwater (Texas fever), and accordingly designated the disease by that name. From 1898 to 1901 it attracted no special attention. About the latter end of 1901, an outbreak of a disease corresponding to that observed by Koch in German East Africa was found amongst a mob of recently imported cattle at Beira ; a number

died, the remainder were removed to Umtali, Rhodesia, where they continued to die. It appears that the cause of death at Beira was redwater (Texas fever), and that the lot which were removed to Rhodesia developed the usual symptoms of the East African disease as a result of being grazed on ground infected by cattle introduced from German East Africa; the disease now attracted more attention, although still associated with redwater (Texas fever) by the first workers. East Coast fever was subsequently introduced into the Transvaal in May, 1902, apparently from Lourenco Marques via the Delagoa Bay Railway, and first appeared at Komatipoort and Nelspruit, and thence eventually spread to other districts and Swaziland. After its introduction into Rhodesia and the Transvaal, and when its study was systematically undertaken, it was found not to be identical with redwater (Texas fever), but to be a disease of its own.

In the experiments with ordinary redwater inoculations it was noticed that the blood of animals which were injected with blood of redwater immune animals, showed on microscopical examination the typical *Babesia bigemina*. When, however, the examination of the blood was continued endoglobular parasites similar to those of *Thieleria parva* were also observed. This phenomenon led some observers to conclude that these organisms are a stage in the life-cycle of *Babesia bigemina*. Others, however, held that the small endoglobular organisms found in the blood of animals had to be connected with East Coast fever. At the Veterinary Conference held in Bloemfontein in 1903,* Professor Koch stated that he had found the parasites of East Coast fever in smears of blood collected from East London cattle, and concluded that that disease existed on the south-eastern coast of Africa, basing his opinion on the presence of the bacillary piroplasms. Theiler, who had carried out some investigations in connection with the appearance of small endoglobular parasites, before East Coast fever was known to the Transvaal, in cattle out of countries known to be free from that disease, considered the piroplasm referred to by Koch was probably a species of its own and not connected with East Coast fever. In the light of our present knowledge it is evident that the parasites observed by Koch were those which were formerly associated with the immune redwater ox, but which are now known to be a distinct species, viz., *Babesia mutans*. Since *Babesia mutans* exists in the Transvaal wherever redwater is found, and it is the exception to find the one unassociated with the other (this association of *Babesia bigeminum* and *Babesia mutans* appears to exist in other countries, e.g. China, Japan, Annam, Madras), and since it is known that Madagascar cattle are immune to both redwater (Texas fever) and mutans, its introduction may be traced back to the importation of cattle from that country.

* Report of the Proceedings of the Conference on Diseases amongst Cattle and other Animals in South Africa; Bloemfontein, 1903.

## Host of the Piroplasm.

Numerous experiments (Theiler) (Lounsbury) have established the fact that the transmission of the parasites which invade the erythrocytes in East Coast fever takes place by the agency of ticks. From the most recent experiments it has been shown that *Rhipicephalus appendiculatus* is the principal transmittor; the affection being carried by nymphae and adults, that *Rt. evertsi*, *Rt. capensis*, *Rh. simus*, and *Rh. nitens* are hosts of *Theileria parva*. Although the present knowledge with regard to the means of transmission of *Babesia mutans* is perhaps still incomplete, recent observations (Theiler *) point to *Rh. appendiculatus* and *Rh. evertsi* as being hosts.

*Course, Symptoms, and Pathological Lesions.*

The *incubative* period of *East Coast fever* averages twelve days, variations being ten days shortest and twenty days longest period; following this a sudden rise of temperature occurs in the majority of cases, reaching 106–107 and remaining high throughout. In a few cases the temperature rises gradually day by day, until the maximum is reached.

The *fever* period averages about thirteen days, variations being six days minimum and twenty days maximum. Consequently the *length of the disease* from date of infection averages twenty-five days.

The *symptoms* during life are not always characteristic; they are subordinate to the seat of lesions. In some instances the animal may appear in normal health up till the time of death, and in such is usually found dead with a discharge of foam from the nostrils, resulting from an oedematous condition of the lungs. In other cases in which the oedema of the lung, although present, is not so pronounced as to cause immediate death pulmonary symptoms, such as coughing and discharge from nostrils, may be noticed. Where bowel lesions are present diarrhœa or a haemorrhagic discharge may be present; depending on their severity, swelling of the lymphatic glands of the head and throat may be detected in a number of cases. Frequently the first symptoms observed will be a discharge from nose and eyes and increased flow of saliva from the mouth, or simply a loss of condition. Haemoglobinurea is not a symptom of the disease, but is the expression of the complication with ordinary redwater (Texas fever). Where the disease is associated with ordinary redwater, as frequently happens in a country in which that disease is known to exist, symptoms of redwater overmask those of East Coast fever.

The *post-mortem* lesions vary. The organs found most constantly affected are the spleen, liver, kidney, and heart. Lung lesions are present in from 30–35 per cent. of cases (Gray).†

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* Transvaal Biological Society Proceedings, 29/3/09.

† East Coast Fever—A Historical Review: Annual Report of the S.A. Association for the Advancement of Science, Grahamstown meeting, 1908.

The following post-mortem appearances were recorded in a case of pure infection with *Theileria parva* :—

*Post-mortem examination of Heifer No.* 683, 21*st March*, 1909.

Age : Two years.
Interim : Five hours after death.
Condition : Fair.
Blood : Not coagulated.
Subcutaneous tissue : Yellow infiltration.
Fat : Yellowish in colour.
Mediastimum : A few small hæmorrhages.
Pericardium : Empty.
Lungs : In inspirium ; pleura whitish ; both anterior lobes and lower half of middle lobe pneumonia, Stad. 11.
Bronchi : White mucus.
Pharynx : Slight hyperaemia; some pus in right tonsillae.
Heart : *Left endocard* yellowish, left ventricle contains blood coagulated.
*Right endocard* yellowish, right ventricle contains blood coagulated.
*Myocard* soft.
*Epicard* yellowish.
Liver : Enlarged, 45 × 35 cm., swollen, brown yellowish discoloration on section ; oedema ; infarcts. Bile thick, dark green.
Spleen : 42 × 16 cm. In ligamentum gastro lienale a haematome surrounded by fibrous tissue.
Stomach : Abomasum liquid contents, folds swollen. Mucosae slight oedema and hyperaemia.
Small intestines : *Jejunum* black discoloration, cross striped. *Ileum* whitish mucus and a few hyperaemic patches.
Large intestines : *Caecum* contracted, folded, slate colour. Valvula ileo caecalis discoloured.
Colon : Folded and black discoloration.
Kidneys : A few small infracts the size of a pea ; capsula easily detached and oedematous infiltration.
Internal lymphatic glands : Swollen and hyperaemic ; white spots in sinus.
Skull : *Brain* slight injection of pia.
*Marrow of bones* : Femur epiphysis soft, oedema and hyperaemic spots.
*Ribs* : Red, bone marrow, soft.

In the majority of cases lesions resembling infarcts are found in the kidneys and sometimes in the liver. They are specific for East Coast fever. In the kidney they may be detected on inspection of the surface of this organ as regular shaped areas or spots, their colour varying from red to white. If recent usually projecting above the surface and

surrounded by a hyperaemic zone, a yellowish zone may be seen within the red zone.

Collaud,* as a result of the examination of sections from kidneys of thirteen bovines sent by Theiler, noted an inflammatory process which he divides into the following stages, viz. :—

(1) Hæmorrhage from the capillaries (through their walls) into the kidney tissue.

(2) Cellular infiltration of the hæmorrhagic areas with leucocytes, lymphocytes and fibroblasts and destruction of the red corpuscles ; the leucocytes eventually disappearing, young connective tissue cells and lymphocytes finally remaining ; the epithelium canaliculaire is also destroyed.

(3) The connective tissue consolidates and invades not only the hæmorrhagic areas but the surrounding tissue as well. These different stages are observed in the same kidney.

According to the author, the *Theileria parvum* secretes a toxine which acts on the endothelium of the vessel walls and on the cells of the renal epithelium. (Hæmorrhages are found in all the organism.) The author proposes the denomination of *Nephritis hæmorrhagica piroplasmatica*. It would thus seem that the process is an inflammatory one leading to regeneration.

In a case of *Babesia mutans* infection, the *incubative* period varies from twenty to forty-five days. The *fever* period is not pronounced, but there is usually a continuous rise of temperature lasting for some weeks, ending in recovery. The *main symptoms* are as follows, viz. :—An animal with *Babesia mutans* may show no outward symptoms at all; in other cases a loss of condition is appreciable, and occasionally the symptoms of an anaemia are pronounced. Death due to pure mutans infection has as yet not been diagnosed. *Post-mortem lesions.*—In cases where piroplasms were found to be present it was usually complicated with other diseases, hence it is difficult to state where the lesions of mutans begin and where they end.

The post-mortem of an animal which was killed at the height of the infection was as follows :—

*Post-mortem examination of Ox* 660, 21*st November*, 1908. *Destroyed.*

Condition : Fair.

Pericard : Contains a little straw-coloured fluid.

Lungs : Normal. Foam at bifurcation of trachea.

Heart : *Left ventricle* empty, endocard ecchymosis.

*Right ventricle.* A small blood coagulum, endocard a few petechiae size of a pin's head.

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* L. Collaud : Beitrage zur Pathologischen Histologie der Niere bei, Rhodesian Redwater der Rinder in Süd Afrika (Piroplasmosis) Inaug. dissert., University Zurich, 1906 ; Bulletin l'Institut Pasteur, t. V, No. 6, page 252, 30/3/07.

Liver : On section a glazed reddish tint, hard, gall-bladder normal, contents yellowish green in colour.

Spleen : Slightly enlarged ; pulpae soft.

Stomachs : *Abomasum*—Mucous membrane patchy, hyperaemia.
*Rumen*—Full of pulpy ingesta, mucosa normal.
*Reticulum*—Contents pulpy, ingesta, mucosa normal

Small intestines : *Jejunum* ; mm. hyperaemia in streaks.
*Ileum* ; mm. hyperaemia slight, in streaks.

Large intestines : *Caecum* normal.
Colon ; mm. hyperaemia.

Kidneys : Right, normal.
Left—A whitish coloured body, size of a pea, resembling an infarct.

Bladder : Normal, contents normal.

## Microscopical Examination of Blood Smears.

When the blood of an animal sick with East Coast fever is microscopically examined after colouration by the method of Giemsa, or other Romanowsky modifications, parasites will be found to make their appearance in the globules a few days after a rise of temperature occurs; it will be observed that the parasites assume various shapes, and that a visible portion of each (nucleus) takes the chromatin stain (red), the remainder (protoplasm) taking the basic stain. The shapes observed will be as follows, viz. :—

Bacillary ring, ovoid (there appears to be a transitional form between the bacillary and ovoid forms). The average measurement of a number of the *bacillary* forms was found to be $1 \cdot 5\mu$ in length. They present the form of a baton with the chromatin concentrated at one extremity. In some cases the chromatin occupies about one-half of the total length of the baton. The cytoplasm may be of the same thickness throughout and straight—clove forms, or it may taper to a point at the extremity opposite the karyosome and be curved—comma form. The ring forms average about $1\mu$ in diameter ; they have a clear central portion (vacuole), and the disposition of the chromatin varies. The ovoid forms have likewise a central clear portion, their length being $1 \cdot 5\mu$ and their thickness $0 \cdot 75\mu$.

I have noted that the preparation of the smear during life exercises an influence on the size of the piroplasm, viz., when the blood corpuscles are much contracted the piroplasms are also found reduced in size. The cause of this phenomenon is in all probability the increase of osmotic pressure in the periglobular liquid due to a certain evaporation which takes place in making the smear.

A similar phenomenon is also noticed with *Babesia bigeminum* after death. In a case of East Coast fever, as already stated, parasites make their appearance a few days after a rise of temperature is recorded. At the beginning of the disease, no corpuscles are found to be affected. As the disease progresses an invasion of the corpuscles takes place. The increase may be slow and never reaches a high percentage ; it may be continuous and reach a high percentage, or it may be almost sudden, invading all the corpuscles.

In the initial or middle stages when the percentage of cells affected in a blood smear is yet small, it resembles that of a smear from *B. mutans* infection. It is here that a definite diagnosis becomes embarrassing. In such a case the only course would be to continue daily the microscopic examinations when an increase of parasites usually takes place. In a case of *Babesia mutans* no notable increase, or only a slow one, is observed. Blood changes, viz., lesions of anaemia will, however, in most cases be recognised with the appearance of this piroplasm. These take the form of anisocytosis, poikilocytosis, basophile, and polychromatic cells (nucleated red cells being rarely found). This is specific for mutans and not for parvum infection. In those cases of East Coast fever in which lesions of anaemia are noted, it is due to the complication with *Babesia bigeminum*, ordinary redwater (Texas fever), and other blood infection.

Other elements found in smears from spleen, kidneys, lymphatic glands, and in rare cases in blood smears of cattle affected with East Coast fever are the so-called Koch's granule. They were first described by Professor Koch. They are contained within a cell-shaped body and vary in size, shape, and numbers in the same smear ; two varieties may be distinguished, viz. :—1st. Those in which the cell-shaped body stains a deep blue tint and with granules varying in size ; the cell-shaped body varies in size from 1·5–9$\mu$ in diameter. 2nd. Here the granules vary in size from 0·5–0·75$\mu$, and the granular matter does not appear as compact as in the former variety.

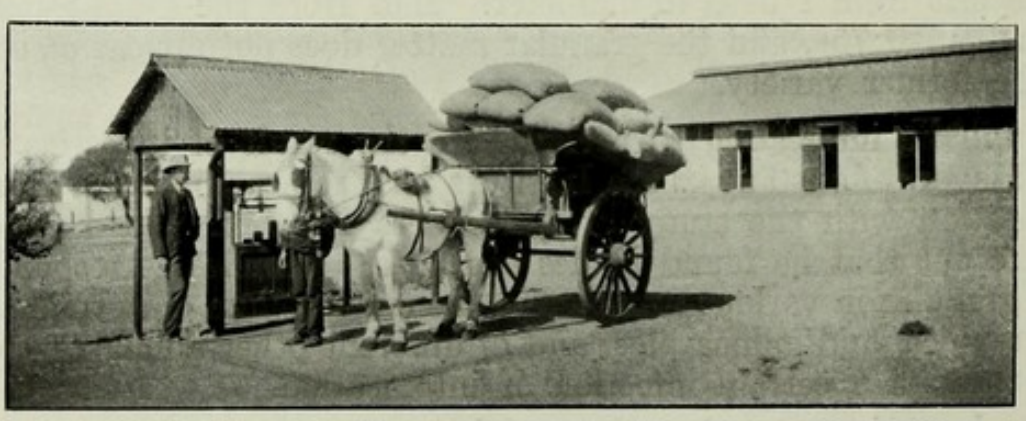
It will be interesting to review here the result of investigations of Martin Mayer,* Assistant in the Institute for Tropical Diseases, Hamburg, in connection with the study of spirochaeta Duttoni. In an end note he points out that he found bodies containing granules corresponding to Koch's granules in various numbers in the endothelium of normal kidneys of cattle, mice, and guinea pigs, and of different other animals which had passed through a certain infection or intoxication.

He also points out that these globules are produced by contraction of a part of the plasma of the cells, and that phagocytes transport these to other cells, and that it seems a new proof that the endothelial cells of the kidneys have a very great importance for destroying parasites and

* Archiv für Schiff und Tropen Hygiene, Bd. XII, No. 22, 1908.

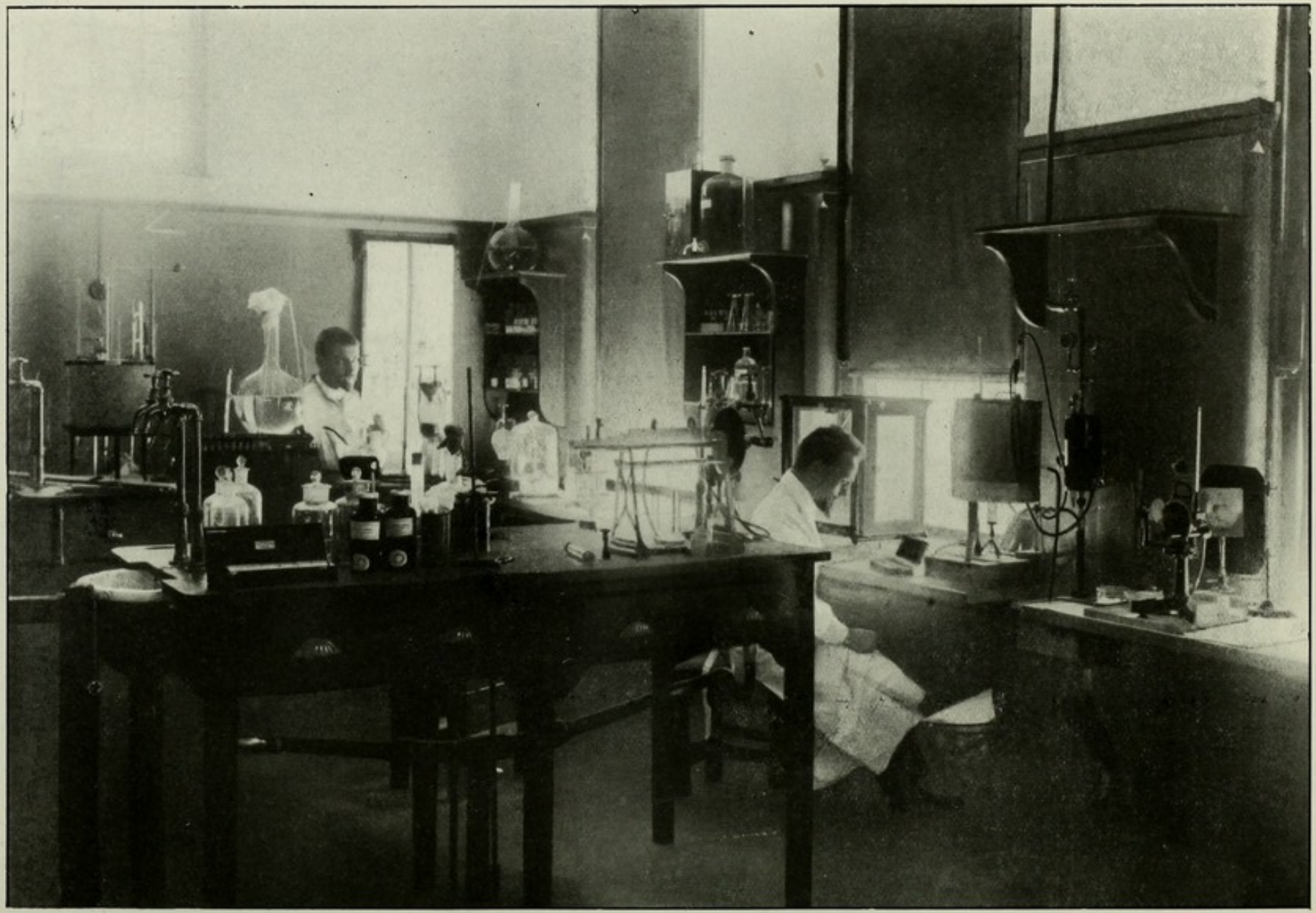
chemical noxious products. It thus appears that Koch's granules have been found in normal kidney smears. This is not in accordance with the result of the examination of smears from normal kidneys of cattle carried out by the writer for the purpose of ascertaining whether the bodies referred to by Koch were present in normal kidney and spleen smears of cattle. A number of each were examined; in all cases the results were negative. On the contrary the examination of a considerable number of smears of spleens of East Coast fever cattle sent in from different districts of the Transvaal always gave positive results. The writer has observed during the course of a microscopical examination of a number of normal smears from cattle minute reddish coloured granular bodies which are found scattered throughout the smear in some cases; in other, arranged in groups. In some instances they appear to be contained within a cell wall, in which case they resemble somewhat the appearance presented by blood plates in normal blood. They differ from Koch's granules in size and also staining reaction.

The constant presence of Koch's granules in the lymphatic glands, in the kidneys and spleen of animals affected with East Coast fever affords a means of basing a diagnosis in those cases, in which, as already pointed out, a microscopical examination does not permit of this. The presence of these granules in the lymphatic glands, kidneys, and spleen is specific for East Coast fever. The Bacteriological Laboratory make use of the fact for the definite diagnosis of all doubtful cases of infection with small piroplasms.

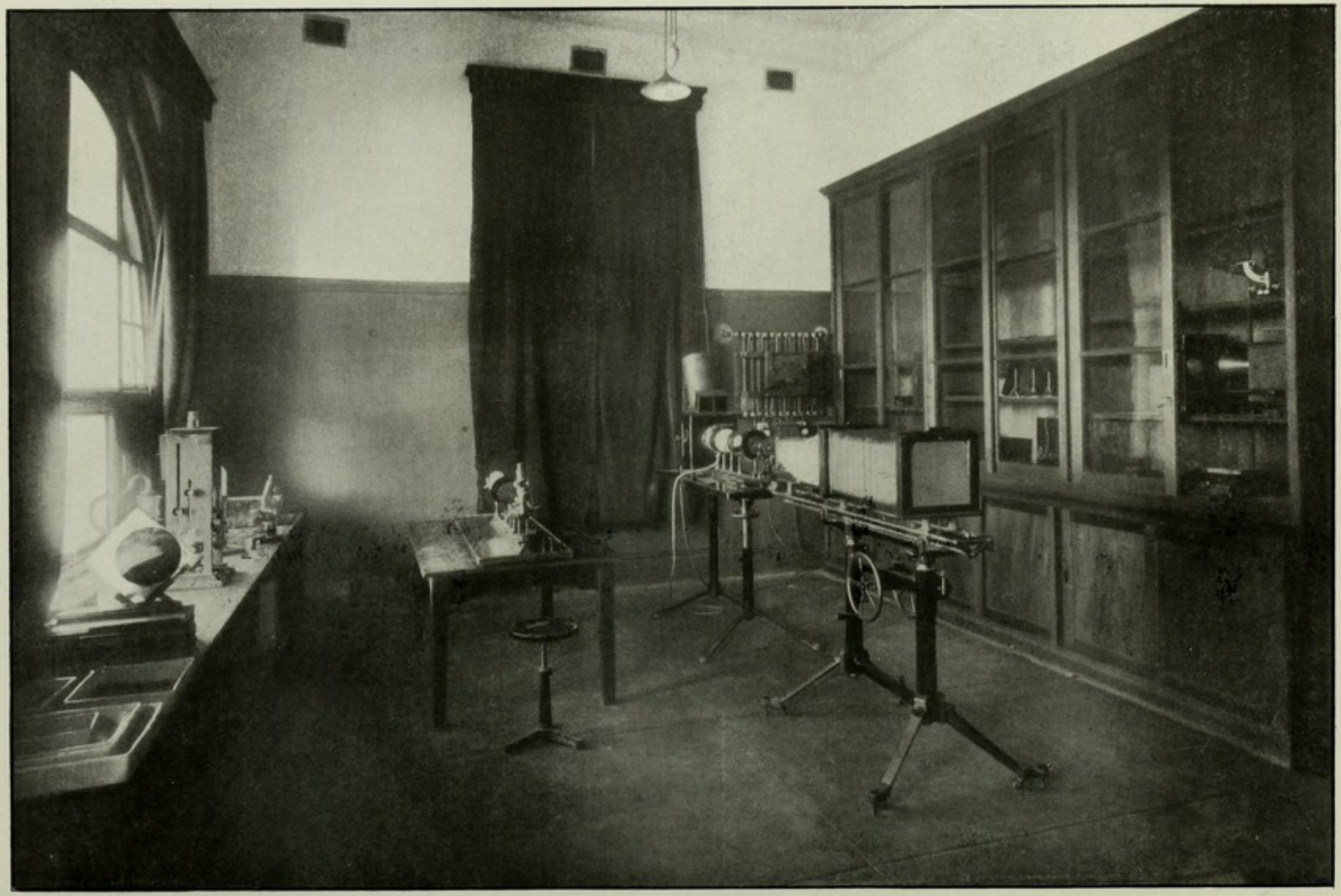


# Haemolysis in Practical Veterinary Science.

By Dr. WALTER FREI.



*The Physical Chemical Laboratory.*



*The Photographic Room.*

# Haemolysis in Practical Veterinary Science.

## I.—INTRODUCTION.

In producing and using anti-sera for curative and preventive purposes, it must be kept in mind that the injection of virulent matter gives rise, not only to specific anti-bodies against the microbes, but also against every proteid substance which accompanies them. Since the virulent matter in horse-sickness, for example, is contained in the blood, and the anti-serum is produced by the injection of such blood, it must be expected that anti-bodies are formed, not only against the micro-organism, but also against the constituents of such blood; the prevalent anti-body is, however, that corresponding to the horse-sickness organism.

The following table may elucidate the theoretical possibilities in passive and active immunisation:—

| *A.* Antigen. | *B.* Gives rise to anti-bodies. | *C.* Result of injection of serum containing the anti-bodies mentioned under *B.* |
|---|---|---|
| 1. Pure living culture of microbes. | Bactericidines, lysines, agglutinines (anti-toxines and precipitines), opsonines. | Bacteriolysis and agglutination. |
| 2. Dead culture or filtrate. | Anti-toxines, precipitines. | Neutralisation of the toxins, precipitation of the bacterial products. |
| 3. Blood or serum containing the virus which is generally ultra-visible. | Anti-bodies as under 1 and 2. In addition haemolysines, haemagglutinines, serum precipitines. | Action as under 1 and 2. In addition: precipitation of serum constituents, **Haemolysis.** |

In the Transvaal the following methods of immunisation are actually in practice:—

A.—*Against Bacterial diseases.*

1. Black Quarter:--Active immunisation by means of a vaccine, that is to say, with an artificially attenuated virus (heated and dried muscle substance)—two injections.
2. Pleuro-pneumonia:—Active immunisation by one injection of a pure culture (in bouillon Martin).

B.—*Against Protozoic diseases.*

3. Piroplasmoses of equines:—Active immunisation with the blood of immune animals.

4. Piroplasmoses of cattle (redwater):—Active immunisation with the blood of immune animals.

C.—*Against diseases caused by ultra-visible organisms.*

5. Blue-tongue in sheep:—Active immunisation by vaccination.

6. Rinderpest:—

(*a*) Simultaneous injection of virus and serum of hyperimmunised oxen.

(*b*) Injection of bile (vaccination).

7. Horse-sickness:—Simultaneous injection of virus and serum of hyperimmunised animals (as yet only applied to mules).

One injection of a small quantity either of

(1) fully virulent matter in order to infect an animal and to obtain virus; or

(2) specific anti-bodies leads to the production of the required anti-bodies and only of a negligible amount of accompanying anti-bodies.

Only the specific rinderpest anti-bodies were observed, even at the hyperimmunisation of oxen, with great quantities of virulent rinderpest blood in order to obtain a strong immune serum, recognised in vivo by the successful treatment of animals; in no instance were clinical or anatomical phenomena due to haemolysis or precipitation recorded. We are therefore confronted by the remarkable fact that cattle do not produce isolysines in their blood, i.e. substances with the property of dissolving cattle blood in vivo or in vitro, even after the injection of enormous quantities of blood.

Goats are able to react with the production of isolysines on intraperitoneal infusion of considerable quantities (800 and 900 c.c.) of dissolved goat blood corpuscles. But these anti-bodies do not appear in all goats treated in the same manner, nor does an isolytic goat-serum dissolve the corpuscles of all goats.*

As in goats, isolysines arise in the blood of horse, mule, or donkey after subcutaneous or intravenous introduction of blood, serum, or peritoneal liquid of one of these three. The serum of animals treated in this way is able to produce haemolysis in an emulsion of equine blood in vitro, and also, as recorded by Theiler,† in the living animal.‡

Theiler hyperimmunised horses, mules, and donkeys already immune (or salted) against the South African horse-sickness, that is to say, he injected them in different manners with large quantities of

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* Ehrlich and Morgenroth, Berl. Klin. Wochenschrift No. 1, 1899.

† Annual Report of the Government Veterinary Bacteriologist, 1903, Transvaal.

‡ In the following paper the conception of isolysines is rather wide, and it would be better, perhaps, to call the active substances in donkey serum which dissolve horse blood—heterolysines.

virulent blood (serum or peritoneal liquid) taken from sick animals. The horse-sickness anti-serum obtained from hyperimmunised animals proved to have effective anti-bodies against horse-sickness virus, but accidentally also isolysines which became active in some injected animals, and killed them by haemolysis.

Hence it was necessary

(1) to recognise a haemolytic serum amongst the immune sera by experiments in a test tube;

(2) to find out the haemolytic limit below which a slightly haemolytic serum could be used without any danger for injection. For this purpose it was advisable to express the haemolytic power by figures which he called the haemolytic index;

(3) to find a method of hyperimmunisation which gives only horse-sickness anti-bodies and no isolysines, or as little as possible.

The experiments were carried out with the object of solving these problems in a practical manner. Dr. Theiler kindly permitted me to use his experimental records. I am here publishing a collection of them arranged from the theoretical standpoint* of haemolysis, which is somewhat different from the abovementioned guiding ideas. It so happened that several experiments which, from my point of view, would have been useful in completing a series of deductions, were not carried out.

## Methodology.

In a series of tubes, containing each 2 c.c. of the sterile serum which had to be tested, were added 0·5 c.c. of defibrinated sterile blood of different animals, horses, or mules. The mixtures were kept at 37° for two hours, then placed in an ice chest over night. The change which took place was recorded as follows:—

*Marks.*

1. Trace—indicates a slight red discolouration of the lowest layer of the serum just above the deposit.
2. Slight—indicates the haemaglobin diffused up to the top of the liquid.
3. Distinct—indicates a deep red discolouration of the entire serum, but the deposit still dark.
4. Complete—indicates the deposit colourless, and the haemaglobin completely diffused out of the corpuscles.

*The Haemolytic Index.*

Example: 10 tubes, containing all the same serum, but blood from 10 different horses.

($a$) Number of tubes showing haemolysis=5 (i.e. the blood of 5 horses is dissolved by this serum).

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* Compare: W. Frei, Zur Theorie der Haemolyse, etc. Zeitschrift fur Infektionskrankheiten, parasit. Krankheiten, Hygiene der Haustiere, 2, 158 and 360, 1907.

(*b*) Number of marks of all 10 tubes together=8 (total of haemolysis degrees).

(*a*) and (*b*) multiplied=$5 \times 8 = 40$.

This figure divided by the number of horses whose blood was used (in this example 10) gives the degree of haemolysis for 10 horses=$\frac{5 \times 8}{10} = 4$.

Again divided by the number of horses whose blood was used, gives the average degree of haemolysis for 1 horse=$\frac{4}{10} = 0{\cdot}4$.

0·4 is the haemolytic index for the serum in question. In this way it was possible to compare the results of different haemolysis tests.

I may mention that for the purpose of drawing conclusions, only those tests in which the blood of at least 10 different horses were tested with one serum, have been utilised.

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## II.—ISOLYSINES IN EQUINES.

### A.—Hyperimmunisation by Subcutaneous Injection of the Virus.

(*a*) *Hyperimmunisation with virulent defibrinated blood.*

1. 11 horses hyperimmunised with virulent defibrinated horse blood.
2. 3 horses „ „ mule blood.
3. 3 mules „ „ horse blood.
4. 4 mules „ „ mule blood.
5. 1 donkey „ „ horse blood.

(*b*) *Hyperimmunisation with virulent serum.*

1. 3 mules hyperimmunised with virulent horse serum.
2. 4 mules „ „ mule serum.
3. 2 donkeys „ „ horse serum.
4. 3 donkeys „ „ mule serum.

(*c*) *Hyperimmunisation with virulent bouillon.*
(From peritoneal cavity.)

1. 2 mules hyperimmunised with virulent horse bouillon.
2. 4 mules „ „ mule bouillon.

### B.—Hyperimmunisation by Intrajugular Infusion of the Virus.

(*d*) *Hyperimmunisation with not defibrinated virulent blood by immediate transfusion into the jugular vein.*

1. Horses infused with virulent horse blood.
2. Mules „ „ mule blood.

It must be kept in mind that the immune serum derived from the hyperimmunised animals was never injected alone, but as the experiments were in connection with horse-sickness, and not haemolysis, a previous simultaneous or subsequent injection of virus took place.

## (*a*) Hyperimmunisation with Virulent Defibrinated Blood.

### 1. *Hyperimmunisation of Horses with Horse Blood.*

*Table* 1.

Haemolysis Test with Serum of Horses Hyperimmunised with Virulent Defibrinated Horse Blood.

| Number of Horse. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | | Haemolysis test in Vivo. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haem. test. c.c. | Time which elapsed between first & last bleeding. | Date of Haemolysis test. | Quotient. | | Index. | | Animals injected. | | Animals suffering from Haemolysis. | | Percentage of Deaths. | |
| | | | | | | | Horse. blood. | Mule. blood. | Horse. blood. | Mule. blood. | H. | M. | H. | M. | H. | M. |
| SH 1 | 5.—23/8/02 | 2,020 | — | — | — | — | — | — | — | — | 3s* | — | 0 | — | — | — |
| | 6.—17/9/02 | 3,520 | — | — | — | — | — | — | — | — | 17si* | 3s* | 0 | 0 | — | — |
| | 7.—21/10/02 | 5,520 | — | — | — | — | — | — | — | — | 2i* | 5s* | 0 | 0 | — | — |
| | 8.—13/11/02 | 8,020 | — | — | — | — | — | — | — | — | 31si* | 8si* | 0 | 0 | — | — |
| | 9.—20/2/03 | 9,020 | — | — | — | — | — | — | — | — | 11si* | 2si* | 0 | 0 | — | — |
| | 10.—11/8/03 | 11,020 | — | — | — | — | — | — | — | — | — | 8si* | 0 | 0 | — | — |
| | 11.—12/9/03 | 13,020 | — | — | — | 26–29/9/03 | $\frac{5\times6}{10\times10}$ | $\frac{5\times8}{10\times10}$ | 0·3 | 0·4 | 2 | — | 0 | — | — | — |
| 147 | — | 10,050 | — | — | — | — | — | — | — | — | 5si* | 1i* | 4 | 0 | 80 | 0 |
| | — | 10,050 | — | — | — | 15–16/9/03 | $\frac{4\times4}{6\times6}$ | $\frac{0}{12\times12}$ | 0·444 | 0 | — | — | — | — | — | — |
| | — | 10,050 | — | — | — | 22/9/03 | $\frac{4\times4}{6\times6}$ | — | 0·667 | — | — | — | — | — | — | — |
| | — | 10,050 | — | — | — | 26/9/03 | $\frac{5\times6}{10\times10}$ | — | 0·3 | — | — | — | — | — | — | — |
| | — | — | — | — | — | 29/9/03 | — | $\frac{5\times10}{10\times10}$ | — | 0·5 | — | — | — | — | — | — |
| | — | 14,050 | — | — | — | 9/10/03 | $\frac{27\times47}{38\times38}$ | $\frac{14\times25}{38\times38}$ | 0·879 | 0·242 | — | — | — | — | — | — |
| 160 | 9.—11/8/03 | 9,510 | — | — | — | 16/9/03 | $\frac{3\times6}{6\times6}$ | $\frac{0}{6\times6}$ | 0·5 | 0 | — | — | — | — | — | — |
| | 9.—11/8/03 | 9,510 | 14/9/03 | 80 | — | 22/9/03 | $\frac{0}{6\times6}$ | — | 0 | — | — | — | — | — | — | — |
| | 9.—11/8/03 | 9,510 | 14/9/03 | 80 | — | 29/9/03 | $\frac{10\times13}{10\times10}$ | — | 1·3 | — | 4s* | 1s* | 0 | 0 | — | — |
| | 11.—30/3/04 | 12,010 | 19/4/04 | 32,880 | 8 m.† | 25/4/04 | $\frac{6\times8}{13\times13}$ | $\frac{8\times9}{12\times12}$ | 0.284 | 0·5 | — | — | — | — | — | — |
| | 11.—30/3/04 | 12,010 | 22/7/04 | 54,380 | 11 m. | 30/8/04 | $\frac{0}{5\times5}$ | $\frac{4\times4}{5\times5}$ | 0 | 0·64 | — | — | — | — | — | — |
| | 11.—30/3/04 | 12,010 | 8/9/04 | 63,880 | 13 m. | 20/9/04 | $\frac{6\times8}{6\times6}$ | $\frac{4\times5}{6\times6}$ | 1·333 | 0·556 | — | — | — | — | — | — |
| | 11.—30/3/04 | 12,010 | 21/9/04 | 67,880 | 13 m. | 26/9/04 | — | $\frac{7\times12}{10\times10}$ | — | 0·84 | — | — | — | — | — | — |

* s = Subcutaneous injection of serum ; i = Intrajugular injection of serum. † m = months.

*Table* 1—(continued).

| Number of Horse. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | Time which elapsed between first and last bleeding. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse. blood. | Mule blood. |
| 172 | 8.—2/9/03 | 7,150 | 5/1/04 | 15,000 | 3 m. | 12/1/04 | $\frac{0}{3 \times 3}$ | $\frac{0}{2 \times 2}$ | 0 | 0 |
| | 9.—12/1/04 | 9,150 | 5/1/04 | 15,000 | 5 m. | 20/3/04 | $\frac{5 \times 5}{13 \times 13}$ | $\frac{9 \times 9}{12 \times 12}$ | 0·148 | 0·563 |
| | 11.—30/3/04 | 10,650 | 22/7/04 | 61,000 | 10 m. | 30/8/04 | $\frac{5 \times 5}{5 \times 5}$ | $\frac{3 \times 3}{5 \times 5}$ | 0·36 | 1·0 |
| | 11.—30/3/04 | 10,650 | 8/9/04 | 71,000 | 11 m. | 20/9/04 | $\frac{2 \times 2}{6 \times 6}$ | $\frac{4 \times 4}{6 \times 6}$ | 0·111 | 0·444 |
| 473 | 2.—13/12/04 | 6,000 | — | — | — | 23/12/04 | — | $\frac{4 \times 5}{14 \times 14}$ | — | 0·1 |
| | — | — | — | — | — | 29/12/04 | — | $\frac{4 \times 11}{4 \times 4}$ | — | 2·75 |
| 545 | 3.—9/2/05 | 8,500 | 24/2/05 | 6,000 | 1 m. | 28/2/05 | $\frac{2 \times 1}{10 \times 10}$ | $\frac{2 \times 1}{10 \times 10}$ | 0·2 | 0·2 |
| 611 | 3.—17/11/04 | 4,500 | — | — | — | 29/11/04 | $\frac{0}{6 \times 6}$ | $\frac{8 \times 11}{23 \times 23}$ | 0 | 0·166 |
| | 3.—17/11/04 | 4,500 | 19/1/05 | 22,500 | 2 m. | 2/2/05 | $\frac{0}{11 \times 11}$ | — | 0 | — |
| | 6.—7/2/05 | 9,000 | 25/2/05 | 35,500 | 3 m. | 28/2/05 | $\frac{1 \times 0·5}{10 \times 10}$ | $\frac{5 \times 4}{10 \times 10}$ | 0·005 | 0·2 |
| 612 | 3.—17/11/04 | 4,500 | — | — | — | 29/11/04 | $\frac{2 \times 3}{6 \times 6}$ | $\frac{9 \times 12}{23 \times 23}$ | 0·167 | 0·204 |
| | 3.—17/11/04 | 4,500 | 13/12/04 | 5,000 | 3 m. | 2/2/05 | $\frac{0}{11 \times 11}$ | — | 0 | — |
| | 6.—7/2/05 | 9,000 | 25/2/05 | 29,000 | 3 m. | 28/2/05 | $\frac{2 \times 1}{10 \times 10}$ | $\frac{5 \times 2·5}{10 \times 10}$ | 0·02 | 0·125 |
| 615 | 2.—13/12/04 | 5,000 | — | — | — | 23/12/04 | — | $\frac{3 \times 4}{14 \times 14}$ | — | 0·061 |
| | 2.—13/12/04 | 5,000 | 13/1/05 | 21,000 | 2 m. | 2/2/05 | $\frac{0}{11 \times 11}$ | — | 0 | — |
| | 4.—7/2/05 | 8,000 | 25/2/05 | 35,000 | 2 m. | 28/2/05 | $\frac{5 \times 2·5}{10 \times 10}$ | $\frac{5 \times 6}{10 \times 10}$ | 0·125 | 0·3 |
| 624 | Not injected | | — | — | — | 2/2/05 | $\frac{0}{11 \times 11}$ | — | 0 | — |
| | 3.—8/2/05 | 8,000 | 24/2/05 | 7,000 | 1 m. | 28/2/05 | $\frac{3 \times 1·5}{10 \times 10}$ | $\frac{3 \times 2·5}{10 \times 10}$ | 0·045 | 0·075 |
| 627 | 3.—17/11/04 | 4,000 | — | — | — | 29/11/04 | $\frac{1 \times 2}{6 \times 6}$ | $\frac{4 \times 6}{23 \times 23}$ | 0·056 | 0·045 |
| | 3.—17/11/04 | 4,000 | — | — | — | 2/2/05 | $\frac{0}{11 \times 11}$ | — | 0 | — |

*Discussion.*

Horse SH 1.—The haemolytic index was higher for mules than for horses. In no instance was an injection of this serum followed by haemolysis. Horse serum with a haemolytic index of 0·3 for horses did not seem to have any harmful effect on horse blood in vivo.

Horse 147.—The serum was in one instance more haemolytic for mules than for horses; in another test vice versa. When injected it killed four horses out of five.

Horse 160.—This serum seems to have been more haemolytic for mule than for horse blood. With an index of 1·3 it did not prove to be haemolytic in vivo. (All animals being injected subcutaneously.) With regard to the rise and disappearance of the isolysines, it may be observed that from the 29th September, 1903, to 25th April, 1904 (seven months), its haemolytic index for horses fell from 1·3 to 0·284, although two injections of blood, totalling 2,500 c.c., had been made. We must consider, therefore, that within the three months which passed from the date of haemolysis test, 29th September, 1903, to the tenth injection, 12th January, 1904, a large amount of the isolysines—perhaps all—had disappeared. I am not able to say that the loss of 32,880 c.c. of blood is the cause of the loss of haemolysines; the haemolytic index for *mules* had, however, increased between 25th April to 26th September, 1904, from 0·5 to 0·84, notwithstanding the withdrawal of 35,000 c.c. blood from the horse, and that no further hyperimmunisation had taken place.

Horse 172.—The haemolytic index was higher for mules than for horses.

Horse 611.—This serum also had a stronger haemolytic power for mule than for horse blood. The haemolytic indices were somewhat increasing from the third to the sixth injection; injections of 4,500 c.c. within three months did not give rise to haemolysines for horses.

Horse 612.—This serum was also more haemolytic for mules than for horses. The activity for horses—adversely to h. 611—decreased, although three more injections were made. Both horses 611 and 612 were injected with equal quantities of blood of the same origin at the same time, and the haemolysis tests were made on the same days; but the quantities of haemolysines which they developed in their blood were different. For instance, the haemolytic index for mule blood 611 = 0·2 for 612 = 0·125 at the same date. The only difference between the horses at this date is that 612 had been bled to the extent of 6,000 c.c. more than 611. (Compare h. 160.)

Whilst the haemolytic index in 611 increased during three months, it decreased in 612. The complete disappearance of haemolysines in 612 within two months is demonstrated by the haemolytic index (for horses) of 0·167 on the 29th November, 1904, and nil on the 2nd February, 1905. These horses clearly show by their parallel treatment the individual variations and differences in the appearance, disappearance, and absolute efficacy of haemolysines.

Horses 615 and 624.—Both gave a serum with higher haemolytic activity for mules than for horses. In 615 the haemolytic index for mules increased. The haemolytic index of 615 was four times higher than that of 624, whilst the amounts of injected blood were equal; from horse 615, however, five times more blood was taken than from 624.

In 627, as in 612, haemolysines disappeared within two months.

*Comparative Table.*

HORSES INJECTED WITH VIRULENT DEFIBRINATED HORSE BLOOD.

| No. of Horse. | No. of Injections. | No. of Animals from which the injected blood was taken. | Total Amount Injected. c.c. | Time which elapsed between first and last Hyperimmunisation. | Total Amount Bled. c.c. | Haemolytic Index. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Horse. | Mule. |
| SH 1 | 11 | 11 | 13,020 | 12½ m. | 43,900 | 0·3 | 0·4 |
| 147 | — | — | 10,050 | — | — | 0·3 | 0· |
| 160 R | 11 | 11 | 12,010 | 7½ m. | 32,880 | 0·284 | 0·5 |
| 172 R | 9 | 9 | 9,150 | 15 m. | 15,000 | 0·148 | 0·563 |
| 473 | 2 | 1 | 6,000 | 2 days | — | — | 0·1 |
| 545 | 3 | 2 | 8,500 | 4 days | 6,000 | 0·02 | 0·02 |
| 611 NoR | 6 | 2 | 9,000 | 3 m. | 35,500 | 0·005 | 0·2 |
| 612 NoR | 6 | 2 | 9,000 | 3 m. | 29,000 | 0·02 | 0·125 |
| 615 NoR | 4 | 2 | 8,000 | 2 m. | 35,000 | 0·125 | 0·3 |
| 627 NoR | 3 | 1 | 4,000 | 3 days | — | — | 0·045 |
| 624 | 3 | 2 | 8,000 | 3 days | 7,000 | 0·045 | 0·075 |

CONCLUSIONS.

1. The most remarkable fact is that from eleven horses hyperimmunised with defibrinated horse blood, the serum of 8 (= 72·7 %) is undoubtedly more haemolytic for mules than for horses. In one instance the haemolytic indices were equal, and in the other two cases the haemolytic tests are not satisfactory enough to make a definite statement.

   It appears, therefore, that the organism of horses "avoids" or is incapable of reacting on the introduction of horse blood by the production of anti-bodies exclusively specific for horse blood. We cannot tell whether the isolysines arising are the same for horses and mules, as the enumerated experiments do not settle this point. It would have to be carried out by specific absorption (according to Ehrlich and Morgenroth).

2. The horses SH 1, 147, 160, and 172, showing the highest haemolytic indices for horse and mule blood, had also been most frequently injected with the greatest quantities of blood, but a strict proportionality between the amount of blood injected and the value of the haemolytic index cannot be demonstrated. The haemolytic index seems rather to a great extent to be independent of the amount of blood injected and to differ individually; for instance, in horse 611, after having received 4,500 c.c. the serum had an index of 0·166 for mules. Horse 612, treated in the same way, even with the blood of the same horses, showed an index of 0·204; the haemolytic index of serum 624 was only 0·075, though the quantity injected into this horse amounted to nearly the double of that injected into horses 611 and 612. On the other hand, although horses 615 and 624 had both received 8,000 c.c., the haemolytic power of serum 615 is three and four times greater respectively than that of serum 624.

3. The haemolytic index varied considerably in the same horse.
   (*a*) The haemolytic index increased when further injections were made (horse 611 for mules and horses, horse 612 for horses, horse 615 for mules and horses).
   (*b*) The haemolytic index decreased, notwithstanding the higher hyperimmunisation (horse 160 for horses, and horse 612 for mules).
   (*c*) The haemolytic index increased without further injection of blood (horse 160 for mules).

4. A serum of which the haemolytic index was 1·3 or lower could safely be injected into horses; it did not produce haemolysis in vivo.

## 2. *Hyperimmunisation of Horses with Mule Blood.*

*Table 2.*

HAEMOLYSIS TEST WITH SERUM OF HORSES HYPERIMMUNISED WITH VIRULENT DEFIBRINATED MULE BLOOD.

| Number of Horse. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse blood. | Mule blood. |
| 264 | 2.—14/9/03 | 1,500 | — | — | — | 29–26/9/03 | $\frac{4\times7}{10\times10}$ | $\frac{7\times8}{10\times10}$ | 0·28 | 0·56 |
| | 3.—11/11/03 | 3,000 | — | — | — | 11/12/03 | $\frac{0}{5\times5}$ | $\frac{0}{5\times5}$ | 0 | 0 |
| | 3.—11/11/03 | 3,000 | — | — | — | 2/2/04 | $\frac{9\times14}{19\times19}$ | $\frac{8\times10}{14\times14}$ | 0·349 | 0·408 |
| | 4.—2/2/05 | 5,000 | 16/3/04 | 3,000 | 6 m. | 20/9/04 | $\frac{4\times7}{6\times6}$ | $\frac{5\times7}{6\times6}$ | 0·778 | 0·972 |
| | 4.—2/2/05 | 5,000 | 16/3/04 | 3,000 | 8 m. | 11/11/04 | — | $\frac{3\times7}{11\times11}$ | — | 0·173 |
| | 4.—2/2/05 | 5,000 | 16/3/04 | 3,000 | 8 m. | 26/11/04 | — | $\frac{3\times3}{9\times9}$ | — | 0·111 |
| 301 | 2.—14/9/03 | 1,500 | — | — | — | 26–29/9/03 | $\frac{5\times8}{10\times10}$ | $\frac{10\times23}{10\times10}$ | 0·4 | 2·3 |
| | 3.—30/12/03 | 3,000 | — | — | — | 30/1/04 | $\frac{5\times5}{5\times5}$ | $\frac{5\times5}{5\times5}$ | 1·0 | 1·0 |
| | 3.—30/12/03 | 3,000 | — | — | — | 2/2/04 | $\frac{8\times8}{23\times23}$ | $\frac{13\times19}{17\times17}$ | 0·121 | 0·845 |
| | 4.—2/2/05 | 5,000 | — | — | — | 20/9/04 | $\frac{1\times1}{6\times6}$ | $\frac{5\times6}{6\times6}$ | 0·028 | 0·833 |
| | 4.—2/2/05 | 5,000 | 21/9/04 | 8,000 | 7 m. | 11/11/04 | — | $\frac{2\times3}{11\times11}$ | — | 0·049 |
| | 4.—2/2/05 | 5,000 | 21/9/04 | 8,000 | 7 m. | 26/11/04 | — | $\frac{4\times4}{9\times9}$ | — | 0·197 |
| 398 | 6.—30/12/04 | 4,050 | 20/1/05 | 5,000 | 8 days | 28/1/05 | $\frac{5\times5}{5\times5}$ | $\frac{5\times9}{5\times5}$ | 1·0 | 1·8 |
| | 6.—30/12/04 | 4,050 | 25/2/05 | 23,000 | 1 m. | 28/2/05 | $\frac{1\times0·5}{10\times10}$ | $\frac{5\times3}{10\times10}$ | 0·005 | 0·15 |

*Discussion.*

Horse 264.—Throughout all the tests the haemolytic index was higher for mules than for horses. It decreased for mules in four months in spite of intercedent injection, increased however for horses, although the horse was not bled. There still remained a haemolytic power for mules in the serum ten months after the last injection.

Horse 301.—This serum was also more haemolytic for mules than for horses, similarly to that of horses 612 and 264. The haemolytic index of serum 301 for mules and horses decreased in four months notwithstanding 3,000 c.c. more blood had been injected and the horse had not been bled. The serum still proved to be haemolytic for mule blood ten months after the last hyperimmunisation. (Compare h. 264.)

Serum of horse 398 was more haemolytic for mules than for horses.

*Comparative Table.*

| No. of Horse. | Number of Injections. | Number of Animals from which the blood was taken. | Total Amount Injected. | Time of Hyperimmunisation. | Total Amount Bled. | Haemolytic Index. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Horse. | Mule. |
| 264 R | 2 | — | 1,500 | 1 month. | 0 | 0·28 | 0·56 |
| | 3 | — | 3,000 | 3 months. | 0 | 0·349 | 0·408 |
| 301 | 2 | 2 | 1,500 | 2 months. | 0 | 0·4 | 2·3 |
| NoR | 3 | 3 | 3,000 | 5 months. | 0 | 0·121 | 0·854 |
| 398 | 6 | 2 | 4,050 | 1 month. | 23,000 | 0·005 | 0·15 |

CONCLUSIONS.

1. Three horses hyperimmunised with defibrinated virulent mule blood gave a serum with stronger haemolytic properties for mule than for horse blood.
2. In two cases the serum preserved its haemolytic power for ten months.
3. The haemolytic index (*a*) decreased in spite of further blood injections for mules and horses (301), (*b*) increased with regard to horses, decreased with regard to mules within four months, in which time one injection took place (264).
4. The haemolytic index is again not dependent on the quantity of blood used for hyperimmunisation and the number of injections.

### 3. *Hyperimmunisation of Mules with Horse Blood.*

*Table 3.*

HAEMOLYSIS TEST WITH SERUM OF MULES HYPERIMMUNISED WITH VIRULENT DEFIBRINATED HORSE BLOOD.

| Number of Mule. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse blood. | Mule blood. |
| 150 | 8.—21/8/03 | 7,550 | — | — | — | 15/9/03 | $\frac{5\times11}{6\times6}$ | $\frac{6\times9}{6\times6}$ | 1·528 | 1·5 |
| | | | | | | 18/9/03 | — | $\frac{5\times13}{6\times6}$ | — | 1·805 |
| | | | | | | 22/9/03 | $\frac{5\times12}{6\times6}$ | — | 1·667 | — |
| | | | | | | 26/9/03 | — | $\frac{9\times23}{10\times10}$ | — | 2·07 |
| | | | | | | 29/9/03 | $\frac{10\times17}{10\times10}$ | — | 1.7 | — |
| | 9.—9/10/03 | 9,550 | 3/11/03 | 6,000 | 12 m. | 11/11/04 | — | $\frac{4\times8}{11\times11}$ | — | 0·264 |
| | 9.—9/10/03 | 9,550 | 3/11/03 | 6,000 | 12 m. | 26/11/04 | — | $\frac{5\times13}{9\times9}$ | — | 0·802 |
| 199 | 8.—12/8/03 | — | — | — | — | 15/9/03 | $\frac{6\times15}{6\times6}$ | $\frac{6\times6}{6\times6}$ | 2·5 | 1·0 |
| | | | | | | 18/9/03 | — | $\frac{5\times9}{6\times6}$ | — | 1·25 |
| | | | | | | 22/9/03 | $\frac{6\times19}{6\times6}$ | — | 3·167 | — |
| | | | | | | 26/9/03 | $\frac{10\times13}{10\times10}$ | $\frac{8\times14}{10\times10}$ | 1·3 | 1·12 |
| | | | | | | 11/11/04 | — | $\frac{3\times7}{11\times11}$ | — | 0·137 |
| | | | | | | 26/11/04 | — | $\frac{4\times10}{9\times9}$ | — | 0·494 |
| 201 | 8.—12/9/03 | 9,150 | — | — | — | 26/9/03 | $\frac{10\times40}{10\times10}$ | $\frac{7\times15}{10\times10}$ | 4·0 | 1·05 |

*Discussion.*

Mule 150.—The serum was more haemolytic for mules than for horses. The haemolytic index for mules decreased in 13½ months to ⅛ of the original value ; or perhaps the haemolysines disappeared and arose again after a further injection. They increased even within 1½ months although no injection took place.

Mule 199.—The serum was more haemolytic for horse blood. The index for mules decreased first and then increased fifteen months after injection.

Mule 201.—The serum of this mule dissolved blood corpuscles of horses much more energetically than of mules.

*Comparative Table.*

| No. of Mule. | No. of Injections. | Number of Animals from which the blood was taken. | Total Amount Injected. | Time of Hyper-immunisation. | Total Amount Bled. | Haemolytic Index. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Horse. | Mule. |
| 150 | 8 | 8 | 7,550 | 9 months | 0 | 1·7 | 2·07 |
| — | 9 | 9 | 9,550 | 11 ,, | 6,000 | — | 0·264 |
| 199 | 8 | 8 | 9,000 | 3 ,, | 0 | 1·3 | 1·12 |
| 201 | 8 | 8 | 9,150 | 7 ,, | 0 | 4·0 | 1·05 |

CONCLUSIONS.

1. Of three mules treated with horse blood two gave a serum with stronger haemolytic properties for horse than for mule blood, the serum of one of them, however, dissolved mule blood better than horse blood.
2. The haemolytic properties of the serum of one mule were still present fifteen months after the last immunisation, they seemed to vary in strength during this time.

## 4. *Hyperimmunisation of Mules with Mule Blood.*

*Table* 4.

HAEMOLYSIS TEST WITH SERUM OF MULES HYPERIMMUNISED WITH VIRULENT DEFIBRINATED MULE BLOOD.

| Number of Mules. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse blood. | Mule blood. |
| 320 | 2.—14/9/03 | 1,500 | — | — | — | 26/9/03 | $\frac{6 \times 12}{10 \times 10}$ | $\frac{7 \times 12}{10 \times 10}$ | 0·72 | 0·84 |
| | 4.—20/1/04 | 5,000 | — | — | — | 29/1/04 | $\frac{5 \times 9}{5 \times 5}$ | $\frac{2 \times 4}{5 \times 5}$ | 1·8 | 0·32 |
| | 4.—20/1/04 | 5,000 | — | — | — | 24/2/04 | $\frac{7 \times 9}{13 \times 13}$ | $\frac{1 \times 1}{8 \times 8}$ | 0·372 | 0·015 |
| 539 | Before hyperimmunisation. | | | — | — | 26/11/04 | — | $\frac{5 \times 8}{20 \times 20}$ | — | 0·1 |
| | 8.—12/2/05 | 12,350 | 25/2/05 | 31,000 | 3 m. | 28/2/05 | $\frac{5 \times 12}{16 \times 16}$ | $\frac{3 \times 6}{14 \times 14}$ | 0·234 | 0·092 |
| | 8.—12/2/05 | 12,350 | 25/2/05 | 31,000 | 4 m. | 7/3/05 | — | $\frac{2 \times 0·5}{7 \times 7}$ | — | 0·02 |
| 552 | Before hyperimmunisation. | | | — | — | 26/11/04 | — | $\frac{1 \times 1}{20 \times 20}$ | — | 0·003 |
| | 9.—12/2/05 | 10,150 | 13/2/05 | 25,000 | 3 m. | 28/2/05 | $\frac{6 \times 8}{16 \times 16}$ | $\frac{7 \times 5·5}{14 \times 14}$ | 0·188 | 0·196 |
| 573 | Before hyperimmunisation. | | | — | — | 26/11/04 | — | $\frac{10 \times 20}{20 \times 20}$ | — | 0·5 |
| | 7.—11/2/05 | 10,500 | 17/2/05 | 20,000 | 3 m. | 28/2/05 | $\frac{9 \times 17}{16 \times 16}$ | $\frac{7 \times 21}{14 \times 14}$ | 0·597 | 0·75 |

*Discussion.*

Mule 320.—The blood dissolving properties of this serum were stronger for mule than for horse blood. Notwithstanding two further injections, the haemolytic index fell within five months. Serum 539 was already haemolytic before the hyperimmunisations were started. After eight injections the serum was more destructive to horse than to mule erythrocytes.

Sera 552 and 573 also dissolved mule blood cells before the immunisation, afterwards the isolysines increased, acting more energetically on mule than horse blood.

*Comparative Table.*

| No. of Mule. | Number of Injections. | Number of Animals from which the blood was taken. | Total Amount Injected. | Time of Hyper-immunisation. | Total Amount Bled. | Haemolytic Index. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Horse. | Mule. |
| 320 | 2 | 2 | 1,500 | 1 month. | 0 | 0·72 | 0·84 |
| — | 4 | 4 | 5,000 | 5 months. | 0 | 0·372 | 0·015 |
| 539 | 8 | 2 | 12,350 | 2½ months. | 31,000 | 0·234 | 0·092 |
| 552 | 9 | 2 | 10,150 | 2½ months. | 25,000 | 0·188 | 0·196 |
| 573 | 7 | 2 | 10,500 | 2½ months. | 20,000 | 0·597 | 0·75 |

539, 552, and 573 were injected with the blood of the same mules and on the same dates.

CONCLUSIONS.

1. Three of the mules injected with mule blood had a serum more haemolytic for mule than for horse blood.
2. The sera of three mules dissolved mule blood before hyperimmunisation. These isolysines were probably provoked by the previous serum and virus injection.

## 5. *Hyperimmunisation of a Donkey with Horse Blood.*

*Table 5.*

HAEMOLYSIS TEST WITH SERUM OF ONE DONKEY HYPERIMMUNISED WITH VIRULENT DEFIBRINATED HORSE BLOOD.

| Number of Donkey. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse blood. | Mule blood. |
| 306 | 4.—2/9/03 | 2,350 | — | — | — | 12/9/03 | $\frac{21 \times 49}{21 \times 21}$ | $\frac{22 \times 47}{22 \times 22}$ | 2·333 | 2·136 |
| | | | | | | 15/9/03 | $\frac{6 \times 24}{6 \times 6}$ | $\frac{6 \times 18}{6 \times 6}$ | 4·0 | 3·0 |
| | | | | | | 18/9/03 | — | $\frac{6 \times 12}{6 \times 6}$ | — | 2·0 |
| | | | | | | 22/9/03 | $\frac{6 \times 16}{6 \times 6}$ | — | 2·667 | — |
| | | | | | | 26/9/03 | $\frac{10 \times 13}{10 \times 10}$ | $\frac{10 \times 17}{10 \times 10}$ | 1·3 | 1·7 |
| | 5.—9/10/03 | 2,850 | 3/12/03 | 8,000 | 13 m. | 11/11/04 | — | $\frac{11 \times 25}{11 \times 11}$ | — | 2·273 |
| | 5.—9/10/03 | 2,850 | 3/12/03 | 8,000 | 13 m. | 26/11/04 | $\frac{9 \times 26}{9 \times 9}$ | — | 2·889 | — |

*Discussion.*—Donkey 306 gave a remarkably haemolytic serum, apparently stronger for the blood corpuscles of horses than of mules. The haemolytic power decreased considerably during one month, but immediately increased above the original value after another blood injection.

## (*b*) Hyperimmunisation with Virulent Serum.

### 1. *Hyperimmunisation of Mules with Horse Serum.*

*Table* 6.

Haemolysis Test with Serum of Mules Hyperimmunised with Virulent Horse Serum.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse blood. | Mule blood. |
| 413 | 9.—15/7/04 | 14,400 | — | — | — | 29/7/04 | 1 × 1 / 19 × 19 | 6 × 6 / 17 × 17 | 0·003 | 0·128 |
| | | | — | — | — | 25/8/04 | 1 × 1 / 5 × 5 | 0 / 5 × 5 | 0·04 | 0 |
| | | | 8/9/04 | 8,000 | 2 m. | 20/9/04 | 0 / 6 × 6 | 3 × 3 / 6 × 6 | 0 | 0·25 |
| 415 | 7.—14/6/04 | 12,000 | — | — | — | 20/6/04 | 13 × 16 / 19 × 19 | 11 × 11 / 17 × 17 | 0·576 | 0·419 |
| | | | — | — | — | 25/8/04 | 1 × 2 / 5 × 5 | 0 / 5 × 5 | 0·08 | 0 |
| | | | 8/9/04 | 9,000 | 2 m. | 20/9/04 | 6 × 8 / 6 × 6 | 5 × 5 / 6 × 6 | 1·333 | 0·694 |
| 479 | 8.—16/7/04 | 18,100 | — | — | — | 29/7/04 | 10 × 11 / 14 × 14 | 6 × 6 / 11 × 11 | 0·561 | 0·298 |
| | 8.—16/7/04 | 18,100 | — | — | — | 25/8/04 | 2 × 2 / 5 × 5 | 0 / 5 × 5 | 0·16 | 0 |
| | 9.—21/9/04 | 20,300 | — | — | — | 11/11/04 | — | 3 × 6 / 11 × 11 | — | 0·148 |

## 2. *Hyperimmunisation of Mules with Mule Serum.*

*Table 7.*

HAEMOLYSIS TEST WITH SERUM OF MULES HYPERIMMUNISED WITH VIRULENT MULE SERUM.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse blood. | Mule blood. | Horse blood. | Mule blood. |
| 368 | 8.—5/5/04 | 10,000 | — | — | — | 15/5/04 | $\frac{4}{11 \times 11}$ | $\frac{0}{11 \times 11}$ | 0·033 | 0 |
| | 8.—5/5/04 | 10,000 | 22/7/04 | 17,000 | 4 m. | 25/8/04 | $\frac{1 \times 1}{5 \times 5}$ | $\frac{0}{5 \times 5}$ | 0·04 | 0 |
| | 8.—5/5/04 | 10,000 | 8/9/04 | 27,000 | 5 m. | 20/9/04 | $\frac{1 \times 1}{6 \times 6}$ | $\frac{0}{6 \times 6}$ | 0·028 | 0 |
| 445 | 7.—7/8/04 | 13,800 | — | — | — | 25/8/04 | $\frac{5 \times 15}{5 \times 5}$ | $\frac{3 \times 9}{5 \times 5}$ | 3·0 | 1·08 |
| | 7.—7/8/04 | 13,800 | — | — | — | 11/11/04 | — | $\frac{8 \times 12}{11 \times 11}$ | — | 0·793 |
| 464 | 5.—6/8/04 | 12,000 | — | — | — | 25/8/04 | $\frac{9}{11 \times 11}$ | $\frac{4}{11 \times 11}$ | 0·074 | 0·033 |
| | 5.—6/8/04 | 12,000 | — | — | — | 30/8/04 | $\frac{1 \times 1}{5 \times 5}$ | $\frac{0}{5 \times 5}$ | 0·04 | 0 |
| | 5.—6/8/04 | 12,000 | 8/9/04 | 14,000 | 2 m. | 20/9/04 | $\frac{2 \times 2}{6 \times 6}$ | $\frac{2 \times 2}{6 \times 6}$ | 0·111 | 0·111 |
| 482 | 6.—7/8/04 | 15,400 | — | — | — | 25/8/04 | $\frac{1 \times 1}{5 \times 5}$ | $\frac{3 \times 6}{5 \times 5}$ | 0·04 | 0·48 |

*Comparative Table to Table 6.*

| No. of Mule. | Number of Injection. | Number of Animals. | Total Amount Injected. | Period of Hyperimmunisation. | Total Amount Bled. | Haemolytic Index. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Horse. | Mule. |
| 413 | 9 | 7 | 14,400 | 2 months | 0 | 0·003 | 0·128 |
| 415 | 7 | 6 | 12,000 | 2½ ,, | 0 | 0·576 | 0·419 |
| 479 | 8 | 7 | 18,100 | 2½ ,, | 0 | 0·561 | 0·298 |

Hyperimmunisation of mules with horse serum still produced haemolytic serum, which in two instances was more active on horse than on mule blood.

*Comparative Table to Table 7.*

| No. of Mule. | Number of Injection. | Total Amount Injected. | Period of Hyper-immunisation. | Total Amount Bled. | Haemolytic Index. | |
|---|---|---|---|---|---|---|
| | | | | | Horse. | Mule. |
| 368 | 8 | 10,000 | 5 months | 0 | 0·033 | 0 |
| 445 | 7 | 13,800 | 3 months | 0 | (3·0) | (1·08) |
| — | 7 | 13,800 | 3 months | — | — | 0·798 |
| 464 | 5 | 12,000 | 3 months | 0 | 0·074 | 0·033 |
| 482 | 6 | 15,400 | 5 months | 0 | (0·04) | (0·48) |

Mules treated subcutaneously with mule serum produced haemolytic serum, which in two cases dissolved horse blood better than mule blood.

## 3. *Hyperimmunisation of Donkeys with Horse Serum.*

*Table* 8.

HAEMOLYSIS TEST WITH SERUM OF DONKEYS HYPERIMMUNISED WITH VIRULENT HORSE SERUM.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse. | Mule. | Horse. | Mule. |
| 381 | 7.—14/7/04 | 7,900 | — | — | — | —/7/04 | $\frac{6 \times 18}{6 \times 6}$ | $\frac{7 \times 25}{7 \times 7}$ | 3·0 | 3·571 |
| | 7.—14/7/04 | 7,900 | — | — | — | 25/8/04 | $\frac{4 \times 12}{4 \times 4}$ | $\frac{5 \times 15}{5 \times 5}$ | 3.0 | 3·0 |
| | 7.—14/7/04 | 7,900 | — | — | — | 11/11/04 | — | $\frac{10 \times 20}{11 \times 11}$ | — | 1·653 |
| | 7.—14/7/04 | 7,900 | — | — | — | 26/11/04 | — | $\frac{8 \times 9}{9 \times 9}$ | — | 0·889 |
| 514 | 8.—14/7/04 | 8,700 | — | — | — | —/7/04 | $\frac{6 \times 10}{6 \times 6}$ | $\frac{5 \times 7}{7 \times 7}$ | 1·667 | 0·714 |
| | 8.—14/7/04 | 8,700 | — | — | — | 25/8/04 | $\frac{4 \times 8}{4 \times 4}$ | $\frac{4 \times 5}{5 \times 5}$ | 2·0 | 0·8 |

*Comparative Table.*

| No. of Donkey. | Number of Injection. | No. of Animals. | Total Amount Injected. | Period of Hyperimmunisation. | Total Amount Bled. | Haemolytic Index. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Horse. | Mule. |
| 381 | 7 | 7 | 7,900 | 2½ months | 0 | (3·0) | (3·571) |
| 514 | 8 | 6 | 8,700 | 2½ ,, | 0 | (1·667) | (0·714) |

CONCLUSION.

Two donkeys injected with horse serum developed isolysines in their blood for horses and mules. The haemolytic index for mules in 381 decreased to the half of its value within fifteen days.

## 4. *Hyperimmunisation of Donkeys with Mule Serum.*

*Table* 9.

HAEMOLYSIS TEST WITH SERUM OF DONKEYS HYPERIMMUNISED WITH VIRULENT MULE SERUM.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse. | Mule. | Horse. | Mule. |
| 379 | 5.—23/6/04 | 5,900 | — | — | — | 30/7/04 | $\frac{1 \times 1}{12 \times 12}$ | $\frac{2 \times 3}{13 \times 13}$ | 0·07 | 0·036 |
| | 5.—23/6/04 | 5,900 | — | — | — | 25/8/04 | $\frac{1 \times 1}{4 \times 4}$ | $\frac{2 \times 3}{4 \times 4}$ | 0·063 | 0·375 |
| | 5.—23/6/04 | 5,900 | 8/9/04 | 5,500 | 2 m. | 20/9/04 | $\frac{0}{6 \times 6}$ | $\frac{0}{6 \times 6}$ | 0 | 0 |
| 515 | 5.—9/8/04 | 6,000 | — | — | — | —/8/04 | $\frac{5 \times 9}{6 \times 6}$ | $\frac{6 \times 10}{7 \times 7}$ | 1·25 | 1·224 |
| | 5.—9/8/04 | 6,000 | — | — | — | 25/8/04 | $\frac{4 \times 8}{4 \times 4}$ | $\frac{5 \times 7}{5 \times 5}$ | 2·0 | 1·4 |
| 516 | 6.—9/8/04 | 6,400 | — | — | — | —/8/04 | $\frac{1 \times 1}{12 \times 12}$ | $\frac{2 \times 2}{13 \times 13}$ | 0·007 | 0·024 |
| | 6.—9/8/04 | 6,400 | — | — | — | 25/8/04 | $\frac{1 \times 1}{4 \times 4}$ | $\frac{2 \times 2}{5 \times 5}$ | 0·063 | 0·16 |
| | 6.—9/8/04 | 6,400 | 8/9/04 | 6,000 | 2 m. | 20/9/04 | $\frac{0}{6 \times 6}$ | $\frac{0}{6 \times 6}$ | 0 | 0 |

*Discussion.*

515 and 516 were injected at the same time and with the same serum of the same mules (516 had one injection more). The haemolytic index in 516, however, appeared to be much lower.

CONCLUSION.

The sera of all three donkeys injected with mule serum were haemolytic for horse and mule blood.

## (*c*) Hyperimmunisation with Virulent Bouillon.

*Table* 10.

Haemolysis Tests with Serum of Mules Hyperimmunised with (*a*) Bouillon from Horse, (*b*) from Mule (Peritoneal Exudate).

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Injection. | Total amount injected. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. | | Index. | |
| | | | | | | | Horse. | Mule. | Horse. | Mule. |
| *a* 469 | 5.—20/8/04 | 11,500 | — | — | — | 27/8/04 | 0<br>5 × 5 | 0<br>5 × 5 | 0 | 0 |
| *a* 501 | 3.—16/7/04 | 6,700 | — | — | — | 27/8/04 | 5 × 5 | 2 × 2 | 0·8 | 0·16 |
| | 4.—10/9/04 | 9,200 | 24/10/04 | 10,000 | 2 m. | 26/11/04 | 5 × 5<br>— | 5 × 5<br>2 × 2<br>9 × 9 | — | 0·049 |
| *b* 492 | 4.—1/8/04 | 8,500 | — | — | — | 27/8/04 | 5 × 6<br>5 × 5 | 1 × 1<br>5 × 5 | 1·2 | 0·04 |
| *b* 496 | 5.—9/8/04 | 8,500 | — | — | — | 27/8/04 | 0<br>5 × 5 | 0<br>5 × 5 | 0 | 0 |
| *b* 509 | 4.—9/8/04 | 9,000 | — | — | — | 27/8/04 | 0<br>5 × 5 | 0<br>5 × 5 | 0 | 0 |

Conclusion.

One mule treated with peritoneal exudate of horses and another injected with peritoneal exudate of mules showed haemolysines in their serum, thus proving that it is possible to produce isolysines by subcutaneous injection of peritoneal liquid.

## Summary of Results from Subcutaneous Injection of Blood, Serum, and Peritoneal Exudate of Horses and Mules into Equines.

The above experiments demonstrated that in every instance isolysines for horses and mules arose.

(1) In horses after injections of horse and mule blood.

(2) In mules after injections of horse and mule blood and serum (except 368 for mules).

(3) In donkeys after injections of horse blood and serum and mule serum.

It was also possible to obtain isolysines for horse and mule blood in mules by subcutaneous injections of horse and mule peritoneal exudate.

It stands to reason that horses would also produce isolysines for horses and mules after injections of horse and mule serum and probably of peritoneal exudate as well.

These experiments give no indication how soon the isolysines arise, but they allow of a conclusion regarding the length of time a serum preserves its dissolving properties. Generally they were present up to six months after the last hyperimmunisation. In three instances (horses 611, 612, and 627) the serum was no longer haemolytic after two and two and a half months respectively, but in two cases (horses 264 and 301) it dissolved mule blood ten months, and in one case even fifteen and a half months after the last injection (mule 199).

In the majority of cases after the injection of mules with horse blood, the isolytic serum was more haemolytic for horse blood.

In the majority of cases after the injection of mules with horse serum the isolytic serum was more haemolytic for horse blood.

In 50 per cent. of cases after the injection of mules with mule serum the isolytic serum was more haemolytic for horse blood.

After the injection of a donkey with horse blood the isolytic serum was more haemolytic for horse blood.

In the majority of cases after the injection of horses with horse blood the isolytic serum was more haemolytic for mule blood.

In all cases after the injection of horses with mule blood the isolytic serum was more haemolytic for mule blood.

It appears, therefore, that the injected animal avoids producing isolysines against the blood corpuscles of the variety to which it belongs. The same phenomenon could be observed after the infusion of mules with mule blood, but it did not take place after the infusion of horses with horse blood. The best isolytic serum, for instance, for horse blood was therefore obtained by injecting donkeys or mules with horse blood. The tables support this idea, for the haemolytic indices were indeed highest of the sera of one donkey, and of mules treated with horse blood it was also seen that injection of defibrinated blood provoked a more intensive production of isolysines than serum alone. The blood corpuscles are of course the best carriers of haemolytic, i.e. isolytic, antigenes.

The haemolytic power of a serum is to a great extent not dependent on the quantity of blood or serum injected, nor on the number of animals the antigenes were derived from; the number of injections and the total time of hyperimmunisation make no difference. Bleedings have apparently no influence on the haemolytic index.

For the rise of isolysines in an animal it is not necessary that it reacts with hyperthermia on hyperimmunisation.

### Animals Hyperimmunised by Infusion.

Hyperimmunisation by infusion was done by connecting the jugular vein of the animal which is suffering from horse-sickness with the corresponding vein of the immune animal which has to be hyperimmunised. This method is simple and expedient, and it was usually continued for six minutes. Measured through a trocar and the pipes in use, about half a litre of blood passed through in a minute. There were usually three infusions made into one animal on two or three succeeding days.

1. *Infusion of Horses with Horse Blood.*

*Table* 11.

HAEMOLYSIS TESTS WITH SERUM OF HORSES HYPERIMMUNISED BY INFUSION OF VIRULENT HORSE BLOOD.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Infusion. | Total amount infused. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. Horse. Respective of | Quotient. Mule. Number Tests. | Index. Horse. | Index. Mule. |
| 290 | 3.—11/4/05 | 7,250 | — | — | — | 20/4/05 | 5 | 5 | 0 | 0 |
| | 4.—25/4/05 | 9,750 | — | — | — | 19/5/05 | 10 | 10 | 0 | 0 |
| | 5.—25/7/05 | 18,750 | 18/7/05 | 30,000 | 3 m. | 9/8/05 | 10 | 10 | 0 | 0 |
| | 6.—20/12/05 | 27,750 | 11/12/05 | 82,000 | 8 m. | 9/1/06 | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | 5 | 0·02 | 0 |
| | 7.—21/1/06 | 36,750 | 15/1/06 | 93,000 | 9 m. | 6/2/06 | 5 | 5 | 0 | 0 |
| 602 | 3.—16/5/05 | 9,500 | — | — | — | 2/6/05 | 5 | 5 | 0 | 0 |
| | 4.—1/8/05 | 18,500 | 18/7/05 | 24,000 | 2 m. | 14/8/05 | 5 | 5 | 0 | 0 |
| | 5.—6/12/05 | 27,500 | — | — | — | — | No | Test | made. | |
| | 6.—20/1/06 | 36,500 | 2/1/06 | 91,000 | 7 m. | 6/2/06 | 5 | 5 | 0 | 0 |
| 615 | 6.—31/7/05 | 17,000 | 25/7/05 | 109,000 | 8 m. | 14/8/05 | 5 | 5 | 0 | 0 |
| | 7.—30/11/05 | 25,500 | 20/11/05 | 164,000 | 12 m. | 12/12/05 | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | 5 | 0·02 | 0 |
| | 8.—12/1/06 | 33,500 | 8/1/06 | 176,000 | 13 m. | 30/1/06 | $\frac{3 \times 2}{5 \times 5}$ | $\frac{2 \times 1{\cdot}5}{5 \times 5}$ | 0·24 | 0·12 |
| | 9.—31/3/06 | 42,500 | 12/3/06 | 191,000 | 15 m. | 23/4/06 | 5 | 5 | 0 | 0 |
| 714 | 1.—11/4/05 | 9,000 | — | — | — | 20/4/05 | 5 | 5 | 0 | 0 |
| | 2.—24/7/05 | 18,000 | 11/7/05 | 36,000 | 3 m. | 7/8/05 | $\frac{5 \times 4}{5 \times 5}$ | $\frac{3 \times 3}{5 \times 5}$ | 0·8 | 0·36 |
| 731 | 1.—11/4/05 | 7,500 | — | — | — | 20/4/05 | 5 | 5 | 0 | 0 |
| | 2.—21/7/05 | 16,500 | 11/7/05 | 34,000 | 3 m. | 9/8/05 | 5 | 5 | 0 | 0 |
| | 3.—22/11/05 | 25,500 | 30/10/05 | 72,000 | 6 m. | 12/12/05 | 5 | 5 | 0 | 0 |
| | 4.—11/1/06 | 34,500 | 8/1/06 | 90,000 | 9 m. | 30/1/06 | 5 | 5 | 0 | 0 |
| 811 | 1.—11/4/05 | 7,000 | — | — | — | 20/4/05 | 5 | 5 | 0 | 0 |
| | 2.—25/7/05 | 16,000 | 11/7/05 | 37,000 | 3 m. | 9/8/05 | 5 | 5 | 0 | 0 |
| | 3.—15/11/05 | 25,000 | 13/11/05 | 87,000 | 7 m. | 28/11/05 | 5 | 5 | 0 | 0 |
| | 4.—12/1/06 | 34,000 | 8/1/06 | 112,000 | 9 m. | 30/1/06 | 5 | 5 | 0 | 0 |
| | 5.—6/4/06 | 43,000 | 12/3/06 | 134,000 | 11 m. | 23/4/06 | 5 | 5 | 0 | 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 812 | 1.—11/4/05 | 9,000 | — | — | — | 20/4/05 | 5 | 5 | 0 | 0 |
| | 2.—25/7/05 | 18,000 | 11/7/05 | 31,000 | 3 m. | 9/8/05 | 5 | 5 | 0 | 0 |
| | 3.—23/11/05 | 27,000 | 27/11/05 | 71,000 | 7 m. | 12/12/05 | 5 | 5 | 0 | 0 |
| | 4.—11/1/06 | 36,000 | 8/1/06 | 85,500 | 9 m. | 30/1/06 | 5 | 5 | 0 | 0 |
| | 5.—6/4/06 | 45,000 | 12/3/06 | 103,500 | 11 m. | 23/4/06 | 5 | 5 | 0 | 0 |
| | 6.—9/10/06 | 54,000 | 1/10/06 | 140,500 | 18 m. | 29/10/06 | 4 × 4 / 5 × 5 | 5 × 5·5 / 5 × 5 | 0·64 | 1·1 |
| | 7.—12/12/06 | 63,000 | 10/12/06 | 152,500 | 20 m. | 8/1/07 | 2 × 1 / 5 × 5 | 5 × 5·5 / 5 × 5 | 0·08 | 1·1 |
| | — | — | 7/1/07 | 156,500 | 21 m. | 15/1/07 | 0 / 5 × 5 | 1 × 1 / 5 × 5 | 0 | 0·04 |
| 869 | 1.—16/5/05 | 9,000 | — | — | — | 2/6/05 | 5 | 5 | 0 | 0 |
| | 2.—1/8/05 | 18,000 | 18/7/05 | 24,000 | 2 m. | 14/8/05 | 5 | 5 | 0 | 0 |
| | 3.—6/12/05 | 27,000 | — | — | — | — | No | Test | made. | |
| | 4.—12/1/06 | 36,000 | 2/1/06 | 83,000 | 7 m. | 30/1/06 | 2 × 1 / 5 × 5 | 1 × 0·5 / 5 × 5 | 0·08 | 0·02 |
| 874 | 1.—11/4/05 | 8,000 | — | 0 | — | 20/4/05 | 5 × 3 / 5 × 5 | 3 × 2 / 5 × 5 | 0·6 | 0·24 |
| 882 | 1.—10/4/05 | 9,000 | — | 0 | — | 20/4/05 | 5 | 5 | 0 | 0 |
| | 2.—25/7/05 | 18,000 | 11/7/05 | 40,000 | 3 m. | 9/8/05 | 5 | 5 | 0 | 0 |
| | 3.—30/11/05 | 27,000 | 4/12/05 | 109,000 | 8 m. | 12/12/05 | 5 | 5 | 0 | 0 |
| | 4.—11/1/06 | 35,500 | 8/1/06 | 122,000 | 9 m. | 30/1/06 | 5 | 5 | 0 | 0 |
| 1055 | 1.—16/5/05 | 9,000 | — | — | — | 2/6/05 | 5 | 5 | 0 | 0 |
| | 2.—22/8/05 | 18,000 | 18/9/05 | 43,000 | 4 m. | 2/10/05 | 1 × 0·5 / 5 × 5 | 1 × 0·5 / 5 × 5 | 0·02 | 0·02 |
| | 2.—22/8/05 | 18,000 | 16/10/05 | 56,000 | 5 m. | 31/10/05 | 5 | 5 | 0 | 0 |
| | 3.—12/12/05 | 27,000 | 11/12/05 | 81,000 | 7 m. | 28/12/05 | 2 × 6 / 5 × 5 | 2 × 6 / 5 × 5 | 0·48 | 0·48 |
| 1056 | 1.—16/5/05 | 9,000 | — | — | — | 2/6/05 | 5 | 5 | 0 | 0 |
| | 2.—23/8/05 | 18,000 | 18/9/05 | 36,000 | 4 m. | 2/10/05 | 5 | 5 | 0 | 0 |
| | 2.—23/8/05 | 18,000 | 16/10/05 | 46,000 | 5 m. | 31/10/05 | 5 | 5 | 0 | 0 |
| | 3.—12/12/05 | 27,000 | 11/12/05 | 61,000 | 7 m. | 28/12/05 | 5 | 5 | 0 | 0 |
| | 4.—6/3/06 | 36,000 | 26/2/06 | 91,000 | 10 m. | 27/3/06 | 5 | 5 | 0 | 0 |
| 1076 | 1.—14/6/05 | 9,000 | 21/8/05 | 33,000 | 3 m. | 2/9/05 | 5 | 5 | 0 | 0 |
| | 2.—6/9/05 | 18,000 | 18/9/05 | 46,000 | 4 m. | 30/9/05 | 5 | 5 | 0 | 0 |
| | 2.—6/9/05 | 18,000 | 27/12/05 | 92,000 | 7 m. | 9/1/06 | 1 × 0·5 / 5 × 5 | 5 | 0·02 | 0 |
| | 3.—11/1/06 | 27,000 | 8/1/06 | 98,000 | 8 m. | 30/1/06 | 5 | 5 | 0 | 0 |
| 1077 | 1.—14/6/05 | 9,000 | 21/8/05 | 31,000 | 3 m. | 2/9/05 | 5 | 5 | 0 | 0 |
| | 2.—6/9/05 | 18,000 | 18/9/05 | 43,000 | 4 m. | 30/9/05 | 3 × 1·5 / 5 × 5 | 0 / 5 × 5 | 0·18 | 0 |
| | 2.—6/9/05 | 18,000 | 18/9/05 | 43,000 | 5 m. | 17/10/05 | 2 × 1 / 5 × 5 | 1 × 0·5 / 5 × 5 | 0·08 | 0·02 |
| | 2.—6/9/05 | 18,000 | 30/10/05 | 56,000 | 7 m. | 21/11/05 | 1 × 1 / 5 × 5 | 4 × 7 / 5 × 5 | 0·04 | 1·12 |

*Table* 11—(*continued*).

HAEMOLYSIS TESTS WITH SERUM OF HORSES HYPERIMMUNISED BY INFUSION OF VIRULENT HORSE BLOOD.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Infusion. | Total amount infused. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. Horse. Respective of | Quotient. Mule. Number Tests. | Index. Horse. | Index. Mule. |
| 1087 | 1.—20/6/05 | 8,500 | — | — | — | 5/7/05 | $\frac{7 \times 5}{10 \times 10}$ | $\frac{4 \times 3}{10 \times 10}$ | 0·35 | 0·12 |
| 1089 | 1.—14/6/05 | 8,500 | — | — | — | 5/7/05 | 10 | 5 | 0 | 0 |
| | 2.—9/9/05 | 17,500 | 28/8/05 | 24,000 | 3 m. | 30/9/05 | 5 | 5 | 0 | 0 |
| 1094 | 1.—14/6/05 | 9,000 | — | — | — | 5/7/05 | 5 | 5 | 0 | 0 |
| | 2.—23/9/05 | 18,000 | 11/9/05 | 37,000 | 4 m. | 10/10/05 | 10 | 5 | 0 | 0 |
| | 3.—20/1/06 | 27.000 | 2/1/06 | 77,000 | 8 m. | 6/2/06 | 5 | 5 | 0 | 0 |
| | 4.—18/5/06 | 36,000 | 30/4/06 | 105,000 | 12 m. | 6/6/06 | 5 | 5 | 0 | 0 |
| 1166 | 4.—21/7/05 | 9,000 | — | — | — | 31/7/05 | $\frac{4 \times 2}{5 \times 5}$ | $\frac{3 \times 1·5}{5 \times 5}$ | 0·32 | 0·18 |
| 1170 | 1.—18/7/05 | 8,500 | — | — | — | 31/7/05 | $\frac{0}{5 \times 5}$ | $\frac{1 \times 0·5}{5 \times 5}$ | 0 | 0·02 |
| | 1.—18/7/05 | 8,500 | 9/10/05 | 32,500 | 3 m. | 24/10/05 | 5 | 5 | 0 | 0 |
| | 2.—8/11/05 | 17,500 | 23/10/05 | 37,500 | 4 m. | 21/11/05 | 5 | 5 | 0 | 0 |
| | 3.—1/2/06 | 26,500 | 2/1/06 | 57,500 | 7 m. | 20/2/06 | 5 | 5 | 0 | 0 |
| | 4.—23/5/06 | 35,500 | 30/4/06 | 73,500 | 11 m. | 19/6/06 | 5 | 5 | 0 | 0 |
| 1174 | 1.—24/7/05 | 8,500 | — | — | — | 7/8/05 | $\frac{5 \times 4·5}{5 \times 5}$ | $\frac{3 \times 2·5}{5 \times 5}$ | 0·9 | 0·3 |
| | 1.—24/7/05 | 8,500 | — | — | — | 19/8/05 | $\frac{5 \times 5}{5 \times 5}$ | $\frac{5 \times 4·5}{5 \times 5}$ | 1·0 | 0·9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1175 | 1.—21/7/05 | 9,000 | — | — | — | 7/8/05 | 5 | 5 | 0 | 0 |
| | 2.—15/11/05 | 18,000 | — | — | — | — | — | — | — | — |
| | 3.—27/2/06 | 27,000 | 19/3/06 | 91,000 | 8 m. | 21/3/06 | 5 | 5 | 0 | 0 |
| | 4.—23/5/06 | 36,000 | 21/5/06 | 103,000 | 11 m. | 19/6/06 | **5** | 5 | 0 | 0 |
| | 5.—23/8/06 | 45,000 | 23/7/06 | 111,000 | 14 m. | 18/9/06 | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | $\frac{2 \times 1}{5 \times 5}$ | 0·02 | 0·08 |
| | 6.—5/12/06 | 54,000 | 18/12/06 | 139,000 | 17 m. | 24/12/06 | $\frac{2 \times 1}{5 \times 5}$ | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | 0·08 | 0·02 |
| 1216 | 1.—16/8/05 | 9,000 | — | — | — | 4/9/05 | 5 | 5 | 0 | 0 |
| | 2.—12/12/05 | 18,000 | 11/12/05 | 48,000 | 4 m. | 28/12/05 | 5 | 5 | 0 | 0 |
| | 3.—20/3/06 | 27,000 | 2/4/06 | 72,000 | 8 m. | 10/4/06 | 5 | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | 0 | 0·02 |
| | 4.—7/6/06 | 36,000 | 5/6/06 | 85,000 | 11 m. | 9/7/06 | 5 | $\frac{2 \times 1{\cdot}5}{5 \times 5}$ | 0 | 0·12 |
| 1224 | 1.—16/8/05 | 9,000 | — | — | — | 29/8/05 | 5 | 5 | 0 | 0 |
| | 2.—18/12/05 | 18,500 | 4/12/05 | 53,000 | 5 m. | 3/1/06 | $\frac{5 \times 15}{5 \times 5}$ | $\frac{3 \times 7}{5 \times 5}$ | 3·0 | 0·84 |
| 1258 | 1.—5/9/05 | 9,000 | — | — | — | 19/9/05 | 5 | 5 | 0 | 0 |
| | 2.—19/12/05 | 17,000 | 11/12/05 | 30,000 | 5 m. | 9/1/06 | 5 | 5 | 0 | 0 |
| | 3.—19/3/06 | 26,000 | 12/2/06 | 47,000 | 8 m. | 10/4/06 | 5 | $\frac{2 \times 1{\cdot}5}{5 \times 5}$ | 0 | 0·12 |
| | 4.—29/6/06 | 35,000 | 18/6/06 | 57,000 | 11 m. | 17/7/06 | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | $\frac{1 \times 0{\cdot}5}{4 \times 4}$ | 0·02 | 0·031 |
| 1263 | 1.—12/9/05 | 9,000 | — | — | — | 27/9/05 | $\frac{4 \times 5{\cdot}5}{5 \times 5}$ | $\frac{2 \times 1{\cdot}5}{5 \times 5}$ | 0·9 | 0·12 |
| | 1.—12/9/05 | 9,000 | — | — | — | 6/11/05 | $\frac{1 \times 0{\cdot}5}{5 \times 5}$ | $\frac{0}{5 \times 5}$ | 0·02 | 0 |
| | 2.—20/12/05 | 18,000 | 22/1/06 | 27,000 | 3 m. | 30/1/06 | $\frac{3 \times 4{\cdot}5}{5 \times 5}$ | $\frac{3 \times 7}{5 \times 5}$ | 0·45 | 0·84 |
| | 3.—20/3/06 | 27,000 | 19/2/06 | 35,000 | 6 m. | 10/4/06 | $\frac{4 \times 4{\cdot}5}{5 \times 5}$ | $\frac{3 \times 2{\cdot}5}{5 \times 5}$ | 0·72 | 0·3 |

## 2. *Infusion of Mules with Mule Blood.*

*Table* 12.

HAEMOLYSIS TESTS WITH SERUM OF MULES HYPERIMMUNISED BY INFUSION OF VIRULENT MULE BLOOD.

| Number of Animal. | Hyperimmunisation. | | Bleedings. | | | Haemolysis test in Vitro. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number and date of the last Infusion. | Total amount infused. c.c. | Last bleeding before Haemolysis test. | Total amount bled up to Haemolysis test. c.c. | During. | Date of Haemolysis test. | Quotient. Horse. Respective Number of Tests. | Quotient. Mule. Respective Number of Tests. | Index. Horse. | Index. Mule. |
| 448 | 2.—24/2/05 | 13,000 | 23/2/05 | 52,000 | 5 m. | 9/3/05 | 5 | 5 | 0 | 0 |
| | 3.—27/6/05 | 22,000 | 13/6/05 | 98,000 | 9 m. | 12/7/05 | 5 | 5 | 0 | 0 |
| | 4.—17/10/05 | 31,000 | 2/10/05 | 137,000 | 12 m. | 31/10/05 | 5 | 5 | 0 | 0 |
| 459 | 1.—21/10/04 | 9,750 | — | — | — | 15/11/04 | — | 9 × 14 / 28 × 28 | — | 0·16 |
| 462 | 2.—16/5/05 | 21,000 | 3/5/05 | 99,000 | 7 m. | 31/5/05 | 5 | 5 | 0 | 0 |
| | 3.—23/8/05 | 30,000 | 8/8/05 | 134,000 | 11 m. | 4/9/05 | 5 | 5 | 0 | 0 |
| | 4.—31/12/05 | 38,500 | 11/12/05 | 177,000 | 15 m. | 16/1/06 | 2 × 1 / 5 × 5 | 1 × 0·5 / 5 × 5 | 0·08 | 0·02 |
| 463 | 3.—16/5/05 | 21,000 | 3/5/05 | 99,000 | 8 m. | 31/5/05 | 5 | 5 | 0 | 0 |
| | 4.—26/8/05 | 30,000 | 8/8/05 | 132,000 | 12 m. | 4/9/05 | 5 | 5 | 0 | 0 |
| 468 | 3.—9/5/05 | 17,500 | 3/5/05 | 100,000 | 8 m. | 20/5/05 | 4 | 4 | 0 | 0 |
| | 4.—23/8/05 | 26,500 | 14/8/05 | 141,000 | 12 m. | 4/9/05 | 5 | 5 | 0 | 0 |
| 483 | 1.—21/10/04 | 8,500 | — | — | — | 15/11/04 | — | 16 × 39 / 28 × 28 | — | 0·796 |
| 485 | 2.—24/2/05 | 15,000 | 23/2/05 | 38,500 | 5 m. | 9/3/05 | 1 × 0·5 / 5 × 5 | 0 / 5 × 5 | 0·02 | 0 |
| | 3.—27/6/05 | 23,500 | 13/6/05 | 85,500 | 9 m. | 12/7/05 | 5 | 10 | 0 | 0 |
| | 4.—17/10/05 | 32,500 | 2/10/05 | 123,500 | 12 m. | 31/10/05 | 5 | 5 | 0 | 0 |
| 530 | 2.—24/2/05 | 10,950 | 23/2/05 | 30,000 | 3 m. | 9/3/05 | 1 × 0·5 / 5 × 5 | 0 / 5 × 5 | 0·02 | 0 |
| | 3.—27/6/05 | 19,450 | 13/6/05 | 70,000 | 7 m. | 12/7/05 | 5 | 10 | 0 | 0 |
| | 4.—18/10/05 | 28,450 | 16/10/05 | 110,000 | 10 m. | 31/10/05 | 5 | 5 | 0 | 0 |
| 539 | 1.—4/12/04 | 5,350 | 14/12/04 | 5,000 | 1 m. | 17/12/04 | — | 6 × 9 / 20 × 20 | — | 0·135 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.—12/2/05 | 12,350 | 13/2/05 | 25,000 | 3 m. | 28/2/05 | 1 × 3<br>4 × 4 | 3 × 3·5<br>9 × 9 | 0·187 | 0·125 |
| 550 | 1.—3/12/04 | 5,850 | 14/12/04 | 5,000 | 1 m. | 17/12/04 | — | 3 × 4<br>20 × 20 | — | 0·03 |
| 552 | 1.—3/12/04 | 4,900 | 14/12/04 | 5.000 | — | 17/12/04 | — | 1 × 1<br>20 × 20 | — | 0·003 |
| | 2.—12/2/05 | 10,150 | 13/2/05 | 25,000 | 3 m. | 28/2/05 | 2 × 3<br>4 × 4 | 4 × 3<br>8 × 8 | 0·375 | 0·187 |
| 554 | 2.—24/2/05 | 10,950 | 23/2/05 | 30,000 | 3 m. | 9/3/05 | 1 × 0·5<br>5 × 5 | 5 | 0·02 | 0 |
| | 3.—28/6/05 | 17,750 | 13/6/05 | 70,000 | 7 m. | 12/7/05 | 5 | 10 | 0 | 0 |
| 558 | 1.—21/10/04 | 8,500 | — | — | — | 15/11/04 | — | 17 × 34<br>28 × 28 | — | 0·737 |
| 559 | 1.—3/12/04 | 6,000 | — | — | — | 17/12/04 | — | 20 | — | 0 |
| | 2.—24/2/05 | 11,000 | — | — | — | 9/3/05 | 3 × 9<br>5 × 5 | 4 × 9<br>5 × 5 | 1·08 | 1·44 |
| 562 | 1.—24/11/04 | 3,500 | — | — | — | 17/12/04 | — | 0<br>8 × 8 | — | 0 |
| | 2.—31/12/04 | 8,500 | — | — | — | 8/1/05 | 3 × 4<br>5 × 5 | 4 × 4<br>5 × 5 | 0·48 | 0·64 |
| 566 | 2.—16/5/05 | 14,700 | 3/5/05 | 47,000 | 5 m. | 31/5/05 | 5 | 5 | 0 | 0 |
| NoR | 3.—5/9/05 | 23,700 | 4/9/05 | 87,000 | 9 m. | 19/9/05 | 5 | 5 | 0 | 0 |
| 567 | 3.—2/6/05 | 16,500 | 16/5/05 | 45,500 | 5 m. | 9/6/05 | 3 × 2<br>5 × 5 | 4 × 3·5<br>5 × 5 | 0·24 | 0·56 |
| | 4.—31/10/05 | 25,500 | 23/10/05 | 55,500 | 10 m. | 21/11/05 | 2 × 3<br>5 × 5 | 5 × 13<br>5 × 5 | 0·24 | 2·6 |
| 568 R | 3.—28/6/05 | 17,000 | 13/6/05 | 62,000 | 7 m. | 12/7/05 | 5 | 5 | 0 | 0 |
| R | 4.—19/10/05 | 26,000 | 16/10/05 | 103,000 | 10 m. | 31/10/05 | 1 × 0·5<br>5 × 5 | 2 × 1<br>5 × 5 | 0·02 | 0·08 |
| 570 R | 3.—28/6/05 | 23,900 | 13/6/05 | 70,000 | 8 m. | 12/7/05 | 5 | 5 | 0 | 0 |
| | 4.—18/10/05 | 32,400 | 2/11/05 | 103,000 | 12 m. | 16/11/05 | 5 | 2 × 1<br>5 × 5 | 0 | 0·08 |
| 572 | 2.—2/6/05 | 16,250 | 16/5/05 | 51,000 | 6 m. | 9/6/05 | 5 | 5 | 0 | 0 |
| | 3.—7/9/05 | 25,250 | 4/9/05 | 87,000 | 9 m. | 30/9/05 | 5 | 5 | 0 | 0 |
| 573 | 1.—3/12/04 | 5,750 | 14/12/04 | 5,000 | — | 17/12/04 | — | 10 × 20<br>28 × 28 | — | 0·268 |

The various haemolytic tests of the serum from the infused animals were made on the corpuscles of five horses and five mules. Whilst from the horses, mules, and donkeys, treated subcutaneously with blood and serum, all gave rise to isolysines in their serum, there were amongst the infused horses only 37·5 per cent., amongst the infused mules 43·5 per cent., with isolytic serum. The transfusion proved to be the best method of hyperimmunisation against horse-sickness, hence it was chosen for the future.

The majority of the sera of the various horses infused with horse blood, and mules with mule blood, was more haemolytic for horses than for mules.

There were a greater number of mule sera more haemolytic for mule than for horse blood corpuscles.

The question arises whether the greater haemolytic index of the majority of horse and mule sera for horse than for mule blood is due to a prominent sensibility of the horse erythrocytes or whether there are in an isolytic serum two different isolysines for horse and mule blood. If this latter should be the case the isolysines for horse blood would be in excess.

The quantities of isolysines in the various horses and mules after immunising by subcutaneous injection or infusion are quite different. There again seems to be no relation between the number of injections, the quantity injected or the number and amount of bleedings and the haemolytic potence of the serum.

One would think that the individual properties of the blood used for injection or infusion have an influence on the quantity of the produced haemolysines ; but it was not possible to find any quantitative similarly in the haemolytic action of the sera of those animals which were injected with the same blood. We must therefore consider that the main factor ruling the quantity (and quality) of the isolysines is the individuality of the injected animal.

Temperature reaction of an animal after hyperimmunisation would be an expression of its sensibility ; it has to be taken into consideration that the degree of hyperthermia might be an indicator for the production of antibodies, in our case isolysines. Indeed, it was found that the majority of animals showing a temperature reaction after infusion and the minority of those not reacting contained isolysines in their blood ; but at the same time a considerable number of sera derived from horses or mules showing thermo reaction was not haemolytic, whilst some of those without rise in temperature was haemolytic.

These facts proved that fever reaction after hyperimmunisation is not always a sign of the production of antibodies and that immune substances also arise, though no alteration of temperature followed the injections.

## Experiments with Serum Mixtures.

Since we know that the immune substances are all colloid systems, it is *a priori* not to be expected that the final haemolytic effect of a mixture of various isolytic sera should correspond with the calculated haemolytic index; or with other words: the final effect of a combination of several haemolytic sera must not be simply the sum of the individual effects of the components, because the sera—though being colloids of the same electric character and belonging to the same species of animals—influence each other. They form colloid compounds with new peculiarities.

Cernovodeann and Henri * found that the haemolytic effect of a mixture of two different sera is more than double the calculated sum of the individual effects of the components. The experiments I wish to communicate here were all made in the same manner, namely: To 2 c.c. of the mixture 1 c.c. of defibrinated blood was added. The haemolysis tests of the components and the respective mixtures were made under the same conditions, the same day, and with the same kinds of blood.

Thus it is possible to compare the haemolytic index of the mixture as it is given by experiment with the calculated haemolytic mixture index which is the quotient:

$$\frac{\textit{Sum of Haemolytic Indices of Components.}}{\textit{Number of Components.}}$$

As the mixtures were made with equal quantities of serum, the calculated index gives the mathematical sum of the haemolytic effects of the components.

*Example.*—Three sera with the indices 0·2, 0·4 and 0·6, mixed together (2 c.c. each) would give a calculated haemolysis 1·2 with a given quantity of blood. Since only 2 c.c. of the mixture are added to a constant quantity of blood, the actual quantity of each serum is only $\frac{1}{3}$ of 2 c.c. and the calculated haemolytic index would be $\frac{1 \cdot 2}{3} = 0 \cdot 4$.

* Comptes Rend. Soc. Biol., No. 11, 1905.

*Table* 13.

EXPERIMENTS WITH SERUM MIXTURES.

| No. | Haemolytic Index. | | Haemolytic Index of Mixture. | | No. of animals injected. | | Percentage of animals suffering from Haemolysis. | |
|---|---|---|---|---|---|---|---|---|
| | Horses. | Mules. | Horses. | Mules. | Horses | Mules. | Horses. | Mules. |
| Horses. | *1st Mixture.* | | | | | | | |
| SH 1 | 0·137 | 0·391 | | | | | | |
| 160 | 0·537 | 0·5 | — | — | — | 20 | — | 60 (died) |
| 172 | 0·148 | 0·563 | | | | | | |
| | Calculated Mixture Index | | 0·274 | 0·458 | | | | |
| Horses. | *2nd Mixture.* | | | | | | | |
| 160 | 1·333 | 0·555 | 0·25 | 0·444 | | | | |
| 172 | 0·111 | 0·444 | | | | | | |
| | | C.M.I. | 0·722 | 0·499 | | | | |
| | | | 0·555 | 0·444 | 1 | 1 | 0 | 100 (haemoglobinuria) |
| | | C.M.I. | 0·562 | 0·701 | | | | |
| 264 | 0·77 | 0·972 | 0·666 | 1·333 | | | | |
| 301 | 0·028 | 0·833 | | | | | | |
| | | C.M.I. | 0·402 | 0·902 | | | | |
| Horses. | *3rd Mixture.* | | | | | | | |
| 473 | — | 0·102 | — | 1·12 | | | | |
| 615 | — | 0·061 | | | | | | |
| | | C.M.I. | — | 0·08 | | | | |
| Horses. | *4th Mixture.* | | | | | | | |
| 611 | 0 | 0·166 | | | | | | |
| 612 | 0·167 | 0·204 | 0·049 | 0·54 | 1 | 1 | 0 | 0 |
| 627 | 0·056 | 0·045 | | | | | | |
| | | C.M.I. | 0·074 | 0·138 | | | | |
| Horses. | *5th Mixture.* | | | | | | | |
| 611 | 0·005 | 0·2 | | | | | | |
| 612 | 0·02 | 0·125 | | | | | | |
| 615 | 0·125 | 0·3 | 0·02 | 0·14 | — | 5 | — | 0 |
| 624 | 0·045 | 0·075 | | | | | | |
| 545 | 0·02 | 0·02 | | | | | | |
| 398 | 0·005 | 0·15 | | | | | | |
| | | C.M.I. | 0·037 | 0·145 | | | | |
| Mules. | *6th Mixture.* | | | | | | | |
| 368 | 0·028 | 0 | | | | | | |
| 413 | 0 | 0·25 | 0·111 | 0·25 | 1 | 1 | 0 | 0 |
| 415 | 1·333 | 0·694 | | | | | | |
| 464 | 0·111 | 0·111 | | | | | | |
| | | C.M.I. | 0·368 | 0·24 | | | | |
| Mules. | *7th Mixture.* | | | | | | | |
| 462 | — | 0·036 | | | | | | |
| 463 | — | 0·123 | — | 0·061 | — | 19 | — | 0 |
| 468 | — | 0·077 | | | | | | |
| | | C.M.I. | — | 0·079 | | | | |
| Mules. | *8th Mixture.* | | | | | | | |
| 448 | — | 0·056 | | | | | | |
| 462 | 0·25 | 0·110 | 0 | 0·003 | 1 | 16 | 0 | 0 |
| 463 | 0·03 | 0·110 | | | | | | |
| 468 | 0·03 | 0·110 | | | | | | |
| | | C.M.I. | >0 | 0·097 | | | | |
| Mules. | *9th Mixture.* | | | | | | | |
| 448 | — | 0·056 | | | | | | |
| 462 | 0·25 | 0·110 | | | | | | |
| 463 | 0·03 | 0·110 | | | | | | |
| 468 | 0·03 | 0·110 | 0·137 | 0·111 | 5 | 9 | 0 | 0 |
| Horses. | | | | | | | | |
| 611 | 0 | 0·166 | | | | | | |
| 612 | 0·167 | 0·204 | | | | | | |
| 627 | 0·056 | 0·045 | | | | | | |
| | | C.M.I. | — | 0·114 | | | | |

*Table* 14.

TABLE OF REAL AND CALCULATED HAEMOLYTIC INDICES OF SERUM MIXTURES.

| No. of Mixture. | Haemolytic Indices of Mixtures. | | | Blood of. |
|---|---|---|---|---|
| | Real I. | | Calculated I. | |
| 2 | 0·25 | < | 0·722 | Horse. |
| | 0·444 | < | 0·499 | Mule. |
| | 0·666 | > | 0·402 | Horse. |
| | 1·333 | > | 0·902 | Mule. |
| | 0·555 | < | 0·562 | Horse. |
| | 0·444 | < | 0·701 | Mule. |
| 3 | 1·12 | > | 0·08 | Mule. |
| 4 | 0·049 | < | 0·074 | Horse. |
| | 0·54 | > | 0·138 | Mule. |
| 5 | 0·02 | < | 0·037 | Horse. |
| | 0·14 | < | 0·145 | Mule. |
| 6 | 0·111 | < | 0·368 | Horse. |
| | 0·25 | > | 0·24 | Mule. |
| 7 | 0·061 | < | 0·079 | Mule. |
| 8 | 0 | < | > 0 | Horse. |
| | 0·003 | < | 0·097 | Mule. |
| 9 | 0·111 | < | 3·114 | Mule. |

The haemolysis produced by a mixture of sera is in about two-thirds of the cases smaller than the average of the individual effects, in contrast to the results obtained by Cernovodeann and Henri.

We have to admit that the isolysines produced by various animals of the same species are different colloids and that they influence each other. It is a well-known fact that colloid combinations have an optimum of efficiency only if mixed in certain proportions, characteristic for the nature of the components. It is possible that isolytic sera would also show maximal effects when mixed in optimal quantities, the proportion of which evidently is not 1 : 1 as in the abovementioned experiments. But as the sera were mixed in this proportion, very likely not being the optimal one the maximal effect could not be obtained from these mixtures. This might explain the variations in the differences between real and calculated haemolytic index and the absence of a constant mathematical relation between both.

On the other hand, it must not be forgotten that quantitatively a reduction of a haemolytic substance does not mean a direct proportional decrease of its blood dissolving effect, as experiments of Cernovodeann and Henri* with serum and myself* with saponine have proved.

The diminution of haemolysis is at first very rapid and becomes slower and slower as the haemolytically active substance decreases. Therefore an equal quantitative reduction (for instance to the half) of two sera of different haemolytic strength involves not a proportional reduction (to one-half) of the effect of each on the same quantity of red blood corpuscles, and the haemolytic result of a mixture of the two halves of these sera need not be the mathematical average of the individual effects.

I am not able to say which of the above given possible explanations holds good, but certainly they both might be applicable. It is, of course, not permissible to draw conclusions from the calculated mixture index with regard to whether a serum mixture will produce haemolysis in vivo.

(Compare indices and results of injections of mixtures 1, 2, and 4.)

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## III.—CLINICAL SYMPTOMS OF HAEMOLYSIS.

The chief symptoms can be forecasted if it is kept in mind

(1) what is the function of the living erythrocytes

(2) what happens with them, when they are dead

We record, therefore,

(*a*) symptoms due to the deficiency of red blood cells ;

(*b*) symptons due to the elimination of the destroyed erythrocytes.

Naturally the symptoms mentioned under (*a*) appear first ; not immediately following the injunction of the destructive serum, but after a certain incubation. This is sometimes, after intrajugular injection, very short—in a few cases only about five minutes, in others a few hours ; it is longer, according to the resorption of the serum, after subcutaneous injection, varying from 1–3 days. After this time the decrease of the number of erythrocytes and icterus are the most striking symptoms, whilst the predominant phenomena which follow immediately an intravenous serum injection are mostly troubles of respiration and heart action. Under normal conditions the organism requires a certain number of erythrocytes which are the carriers of a defined amount of oxygen from the lungs to the tissues which is wanted by the latter. As the frequency of pulse and respiration largely depends on the extent or intensity of oxygen metabolism in the cells, a numerical decrease of red blood corpuscles, as it happens after haemolysis, consequently emphasises itself by acceleration of respiration and heart action, the haemaglobine dissolved in the plasma being unable to transmit oxygen. Both symptoms sometimes take place very soon after intrajugular injection of isolytic serum. In such instances the horse

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*Loco cit.

falls, breathing rapidly with wide open nostrils, the head being stretched straight forwards. The pulse is weak and very quick, tremor of muscles may be noted, probably due to the sudden alteration of oxygen metabolism.

I observed quite similar symptoms after infusion of distilled water into the jugular vein, whereby haemolysis by sub-normal osmotic pressure (hypotony) is caused.

Acceleration of pulse and respiration are well-known symptoms in piroplasmosis of horse and dog, and human medical science records inclinations to forced respiration of patients suffering from anæmia.* Haemolysis is, of course, the principle of both diseases.

Corresponding with the progress of artificial haemolysis and anæmia the animals lose in condition from day to day and become weak, as a result of which they frequently lie down. The mucous membranes are pale, on account of oligocythaemia and yellow on account of icterus ; the same is the case in piroplasmosis. Sometimes it was observed that a horse suddenly, during or immediately after intrajugular injection, fell down, arose after a few minutes and could walk round ; the next day, however, was found lying on the ground unable to rise, and so on, corresponding with the *periodical* appearance and disappearance of icterus and red urine.

I want to draw attention to the fact that *paralysis and loss of control of the hind quarters* was found in some cases of haemolysis due to isolysines, sometimes in piroplasmosis of dogs and in haemoglobinaemia (lumbago) of horses and cattle. This analogy points to a unitary cause in all three instances—haemolysis.

Rise in temperature during an attack of haemolysis was recorded for horses. Fever was also observed in human beings after transfusion.

*Blood examinations* were made by means of three methods, which gave indications on :

(1) Number and shape of red blood corpuscles.

(2) Viscosity or internal friction of the blood.

*The number of erythrocytes* (obtained first by counting with Zeiss-Abbe apparatus) shows intensity and rapidity of the action of the isolysines. The destruction commences on the first day, or sooner or later according to the haemolytic strength of the serum. It is not always continual but shows periods of increase and decrease. The number of erythrocytes gives no prognostic indication as to exitus, for an animal might die in spite of a but very slight decrease of the quantity of the red blood corpuscles ; on the other hand, an animal can recover though its erythrocytes had diminished in number to $\frac{1}{3}$. The eventual exitus lethalis does not always occur when the number of blood cells is at a minimum : in some instances it followed after the number had increased again. (Compare examples given later.) Hence there must still be some other factors as cause of death.

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* Kraus, Lubarsch-Ostertag, Ergebu. 3, 416, 1896.

Lately, instead of counting the red blood corpuscles their volume percentage was used (by means of haematocrit and centrifugalisation) to find the number of erythrocytes, which, as numerous comparative experiments have proved is $\frac{\text{Vol}}{4{\cdot}8}$

As the *viscosity* of blood is chiefly due to the amount of blood corpuscles contained in it, the measurement of the internal friction also gives an idea of the degree of destruction, and series of figures obtained from blood examinations on horses suffering from isolytic haemolysis, piroplasmosis, or artificial anæmia demonstrate that the viscosity of blood is subnormal and goes parallel with the volume of red blood corpuscles.

There is no doubt that physico-chemical researches on the serum of animals showing haemolysis from the injection of haemolytic serum would have brought forward results similar to those we observed in piroplasmosis in horses, namely, alterations of specific gravity, electric conductivity, depression of freezing point, internal friction, capillarity, etc., because the products of the destruction of erythrocytes circulating in the plasma for a certain time influence the osmotic concentration and the structure of the colloids of the serum.

The main factor producing the second group of clinical systems is the haemoglobin.

Haemolysis means not only a diffusion of haemoglobin but also of salts out of the stroma through the membrane of the red blood corpuscles. These three residues, stroma, haemoglobin, and salts have to be eliminated out of the blood. The organs which come in consideration for this function are : 1, liver ; 2, kidneys ; 3, spleen ; 4, bone marrow.

Erythrocytes which have been killed by any haemolytic substance are phagocytosed, and those still containing haemoglobin are deposited in the liver. The haemoglobin dissolved in the plasma is also kept back and carried by the circulation. The haemoglobin is then decomposed : one part of the products which contains the iron (haemosiderine iron-albumine) is either taken off by phagocytes and deposited in liver, spleen, and bone marrow (Biondi*) or discharged in the urine. The part free of iron is the basis of the bile pigments (bilirubine and biliverdin) which are sent partially into the duodenum, whilst the other part after a further chemical process (bilirubine-urobiline) goes through the kidneys into the urine. Such is the case already under normal circumstances. (Several authors found in cases of haemolysis an increase of bile pigments.†)

When the accumulation of haemoglobin and the production of bile pigments has reached a certain supernormal degree, a greater or smaller amount of bile passes over—by a mechanism, unknown at present—into the blood (cholaemia). These (colloidal) pigments are absorbed by the tissues and thus give rise to the symptom called icterus (conjunctiva, mucosae). The presence of bile pigments in the blood is soon

* Ziegler, Beitrage 18th Jan., 1895 ; cit. Schmidt, Lubarsch-Ostertag, Ergebu. 3, 542, 1897.
† Oswald, Chem. Pathalog. Leipzig, 1907, 127.

followed by excretion of a part of them through the kidneys. (Choluria, bilirubinuria.)

If the destruction of blood corpuscles proceeds or increases the liver is unable to decompose the entire quantity of haemoglobin which it receives, and so the surplus of this latter goes, as it is, into the bile and is mixed with the faeces. Then a further increase of the haemolysis emphasises itself by haemoglobinuria followed by renal symptoms.

In cases of (haemolytic) icterus, simultaneously with the bile pigments, salts of the bile acids—especially sodium glycocholicum and tauro-cholicum are also in the blood.

As the latter salt itself is able to destroy blood corpuscles, icterus as a symptom is at the same time a pathogenic factor of haemolysis, under the supposition that the concentration of these salts in the blood is high enough, which is perhaps seldom the case. More important, however, is the influence of taurocholic salts on the heart and on the kidneys, a protraction of frequency being one of the effects on the former. After all, the icterus influences the liver itself, and haemolytic sera are also able to affect liver cells.* As the "internal" function of this organ is enormously important, specially for the nitrogen and hydrocarbonate metabolism and the destruction of poisons, interruption or disturbance of the normal liver action influences the health of the entire organism.

Haemoglobin which is not retained in the liver, bile pigments and salts of bile acids circulating in the blood, have to be eliminated by the kidneys and thus give rise to a complex or symptoms due to affections of the kidneys : (1) haemoglobinuria, (2) albuminuria, (3) choluria, (4) haematuria.

(1) Albuminuria was found in slight cases of haemolysis as a consequence of the increased blood destruction. The most striking and common symptom of haemolysis (besides jaundice) is the appearance of haemoglobin in the urine. Subsequently the latter becomes stained from a slight to a deep dark red colour.

If the inflammation of the kidneys is acute, epithelial cells may be found in the urine, and a more intense destruction of them *in situ* is followed by the escape of blood cells into the tubuli (haematuria). In such cases the quantity of urine is very small (anuria, uraemia), and death is the general exitus. (Compare the following examples.)

Clinical symptoms resulting from the affection of spleen and bone marrow have not yet been recorded.

The leading idea for the above classification of the symptoms of haemolysis is the physiological course of life, function, and death of a red blood corpuscle. It enables us to derive the following symptoms of intensity and chronological succession of the clinical symptoms of haemolysis, which are in accordance with the recorded facts :—

1. Very slight destruction of erythrocytes. Very small decrease of their number and volume is the only symptom. (Eventually slight albuminuria).

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* Kretz, Lubarsch-Ostertag, Ergebu. 8, II, 495, 1902.

2. Considerable haemolysis and decrease of the number of erythrocytes. Super-production of bile. Transmission of bile pigments into the blood, icterus.
3. Strong haemolysis (when more than $\frac{1}{6}$ of the haemoglobin of the body is dissolved in the plasma) elimination of haemoglobin through the kidneys : *Red urine.*
   Deaths very frequent.
4. Enormous haemolysis, kidneys strongly affected ; red blood cells in the urine ; *haematuria, oliguria, anuria, uraemia, exitus lethalis.*

EXAMPLES.*

Mule 356.—Injected on the 13th November, 1903, with a mixture of different sera.

| | | | |
|---|---|---|---|
| 14/11/03, | number of blood | corpuscles, | 6,100,000. |
| 15/11/03, | ,, | ,, | 2,640,000 Red Urine. |
| 16/11/03, | ,, | ,, | 2,560,000 ,, |
| 17/11/03, | ,, | ,, | 3,366,000 ,, |
| 18/11/03, | ,, | ,, | 4,560,000 ,, |
| 19/11/03, | ,, | ,, | 4,860,000 ,, |
| 20/11/03, | ,, | ,, | 4,246,000 ,, |
| 21/11/03, | ,, | ,, | 4,100,000 ,, |

Mule 356 died on the 21st November, 1903. This animal suddenly showed an alarming dyspnoe, accompanied by a whistling and trumpeting sound. It died within an hour after these symptons had started.

Horse 348.—Injected on the 20th November, 1903, with a mixture of different sera. The count of red blood corpuscles was as follows :—

| | | | |
|---|---|---|---|
| 19/11/03, | blood | count, | 6,020,000 per m.m. |
| 20/11/03, | ,, | | 6,332,000 |
| 21/11/03, | ,, | | 4,706,000 |
| 22/11/03, | ,, | | 3,820,000 |

There was a distinct jaundiced condition of the mucous membrane of the eyes, on which several blood spots were also noticed. Red urine was voided.

| | | | |
|---|---|---|---|
| 23/11/03, | blood | count, | 3,006,000 per m.m. |
| 24/11/03, | ,, | | 3,506,000 |
| 25/11/03, | ,, | | 3,966,000 |
| 26/11/03, | ,, | | 3,392,000 |
| 27/11/03, | ,, | | 4,632,000 |

Urine was again clear ; Esbach's albumen test gave a strong precipitation.

| | | | |
|---|---|---|---|
| 28/11/03, | blood | count, | 4,126,000 |
| 29/11/03, | ,, | | 4,612,000 |

Albumen was less in the urine than on 27/11/03.

| | | | |
|---|---|---|---|
| 30/11/03, | blood | count, | 5,552,000 |
| 1/12/03, | ,, | | 4,320,000 |

* Copied from Ann. Rep. Govt. Vet. Bact., 1903-04.

The urine was again red and contained a large amount of albumen. The horse had symptoms of colic; it lay down at intervals.

2/12/03, blood count, 3,906,000 per m.m.$^3$
3/12/03, „ 4,846,000
4/12/03, „ 5,066,000
5/12/03, „ 5,152,000

Urine was again clear from the 3rd December, 1903.

6/12/03, blood count, 5,050,000

This animal recovered.

Horse 360.—Injected on the 20th November, 1903, with a mixture of different sera.

19/11/03, count of red corpuscles, 7,650,000 per m.m.$^3$
20/11/03, „ „ 7,416,000
21/11/03, „ „ 7,320,000
22/11/03, „ „ 6,826,000
23/11/03, „ „ 6,612,000

A slight jaundiced condition of the mucous membrane of the eyes was noticeable.

24/11/03, count of red corpuscles, 6,494,000
25/11/03, „ „ 6,850,000

Red urine was noticed since the day previous.

26/11/03, count of red corpuscles, 7,080,000

Urine had cleared up since previous evening, but still contained albumen.

27/11/03, count of red corpuscles, 5,646,000

Urine still clear.

28/11/03, count of red corpuscles, 5,906,000

The urine of the previous evening was red; of this date (morning) it was dark brown, containing much albumen.

29/11/03, count of red corpuscles, 7,060,000

The urine of the previous evening was again red.

30/11/03, count of red corpuscles, 7,080,000

The urine was clear and contained but little albumen.

1/12/03, count of red corpuscles, 6,352,000
3/12/03, „ „ 6,494,000
4/12/03, „ „ 6,180,000
5/12/03, „ „ 5,086,000

The horse died on the 5th December, 1903.

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## IV.—Pathological Anatomical Lesions of Haemolysis.

According to the clinical symptoms, we distinguish among the pathological anatomical lesions of haemolysis:

1. Alterations of the blood.
2. Alterations of spleen, bone marrow.
3. Alterations of the liver.
4. Alterations of the kidneys.

1. *The Blood.*

After every serious attack of haemolysis the blood is watery and thin, thus showing the loss of corpuscular elements. Sometimes it is found well coagulated and in other cases not. In the former instances the serum is red stained by the haemoglobin dissolved in it, and so is the plasma clot when the corpuscles are deposited before coagulation. In cases of cholaemia (and general jaundice) plasma clot and serum have a brownish hue.

Obturations of blood vessels by residues of erythrocytes or conglomerations of agglutinated blood corpuscles are in great probability the cause of the infarcts which are met with in lungs, kidneys, and spleen. It is stated by several authors* that in cases of poisoning with specific blood poisons the blood coagulates easier, and even in the living animal coagulations take place and in this way blood vessels can be blocked and infarcts and thrombi due to stasis produced.†

2. *Spleen.*

The spleen generally is found congested. Spleen tumour has been observed not only in cases of isolysine-haemolysis but also in piroplasmotic infections, malaria, and infections with bacillus typhi, spirochaete recurrentis, streptococci, and staphylococci, that is to say in all diseases accompanied by destruction of red blood corpuscles and also after experimental poisoning with the very haemolytic potassium and sodium chloricum‡ and toluylendiamin.

There is now ample evidence that the destruction of erythrocytes alone, without infection is sufficient to produce splenitis and spleen tumor; but the question remains, which of the products of the decomposition of blood corpuscles is the real cause of the splenitis. Heinz§ says that the morphological residues of the destroyed blood corpuscles are chiefly deposited in spleen and liver, that the spleen cells take the altered blood cells, dissolve them, keep the pigment back whereby the spleen swells up. According to Biondi (l.c.) the pigment containing the iron which is a derivative of haemoglobin is made in the liver and carried into the spleen by leucocytes, and after being taken by the spleen cells it might act on them as stimulus for hyperplastic and hypertrophic processes.

3. *Liver.*

The liver is, after the blood glands, the first affected gland, because the chief decomposition of haemoglobin takes place in it. This work being very much too great for the liver in cases of pathologically extended destruction of erythrocytes leads to degenerative and regenerative processes in this organ. Therefore it is found in almost every case

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* Cit. Kionka, Lubarsch-Ostertag, Ergebn. 7, 515, 1900-1901.

† Several authors go as far as to take the pathological anatomical phenomenon of obturation of vessels or infarcts as one of the specific criteria and even a diagnosticum for blood poisons (sulphites, argentum colloidale, CO, saturnism, bismut phosphoric, salicylic acid, *Kionka*; glycerin, carbolic acid, pyrogallic acid, anilin and derivates, *Silberman*; arsen, Ba $Cl_2$, phosphorus, *Heilborn*; arsenic, *Heinz*; and others) (Compare Kionka, l.c.) Affections of the endothelia of the vessels however have to be taken into consideration.

‡ Jawein, Virchows Archiv, 161, 3, 1900.

§ Cit. Kionka, Lubarsch-Ostertag, Erg. 7, 506, 1901.

of haemolysis congested, very often soft, friable, enlarged, in the majority of instances jaundiced, brownish or greenish discoloured with an increased quantity of bile.

4. *Kidneys.*

Haemoglobin is noxious to the kidneys. Parenchymatous degenerations of these organs were observed after haemolysis due to the injection of distilled water (own experiment), in haemoglobinuria paroxysmalis hominis in the haemoglobinuria paralytica of horses (lumbago), in all diseases which occur with haemolysis, and finally in cases of haemolysis after injection of isolytic serum. In connection with this "serum disease" the following lesions were recorded :—Adherent capsula, congestion and dark appearance of the paremchym, abnormal size and weight, oedema and friability, dark radial stripes.

Examples* : Horse 297.—Injected on 6th July, 1903, with serum of horse 147. This animal showed similar symptoms as those described in horse 298. It was better the next day (7/7/03), but would not eat. On 8th July, 1903, haemoglobinuria was noticed for the first time. The mucous membranes were yellow. There was loss of condition. The animal laid down almost the whole of the 9th July, 1903.

Horse 297 died on 9th July, 1903. Post-mortem was made one hour and a half after death. The condition of the animal was good. Rigor mortis was present. There was a general jaundiced condition of the flesh and the serous membranes. The blood was coagulated and separated into black and white clots ; the plasmatic clot had a somewhat brownish hue. The heartbag was filled with dark yellow liquid. The heart muscle was pale. There were no echymosae on the endocard. The liver was dark green in colour. The spleen was enormously enlarged, its weight being 12 pounds ; the pulpa was soft. The kidneys were uniformly dark red, with haemorrhagic infarcts in the cortex. The urine was black. The mucous membrane of the stomach was covered with blackish mucous (coagulated blood). The intestines were normal.

Horse 298.—Injected on 6th July, 1903, with serum horse 147. The injection was made into the lower part of the jugular vein. Soon after injection the horse lost control of the hind quarters, staggered for a few minutes, and dropped suddenly, neighing repeatedly. The pulse was rapid, the respiration was accelerated. After a lapse of a few minutes the horse rose again ; it now kept the head stretched out ; it breathed quickly with wide open nostrils. In the afternoon of the same day the horse went down again. It showed tremor of the muscles ; it moaned and had a rapid respiration. The next day (7/7/03) horse 298 seemed to be better. On the 8th July, 1903, horse 298 lay down again. The mucous membranes had a yellowish colour. The horse tried to stale repeatedly. Red urine was voided. The animal lost condition from day to day until death.

Horse 298 died at 1 o'clock p.m. on 10th July, 1903. Post-mortem was made one hour later. The condition of the cadaver was fair

* Copied from Ann. Rep. Govt. Vet. Bact., 1903-04.

There was a general jaundiced condition of the flesh, the serous membranes, and all organs. The lungs were oedematous and a few haemorrhagic infarcts were present. The blood of the heart ventricles was not completely coagulated. A few haemorrhagic spots were noticed on the endocard of the left ventricle. The heart muscle was pale. There was but little liquid in the heartbag. The spleen was slightly enlarged, there were several haematoms in the tissue of the spleen. The pulpa was black. A white thrombus was found in the splenic vein. The liver was enormously enlarged. The tissue was friable, of a green-yellow-reddish colour ; it was studded with white spots, which on close examination proved to be small abscesses ; minute haemorrhagic spots were also present. The kidneys were enormously enlarged, almost spherical. The capsula was infiltrated. The tissue of the kidneys was black, oedematous, and friable. The mucosa of the stomach was uniformly reddened and swollen ; that of the duodenum was also thickened and red stripes across the mucosa were present. The mucous membrane of the coscum and colon were slate-coloured. The bladder was empty. The lymphatic glands were not enlarged.

Microscopical examination of smears of the different organs proved the absence of endoglobular parasites. The small abscesses in the liver contained a bipolar bacterium.

The above-described complex of clinical symptoms and pathological anatomical lesions, which are met in cases of haemolysis due to the injection of isolytic serum, has to be expected more or less complete in all diseases where destruction of erythrocytes takes place, namely in :

1. Various poisonings with saponine, digitoxine, cyclamine, colloidal metals and metal hydro-oxydes; acids, $HgCl_2$, $KClO_3$, $NaClO_3$, $BaCl_2$, As, $As_2O_3$, P, and many others.
2. After influence of very low and very high temperatures.
3. After infections with microbes secerning haemolytic toxines : strepto—and staphylo-cocci, b. anthracis, b. typhi, tetani, bact. cholorae a.o.
4. After poisonings with snake venoms.
5. After infections with piroplasms; they are all endoglobular parasites and are specially important for South Africa.

In cattle: Piroplasma bigeminum (Texas fever, ordinary redwater).
Piroplasma mutans (gall sickness).
Piroplasma parvum (East coast fever, Rhodesian redwater), haemolysis clinically seldom observed.

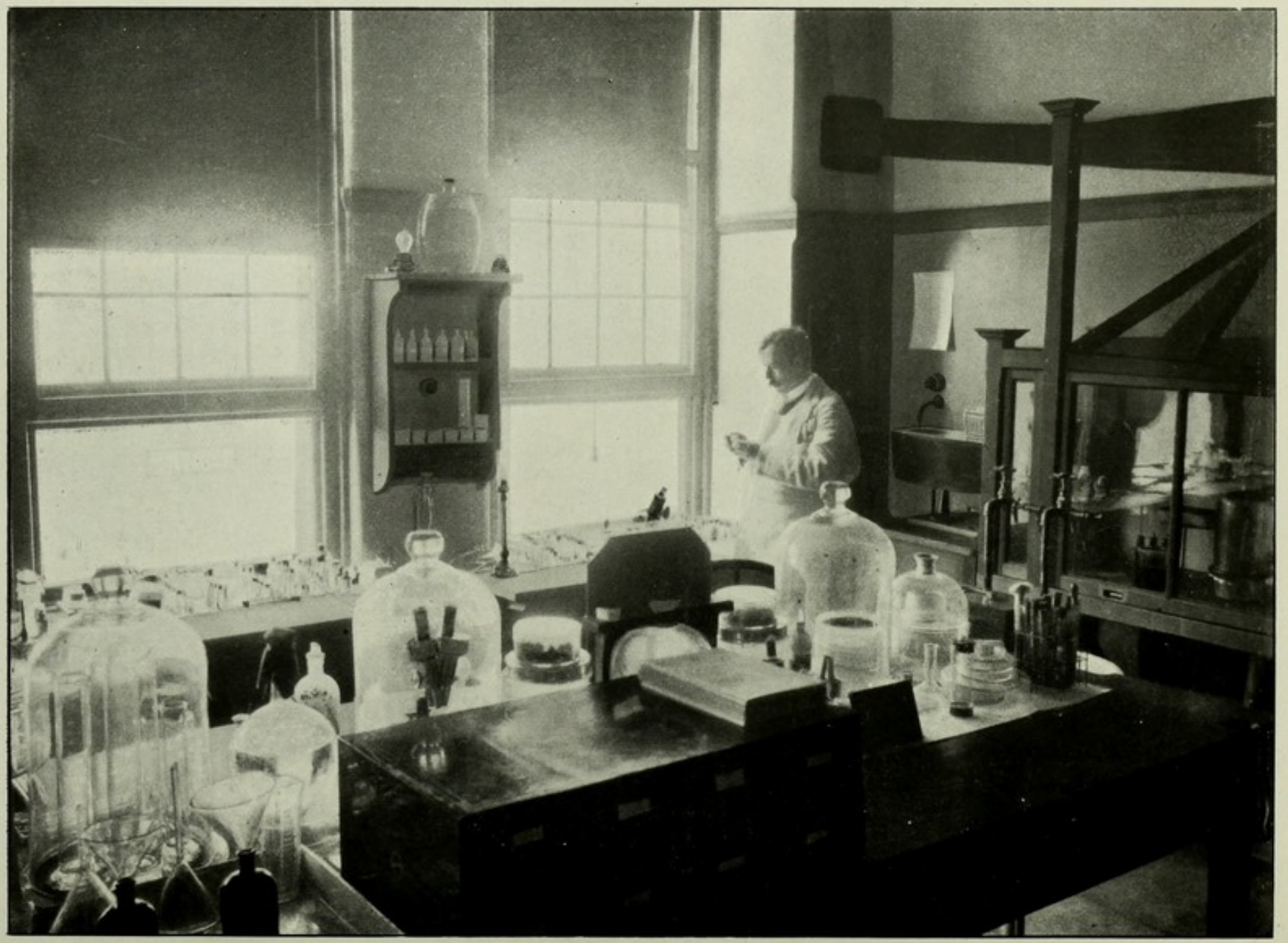
In equines : Piroplasma equi (biliary fever).
(Horses, mules, donkeys, zebras.)

In dogs : Piroplasma canis (biliary fever).

The clinical pathological anatomical appearance of these diseases is of course not always the same as it is seen in cases of serum-haemolysis, but mixed with other local symptoms of intestinal, nervous, or cardiac nature. This applies specially to diseases due to microbes which secrete other toxines besides haemolysines (tetanus, typhus, anthrax, etc.)

# The Anatomy of Stilesia Centripunctata.

By Dr. LEWIS H. GOUGH.



*Zoological Laboratory.*

# The Anatomy of Stilesia Centripunctata (Rivolta).

By Lewis Henry Gough, Ph.D.

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*Stilesia centripunctata* (Rivolta) is a parasite of the jejunum of sheep and, according to Von Linstow (Compendum der Helmenthologie, 1889), of cattle, but there is internal evidence that this statement is due to an error, as he does not quote it as a parasite of sheep. Linstow, however, is there citing Rivolta, who found it in sheep.

Its geographical distribution appears to be somewhat limited, as, apart from South Africa, I have only seen records from Italy (Rivolta, Mattozzi) and Algeria (Neumann, Tempère).

In South Africa (Transvaal) it is absolutely the commonest cestode found in the intestine of sheep; several specimens are usually found together—it is, however, rarer than the liver tape-worm, *Stilesia hepatica*, Wolfh.

The parasite is almost entirely restricted to the continent of Africa and to Southern Europe—to the Mediterranean and Aethiopean regions, but especially to the latter.

The worm was first described as *Taenia centripunctata* by Rivolta in 1874 (Sopra alcune Specie di Tenie della Pecora, Pisa), it was again mentioned by Perroncito in 1882 (I Parassiti dell' Uomo e degli Animali Utili, Milano), and again in 1886 (Trattato teorico-pratico sulle malattie piu comuni degli Animali domestici dal punto di vista agricolo, commerciale ed igienico, Torino). In the same year (1886) Railliet refers to it in the first edition of Elements de Zoologie Medicale et Agricole, Paris. In 1888 it was again referred to by Neumann in the first edition of Traité des Maladies parasitaires non microbiennes des Animaux domestiques, Paris.

These records or references all appear to have been based on the same material, or to have been quoted from one paper.

In 1891 Neumann obtained fresh material, and was able to supplement the details given by the earlier authors. (Observations sur les Ténias du Monton: C.R. Soc. Hist. Nat., 1891, 18 Mars.)

The revised diagnosis is found also in the second edition of the Traité des Maladies parasitaires 1892, and in Railliet's second edition of Traité de Zoologie Médicale et Agricole, 1893. In the same year Stiles gives a description, partly original, from Neumann's material, partly abstracted, in "A Revision of the Adult Cestodes of Cattle, Sheep, and Allied Animals," being the best description of the worm published hitherto.

Since Stiles I only find one further reference (using the Zoological Record), by Tempère, in the Micrographe Préparateur, 1904, p. 27, which, however, is evidently only an abstract from Neumann, the illustration given being simply a copy of Neumann's figure of the Scolex. Most of the older literature is inaccessible to me (working in South Africa), and has been quoted from Stiles.

## General Appearance.

*Stilesia centripunctata* (Rivolta) is macroscopically instantly recognisable among the parasites of sheep by the following details. It differs from the species of *Moniezia* and *Thysanosoma* by its small breadth as compared to its length, the extreme breadth not frequently exceeding 3 mm.; its greatest breadth is usually near the scolex, the posterior portion of the strobila being almost round in section.

It differs macroscopically from the other species of *Stilesia* by its greater length, and also in the following particular. The strobila from 10 cm. distance from the scolex onwards appears to consist of five longitudinal bands, a moderately transparent one laterally on each side, within this a very transparent band on each side, enclosing medially an opaque band.

The moderately transparent portions are the lateral fields; the very transparent bands are caused by the extremely wide ventral longitudinal canals; the opaqueness of the median band is caused by the female genitalia and their supporting tissues.

In life the worm has a gelatinous appearance (except along the median line, which is somewhat opaque); it is slightly yellowish or greyish.

## Dimensions.

Perroncito (quoted from Stiles' "Revision") gives the following dimensions:—

Total length 2·75—2·84 m.

Width 10 cm. from scolex 2—4 mm., 50 cm. from head 2—3 mm. 150 cm. from head 1.55 mm., terminally 1 mm.

Neumann, Traité, second edition, gives approximately the same dimensions. Stiles states the total length to be nearly 3 m., at 10 cm. from head the segments are 2—3 mm. wide and grow narrower from here onwards, posterior segments being scarcely 1 mm. wide. Tempère (1904) evidently quotes the length from Neumann or Perroncito, giving it at 2·75 m.—2·85 m., segments 10 cm. from scolex being 2—4 mm., at 150 cm. from scolex 1 mm. He adds that this worm differs from other Taeniadae in that its segments are widest near the head,

A specimen taken 4th June, 1909, measures 202 cm. (after fixing in sublimate), it contains mature oncosphaeres. The total length must, therefore, vary from 2—3 m.

As to the breadth, this varies very considerably according to the state of contraction; the greatest breadth is usually, but not always, attained close to the scolex. One specimen measured 2·8 mm. at 10 cm. from scolex, 3·2 mm. at 20 cm. from scolex. Another measured 2·5 mm. directly behind the scolex (normal value), but at 2 cm. from scolex fell off to 0·5 mm.

The posterior portion is always very narrow and thick, almost round in section, measuring at the most 1 mm. broad. Anteriorly the segments are always much broader than thick. All segments are much broader than long, the ratio of breadth to length varying very considerably according to contraction; at normal contraction, sections passing vertically to the long axis in the posterior half of the stobila will almost always pass through genitalia belonging to more than one segment, the anterior and posterior surfaces of each maturing segment not being flat, but having projections caused by the bulky female organs, or indentations caused by those of the preceding or following segments. The scolex measures, according to Perroncito, 2 mm., Neumann gives the measurements as 1·5—2 mm.; there are some discrepancies in the literature concerning the scolex.

Neumann figures it with large cylindrical suckers directed, parallel to each other, anteriorly; Tempère follows Neumann and copies his figure, as does Railliet.

Stiles states that the scolex in his possession has its suckers situated at its four corners and that they are directed diagonally forwards.

I have had the opportunity of examining several scolices, over twenty, and in every case they agree with Stiles' description (Fig. 1). Sections also show the suckers to be directed outwards and forwards.

The size of the scolex also varies fairly considerably: five specimens were measured:—

| | Length. | Breadth. |
|---|---|---|
| *a.* | 1·5 mm. | 2·3 mm. |
| *b.* | 3·1 mm. | 2·8 mm. |
| *c.* | 1·8 mm. | 2·4 mm. |
| *d.* | 1·5 mm. | 2·0 mm. |
| *e.* | 1·8 mm. | 1·5 mm. |

Specimen *b* was the largest, *e* one of the smallest I have seen.

The segments are hardly distinguishable by the naked eye; in several specimens in which segmentation was apparently visible magnification proved that what appeared at first sight to be segments was in reality caused by the grouping together of four, five, or more segments, due to contraction.

The genital openings are not prominent, and are very slightly developed, as compared with those of other Anoplocephalids. The genital openings are irregularly alternate.

## Anatomy.

*The Cuticula.*—

The cuticula on scolex and strobila everywhere presents a similar appearance, and is composed of three distinct layers, differing from each other in thickness, structure and in their behaviour towards staining reagents.

For the study of the cuticula, specimens fixed with silver-nitrate and developed with hydroquinone give on the whole the best results. (Method : Ramony Cajal for nervous tissue.)

In a specimen fixed in this manner and stained with haematoxylin counterstained with orange *g*, the cuticula at 15 cm. from the scolex is about 4·5 μ thick ; of this the outer layer accounts for about 1·5 μ, the middle for 3 μ, the innermost layer is extremely thin. The outermost layer stains in this specimen darker than the middle, and has a bluish tinge due to the haematoxylin. Excepting for a single row of minute black granules on its external surface, it is apparently structureless. The middle layer stains only with the orange ; it is not homogenous, as it contains in its entire thickness very numerous, minute, black granules. There is no definite arrangement for these granules, and they are equally abundant in all parts of the layer. The innermost layer is, as already stated, extremely thin, and stains black according to this method. I consider it to be structureless.

Material fixed in Zenker's solution, but stained in the same way, does not show as much detail. The outer layer is dark blue, taking almost the same colour as the chromatin of the nuclei. It does not appear homogenous, however, but seems to be traversed by fine pore-canals.

In the middle layer the granules are not differentiated, the entire thickness of the layer being almost homogenous ; very careful examination shows, however, lighter spots corresponding to the granules, but as these are very minute, they are extremely difficult to make out. The innermost layer does not differentiate, or appears as a lighter orange line between the middle layer and the muscles running parallel to the cuticula. Material fixed in formaline (4 per cent.), but stained in the same way, gives the same results. The cuticula of *S. centripunctata* is smooth, unlike that of *S. hepatica*, which is villose.

*The Subcuticula.*

The subcuticula is best studied on not too contracted pieces fixed with Zenker's fluid and stained with haematoxylin and orange *g* ; according to the state of contraction or extension it, offers rather different pictures. (Fig. 2.)

In places where the cells are separated owing to expansion of the worm, the subcuticula is seen to consist of elongated cells, rich in protoplasm, standing at right angles to the cuticula ; these cells are in contact with the innermost cuticula layer by three, four, or more processes, which are again often branched before reaching the cuticula. These processes appear occasionally to form anastomoses with processes from the same or other cells, before inserting in the cuticula. The proximal end of the cell terminates in a single elongate process, which is continued into a long fibrilla; these fibrillae can sometimes be traced continuously through the ring-muscle into the middle layer of the worm and from thence to the cuticula of the opposite side, sometimes interrupted on their course by the spindle-shaped cells belonging to the transverse-muscle system.

The fibrillae of several adjacent cells appear to close together into definite bundles before penetrating the longitudinal muscles ; this is best seen on more contracted portions.

The subcuticula cells appear to have traces of a membrane, are very distinctly outlined, and their plasma appears to be slightly denser along the walls of the cells.

The nuclei of the subcuticula cells consist of small vesicles ; the membrane surrounding the nucleus stains quite distinctly ; each nucleus contains several round chromatine bodies, often five or six in addition to the nucleolus, which is about twice the size of the chromatine bodies.

The measurement of a few subcuticular cells is (Zenker's solution—haematoxylin orange—20 cm. from scolex) :—

| | | | | | | |
|---|---|---|---|---|---|---|
| (*a*) Total length of cell from insertion in cuticula to attachment to fibrilla.. | 57 μ | 42 μ | 36 μ | 42 μ | 60 μ | 51 μ |
| (*b*) Greatest width of same cell | 3 μ | 4·5 μ | 6 μ | 4 μ | 4 μ | 4 μ |
| (*c*) Length of nucleus.. .. | 6 μ | 6 μ | 5 μ | 5 μ | 5 μ | 6 μ |
| (*d*) Breadth of nucleus .. | 3 μ | 4·5 μ | 4·5 μ | 4 μ | 4 μ | 3 μ |

Only such cells were measured whose insertion in the cuticula and attachment to the fibrilla could be made out. The attachment to the fibrilla is only able to be found in cells lying entirely in the plane of the section.

Between the just described subcuticula cells and the cuticula, two layers of muscle fibrillae are to be found running parallel to the cuticula.

The outermost of these runs horizontally and forms an outer ring muscle layer; the inner one runs at right angles to it in the direction of the long axis of the worm. The outermost longitudinal fibres pass between the circular fibres. These systems are best studied on sections parallel to the surface of the cuticula, but can be made out also on transverse sections.

*Calcareous Corpuscles.*

*Stilesia centripunctata* does not seem to possess any calcareous bodies.

*Nervous System.*

The two lateral nerves, which in the strobila lie midway between the ventral canal and the lateral margin, on entering the scolex converge somewhat and pass in well within the rhomb-shaped area enclosed by the suckers. On transverse sections shortly after the cavity of the suckers is reached a rhomboidal commissure is visible, whose angles project towards the interacetabular spaces and form ganglia there.

Directly anterior to the rhomboidal commissure, transverse commissures connect the four ganglia of the rhomboidal commissure; these two transverse commissures are fused in the centre and form a cross.

From each of the four ganglia in the angles of the rhomboidal commissure four nerves originate, one laterally going to each of the two adjacent suckers, the other two ascending, at first towards the summit of the scolex, then bending over and forming loops with each other, the right ventral fusing with the right lower (ventral) lateral, the upper (dorsal) right lateral with the right dorsal, the left dorsal with the upper left lateral, and the lower lateral with the left ventral.

A large median plate, poor in ganglion cells, appears to lie further anteriorly, just below the anterior loops of the lateral canals, but I have not been able to trace the connection of the plate with any of the eight nerves (four loops).

The lateral nerves on transverse sections fixed with silver nitrate appear to consist of a dark network, containing many small black granulations (precipitate of silver ?) on the meshes. The meshes become rather denser towards the margins. There is no membrane between the nerve and the parenchyma. Small cells, glia cells, are frequent, especially on the circumference of the nerve; some are, however, situated in the depth. These glia cells are very poor in protoplasm, only their vesicular nuclei with a large nucleolus being visible. The nucleus measures about 4·5 $\mu$ by 3 $\mu$. Where the nerve is not cut transversely, but obliquely or longitudinally, the network is seen to consist of more or less parallel fibres. Large ganglion cells are very frequent in the rhomboidal and transverse commissures and in the nerves originating there, and also in the body of the suckers. (Fig. 3.)

The ganglionic cells in the nerves are elongate, fusiform, and in some cases appear to be bipolar.

Their long axis always lies parallel to the axis of the nerve. These cells vary in size, their apparent total length in a section, of course, depending on the angle in which the plane of the section has cut them.

The processes originating from these cells can often be traced for a long distance in the nerve. The protoplasm of these nerve-cells is almost invariably filled with short rod-shaped bodies, arranged serially, each series more or less parallel to the next, staining dark blue with haematoxylin (tigroid bodies). These structures also occur for some distance along the nerve processes. The nucleus is oval, vesicular, staining very

lightly with haematoxylin, 9 μ long by 4·5 μ wide; it contains a large, round, dark nucleolus, not quite 3 μ in diameter, and up to four smaller bodies, staining equally dark.

The total length of one of the fusiform nerve-cells is 30 μ, its fibril can be followed on one side for further 36 μ, breadth about 9 μ. These cells are evidently conducting elements. Larger, more or less rounded, ganglion cells are found in the ganglia at the origin of the nerves. I cannot state definitely whether they are multipolar or unipolar. In these cells the tigroid bodies appear to be absent, the protoplasm staining blue with haematoxylin, and presenting a spongy or reticulated appearance, dark meshes enclosing a lighter ground.

The nucleus is round, rather pale, but contains a very large dark nucleolus and several (six or more) smaller dark staining bodies. One such cell measured 27 μ in diameter, its nucleus 12 μ, the nucleolus 3 μ.

Large ganglion cells are also found in the body of the suckers, lying between the bundles of radial fibres, usually closer to the body side than to the cavity of the sucker, and often form an almost definite layer. (Fig. 4.) These ganglion cells do not contain tigroid bodies; their protoplasm has the same reticulated or spongy appearance as the ganglion cells of the scolex ganglia. The nuclei are vesicular, their membrane very distinct, their body not taking stain, their shape is round to oval. The size of one of these cells measures 18 μ across, the nucleus 9 μ by 6 μ, nucleolus 3 μ. Besides the nucleolus there are a few (two or three) smaller dark staining chromatine bodies, often very closely approached to each other.

These cells and the bipolar nerve-cells in the commissures described above are evidently the "Myoblasts" of Pinter and Zschokke. They are evidently not muscle cells; their appearance is, if possible, even more typical for nerve elements in *Anoplocephala magna*, Abilg., than in *Stilesia*, owing to their larger size. (Fig. 5.) One specimen measured in *Anoplocephala magna*: nucleus 12 μ long by 9 μ broad, vesicular, nucleolus distinct, round, 4 μ in diameter. Protoplasma staining dark with haematoxylin, reticulated or spongy in appearance, distally the meshes arrange themselves more and more to parallel threads and finally lose themselves in typical neurofibrills, which can be traced some distance. No cell membrane. Total length (exclusive fibrillae) 75 μ, breadth 15 μ; the neurofibrillae could be traced as a definite thread for 36 μ on one side, 60 μ on the other. The fibrillae of the longer side could be seen to divide at their end and to connect with other similar ganglionic cells. In *Anoplocephala* as in *Stilesia*, these cells are restricted more or less to a definite zone, near the middle of the radial fibres, and form a definite nervous system in the suckers.

In the strobila on horizontal sections one sees occasionally nerves proceeding from the lateral nerve towards the lateral margin, accompanying the genital ducts. They are, however, hard to find, and cannot be followed for any distance.

## The Musculature.

The transverse and longitudinal muscles between the cuticula and subcuticula have already been mentioned, and it has already been stated that I consider the subcuticula itself to belong to the contractile elements.

The longitudinal, transverse, and dorsoventral systems of muscles (*Parenchym muskeln*, Braun) are found in characteristic arrangement in the strobila. The longitudinal muscles are the most developed of the three systems. They lie in a single layer between the subcuticula and the transverse muscles, being divided into rather indistinct bundles by the dorsoventral muscles. The longitudinal muscle layer is broken laterally on both sides.

The fibres run continuously, through more than one segment, parallel with each other. They are roundish on transverse sections, often enclosing a hollow, appearing then annuliform. Myoblasts are fairly frequent.

The transverse muscles form a much weaker layer than the longitudinal, consisting both of fewer and of thinner fibres. The dorsal and ventral plates of transverse fibres meet laterally, but the fibres of either plate do not pass round into the other plate. Myoblasts are frequent.

The dorsoventral is the weakest system, being composed of single fibres; the fibres are thickest in the lateral fields. The fibres of this system appear to attach to the fibrillae already mentioned as continuing the subcuticular cells. Myoblasts are very frequent.

The musculature of the scolex is more complicated, but consists of fibres derived from these three systems. At the base of the scolex the longitudinal fibres of dorsal and ventral series approach so close as almost to meet laterally.

The transverse muscles form a much thicker and denser layer, and the dorsoventral muscles become more prominent and more frequent. Entering the scolex their arrangement and direction becomes changed, owing to the other organs contained in the scolex. Without going into detail, the following systems of muscles can be mentioned as occurring in the scolex:—

(1) A diagonal cross system anterior to the terminal loops of the longitudinal canals; it covers the entire anterior surface of the scolex, passing from the front of the left ventral to the right dorsal, and from the right ventral to the left dorsal sucker.

(2) A second diagonal cross system composed of four bundles, each consisting of a few fibres; it is situated just behind the terminal loops of the longitudinal canals and runs from sucker to sucker in such a way that (1) fibres from the lateral face of the right ventral sucker run to the lateral face of the left dorsal sucker; (2) fibres from the lateral face of the right ventral sucker run to the median face of the left dorsal sucker; (3) fibres from the median face of the left ventral sucker run to the

lateral face of the right dorsal sucker; and (4) fibres from the lateral face of the left ventral sucker run to the median face of the right dorsal sucker.

(3) A third cross system orthagonal, running dorsoventral and latero-lateral, is situated just behind the second diagonal system. Behind this third muscle-cross the great nerve commissures occupy the central space between the suckers.

(4) A fourth cross system is found (behind the great nerve commissures) at the base of the suckers; the fibres of this system insert on the suckers in such a way that: (1) the median faces of the two right suckers, (2) the median faces of the two left suckers, (3) the lateral faces of the two dorsal suckers, and (4) the lateral faces of the two ventral suckers are connected.

(5) Behind this there appears to be a muscular connection between (1) the right sides of the ventral suckers; (2) the left sides of the ventral suckers; (3) the right side of the dorsal suckers; (4) the left sides of the dorsal suckers. I did not notice this system to connect the dorsal and ventral suckers.

## The Suckers.

The suckers are large and are pointed diagonally outwards and forwards. A ring formed by a fold of the subcuticula projects beyond the margin of the suckers, giving them greater depth. Externally they are covered with cuticula, similar to that covering the rest of the worm; internally they are separated from the scolex by a very thin membrane.

The muscle fibres composing the suckers are arranged in the usual manner. The radial fibres are well developed, arranged in small bundles in the middle zone; these bundles spread out slightly both exteriorly and interiorly. The ganglion cells already referred to are mostly situated at from the middle to the inner third of the radial fibres.

A thick layer of muscles is found on the membrane separating the suckers from the scolex. These muscles run parallel to the membrane and are seen lengthwise on transverse sections; the radial fibres pass between these fibres to attach themselves to the membrane. Above this layer proceeding away from the delimiting membrane, one finds cross-sections of muscles running also parallel to the membrane, but at right angles to the first layer. These muscles all show a hollow in their middle (or appear annuliform on section). About midway between the two curved surfaces of the sucker (still examining a transverse section) one finds very fine fibrillae, probably muscular, which run from margin to margin of the sucker. They do not keep the exact middle between the cuticula and membrane, but in the centre of the sucker approach the membrane.

A ring muscle, or sphincter, is seen at the margins of the sucker. On transverse sections one only finds cross sections of its muscle-fibres. This

muscle seems to be a further development of the second layer from the membrane mentioned above, as on transverse sections it is continued into it. Its fibres are also found under the cuticula, fewest in the middle of the sucker, in greatest abundance at the margins.

All the muscles of the suckers are embedded in parenchym. A layer of cells, with small round nuclei, measuring on an average about 4·5 $\mu$, is found between the radial fibres quite close to the cuticula. These cells are rather poor in plasma, their nucleus is vesicular, contains a fairly large nucleolus and one or two chromatin bodies. These cells may represent a much modified subcuticula, for which their arrangement in a definite layer under the cuticula and the cuticular muscles (ring muscle) would seem to speak; on the other hand, their plasma bodies do not resemble the normal subcuticular cells of this worm.

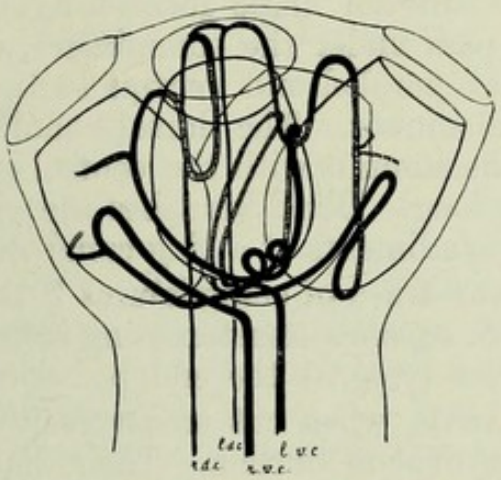
### The Longitudinal Canals.

The longitudinal canals have already been shown to be very conspicuous in the posterior part of the strobila. It is, however, only the ventral canals which one sees; the dorsal canals become rudimentary at 50 cm. from the scolex, though they can be traced as a solid line of tissue throughout the strobila. In the proglottids, the dorsal canal gets displaced and runs laterally and internally to the ventral canal. In the base of the scolex we find them lying dorsal and ventral to each other; even here the difference in calibre is very apparent.

The left ventral canal on reaching the level of the base of the suckers turns at right angles and proceeds horizontally to the base of the left dorsal sucker, rising vertically and laterally to the sucker to just above the first sections showing the lumen of the sucker; it then turns over and proceeds downwards parallel to its ascent; arrived near the base of the sucker it gives off a short branch which terminates abruptly in about the same height as the loop just mentioned (i.e. after the first sections showing the lumen of the sucker).

The main canal, however, twists around, internal to its initial horizontal portion and almost parallel to it, crossing the scolex externally to the dorsal canal; arrived at the base of the left ventral sucker, it ascends the median side of the sucker until the external opening of the sucker is reached, it then runs downwards parallel to its ascent, until the base of the sucker is reached; passing round the external face of the base of the sucker and forming a few spiral loops at its base, it ascends the lateral inner side of the sucker as far as the cross commissure, then it sends off a branch which proceeds a short distance to the outer surface of the dorsal sucker, where it terminates abruptly. The main canal, however, continues ascending just between the two suckers, after making a single spiral loop, to the summit of the scolex, where it bends over, descends in the inner angle formed by the two suckers until the cross commissure is reached; here it bends inwards, then upwards, and runs parallel and ventral to the

dorsal canal until the summit of the scolex is reached; here it again bends over horizontally and dorsally to join the left dorsal canal in a simple loop.



*Explanation of Figure.*—r.v.c. right ventral canal.
l.v.c. left ventral canal.
r.d.c. right dorsal canal.
l.d.c. left dorsal canal.
The scolex is seen obliquely, the left central sucker facing the observer.

The right ventral canal, having reached the base of the suckers, also bends over dorsally and horizontally, running to the right dorsal sucker, then it ascends the median internal face of the right dorsal sucker and runs almost the whole length of the sucker, veering over more and more to the right until it at last appears on the dorsal summit of the sucker; then it turns over, descends parallel to the direction it came, until the base of the sucker is again reached; it now turns and runs horizontally through the scolex, remaining exterior to the dorsal canal but interior to its first horizontal winding; arrived at the median internal base of the right ventral sucker, it ascends and descends its internal ventral side, reaching the height of the opening of the sucker. Passing around the base of the right ventral sucker on its posterior surface, it ascends the outer angle of the two right suckers; at about the same level as the left canal branches, the right canal does the same, but unlike the left canal, it sends off two ramifications, one to the outer surface of the dorsal, the other to the outer surface of the ventral sucker. After the branches have been given off, it ascends almost to the summit, bends over laterally and inwards, descends in the inner angle of the right suckers until the cross commissure is reached; here it turns inwards, passes under the nerve commissure, then proceeds in a straight line anteriorly, parallel, and ventral to the dorsal canal; arrived at the summit it bends over dorsally and joins the dorsal canal. The two dorsal canals ascend the scolex in a straight line until they are joined to the ventral canals; the right and left dorsal canals are connected at the summit by a short loop.

The course of these canals can be summed up as follows: The ventral canals on entering the scolex pass over to the dorsal suckers, run up and

down their exterior surfaces, recross the scolex, run up and down the median surfaces of the ventral suckers, pass round the base of the same suckers, run up the outer lateral angle formed by the dorsal and ventral suckers, cross over the anterior angle formed by the same suckers, run down the inner angle, pass under the commissure, and then vertically to the summit of the scolex parallel and ventral to the dorsal canal, joining the dorsal canal at the summit. The dorsal canal runs straight through the scolex, uniting at the summit with the ventral canals, and joined near the summit by a short loop. Blind branches are given off by both right and left canals (perhaps rudiments of a ring canal ?).

I have not found any trace of commissures between the lateral canals in the proglottids ; in *S. hepatica* anastomosing canals connect the ventral canals, these commissures lying at the end posterior of the segment.

The longitudinal canals, when cut transversely to their long axis are more or less circular or oval in outline. Their diameter varies considerably in various parts of the body, as can be seen from the following :

| | Dorsal. | Ventral. |
|---|---|---|
| Scolex, at apex .. .. .. | 72 μ | 72 μ |
| ,, at base .. .. .. | 32 μ | 128 μ |
| Strobila, near scolex .. .. .. | 16 μ × 32 μ | 96 μ × 48 μ |
| ,, 10 cm. from scolex .. | 12 μ × 24 μ | 32 μ × 80 μ |
| ,, 20 cm. ,, .. | 28 μ × 20 μ | 96 μ × 96 μ |
| ,, 30 cm. ,, .. | 4 μ × 4 μ | 112 μ × 72 μ |
| ,, 40 cm. ,, .. | obliterated | 120 μ × 80 μ |
| ,, 50 cm. ,, .. | ,, | 80 μ × 110 μ |
| ,, 60 cm. ,, .. | ,, | 84 μ × 48 μ |
| ,, 70 cm. ,, .. | ,, | 160 μ × 240 μ |

Segments with nearly ripe ova obliterated 192 μ × 80 μ. The walls of the lateral canals are formed of a membrane, staining with orange *g*. In the apex of the scolex, in sections tangential to the course of the canals a very fine cross-striping becomes apparent; the stripes are 2 μ across, separated from each other by an almost invisible fine dark line. I have been able to find this structure in material fixed in Zenker's solution or formaline, stained with haematoxylin and orange *g*. The walls of the canals are covered with a layer of cells, probably belonging to the parenchym. In the strobila the cross-stripes are much harder to make out.

On sections in the proglottids the dorsal and ventral canals differ considerably.

The ventral canals have thin walls, and the nuclei of the surrounding parenchym cells lie quite close and tangentially to it. The dorsal canals, on the other hand, have much thicker walls, often thicker than the lumen ; these walls have three different layers, the innermost very thin, dark ; the middle about 1 μ thick staining orange, the outer about 2 μ thick, staining with haematoxylin ; they are surrounded by closely radially arranged

club-shaped structures, thickest at the outer end, staining lightly with haematoxylin, total length about $4\frac{1}{2}$ $\mu$, thickness perhaps $\frac{1}{2}$ $\mu$ or less. (Zenker, haematoxylin, orange *g*, 40 cm. from scolex.)

## The Genital Apparatus.

The first trace of the genital apparatus can be seen in total preparations at a distance of about 1 cm. from the scolex; here one notices a narrow unbroken band of darker stained tissue running down the chief axis of the strobila; this band becomes more distinct by the time the 10th cm. from the scolex is reached. Between the 12th and 20th cm. the testes begin to appear, filling the dorsal portion of the field between the ventral canal and the lateral nerve, and between the ventral canal to just beyond the dorsal canal; there is no space for testes dorsally to the ventral canal. The central dark stripe also begins to lose its continuity and to break up into a series of transverse lines. Between the 20th and 30th cm. a great deal of development takes place. The median dark stripe disappears, the testicles become much more conspicuous, occupying the outer quarter of the median field, but not passing the nerve laterally. In each segment one now sees a dark strip of tissue, irregularly alternating to right or left, commencing just before the median line, slightly swollen at its internal end, clearly traceable as far as the lateral canal; very careful examination shows these transverse lines of tissue to be continued to the opposite side by a very fine canal, running to the testes (*vas differens*). At 40 cm., the testes remaining as they were, these strips of dark staining tissue have still further developed. Just to the right or left of the median line, according to whether the segment opens to the right or left, very distinct round clumps of tissue are seen (ovaria); to each side of these clumps the dark tissue becomes fusiform and stains less darkly; it is connected by a mere thread across the ventral canal to the anlage of the vagina and cirrhus apparatus, which lies as a long, slightly pointed strip between the ventral canal and margin, without, however, reaching the margin. At 50 cm. the chief difference seen is that the cirrus has somewhat approached the margin, which is reached at 60 cm. At 70 cm. we notice the anlage of the uterus crossing the median field. As the uterus becomes larger and broader and fills with eggs, the ovary atrophies and finally disappears from view on total preparations about 40 cm. further on. (The exact distance cannot be given, as the specimens studied consist of various fragments.) At about 95 cm. from the scolex a strip of dark staining fibrillar connective tissue begins to develop between the uteri of every two consecutive segments, firmly holding them in position. At 130 cm. from the scolex, the uteri have increased still more in size, and in contracted material three or more segments can be seen to pass over a single uterus; that is to say, the uterus of each segment bulges into the two adjacent and sometimes even into still more distant segments. At this stage Wolffhügel's " Faserknaeüel " (*Stilesia hepatica*, p. 10), is to be seen

on transverse sections, in typical arrangement as described by Wolffhügel, only that (in *S. hepatica* and in *S. centripunctata*) it is connected with the uterus and not with the ovary. Out of this organ the egg-pouches develop; the eggs leaving the uterus, which atrophies, finally collect in the egg-pouches.

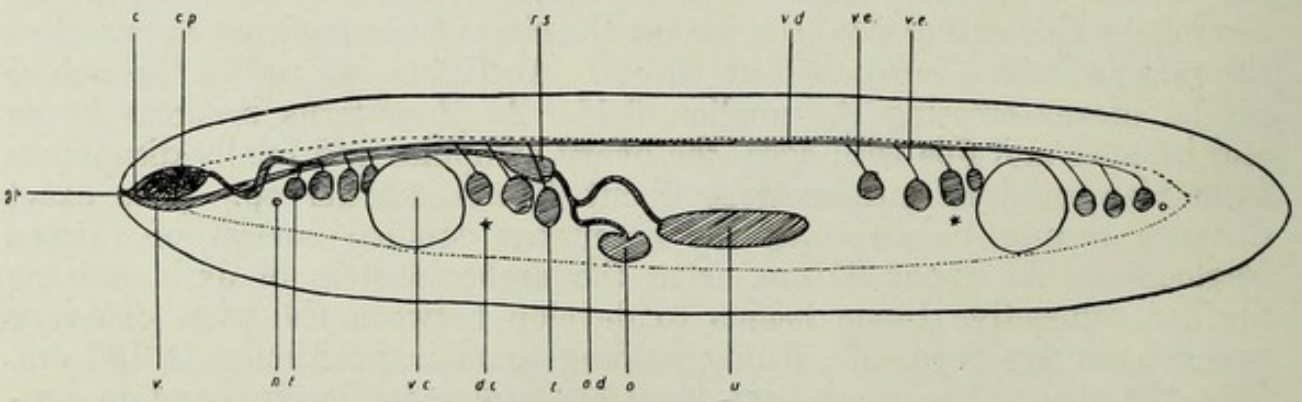
### Topography of a Segment at Male Sexual Ripeness.

The genital organs, as has already been explained, open irregularly alternately to the right or left.

At the stage when the spermatozoa are maturing and the first have reached the receptaculum seminis, the genital organs, which all lie in the transverse plain, are composed as follows :—

There are three, four, or more testes on each side of both right and left ventral canals, lying slightly dorsally ; all contain spermatozoa and also spermatoblasts. *Vasa efferentia* can be found upon very careful search; they appear to run from the testicle direct to the *vas deferens*. The *vas deferens* crosses the dorsal side of the median zone, quite close to the transverse muscles, from the aporose to the pore side. Just before reaching the cirrhus pouch, laterally to the ventral canal, it becomes much dilated and crammed with spermatozoa and is no longer straight, but wound in a few spirals. Having entered the cirrhus pouch it is wound up in a number of convolutions, still filled with spermatozoa ; the terminal portion is straight, easily distinguishable by the dark blue staining of the ciliated layer next to its lumen. The length of this ciliated portion is 63 $\mu$ ; breadth about 3 $\mu$. The genital pore is extremely small, probably no longer functional. The end of the cirrhus bends over and joins the vagina; it is not introduced into the vagina, but appears to be fused to the end of it. (Fig. 6.) There is a short, very narrow connection between vagina and pore.

Schematic Diagram of the Principal Organs at Male Sexual Maturity.




*Explanation of Figure.*

| | | |
|---|---|---|
| v. vagina. | t. testicle. | r.s. receptaculum seminis. |
| c.p. cirrhus pouch. | o. ovarium. | n. nerve. |
| g.o. genital aperture. | o.d. oviduct. | v.c. ventral canal. |
| v.d. vas deferens. | u.d. uterine duct. | d.c. dorsal canal. |
| v.e. vas efferens. | u. uterus. | |

The vagina runs straight from the pore to dorsal of the lateral canal and testes. On transverse sections I find that on one side of the body the cirrhus is dorsal to the vagina, on the other ventral to it. (The main axis of these sections being uncertain, I cannot state which is right and left.) Arrived in the median field it proceeds dorsally to the testes, but ventrally to the *vas deferens*, forming a *receptaculum seminis* dorsal to the most median testes. The *receptaculum seminis* is continued inwards by a single canal, the fertilisation canal, which, however, soon branches, one branch being the oviduct, the other the uterine duct.

The ovary is single, somewhat bean-shaped. There is [as in *St.* (*globipunctata* and ?) *hepatica*] neither vitelline gland nor shell gland. The uterus at this stage forms a straight, cylindrical chamber, lying in the transverse axis of the proglottis. At this stage the first eggs are finding their way over into the uterus, so that it is evident that the testes and the ovary ripen simultaneously ; in *S. hepatica* the ovary ripens before the testes are quite mature.

### Spermatogenesis.

The spermatogenesis is rather difficult to follow for several reasons. In the first place it has to be studied on section series, then it is rather confusing that several stages can occur at the same time in one and the same testis, and finally one has to distinguish between the development of two different kinds of cells, but which both appear to have a common origin.

As already remarked by Child (Amitosis in Moniezia), it is impossible to distinguish the cells from which the testes arise from the parenchym cells. The first stage at which one can feel certain is one at which two or more cells are found lying together in definite arrangement and apparently surrounded by a membrane.

The youngest cells seen (urogenital cells, wall and basal cells) form a syncytiium of four nuclei in a testicle—diameter of testicle, 9 $\mu$; nucleus 6 $\mu \times 4\frac{1}{2}$ $\mu$ cytoplasm stains blue ; nucleus blue, same depth of colour as cytoplasm ; nucleolus minute, at the most 1 $\mu$, very dark ; chromatin in small round masses lying on the surface of the nucleus. The arrangement of the nuclei not radial.

On the same sections one finds testicles which have developed further, and on which the division into the two mentioned types cells have taken place. These can be classed by their appearance into " yellow " or acid cells and blue or basal cells, according to their staining properties. The " yellow " cells generally stain yellow with orange *g*, they are devoid of cytoplasma or nearly so, a very large nucleolus, measuring up to 3 $\mu$ (sometimes smaller in younger stages). The chromatin in young stages in globules, later band-shaped, finally absent. The nucleolus in sections first stained with borax carmine, then haematoxylin and orange *g*, has a

decidedly ruby red tinge, not noticeable in my sections in any other type of cell in the whole worm.

These yellow cells are found in the testicles during the younger stages of development; they disappear before maturity and apparently are concerned with the nutrition of the blue cells which finally produce the spermatozoa.

The " blue " cells differ very much in size during the various stages they pass through ; their cytoplasm is, when present, always blue (except detritus remaining after formation of spermatozoa, which eventually turns yellow). Their nucleolus never reaches the size of that of the " acid " cells.

For some time, and until the testes have reached a fair state of development, the multiplication of both types of cells appears to take place by amitotic division of the cells into two, the resultant cells not arranging themselves radially (spermatogonia).

After these preliminary divisions, one finds both blue and yellow cells undergoing division, by which the resultant new cells (spermagenes) lie arranged radially (Fig. 7), connected in the centre by thin filaments of protoplasm, yellow in the case of " acid " cells, blue in the case of the normal spermagene.

This stage resembles the " first phase of proliferation " Bugnion and Popoff saw in the spermatogenesis of *Lombricius agricola* (Bugnion and Popoff, La Spermatogenèse du Lombric, Zool. Congress, Berne, 1904). It is followed as in the earth-worm by a phase of dissociation, especially marked in the case of the acid cells which separate entirely. The blue cells (spermatocytes 1), on the other hand, remain connected to the centre of radiation by their protoplasmic processes for some time, increasing in size ; their nuclei becoming denser and darker as the cells increase in size, but without changing in magnitude (nuclei soon after proliferation $4\frac{1}{2}$ $\mu$, after increase of cell $4\frac{1}{2}$ $\mu$). After proliferation chromatin in small peripheral globules, after growth of cell in larger masses filling the nucleus.

The basal cells do not seem to divide again, but degenerate and finally disappear.

Just before the second proliferation of the spermatocytes the chromatin again changes, first forming small globules, then stretching itself out into threads. The next change appears to be that the cells forming one of the radial figures left after the first proliferation and dissociation fuse, the nuclei resolve themselves into chromatin threads and wander into the interior of the syncytium, all traces of the single component cells becoming lost. The nucleoli remain visible until the chromatin has drawn itself out into threads ; it then also appears to form threads. The chromatin then seems to break up into short lengths, each of which at the surface forms the nucleus of a new cell (spermatid). These cells after further division give rise to spermatozoa. A large quantity of cytoplasma and

a few loops of the chromatin remain behind in the middle and form a cytophore. The cytophore can persist for some time, eventually all trace of its nucleus disappears, the protoplasm changes its reaction, staining orange, and finally it disappears when the spermatozoa mature.

I have not been able to compare with any data given by other observers than those quoted, owing to inaccessibility of the literature.

## Further Development of the Female Organs.

The Oogenesis in *Stilesia* would be doubly interesting to follow, for, owing to the absence of vitelline glands, it differs from that of most other cestodes.

In the earliest stages the ovarian nuclei form a syncytium and divide amitotically as described by Child for Moniezia; the first mitoses were observed at about 50 cm. from the scolex, at a stage at which the oviduct and uterus were yet solid masses of tissue without apparent lumen.

At this stage, the cell boundaries are becoming distinct. As development progresses, mitoses become more frequent, and finally all cell division appears to take place by mitosis. In the ripe ovary (80 cm. from scolex) mitoses are frequently observed, four chromosomes appearing to be the normal number. In the case of the ovarian ova, the resultant cells seem to separate as soon as the mitosis are over. Mature ovarian ova measure approximately 20 $\mu \times 16$ $\mu$; their nuclei are round and about 8 $\mu$ in diameter. Fertilisation probably takes place in (the oviduct or) the uterine duct. The passage of eggs through the ovi-uterine duct must be a very quick one, as in no case have I found an ovum traversing either of the two ducts; this although many segments have been examined on sections in stages in which the ovarium is in full function, and the uterus is filling with ova.

Arrived in the uterus mitoses commence again, but the resultant nuclei remain embedded in a syncytium; such cells with two or four nuclei are very common; the number of chromosomes seems to have increased—I believe that there are eight—but they are very difficult to count; in any case four is no longer the normal, but a higher number is always present. Young uterine ova measure approximately the same as the mature ovarian ova, their nuclei however decrease in size as division progresses; two in one young ovum measured 6 $\mu$ in diameter. It may here be stated, that I have sometimes found ova similar to those in the ovary or to those just arrived in the uterus, free in the parenchym of the body, occasionally in large numbers. Just at this part, my material is unfortunately not sufficiently well preserved to enable the further changes to the ova to be followed in detail.

The maturest ova which I have seen have a thin membrane, and lie in egg-pouches, no longer in the uterus.

After the stage at which fertilisation takes place, several changes occur in the female genitalia; in the first place a layer of dense fibrous supporting (or connective) tissue is formed anteriorly to each uterus.

On sagittal sections, it sometimes appears to be both anterior and posterior, but in such cases I believe that this is only apparently the case and in reality the pads of fibrous tissue belong to separate segments. In sagittal sections one often finds two pads between two adjacent uteri; on a horizontal section it would be seen that the uterus of a third segment has been crowded out to the side between the two uteri visible on the section. Owing to bad preservation of my material just at this part of the worm I cannot venture any statement as to the origin of the pads of fibrous tissue.

The uterus after having received the fertilised ova undergoes several changes; at the time when first filling with ova, it is simply a wide transverse tube. The ova at a slightly later stage appear to be surrounded by septa formed by cells originating from the uterine walls. (Rather macerated material.) With the disappearance of testicles and ovary the segments lose breadth and contract latero-laterally, forcing the uterus to become more and more globular.

At this stage an organ resembling "Wolffhügel's Faserknaüel" becomes apparent; its early stages are unfortunately lost to me on account of the maceration the material has undergone just at this part. In *S. centripunctata* it has much the same appearance in certain stages as in *S. hepatica*; in both species it is an outgrowth of the uterus, not of the ovarum. On transverse sections (about 120 cm. from scolex) one notices the following changes:—The ova all lie on that side of the uterus nearest to the surface of the worm, in one segment perhaps dorsally, on the next it may be ventrally, according to the position of the uterus, which again depends on the pressure from the uteri proceeding and following it in the strobila.

On the other side of the uterus is a mass of fibrous tissue with radial or parallel fibres, which in its further development gives rise to the Faserknaüel, which in turn develops into egg-pouches. (Fig. 8.) This mass of fibrous tissue is in early stages arranged within the uterus converging towards the side opposite to the ova; it passes beyond the uterus into the parenchym of the body, still converging; the opening it passes through is at first large, later this contracts, thus forcing the fibres to form a double radiation, one in the uterus, the other outside it. At first nuclei are very numerous, later on they become rarer. (Fig. 9.) The portion outside the uterus grows larger and more globular, the fibres arrange themselves so as to form a number of pockets, the uterus itself during this process diminishes in width. (Fig. 10.) Then we see ova appearing in the pockets of the fibrous organ. (Fig. 11.) As they disappear at the same time from the uterus, there is no doubt from where they came. Finally all the ova are in the pockets of the organ and the original uterus atrophies. (Fig. 12.) The fibres of the egg-pouch form an outer circular layer around the inner pouches, which themselves consist of fibres. In each case the fibres seem to be arranged so as to form lamellae.

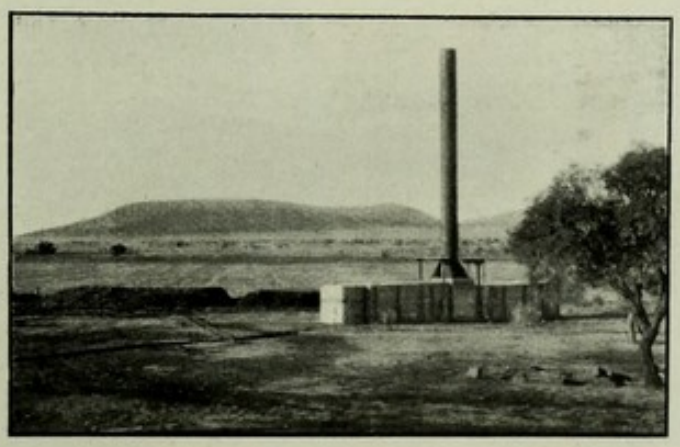
Before leaving the uterus, the ova are already provided with their shell. They are globular, measuring 24 $\mu$ to 28 $\mu$ in diameter (from specimen macerated with 10 per cent. potash), and are devoid of projections or spines of any kind. The pockets in which the eggs are finally enclosed, resemble to a very great degree those figured by Lungwitz for Taenia Ovilla (*Thysanosoma giardi*).

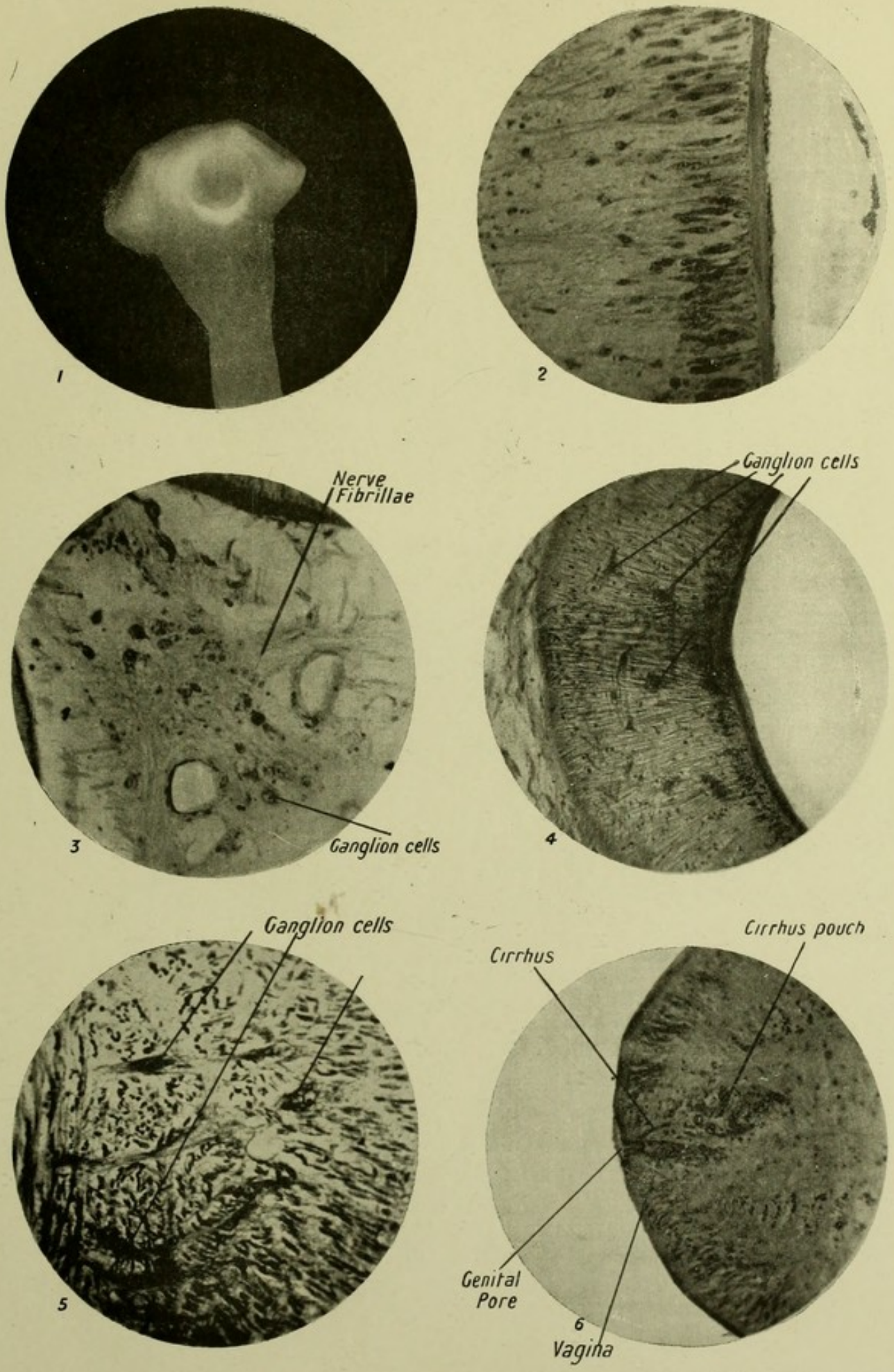
### Influence of Host.

I have not observed any lesions attributable to the presence of the worm at post-mortems of sheep infested with *Stilesia centripunctata*. The suckers are, however, often filled with epithelial elements.

### Specific Diagnosis.

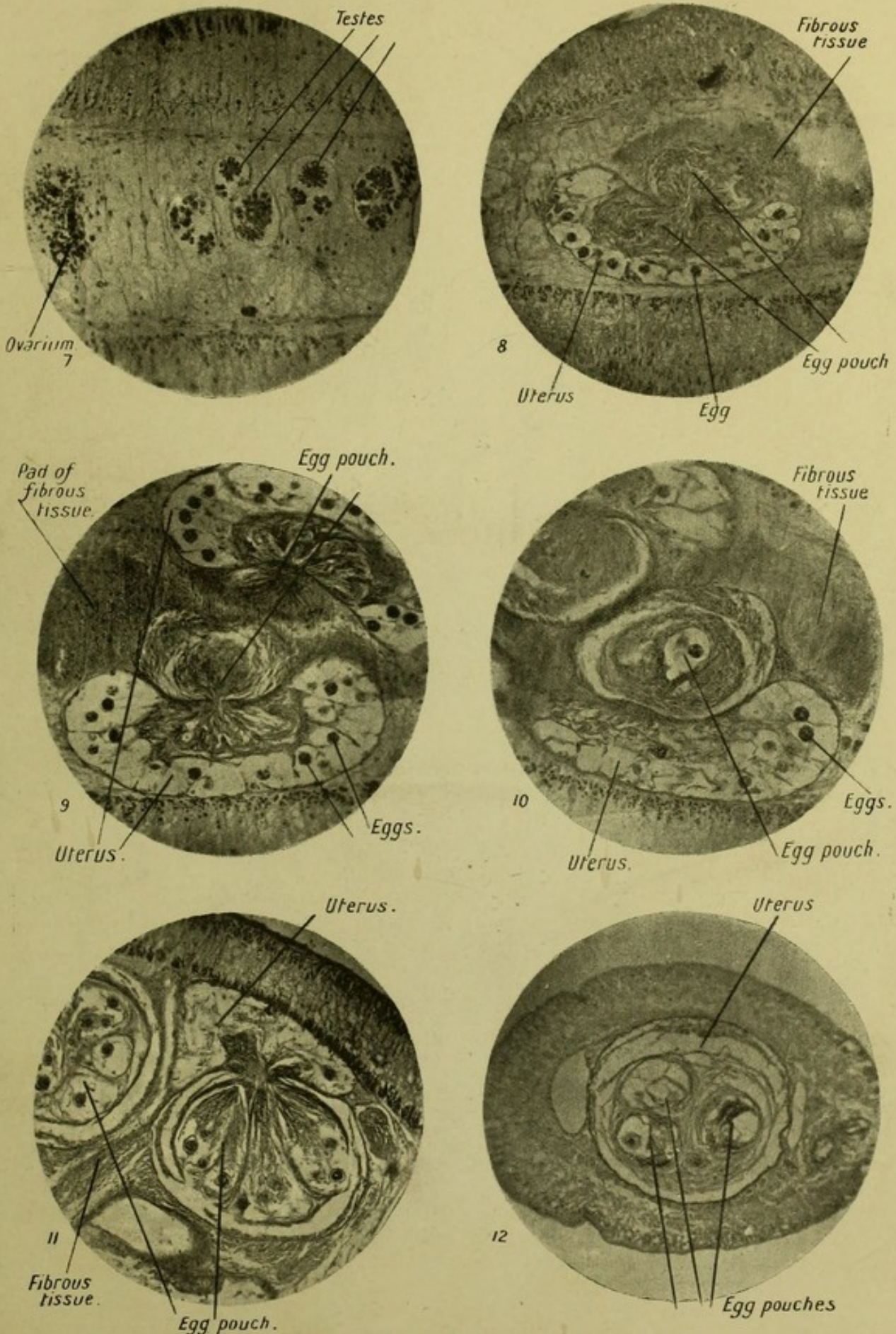
*Stilesia centripunctata* (Riv. 1874), Railliet, 1895. Head large, varying from 1·5 mm. to 3·1 mm. long, by 1·0 mm. to 2·4 mm. broad. Suckers, large, directed diagonally outwards. Strobila from 2 to 3 metres long. Segments 10 cm. from scolex measure from 2 to 3 mm. wide at 20 cm. up to 3·2 mm.; growing narrower posteriorly, the strobila loses its flatness and finally becomes almost round in section, measuring about 1 mm. in diameter terminally. The greatest width is often, but not invariably, close to the scolex. Segments are always much thicker than long, and anteriorly much broader then thick. Genital openings irregularly alternate; uterus transverse in median portion of field, mature ova in egg-pouches arising out of fibrous organ. Testicles extend from a short distance from each end of uterus to almost to the lateral nerve. The genital canals pass dorsally to the nerve and to the dorsal and ventral canals. The *vas deferens* passes dorsally across the segment, dorsal to lateral canals, nerves, vagina spermiduct, *receptaculum seminis*, and uterus. The cirrhus is becoming rudimentary, opening directly into the vagina. No vitelline gland nor shell gland. Ova round, without spines or other projections, measuring 24 to 28 $\mu$. Development unknown; host sheep, Italy and Africa; habitat in the small intestines.






1. Scolex, half expanded (Formaline specimen).
2. Subcuticula and cuticula.
3. Ganglion in rhomboidal commissure.
4. Section through acetabulum, showing ganglion cells.
5. Section of sucker of *Anoplocephala magna* Abilgaard to show ganglion cells.
6. Genital openings, to show connection between cirrhus and vagina..

All figures (except 1) were from specimens fixed in Zenker's fluid and stained with Haematoxylin (Ehrlich), counterstained with orange *g*; average thickness of sections 4 $\mu$.




7. Testicles with various stages of spermato-genesis and anlage of ovarium.
8, 9, 10, 11, 12. Consecutive stages in the development of the egg-pouches.

# Notes on the Pathological Anatomy of Pleuro-Pneumonia.

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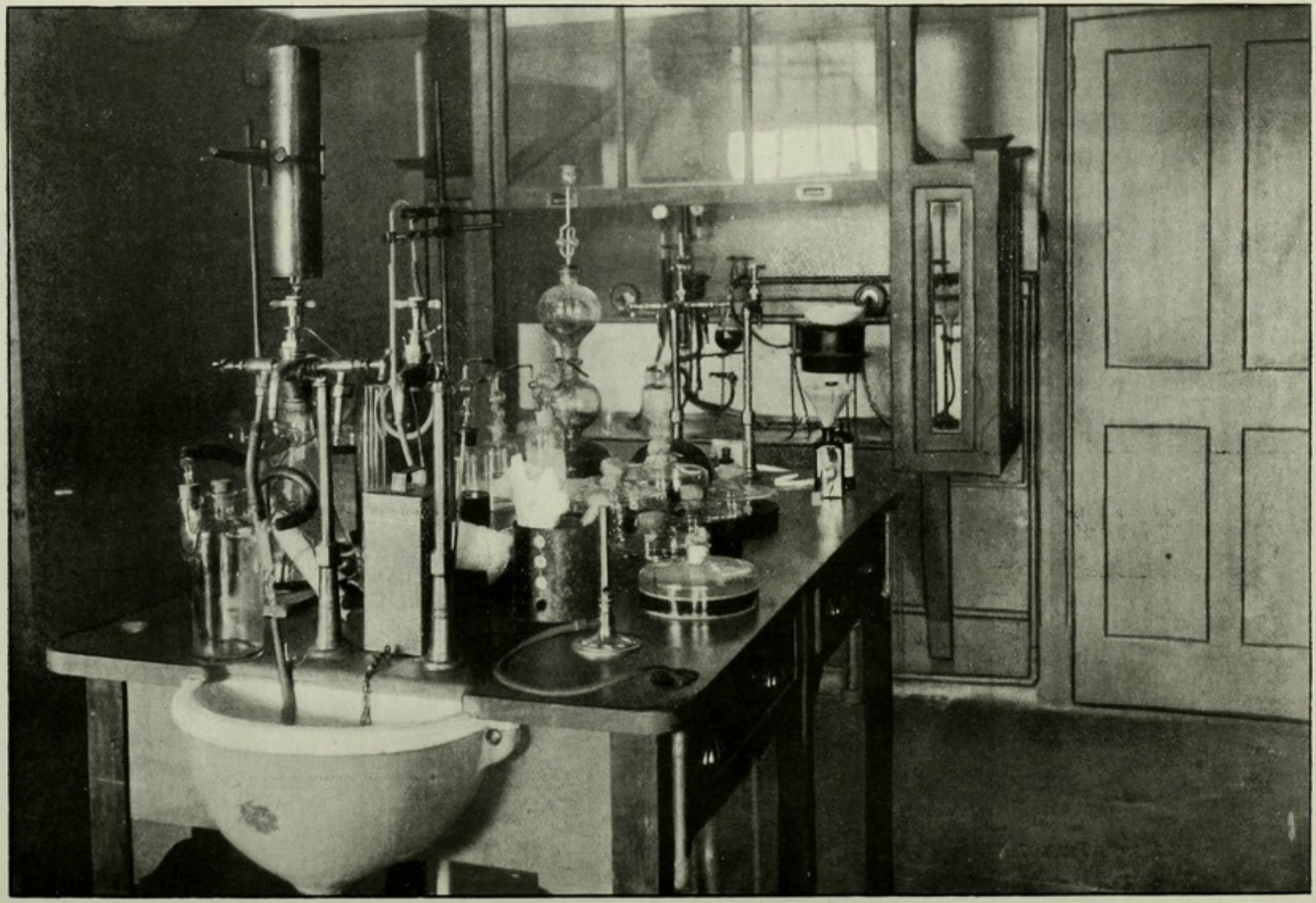
By Dr. K. F. MEYER.

*The Pathological Laboratory.*



*The Bacteriological Laboratory.*

# Notes on the Pathological Anatomy of Pleuro-Pneumonia Contagiosa Bovum.

By Dr. K. F. Meyer.

No attention has been paid to the lesions caused by the virus of pleuro-pneumonia in the lungs and in the tissues generally, since the classical investigations of Sussdorf,* Pourcelot,† M'Fadyean,‡ and Csokor.§ This is all the more astonishing, for since the discovery of Nocard of the micro-organism of pleuro-pneumonia (which was made since the publication of the papers cited above) we are now able to verify the histological lesions with the aid of pure cultures.

It is difficult to assign any reason why such investigations have not been carried out. It may be that, thanks to energetic prophylactic measures, this once so fatal disease has received such a check that suitable material for investigation has become unobtainable; or else, that in consequence of the mutual interdependence of modern research, which all seems to tend in the same direction, workers have left this problem to take up more sensational ones, which might be described as the fashion of the day. I am, however, of the opinion, that it will be amply rewarded if we take up the study of old, well established phenomena from the modern points of view, especially considering the results and technique elaborated by V. Prowazek‖ and Lipschutz¶ for visible filterable micro-organisms.

From the observation of various changes which are absolutely specific for this disease, one is forced to draw conclusions as to the effect of the noxes of the infection. These changes will accordingly be employable in diagnosing the disease.

The macroscopic lesions in the lungs in pleuro-pneumonia (exudative fibrinous pneumonia and pleuritis) cannot be used to diagnose the disease, as they can appear primarily or secondarily as lesions due to other diseases; the various histological changes on the other hand are quite specific. This can be seen to a limited extent from the already existing literature on this subject. It is very useful to be able to make a diagnosis of the disease entirely from the histological lesions, without having to wait for the result of cultures, especially here, where pleuro-puenmonia is of fairly frequent occurrence.

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*Deutsche Ztschrift f. Tiermedizin, 1879, V. Bd., page 358.
†Lyon Médical, 1881, page 145.
‡The Journal of Comparative Pathology, 4/10/1891, page 333.
§Wiener Klinische Wochenschrift, 1894, page 964, and Lehrbuch der Gerichtlichen Tierheilkunde 2. Hâlfte, Wien 1891, page 415.
‖ Arbeitenaus dem Kaiserlich, Gesundheitsamt 1905–06, Munch. Mediz. Wochenschrift, 1908.
¶ Centralblatt f. Bacteriologie, Bd. XLVIII, 1908, Heft 1, page 79.

It is our intention to show that histological diagnosis is both possible and practical.

Both the pathological anatomical collections of the Government Veterinary Bacteriological Laboratory, and material collected during the investigation of an extremely virulent pleuro-pneumonia virus, offered special facilities for the study of the histology of the lesions of the disease, and the more recent cases have given us a specially valuable statistical material. Unless otherwise stated, the material, on which this paper is based, has been treated with Kayserling, or with Orth's liquid (Formol-Mueller), passed through alcohol to xylol to paraffin. And it may be stated here that Orth's method gives splendid results; provided that the tissues have been sufficiently washed out they take all the most recent stains and give perfect illustrative preparations.

The study of the lesions is best carried out proceeding from the already known to the new, and drawing one's conclusions from recognised data of the lungs to compare the other phenomena connected with pleuro-pneumonia. Unless otherwise stated, corrections will only be necessary with regard to microscopical details.

In a paper "On Microscopically Visible Filterable Vira," Lipschutz deplores without reason the absence of histological records in pleuro-pneumonia, and expresses the wish that investigations should be made with the object to demonstrate the presence of "Einschlusse" (inclusions in cells), or of specific products of degeneration in epithelial cells. I have paid special attention to this question as far as possible on hardened material. I intend to return to this problem elsewhere.

My object in this paper is not to study the alterations in the cells themselves, but the changes in the histological complexes. First taking the microscopical lesions I will then go over to the microscopical details.

## Macroscopical Lesions in the Lungs.

All the lobes of the lungs examined were preserved in Kayserling 3, and consequently gave very clear insight into the structure of the morbid changes. It would carry us too far should I analyse in detail each of the pieces at my disposal, especially as we are dealing with well-known data. The general lesions that can be described from about ten lungs and from a similar number of pieces, most of which are taken from chronic cases, or such with high fever, are as follows:—

The pleura has in most cases undergone the different known changes. The fibrinous, plaque-like, thick deposits on the pleura pulmonalis of the anterior and middle lobe are most conspicuous; their fibrous consistence points to fibrine. They are easily ruptured, frequently white in the middle, with filamentous surroundings, outlined with dark yellow, sometimes, however, only straw-coloured irregular contours, which are frequently haemorrhagic infiltrated.

The thickness usually varies from 0·5 mm. to 4 mm.; 6 mm. to 8 mm. is, however, no rarity. The subjacent pulmonary tissue is always oedematous and atelectatic.

The pleura costalis was only in one case attached to the pleura pulmonalis by fibres. The pleura diaphragmatica is only very rarely attached in a similar manner to the diaphragm. The spongy tissue of the fibrinous deposit is completely filled with a sero-yellowish fluid. The pleural cavity itself is filled with a reddish-yellow serum, containing scattered flakes of fibrine. This fluid, the virus par excellence, coagulates fairly readily when in contact with the atmosphere, but expresses a large quantity of serous liquid. On removing the fibrinous deposit, the pleura appears as a much thickened, opaque, whitish membrane, which gradually merges into the diffuse grey pleura of the whole lung; these discolourations are often pigmented, and streaked with small rarely ramificated blood vessels. Wherever the septum mediastinale is preserved, one finds yellow gelatinous masses, which separate the cava pleuralia in the form of tumours. On incision a considerable quantity of fluid escapes. In two cases the oesophagus was deflected by infiltration of solid masses of fibrine, the exudative process having here also affected that organ. As a consequence, a pericarditis fibrinosa *per contiguitatem* had arisen.

The changes are very varied in the cases under review. Frequently only small reticulations of fibrine and capillary ektasia are present, giving the pleura the appearance of a simple pleuritis sicca. This can be considered to be, from the point of view of comparative anatomy, the secondary consequence of the subjacent croupous pneumonia. Through Nocard's researches it was demonstrated that the affection of the interstitial tissue is primary, and that the fibrinous pleuritis arises secondarily *per continuitatem*. It is therefore clear that when the fibrinous exudation has reached the great extent it does, and the serous exudate has become prominent, the process of the disease has already reached an advanced stage. I paid special attention to the question if the portions adjacent to the pleura showed an older stage of pneumonia than the pleura itself; this could be demonstrated to be the case in 80 per cent. of the cases. I cannot go into the question here, why the interstitial tissue of the lungs or its lymphatic vessels must be the points of attack of the pleuro-pneumonia virus, as has been demonstrated experimentally and comparatively. As shall be shown further on, the pleura behaves in just the same manner towards the virus of pleuro-pneumonia, on account of its wealth of lymphatics, as do the serous membranes generally.

The morbid portion of the lungs is conspicuous on account of its great roundness and tougher consistence, it is consequently very easy to separate the diseased tumescent portions from the normally collapsed tissues of the healthy parts. The diseased lobes attain dimensions such as are otherwise never observed. The condition of the lungs remind me

very forcibly of those of horses suffering from the pulmonary form of horse-sickness.

These mostly circumscript affections of the lungs are usually covered smoothly by the pleura. The parenchym is compact, extremely elastic, and heavier than water. In some cases it is also very friable. The changes in elasticity are so variable that in some cases the affected parts are soft and pliable, in other cases so tough and hard that a distinct grating sound is emitted on incision. A variable quantity of sanguineus fluid, or of yellowish-reddish, slowly coagulating, serum-like liquid runs from the section.

On the surface of the cut appears the well-known appearance of interstitial pneumonia, specific in such dimension for pleuro-pneumonia. Broad, whitish-grey bands of connective tissue surround interlobularly, interalveolarly, and peribronchially the consequitive stages of croupous pneumonia. Such an appearance is described in pathologic anatomy as " marbled." The name " pleuro-pneumonia marbling " is generally applied on account of the thickened lymphatic vessels traversing the hepatised lung tissues like the veins in marble. Large and small dark and light lobuli group themselves to an artistic mosaic, not without regularity, although the point of departure of the infection is often hard enough to locate. It must be considered of importance that in 80 per cent. of the specimens, the stage of dark red friable, or haemorrhagic, hepatisation has been found. Blackish-red lobuli are seen in the tough, atelectatic tissue, only rarely penetrated by thick, thrombotised blood vessels. The surface of the sections is usually coarsely granulated. Close by one invariably finds lobuli in the stage of grey hepatisation. I cannot say that it is difficult to find such places, as Sussdorf states. Such groups of lobuli are often in dry necrosis. They are dark brownish-grey, and as compared to the other lobuli conspicuously dry. I do not find that they are always surrounded by dark lobules in my material. One frequently finds patches (from 1 cm. to 20 cm. in diameter) showing oedematous infiltration, and immediately alongside of them patches in advanced necrosis. It is reserved for experiments to show the reason of such differences. The primary condition (stage of "engouement") of the lobuli is always found; it is conspicuous that it is always arranged so as to surround the dark red patches. Healthy lobules, or such with a slight crackling oedema, are frequently interspersed among the diseased ones. We possess specimens of lungs in which single dark red hepatised lobuli are scattered on otherwise absolutely healthy tissue, but the expression " absolutely healthy " must not be taken literally, because the interstities are always somewhat enlarged and infiltrated. The older the stages, the more frequent is necrosis and sequestration. It has been demonstrated specific for pleuro-pneumonia that several lobuli, or even a whole lobe, necrotises and becomes separated in consequence of a

demarcating inflammation by energetic capsulation from apparently healthy or freshly hepatised tissue.

It is a peculiarity of the virus of pleuro-pneumonia and its toxines to facilitate the closure of all nutrative channels by the thrombosing of the blood vessels and the progressive-productive processes of the interstitia, thus forcing necrosis to take place. Even if in certain portions, through *resolutio*, a *restitutio ad integrum* takes place, bands of connective tissue remain behind along the septa and bronchi. A sequester appears as a brownish-grey mass surrounded by a granular slippery capsule, from which portions of blood vessels and thick fragments of bronchi project. Mummification of sequesters often takes place, and if bacteria from outside penetrate, fermentation processes can take place, and deliquation often causes the whole to anneal.

Sussdorf observed such cicatrisation of small foci in protracted cases of lung-sickness. Two such cases are in our collection. The sequester is always intimately connected with the thickened septa. This stage of pleuro-pneumonia corresponds to a *Pneumonia dissecans*.

The bronchi are always more or less affected, the peribronchial tissues are during the initial stages oedematous; later on changes take place in their lymph vessels, with exudation of fibrine, followed the formation by neoplastic connective tissue. For this reason the bronchi appear in chronic cases as thick, rigid tubes, sometimes containing plugs of fibrine, close beside them one usually finds blood vessels containing a thrombus, whose walls present the same thickened appearance. I have not yet seen any direct bronchioectasia as described by Sussdorf.

The mucous membranes of the largest bronchi are usually somewhat swollen, with small superficial haemorrhages. The nearer they are to the diseased lobuli, the more readily are they drawn into the morbid process; they then contain slimy, crumbly, friable plugs.

Besides all these manifestations of exudative pneumonia, closer attention must be given to the interstitium of the interlobular, interalveolar, and of the peribronchial tissue.

Pleuro-pneumonia virus reaches the lungs by inhalation, as has been demonstrated by experiment; arrived there, its pathogenic influence is first felt by the lymph vessels. Without going into the question whether the processes take their origin in the lymph vessels of the interstities or of the pleura, it is certain that its dissemination is closely connected with their course. In the beginning of the disease, one finds the interstities broadened, waxy, yellowish-white, greatly infiltrated, covered with a reticulation of haemorrhages.

The margin of such parts are white, consolidated, but the interior is filled with small cavities and crevices, containing a clear, amber-coloured fluid or fibrinous coagulum. In later stages, which can often be found on the same section, the walls of the cavities or varices, which are nothing

else than lymph vessels or ducts, have generally become thicker and more callous. The rounded cavities are turgid with exudate, often even filled with small, firm, friable, dark yellow thrombus. One can cut them open and squeeze out the contents.

The septa look like rosaries in consequence of the transparent hollows. As the processes becomes older the appearance also gets more uniform ; the neoplastic connective tissue takes up most of the space, and the septa becomes broader, opaquer, and more callous. Septa 3 cm. thick are not rare. The pulmonary tissue is divided up into more or less square areas by the interstities, so as to give a fanciful resemblance to a chess board.

The septa close up to the greyish-white, much-thickened subserosa if the pleura is affected, and if its morbid changes take place *pari passu* with those in the lungs. On removing the yellowish-white attached exudate, one recognises broad opaque bands of connective tissues corresponding to the outlines of the lobuli.

A remark on the modus of infection may here be interesting. In a specimen of a lung of an ox (belonging to the herd of this laboratory) a circumscript serous fibrinous infiltration of the peribronchial lymphatic vessels was found.

The processes seemed to have the tendency to spread from the major bronchus to the interalveolar tissue. I feel inclined to believe that most pleuro-pneumonia infections originate in the bronchial and peribronchial lymph vessels and spread *per continuitatem* to the rest of the lung. Be that as it may, it is certain that in the initial stages we have before us pathologic-anatomically an *interstitial pneumonia* due to *serous fibrinous lymphangitis*. The inflammation, although originally a purely interstitial disease, an uncomplicated pulmonal lymphangitis, soon spreads to the neighbouring alveoli, giving the appearance in chronic cases of a croupous pneumonia. Unmixed pleurogene pneumonia seem to be rare, that is to say pneumonias in which the process has spread secondarily from the pleura, although it has been postulated that only in such cases does one find the well-known characteristic macroscopic lesions. It is certain that the interstitial pneumonia of lung-sickness is not a recuperative reparatory process, such as pneumonias caused by foreign bodies or traumatic pneumonias or abscesses are, for the changes in the septa take place at a period when there can be no question of reconvalescence.

Deposits of fibrine in the vessels of the interstities are caused by the influence of the exudation, which spreads from the lymph vessels to the perivascular tissue ; the blood vessels being very often thrombosed. The same fate awaits the pulmonary blood vessels, which, compressed by infiltrated lung tissues, can pass through all stages of thrombosis and resolution. Dark red thrombus, attached to the intima are most frequently surrounded by the adventitia and perivascular connective tissue as a tough yellowish-grey ring. Filiform or lobular appendages of the intima

of the pulmonary veins as described by Sussdorf have not been observed in my material.

Although the changes due to lung-sickness are not restricted to the pulmonary tissue, yet the lesions in the lungs are of sufficient value to be of great help in diagnosing pleuro-pneumonia macroscopically.

1. The interstitia, i.e. the interalveolar, the interlobular, and peribronchial connective tissues are the seat of a primary inflammation of serofibrinous character. The chief changes due to inflammation take place in the connective tissue, therefore pleuro-pneumonia can rightly be said to be an *interstitial pneumonia.*

2. The disease follows the course of the lymphatic vessels very closely and affect the lymphatic vessels in the first place ; having recourse to the microscopic examination, we must diagnose a *lymphangitis serofibrinosa.*

3. The thickening of the septa (which can reach 8 cm.), and the so-called marbling of sections have certainly some diagnostic value, provided that the histological details are in agreement.

One will never find the described stratified arrangement of cavities and fissures in traumatic pneumonia, so common in cattle in consequence of perforation by foreign bodies ; quite independently of the total difference in histological details.

4. After sequestration has taken place diagnosis is no longer possible except by microscopical investigation.

## Microscopical Lesions in the Lungs.

The specimens which were preserved as already mentioned in formaline or Orth's liquid, were examined in paraffine or celloidin sections, after staining with haemalum-orange, Van Gieson, May-Grunwald Fischer, Heidenhains-haematoxyline, fibrine staining after Weigert and Kockel* or after Fraenkel† with Best's carmine for glycogen elastic fibrillae after Fraenkel and Mallory. Each section was carefully analysed in order to facilitate comprehension and to be able to make comparisons.

In looking through many specimens, one finds great diversity in details according to whether the sections were taken from pieces of recently diseased lobuli, or from such of more or less chronic cases. However they all have in common that they are stages of a croupous pneumonia, and show conditions that are known already and which have been described elsewhere. It will be readily understood that the specificity of the histological lesions of pleuro-pneumonia will not be found here, although an observer, who has much to do with such specimens might find out details which could help towards a diagnosis. It was shown in the microscopical anatomy of pleuro-pneumonia that all three stages of

* Centralbl f. All. Patholog., Bd. 10.
† Muench. Mediz. Wochenschrift No. 50, 1908, p. 2634.

croupous pneumonia are found, the height of the condensation or grey hepatisation being the most numerous.

More or less homogenous loose fibrinous masses mixed with flakes of albumen fill the alveoli, the fibrine threads being shown wonderfully distinctly by fibrine staining. Near the septs, the alveolar plugs appear denser, owing to the accumulation of polynuclear neutrophile leucocytes and red blood corpuscles.

Alveolar anastomoses are often found in such places near the septa because the distribution of fibrine is more regular. In certain stages of the disease the darkest and most nucleated alveoli are situated in the immediate vicinity of the bronchioli, bronchi, and blood vessels, and in degenerative processes which will be described further on. However, one can state generally, that in classical cases, fibrine exudation always takes the first place. Frequent emigration and desquamation in the alveoli, which lie just aposed to the interstitial connective tissue, is so great that one immediately recognises that the inflammation is most acute there.

It is clear that these places are those which in advanced stages show the first signs of degeneration or restitution. Detached and fatty-degenerated endothelial cells are enclosed in the fibrine, and form the more or less granular-flaky contents of the alveoli. In some of the examined pieces of pulmonary tissue a diathesis with erythrocytes was prominent, so that one could believe that what one saw was a direct haemorrhage, on account of the engoument and effacement of the septs. Such haemorrhages are poor in fibrine. These haematomes are separated from the croupous infiltrated lung tissue by a dense wall of polynuclear leucocytes and lymphocytes embedded in fibrine. In advanced stages one find large tracts of the lung poor in chromatine and impossible to stain.

Analyses show such places to be necrotic, and to contain only scattered fibrine elements.

A demarcating inflammation is localised at the edges of such a focus : Karyolytic nuclear elements distension of the capillaries, large collections of leucocytes with phenomena of emigration.

That the process is of old standing is shown by young granulation tissue originating from the nearest septa. Direct organisation processes of such places are rare, consisting of large protoplasmatic cells, which errode the trabecular masses of the sequester. The alveolar exudates become richer in connective tissue as organisation advances. The alveolar epithelia merit special consideration. The latest researches of Lipschutz* on filterable and visible micro-organisms show certain histological peculiarities which are produced by the action of such organisms on the

* Loco cit.

epithelial tissues. No inclusions similar to those found in variola and pigeon pox were seen in the cells, despite the fact that all the proper methods for staining them were used.

All attempts to render the germs visible in sections were in vain, although they are easily stained and demonstrated in smears treated with gentian violet. Some of the specimens, it is true, showed fine dust-like inclusions in the cells, but before I have elaborated a specific method of staining, I do not care to regard this as being the stained lung-sickness microbe.

It is remarkable that the walls of the alveoles do not show any inflammatory changes. In young stages, the alveolar capillaries are filled with star-shaped fibrine deposits; in other parts, they are enlarged and crammed with erythrocytes. The larger arteries and veins are mostly choked with fibrine. Mixed or pure leucocyte-thrombus are frequent. In advanced stages, dam-like collections of leucocytes are observed, they are peculiar to pleuro-pneumonia and will be described under the heading interstitial connective tissue.

The bronchioli and bronchi which have become affected show strong epithelial desquamation and infiltration of the mucosa and subserous connective tissue by leucocytes. Thick, mixed fibrinous fillings are rare. Distinct dendritic injections formed only in two cases the cause of the stepwise advance of the inflammation. The alveoles have characteristics very different and changeable. The structure of the interstities of the alveoles however, is absolutely constant in pleuro-pneumonia. I came to recognise this peculiarity not from examination of the lungs in the first place, but through comparison with the lesions in the other organs.

A rich comparative material of true croupous pneumonia and other secondary pneumonias showed that the lesions in the structure of the interstities of the alveoles were not the same as in pleuro-pneumonia. The histological changes which principally take place around the blood vessels are also found in the muscles, synovial membranes, and serous membranes.

In the beginning of the disease the endothelial tubiform lymphatic vessels in the widened interstities of the lobuli and the peribronchial septa are greatly dilated. Frequently they are only filled with a finely granulated albumen coagulum, frequently also strongly infiltrated with fibrine. The walls of the vessels are absolutely free from pathological changes. The typical specific changes soon supervene. The walls of the distended lymphatic vessels become filled with leucocytes and consequently indistinct. An exudate rich in fibrine spreads along the lymph ducts through the whole interstitium, and condensing, encircles capillaries, greater arteries and veins. The distance from the walls of

the vessel to the outer aspect of the condensation in every direction measures about 150 μ.*

Fibrine tinction gives results which are so characteristic that it is impossible to make a mistake in the diagnosis. All kinds of changes can take place in the lymph vessels, independent of the typical lesions; these changes can present themselves as *lymphothrombosis*, or *lymphangoitis haemorrhagica*; in the last case the vessels contain thrombus rich in fibrine and erythrocytes. If such strictures of lymph vessels take place in peribronchial tissue, the capillaries and bronchioles become obstructed by them, and all the lesions of a *bronchiolitis sero-fibrinosa* appear in the latter.

The incrustations of fibrine around the blood vessels can be replaced in later stages by thick fibrillae of connective tissue; this, however, does not seem to occur frequently. More usually dense crowds of leucocytes take the place of the fibrine, which in turn undergo regressive metamorphoses: karyorhexis, karyolysis, and protoplasmolysis change the whole finely granular mass into untingable flakes of granules, which are in part resorbed and carried away by emigrant leucocytes, and a regenerative process sets in, by which the defect is replaced by fibrillae of connective tissue.

Should a portion remain behind as detritus, it fills fissure between the sequester formed as just described and the surrounding layer of connective tissue. The connective tissue of the capsule usually merges into that of the interstities; in the immediate vicinity of the sequesters the characteristic alterations of the lymph vessels with the typical incrustations of fibrine are however demonstrable.

Such conditions appear regularly in chronic cases of pleuropneumonia, and in the light of what has been said already can well serve as basis for diagnosis.

The walls of the lymph vessels merit special attention, for we have to decide whether we have to do with a lymphangitis or not. This question is the more important, as Csokor is of the opinion that it is a *productive meso* and *periarteriitis* of the capillaries of the interstitial tissue, of which the enormous stasis in the lymphatic vessels is a consequence. Perfectly fresh foci from the lungs would be most suitable for the study of this problem; but as such stages are rare we are obliged to have recourse to foci derived from other tissues, and to make our deductions by comparative anatomical methods.

I had the good fortune to find a perfectly recent focus in one specimen, it being a dilation of a lymphatic capillary. Close beside one sees in the lumina of the vessels slight exudation and coagulation together with strong imbibition and enlargement of endothelial cells. The walls

*μ Mikron.

of the capillaries being very thin, enables the process to spread easily all round. The perivascular connective tissue is hyperaemic and is depositing fibrine. The wall of the lymph vessel is infiltrated, and dense crowds of leucocytes surround the lumina of the larger vessels. These phenomena, which are found to have changed still further in older stages, are true perilymphangitic processes. At the same time no changes have taken place in the blood vessels or their capillaries. Only after the exudation has reached huge dimensions and the supporting tissue has been pushed apart, and after subsequent invasion of fibroplasts, we find stasis in the blood vessels. *Pari passu* with the advance of the disease, the capillaries of the interstitities become more and more dilated and distended with erythrocytes.

The denser the neoplastic connective tissue of the perilymphangitis, the more readily are the walls of the blood capillaries damaged by the toxine of pleuro-pneumonia virus. Exudation of fibrine and formation of thrombus are the consequence. Disturbances in the circulation and consequitive lesions of the walls of the vessels are responsible for the development of these phenomena. In the smaller vessels one usually finds small mixed thrombus with layers of fibrine on the walls; in the larger, trabecular masses fill the lumen.

The streaks of blood-platelets are flanked by walls or rings of leucocytes, from which fibrine mixed with erythrocytes form loops connecting the various streaks. In older stages one finds thick neoplastic connective tissue around the pulmonary veins, perforated and penetrated by loose fibrine and leucocytes and lymphocytes. The leucocytes soon fall a prey to karyolysis and karyorhexis; plasmorhexis is sometimes also observed. When such changes are found one also sees organisatory processes in the thrombus; except in the thrombosised vessels where the intima is involved in the organisatory process, one only finds the adventitia of all three layers of the walls of the vessels affected.

It is more densely nucleated around the vasa vasorum, even lymphatic elements being accumulated in such places. An increase in elastic fibres is not rare. During the appearance of these disturbances, the media is not in any way affected. In strongly thrombosised vessels one finds small nucleated foci in this portion of the wall. It was interesting to demonstrate Dürck's* fibrilles by means of Weigert's iron haematoxylin stain†; they could be recognised running through the media, not unlike telegraph wires. That these bristle-like fibrillae, which are so readily damaged, are found intact shows that the walls of the vessels are affected secondarily.

The basis of the foci of leucocytes is always a lymph space, although the walls of the spaces are often hardly possible to demonstrate. Necrosis

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*H. Dürck, Virchows Archiv, bd. 189, 1907, Heft 1, page 62.

†Art. Weigert, "Nervenfasern," Enzyklopädie der Mikroscopischen Technik, page 942.

in the walls of the vessels is very rare. The elastic tissues of the intima and media are interrupted by the necrotic elements. Direct dissolution of the media was observed in vessels near to large necrotic portions of the lungs, here nucleated flaky masses, in which remains of elastic elements stain faintly, are seen. In later stages these rudiments are replaced by elements of connective tissue.

Reviewing the above, we find that an arteriitis as described by Csokor is really present, but the arteriitis is due in the first place to the lymphangitis, as is seen from the lesions in the adventitia and media. The disease is restricted to these two layers and spreads along the course of the lymph spaces of the vessels, demonstrating the inflammation per continuitatem. It may be that the pleuro-pneumonia virus acts primarily, or it may be that small embolic infarcts in the lymph spaces of the walls of the capillaries and blood vessels incite the described regressive processes.

*The Pleura.*

I intend to point out in short the changes which take place directly in or underneath the intermediate zone of fibrine and pleural tissue. The fibrine itself usually lies in small pitlike depressions, from which its filaments grow outwards in great quantities, covering eventually the whole of the pleura. In the zone between fibrine and pleura, one sometimes find a epithelial layer with large nuclei, of this occasionally only the detritus remains. The epithelial layer becomes more and more destroyed as the pneumonic process spreads further over the pleura.

But we usually have to do with old pleuritis, and in such cases the epithelium is replaced by a layer of tissue of loose tough texture containing nuclei, and attached to the serous membrane. Wide vessels crammed with erythrocytes bore their way vertically to the surface through the masses of fibrine. These vessels are often ramified and extremely thin walled. The endothelia of these vessels have frequently suffered slight imbibition, their nuclei are vesicular and the chromatine attached to the nuclear membrane. The walls are interspersed by frequent polymorphous leucocytes, rare fusiform connective elements, and lymphocytes; the whole is held together by fibrillar interstitial tissue.

Towards the lungs the fibrinous layer becomes arranged in parallel layers, which have probably been exudated and coagulated *in situ*. Around these layers leucocytes collect and organisation commences. The damaged epithelia are, as is to be expected, rich in cells. The blood vessels are dilated, often much ramified and even tangled. Here too an incrustation of fibrine mixed with leucocytes, undergoing karyolysis and karyorhexis is found around the vessels.

As the process grows older more and more of the fibrine is dissolved and replaced by organisatory tissues. The well-known retae of fibrine merge slowly into the supporting tissue of the serosa. As consequence of

the opposition of connective tissue to the lymph vessels, a great thickening of the pleura appears, which gradually goes over into the thickened interstities of the lobuli.

The adjacent alveoli are in the stage of carnification, or have only reached stage one of pneumonia.

Contrary to what was seen in the interstities, there is a very great number of lymphocytes in the spaces of the tissue, which must be regarded as emigrants from the small subpleural lymphatic glands.

### Peritoneum.

Almost the same lesions were found in the peritoneal walls of a serofibrinose peritonitis which was experimentally produced by intraperitoneal injection of a pleuro-pneumonia culture. Another specimen was taken from case 9, which will be described below with its macroscopic lesions. The stages observed by me had strong infiltration and regeneration of the intermediate layers. In some places in the peritoneum granulation tissues are found, the membrana propria and the endothelial layer have disappeared; instead of them there is a layer of fibrine undergoing organisation, just as in the pleura. The subserosa and its lobules of fat are extremely rich in capillaries and foci of infiltration; margins of leucocyte emigrants are very frequent. The quantity of plasma cells is more than normal.

I believe that I am right in stating that the action of pleuro-pneumonia virus on serous membranes is the same as on the interstities of the lungs, and that the lymph spaces and lymph vessels are the starting point of the inflammatory process, in which all stages of lymphangitis can be observed.

I will now quote several post-mortem reports, material collected at which serve as basis for further histological investigations. It will be shown that inflammatory processes of the nature of pleuro-pneumonia can establish themselves in the muscles, in the subcutaneous connective tissues, in the capsules of joints and in the synovialis, these processes being really specific of the disease.

*CASE* 1.—Ox 671 was injected for experimental purposes in the right side of dewlap with two different vira, originating from two different spontaneous cases, separated in time and locality, but both from the Transvaal. The animal was slaughtered on the 16th day, a large oedematous swelling having appeared at the site of injection.

*Post-mortem Report*: On the right side of the dewlap, from the spina scapulae to the fourth cervical vertebra and on the left side to the fossa jugularis is a huge swelling. The skin over the swelling is drawn tight; there are excoriations and bald patches at the bottom of the dewlap. The tumour is hard, very turgescent. Impression of the finger leaves a distinct pit in some places. On section a large quantity of limpid

yellowish fluid rich in albumen escapes which flows from the tumour as water would from a sponge. The subcutaneous tissue is thickened and separated from the fascias of the muscles ; in it one observes the swollen lymph vessels. The muscles (mm. cleido-occipitalis, subclavius hom.; pectoralis profundus, biceps brachii, and a small part of the m. sternocephalicus) are distended by a similar jelly-like trembling tissue. The muscles themselves are very moist, tough, and rich in connective tissue, or spongy and with a characteristic appearance on the section. The fibre bundles contain localised yellowish-white cavities with gelatinous masses, which send radial prolongations into the apparently healthy muscle tissue. In other places one finds dense masses of connective tissue around large thrombosised vessels. The following changes are frequent on transverse sections. The perimysium internum is replaced by a spongy tissue, which forces the primary fascules of muscles apart. In these layers of oedematous tissue one observes long reddish-brown, frequently very sanguineus, stripes and patches, or wavelike lines transversing the muscle. In such foci one only rarely finds small thrombosised veins. The described haemorrhagic stripes are regularly $\frac{1}{2}$ mm. to 1 mm. broad, but vary in length. Around the streaks the tissues are apparently condensed, and whitish-yellow discoloured. The healthy muscles next to the diseased ones do not show any capillary haemorrhagies, whereas the affected ones appear dotted with small red dots. In the musculus sternocephalicus the changes are most pregnant, as the thickness of the modified subcutaneous connective tissue is greatest here. Large thrombus or actually necrotic foci are absent. The left praescapular gland is enlarged 18 $\times$ $6\frac{1}{2}$ cm. and of rigid consistence. A large quantity of yellowish serous fluid, mixed with blood, flows from the section. The capsule is thickened and infiltrated, its spongy connective tissue is $2\frac{1}{2}$ cm. thick. The vessels entering the hylus are thrombosised. The adjacent sinus of the lymph gland are very haemorrhagic. The medulla is granulated and extruding, single yellowish white points being prominent. The vena circumflexa scapulae is thrombosised, and appears as a thick black cord with greyish-whitish surroundings. The lungs are normal, the liver is soft and shows a slight stasis in the vena porta ; the abdominal organs with the exception of the kidneys are normal. The perirenal fat consists of a yellowish gelatinous mass, the capsule is easily removed on section, the cortex is slightly oedematous, glomeruli injected and slight hyaline degeneration.

*Pathological-Anatomical Diagnosis*: Myositis serosa and haemorrhagica, thrombolymphangitis and plebitis, lymphadenitis haemorrhagica, nephritis parenchymatosa as consequence of intoxication with pleuro-pneumonia virus.

*CASE* 2.—Calf " Dalton," derived from a farm on which about forty sucking calves and oxen died from pleuro-pneumonia in consequence of prophylactic injection with collected pleuritic exudate. The strain was very virulent, as was proved experimentally.

*Post-mortem Report*: Body well nourished, carpal and tarsal joints extremely swollen, skin in tension over the joints, strong fluctuations noticeable. The left carpal joint contains a large quantity of light yellow, slightly slimy, fluid with small threads of fibrine. The articulatio antebracheo-carpalis has slight defects of the cartilage, capillary ramifications and easily removable deposits of fibrine. The intercarpeal articulation contains larger flakes of fibrine which are specially located in places where there is least friction. The articulatory capsules of these joints are extremely moist and thickened; their synovial coating forms folds in many places; the same lesions were found in other joints. In the left talo-crural articulation the deposit of fibrine is membranaceous. The cartilage under it has two round circumscript defects; 3½ c.c. synovial fluid could be collected from this joint alone by means of the syringe. The ligaments of all the joints show serous imbibition to a great degree. The tendovagina of the left musculus digitalis profundus is thickened and rich in fluid. The internal coating has small punctiform haemorrhagies. The atlantoepistrophical articulation is naturally strongly involved on account of its frequent motion when the calf was sucking. The capsule of the joint is thickened by a fibrinous exudate.

*Pathological-Anatomical Diagnosis*: Polysynovitis and Tendovaginits serofibrinosa, as a sequel of pleuro-pneumonia.

*CASE* 3.—Ox 672 was injected on the right side of the dewlap with 5 c.c. fluid (one part fluid derived from the joints of the last case diluted with ten parts of bouillon Martin). On the seventh day after injection a large oedematous swelling appeared, synchronising with a rise of temperature. On the tenth day two pieces of the base of the dewlap, from surface and from depth, were excised to enable histological diagnosis to be made. The animal was slaughtered on the twelfth day for experimental purposes.

*Post-mortem Report*: The right side of the dewlap shows a tumour 50 cm. by 25 cm. The subcutaneous tissue is spongy, filled with a yellowish serous fluid which coagulates quickly. The muscles and intra-muscular tissue shows the usual lesions. The humor of the deeper layers contains flakes of fibrine. Abomasus hyperaemic, mucosa swollen and covered with slightly bile-stained mucus; omasus normal; contents of reticulum very dry; rumen normal, containing a small quantity of food. Coecum and colon normal; ileum slightly congested; jejunum, mucosa swollen, diffuse ramifications of the blood vessels and hyperaemia of the folds. Mesenterial lymphatic gland swollen, somewhat soft; right lobe of lungs in posterior part not collapsed; pleura rather whitish, milky, tough. The tissues of the other portions of the lungs normal. Foam in trachea. Heart contracted, left ventricle empty, endocard normal; right ventricle empty, endocard a few white patches. Liver normal; spleen slightly swollen, pulpa soft, trabecles distinct. Kidneys slight hyperaemia of cortex, in two lobuli a few small white points of the character of infarcts. Synovia

of trochanter, joints increased and mixed with fibrous filaments. In the right carpal joint increase of synovia, two small defects in the cartilage. Fetlock joint normal. Left elbow joint capsule infiltrated thickened, synovia slightly increased. The left joint of acetabulum, synovia increased with fibrous flakes. The ligamenta surrounded by haemorrhagic and slightly swollen tissue. Left knee joint, synovia increased, capsule distended, strongly infiltrated, the intermediate cartilage thickened and hyperaemic tendovagina musculi quadricipis distended with a yellowish liquid. Right tarsal joint, synovia increased, cartilage normal. Left knee joint, synovia slightly increased, capsule infiltrated, the neighbouring intramuscular tissue shows the usual lesions. Left praescapular lymphatic gland enlarged to five times normal size, with haemorrhagic infiltration of the sinus. Capsule thickened 1·3 cm., lympoglandulae cervicales caudales swollen and haemorrhagic.

*Diagnosis*: Myositis et synovitis serofibrinosa specifica.

*CASE* 4.—Ox 688 was injected at the tip of the tail with 0·5 c.c. of a pure culture of pleuro-pneumonia. After fourteen days a rather small local swelling at point of injection appears, when a small piece was removed by operation.

*Macroscopically* the piece of the tail showed a very loose structure of the different tissues, in which a lymph-like fluid had collected. Between muscles and subcutis were small lymph spaces, surrounded by dark, bloodstained walls. The structure of the muscles was loose, they were discoloured, often more or less grey or white. The whitish parts were harder and more densely fibrous.

*CASE* 5.—Ox 675 injected with 2 c.c. pleuro-pneumonia culture 2 cm. above the tip of the tail. Temperature rises. The lower end of the tail was amputated on the sixteenth day and histologically examined.

*Macroscopically* the intramuscular tissue of the musculus coccygeus was broadened, a fibrous tissue replaces muscular tissue in part, in places small thrombus, bone normal.

*CASE* 6.—Ox 670, injected on the right side of the dewlap with 2 c.c. Martin's boullion culture which was made by rubbing in a mortar fibrine flakes obtained from the talo-crural joint of calf "Dalton." On the eighth day fever and swelling at the point of injection were observed, on the twelfth day polyarthritis. The animal was slaughtered in collapse temperature.

*Post-mortem Report*: On the dewlap a tumour 20 cm. × 10 cm., the skin above it is taut, the consistence hard, only in places fluctuating and oedematous. On section an enormous quantity of clear yellow fluid flows from the spongy but tough tissue. In many places are small dilations of the lymph vessels and large and small thrombus of the blood vessels. The muscles (mm. pectoralis profundus, brachiocephalieus, sternocleidomastoideus, and biceps brachii) are changed, on section tough,

and filled with many small cavities containing fluid. The muscle fascules are separated and atrophic. The intramuscular tissue and the tissues surrounding the trachea and oesophagus have the same lesions of pleuro-pneumonia. In the musculus sternocephalicus, at 6½ cm. from the sternum, is a necrotic focus of the size of a hen's egg. A reddish-yellow compact mass of tissue is situated in the middle of a very humid tube of muscle, which has all the various changes usual in myositis. A mixed thrombus, 25 cm. long, attached to the wall, is situated in the vena jugularis dextra. The musculus sternomandibularis is extremely haemorrhagic, the areas surrounding the blood vessels being especially conspicuous. The oesophagus is very oedematous, and its mucosa is loosened. The mucosa of pharynx and larynx is swollen and covered with capillary extasia. The retropharyngeal lymph glands are swollen, and dripping; so are the lymphoglandulae cervicales mediae et caudales. The right praescapular lymph gland measures 12 × 4 cm. The capsule is thickened, and the hylus vessels leading into is thrombosised. The sinus are slightly dilated, the follicles and follicular trabeculae protude. The yellowish jelly-like infiltration of the subcutis extends from the mandibula along the neck of the tenth rib. In the omentum maius there are irregular haemorrhages, the mucosa of the abomasus is swollen and diffusely hyperaemic. The other parts of the stomach are normal. Coecum empty, some haemorrhagic spots. Mesenterial lymph glands swollen; colon pigmented with a few calcareous parasitic nodules or such containing pus. Ileum, mucosa slightly swollen; jejunum bloody, jelly-like contents, and longitudinal stripes. Lungs in inspirium pleura white. Severe oedematous infiltration of the mediastinum. On the apex of the heart a few subepicardial ecchymoses. Kidneys, the capsular fat slightly infiltrated, glomeruli injected. All joints, but especially the left articulatio metacarpophalangea are swollen. The capsules are distended by a light yellow slimy fluid containing flakes of fibrine. The synovialis only slightly reddened, but thickened and folded. The surface of the cartilages are everywhere intact. Some of the vaginae of the tendons are dilated and filled with fibrinous exudate.

*Pathological-Anatomical Diagnosis*: Myositis, synovitis, and tendovaginitis serofibrinosa specifica. Intoxication by pleuro-pneumonia virus.

*CASE* 7.—Heifer 674, injected with 2 c.c. boullion culture at the tip of the tail; considerable swelling of the whole tail; skin lifted off up to the third caudal vertebra; in places necrotic, and in others covered with small abscesses. The left anal lymph gland is the size of a hen's egg. The tail was amputated at the second vertebra and the gland extirpated.

*Macroscopically* the amputated part showed a considerable thickening, the skin had peeled from the subcutis over a surface of 30 cm., the hairs had fallen off in parts, and at the lower end of the tail they were replaced by small holes filled with creamy pus. The tip was dry, leathery,

and at 15 cm. from its end was encircled by a distinct haemorrhagic demarcation. On section the musculi coccygei showed pronounced atrophy of the fasicules of muscles, sero-fibrinous imbibition of the intramuscular tissue, and in a few places slight superficial suppuration. The vertebrae and the intervertebral cartilages are intact, a slight separation of the periost is only remarked in two places directly under intramuscular abscesses. Towards the base of the tail the lesions become gradually less pronounced and the course of the affection along the lymph vessels of the subcutaneous and intermuscular tissues is evident. The lymph gland weighs 22·2 grams, its capsule is slightly haemorrhagic, infiltrated, and thickened. A large quantity of fluid flows on section, the gland is otherwise absolutely normal in appearance with exception of the dark red infiltrated marginal sinus.

### Microscopical Lesions of the Subcutaneous Tissues.

Specimens taken from cases 1 and 3 serve as a basis for my investigations on the lesions in the dewlap; cases 5 and 7 for those in the caudal apex. It is a notable fact that although the skin of the dewlap is hardly thickened, measuring 8·5 mm., it measures often over 1 cm. on the tail, which would normally be less than that of the dewlap. Bacteria can be demonstrated, the cracks and ruptures due to the oedema gave them access and a suitable substratum to multiply on, facilitating the formation of abscesses.

As far as it is present the *stratum corneum* presents advanced desquamation and flask-shaped masses in a fibrous tissue rich in leucocytes. These masses consist of necrotic detritus, and lie in small holes in the epidermis close to the *stratum lucidum*. Dense small celled foci of infiltration and young deeply staining tissues separate them from the intact cells of the stratum lucidum. The infiltration increases in proportion to the suppuration. In places the whole of the *corium*, together with the epidermis, is necrotic; only a few stainable nuclei are seen in the innermost layers of the *stratum reticulare*, surrounded by karyolytic and plasmolytic elements. Externally this layer is covered by a stratum of faintly staining fibrine. Where the pleuro-pneumonia virus has acted solely on the deeper layers, and the pressure caused by the oedema has left the surface of the skin undamaged, the *stratum basale* contains a few foci of leucocytes, as sign of a coming inflammation.

The *corpora papillares* are full of emigrants, the blood vessels distended, a few lymph spaces are remarkable on account of their great size and coagulated contents, employing fibrine staining. The lesions become more peculiar, but also more familiar in the deeper layers of the subcutaneous tissue where the follicles of hairs become more frequent. Darkly staining margins of leucocytes surround the capillaries, whose walls are almost always slightly thickened; between these emigrants, and closely attached to them, is a fine reticulation of fibrine. The single fibres of

the subcutaneous tissue are separated by minutely granulated masses, except in such places or around hair follicles. The cells are rich in protoplasm and enlarged. On sections through pieces of the tail the changes in the muscles and in the fat are most prominent. As I intend to deal with the muscles at greater length further on, I will now only make a few remarks about the lesions in the adipose tissue. The margins of the globular tissue consist of dense tissue filled with elastic fibres and very many nuclei. In some sections the masses of leucocyts have the appearance of a demarcating line, in other places they seem to originate from a recent invasion. In the first case one finds fragments of nuclei and fibrino, in the second case irregular tangled capillaries surrounded at a distance of 20 $\mu$ to 60 $\mu$ by a ring of fibrine. Where the fat approaches the fascia a layer containing a great number of nuclei separates the two tissues, and the fascia itself is weakened by friable masses. It must, however, be pointed out, that ischaemic necrosis is only found at the base of the tail, a distinct lymphangitis is usual nearer the apex.

In accordance with the anatomical structure is the reticulation of the papillary stratum ; all processes of infection are passed through in this layer of the skin. Later on one finds crowds of leucocytes around the hair follicles. The necrotic parts are separated by the usual demarcatory processes ; in their immediate surroundings the capillary vessels are thrombotised, and frequently even in resorption. Such places are not frequently found unmixed, usually there are regeneratory foci forming neoplastic connective tissue. Quite close to them, young spindle-shaped cells are forcing themselves between the leucocytes and deposits of exudate which in later stages replace the described lesions by proliferation. In such a condition it is difficult to say histologically what irritation has been caused by the layers of connective tissue between and around the muscles. The condensation of the nucleated tissue around the blood vessels in the subcutaneous strata may perhaps show that an inflammatory process has existed around the vessels.

It is interesting to demonstrate that whichever tissue may have been the seat of injection of pleuro-pneumonia virus it always shows exactly the same changes as have been described as being found in the interstities of the lungs under natural infection. Practical experience shows that after a period of incubation of twelve to fifteen days, the swelling creeps along the course of the lymph vessels centripetally, starting from the point of injection, and cause the usual complications, such as necrosis of the apex of the tail, peritonitis, etc.

It has been stated that contamination of the vaccine is the cause of complications. I quite disagree with such a notion, for, working with absolute pure cultures and taking all aseptic precautions, we yet cannot avoid having losses. Individual disposition, or weakening of the constitution, form the cause of the misfortune.

In South Africa there are strains of cattle which, when inoculated with pleuro-pneumonia vaccine, invariably succumb to a rapid progressive lymphangitis and peritonitis. The practitioner must help nature by cauterising the skin around the tail above the swelling caused by injection, thus causing an acute inflammation of the blood vessels and stopping the disease from progressing towards the body of the animal. Another proof that the virus does not follow the blood vessels, for if it did, we would expect that it would be favoured by the inflammation. Contamination of the virus with bacteria can be shown to be of little importance ; from a vaccine which produced absolutely local lesions, no less than twelve species of bacteria were isolated.

## The Microscopical Lesions in the Musculature.

Pieces of muscles of cases 1, 3, 4, and 6 were examined. In order to obtain comparable specimens, sections were made in each case of the *musculus sternocleidomastoideus*, preparations of other muscles were also made, especially when the macroscopic lesions were in any way remarkable. The first changes observed in any muscle are the great thickening of the subfascial connective tissue, the next alteration observed is that the *perimysum externum* becomes involved ; here one remarks bands of a finely fibrillar tissue, which all stain with the acid components of the tinction employed, and which can to some extent be demonstrated by fibrine staining, crossing and recrossing the field of vision. In this spongy coarsely reticulated tissue one remarks isolated deep staining complexes, these last usually contain rarely ramified blood vessels distended with blood corpuscles. The vessels are surrounded by polynuculear leucocytes which are arranged radially around their axis. The size of the radius is remarkably constant, it averages 50 $\mu$. On the edges of such complexes the intermediate fibrine condenses to form a dense layer. In the meshes of the fibrine the polynuclear leucocytes readily break up ; in older stages karyorhexis is very pronounced. The blood vessels are still perfectly normal at this stage, except perhaps that the intravascular leucocytes collect towards the walls and a slight diapedesis of the erythrocytes is taking place. The perimysium is not alone the starting point of the inflammatory processes in the muscles ; the *secondary muscle fasciles* are strongly invaded by leucocytes on their surface where they are in contact with morbid tissue of the *perimysium internum*. The invasion proceeds gradually towards the centre ; however, one sometimes finds places where leucocytes crowding in between the secondary fasicles towards the centre, surround a capillary and there produce the usual lesions. It is remarkable that each muscle fibrilla is separated from its neighbours by a typical finely fibrous tissue. Here and there are places where the muscles have entirely atrophied, their former presence being indicated by the hollows they have left behind. Those fasciles of the muscle lying

nearest to the perimysium are poorest in fibres, but are filled with young connective elements derived from the perimysium. The component muscle fibres are swollen and vacuolised, frequently hardly recognisable. The muscle and connective nuclei are, on the contrary, well preserved. It is interesting to find places where the thickness of the incrustation of leucocytes around the capillaries can be measured. As the capillaries are situated in the perimysium of the fibres, the emigration of leucocytes caused by the inflammation must necessarily follow the fibre perimysium. An invasion of leucocytes takes place between the fibres of the muscle radially around the capillary, forming a ring whose inner diameter is 100 $\mu$., and whose thickness is of greater or less dimensions according to the size of the blood vessel. The dark, dense net of fibres readily undergoes regressive metamorphosis and proliferation.

We have shown that under the influence of the oedema the muscle fibres become vacuolised by the formation of fissures and cavities, which are only products of a widening of the intercolumnar spaces : the lesions all point to pronounced lymphangitis.

The plasmatic substance of the fibrillae, and the fibrillae themselves to a certain extent, dissolves under the influence of pleuro-pneumonia toxine corresponding to the yellow-brown foci of the macroscopic lesions. In fresh specimens one notices here and there processes of hyaline degenerations, and in such cases it is sometimes possible to demonstrate fat by Sudan III staining. The yellow-brown foci, just mentioned, prove on examination to be old stages of the lesions noted above as occurring around blood vessels, but here the blood vessels have proliferated on account of the action of the virus and now form complexes of much twisted, recently formed, epithelial tubes. Between the vessel and the incrustation there are a large number of young cells of connective tissue, but the incrustation itself forms a kind of demarcation in which a detritus of nuclei erythrocytes, pigments, blood-platelets, etc., transversed by a few connective fibrillae form a dividing line from the spongy tissue described above. Muscle fibres are nowhere recognisable. A few elastic fibres may perhaps have once belonged to the perimysium.

This stage is conspicuous on account of the great proliferation of blood vessels and connective tissue. Whether the process could advance further cannot be stated, because animals succumb to the infection at this stage ; it continues in the tail until a cicatrice remains. These lesions are however very rare, usually necrosis takes place before they have time to develop on account of the thrombus in the blood vessels. Around the necrotic detritus, which is very easily stained with eosine or with picric acid, and then appears absolutely structureless, circular layers of blood pigment, and externally to them the usual circular or long oval incrustations of leucocytes are formed. The muscle lying nearest to the necrotic portions are more filled with round cells than those further off.

It is now evident that the diseased process (phlegmon) which arises under the influence of pleuro-pneumonia virus is absolutely specific. Regeneration, which takes place so readily in other diseases of the muscles, does not happen here, for all the phenomena following the myositis tend towards cicatrisation. Interruption of circulation is here the cause of all changes, and the tangled plexus of blood vessels represent nothing else than collateralia seeking in vain (as far as can be seen on my sections) for a connection.

### Microscopic Lesions of the Lymph-Glands.

The most prominent lesions in the lymph glands mentioned in cases 1, 3, and 6 were in the capsule and the sinus, they compliment the observations made in the other organs in the most remarkable manner.

The capsules measure 4·6 to 6·1 mm. ; their structure is fibrous and stratified, and yet contains but few connective elements. The vasa afferentia which normally increase in size in the capsule are enormously enlarged, and their ramifications nearly fill the whole tissue ; the cavities thus formed contain fibrine and thick deposits of leucocytes on their walls. The arteries which branch in the capsule are distended with erythrocytes and surrounded with the small cellular deposit usual in pleuro-pneumonia. The lesions would have been absolutely similar to those in the lungs and the muscles had it not been that they were complicated by the sero- and leucocytotactic inter-action of stratified thrombus on the hylus causing a large deposit of pigment in the form of haemosyderin crystals, and had it not been for the very small quantity of connective elements formed. In the tissue of the glands the sinus of the cortex are remarkable ; these are filled with granulated albuminous matter, provided irritation is not caused by the proximity of an inflammatory process ; if this is however the case they form bands or domes of round cells around the lymph follicles. The medulla becomes looser towards the centre of the gland and abnormalities become rarer at the same time. If a few of the blood vessels in the trabeculae were not thrombosised, the lesions would be those of acute lymphadenitis.

In consequence of these conditions we find changes around the blood vessels as usual in pleuro-pneumonia. The changes taking place in the lymph vessels are dependent on the conditions surrounding them ; the vessels in the capsule and those immediately below and above it are the first to suffer. The typical incrustations around the blood vessels are not wanting.

### The Microscopical Lesions of the Capsules of the Articulations and of the Synovialis.

Macroscopic lesions were present in the joints described, although no suggestion was made as to their origin. Infection *per continuitatem* is out of the question, except in one case in which it occurred in the articulatio scapulo-humeralis ; it must be supposed to take place by

lymphogene or haematogene channels in the majority of cases. According to Theiler* it is possible to produce acute pleuro-pneumonia of the peritoneum with all the characteristic lesions by intraperitoneal injection of the blood of a calf suffering from the natural disease. Hutcheon† goes a long way to prove the lymphogene or haematogene transportation of the virus ; he states that if a calf suffering from pleuro-pneumonia be castrated or ear marked, typical pleuro-pneumonia tumours will be found at the seat of the wound.

Except for a slight desquamation of the epithelia of the synovialis no remarkable lesions were present. A small deposit of fibrine on the defects was demonstrable by staining. The *plicae synoviales* are free from villi, but it is not possible to state whether the changes are constant, as only a small portion of the synovialis was at my disposal. From the physiological point of view this would be possible, for the negative pressure in the articulation is reduced. Those capsules of joints which have been invaded by the way of the blood vessels present quite different lesions. In normal histology one learns that the *stratum synovialis* is easily destroyed, and that it sooner succumbs to inflammatory processes than any other tissue. The great quantity of capillaries and large number of cells it contains present, of course, great possibilities for attack ; the pleuro-pneumonia process naturally acts on these membranes. A deposit of fibrine usually containing crowds of leucocytes is exudated on the denuded membrane of the synovialis. The plicae are reflected, often adhering to each other, or two or more are enveloped in masses of exudate. The capillaries are distended, and form dense reticulations and loops. These lesions are not found on all parts of the synovial membrane. The underlying connective tissue is loosened, filled with leucocytes and its otherwise regular arrangement is disturbed, the fibrillae running in all directions. An invasion of emigrants following the course of the capillaries takes place only in the lower stratae of the synovialis, the upper layers remaining free. In the fibrosa the lesions are nearly the same, only there is less infiltration. The parasynovial tissue is not affected, so that the haematogenic nature of the infecti on is evident. Here it becomes clear that, when pleuro-pneumonia virus is exuded by any means, the accompanying lesions are totally different to those we have hitherto studied. It is consequently easy to understand that these changes readily heal, in contrast to the other lesions, where the blocking of the blood vessels causes necrosis and requires peripheric restitution.

### Macroscopic Lesions in the Intestines.

Shortly before completion of this paper I had the good fortune to observe the following case.

*CASE* 10.—Ox 662, injected with 0·5 c.c. culture from virus "Dalton," $2\frac{1}{2}$ cm. from apex of tail.

* The Transvaal Agricultural Journal, Vol. II, No. 7, 1904, page 360.

† *Vide* Theiler, loco cit., page 361.

*Clinical Symptoms*: Fourteen days after injection a large swelling was observed 10 cm. from the tip of the tail: on the 16th day the tumour had progressed past the base of the tail and invaded the perianal tissue. (See figure.) The anal mucosa was oedematous and painful, causing incessant straining. Both anal lymph glands were swollen and hard. It may here be mentioned that twenty-six control animals only showed local lesions. Killed shortly before death.

*Post-mortem Report*: Body in fair condition; tail greatly swollen, skin of basal portion with many cracks, resembles bark. A large tumour extends from the base of the tail to the regio pubica. Anus open, mucosa jelly-like, with small superficial haemorrhagies. The subcutaneous connective tissues form a tough layer containing large cavities, from which a yellowish liquid flows which coagulates slowly. The tissue extends to the musculus glutaeus, semimembranosus and semitendinosus, both muscles having their perimysium internum extensively thickened. The lymph glands on the tubera ischiadica are enclosed in a dense envelope of connective tissue, closely attached to the capsule. The glands, which equal a hen's egg in size, are sappy on section. From here, the oedematous infiltration extends to the sphincter ani, passes upwards to the musculus bulbo-cavernosus and ischiocavernosus, and disappears gradually in an oedema of the subcutis or near the middle of the crura. The pelvic cavity has following lesions. The urinary bladder projects into the peritoneal cavity, surrounded by a thick ring of oedematous fat tissue, its diameter is more than 30 cm. The serosa, the lower portions of the excavatio-rectovesicalis and the parietal folds of the pelvis are covered with a dense coating of fibrine, or with fibrous fibrine. The cervix vesicae is connected to the rectum by a mass of fibrine. The ligamenta lateralia vesicae are 2 cm. thick and deeply covered with fibrine. The peritoneum is either diffusely hyperaemic or shows arborisation of the capillaries. The bladder contains 3 litres of a clear, dark yellowish urine. The mucosa of the trigonium lieutaudii is slightly oedematous and contains haemorrhagic spots. The urethra is slightly dilated before the obstruction, its mucosa is slightly swollen and streaked longitudinally. At the collicus seminalis the pressure of the oedematous fibrinous perianal fatty tissue closes the urethra. The mucosa is pale, with a few ecchymoses. The whole urethra and its corpora cavernosa are covered with a spongy tissue of fibrous character. The left kidney is raised out of the capsule fat, weighs 920 gr., some of the lobuli are irregularly swollen, the corresponding urethra is slightly dilated. The capsule strips easily; in one of the middle lobuli is an irregularly outlined yellowish-white patch 7 cm. broad, carrying in its centre a few haemorrhagic patches. This part proves on section to be an infarct, with a remarkably strong injection of the glomeruli. The cortex of the other parts of the kidney is oligaemic, the medulla alone is slightly congested. In the slightly flattened papillae renales whitish-yellow stripes converge towards the apex of the papillae.

Histo-chemically the white stripes prove to be infarcts of uric acid. The pelves and calices renales are dilated, and contain friable concrementa. The whole kidney is moist and friable. The right kidney weighs 760 grams, has a few infarcts and the same signs of stasis as the left. The connective tissue around the aorta is gelatinously infiltrated as far as the arteria mesenterica anterior, and covered with fibrine deposits which gradually merge into the perirectal fatty tissue. From the anus cranially over a distance of 60 cm. the walls of the rectum are 8 cm. thick, 1½ cm. of which belong to the thickened serosa, 3½ cm. to the muscularis and submucosa, the remainder to the mucosa. This coarse tube, with its thick walls, hardly resembles a rectum ; its walls are full of small caverns containing a yellow, quickly coagulating exudate ; the cavities are mostly situated between the fasicles of the circular muscle. The mucosa is folded into six thick swollen folds each 1½ cm. high, perpendicularly above these are narrow small folds, all strongly hyperaemic. This diseased portion passes gradually into the healthy rectum. The lymph glands are greatly enlarged, soft and succulent. Some blood vessels of the mesentery and of the serosa are thrombotised. The anal musculature is strongly infiltrated, its muscle fibres are smothered by fibrous tissue. The abomasus is oedematous in the folds, and hyperaemic. The duodenum contains bile stained jelly-like contents, its mucosa is swollen and has a few hyperaemic patches. The jejunum is slightly swollen and congested, in the ileum the mucosa is slightly reddish or yellowish with adhaerint mucus. Coecum and colon are swollen and contain many haemorrhagic patches. Spleen 50 cm. × 15 cm. × 5 cm., irregularly swollen, margins round, on section dark red, slightly protuding. The liver has in an old cicatrix on the diaphragm side in the middle lobe of the capsula Glissoni a few fibrinous flakes. Colour dark brown with groups of clay coloured lobuli, grates on cutting, hard. Pleura not folded, rather whitish, slight oedema. All other organs normal.

*Pathological Anatomical Diagnosis*: Pleuro-pneumonic inflammation of the rectum, of the tail, muscles of pelvis, perirectal and perianal connective tissue, peritonitis fibrinosa, hydronephrosis, lymphadenitis serosa, cirrhosis and fatty degeneration of the liver.

### Microscopical Lesions of the Rectum.

Of all the layers of the intestine the mucosa is least altered, its epithelium is nearly intact and consists for the greater part of mucous goblet cells, whose granular contents are connected with the coating of mucine by mucus almost free from epithelial elements.

Here and there a slight dissociation of the periglandular connective tissue with a few swollen endothelial cells may be observed. The proportional thickness of the mucosa and of the muscularis mucosae are, however, absolutely normal. Under the epithelial layer are a few enlarged

capillaries, crammed with erythrocyctes. The submucosa is in progressive stages of inflammatory swelling; one instantly recognises its being a granulation tissue by its wealth in vessels and its dense network of connective tissue. Cavities and fissures filled with plasmatic cells poor in chromatin are situated directly below the muscularis mucosae; these elements are evidently the overgrown, desquamated, endothelia of the lymph vessels. Small crowds of leucocytes surround blood vessels close to necrotic foci, whilst regeneratory processes are scattered here and there with multiplication of the connective cells and lymphocytes, which last originate from the follicular apparatus of the intestines as shall be demonstrated later. The centre of the inflammation, namely, the plexus containing lymph vessels between the circular and longitudinal layers of muscles follows this relatively narrow zone; it is remarkable on account of the large number of cavities containing fibrine and leucocytes, and measures 1·2 cm. to 1·3 cm. in diameter. Directly under the submucosa are irregular groups of muscles and scattered muscle fibres, which can easily be recognised as the remains of the circular muscles; cavities containing more or less dense endothelial elements as already described form layers around these. In places the epithelial accumulations are so compact that at first sight they have the appearance of carcinomatous infiltration. It is chiefly connective elements which surround in great quantities the large cells in the denser parts; arteries and veins thickened by connective tissue and encrusted with leucocytes are not rare. Dense tangles of vessels or ramifications of such bring variety into the image.

Large lymph spaces and emigration phenomena, as found in the subcutis, are seen embedded in fibrine and opposed to the longitudinal musculature. Disregarding their contents, the large round cavities are readily recognised on account of their walls to be lymph vessels. Dense bands of leucocytes follow in waved lines the course of the blood vessels around these lymph spaces. The longitudinal musculature is disrupted, and its fasicles lie in a net-like tissue, which merges into the thickened serosa. Similar cavities are found in the serosa, only here the tissue immediately surrounding them is more packed with leucocytes and, consequently, shows older stages of lymphangitis. The epithelium of the serosa has totally disappeared, fibrinous deposits, in all stages as described for the pleura, have taken its place.

These observations serve as further proof that pleuro-pneumonia infection follows the course of the lymph vessels. The lymph plexus between the two layers of muscles which is involved in all affections of the intestine, is also here the centrum of the pathological changes. The large protoplasmatic cells can, following Saltykov (Zeitschr. fur Heilkunde, 1900), be regarded as descendants of elements belonging to the lymph vessels. As they throw light on problems discussed above, I reserve the special description of them for a future paper.

## Casual Infection of Various Organs. (*See Plate* 1.)

In South Africa drenching is the favourite way for immunising. This absolutely unscientific method consists of pouring pleuro-pneumonia virus, either pure or diluted, from a bottle into the slightly opened mouth of the animal. Since Nocard's investigations have shown that pleuro-pneumonia virus remains absolutely inactive when passed through the stomach, it is strange that such a procedure should be employed and confer a certain immunity. It has been possible to demonstrate that this method is frequently accompanied by complications; sometimes pleuro-pneumonia was the result, sometimes pathological anatomical lesions appeared, which were caused by the virus entering a defect in the tissue and there producing the usual progressive process. Logically one can expect the most dangerous pathological changes; I wish, however, to draw attention to one case only, referred to already by Theiler*; a specimen from this case is preserved in our museum.

The specimen is a slice through the trachea, oesophagus, etc. The virus must have entered near the base of the tongue, for according to the post-mortem record, a huge oedematous swelling was found from the base of the tongue, along the throat as far as the bifurcation of the trachea. Clinically, the animal showed blocking of the oesophagus, which was seen by the animal bringing up through its nostrils the water it had drunk. From the specimen, following details have been made out:—One of the rings of cartilage, probably the thirtieth, judging from the position of the oesophagus, is surrounded in a loose spongy connective tissue, or better, in a fibrous tissue. Single groups of fat lobules are intruded between the gelatinous lymph spaces. The upper margin of the tracheal ring is interrupted by a space of $1\frac{1}{2}$ mm., through which the peritracheal tissue communicates by an isthmus with a tumour protruding 2·4 cm. into the trachea and spreading to the middle of both of its sides. The tumour consists of a coarse fibrous supporting tissue, containing broad lymph spaces with thickened walls; the whole is covered by a strongly folded epithelium-like layer. The mucosa of the trachea is slightly thickened, its structure is similar, though not developed to the same degree. The oesophagus is difficult to recognise as such, for everywhere a similar tumour protrudes itself within the otherwise normally folded mucosa. The lesions observed macroscopically in the mucosa are sponge-like plicae, covered by a fine white membrane, below this a jelly-like filling. The muscularis is thickened, its fibres are dissociated, in structure it appears honeycombed and it is almost separated from the fibrosa by a few lymph spaces. Two blood vessels close by are thrombosised; their walls are greatly thickened.

The intermuscular tissue of the M. sternohyoideus and sternomandibularis is dissociated in the usual manner. The tissues of the fossa

* Loco cit., pages 361 and 366.

jugularis are enlarged and metamorphosed into a gelatinous mass. Several larger blood vessels are thrombosised here. It is evident that the specimen is a portion of a peritracheal and perioesophagal oedema.

*Microscopical Lesions.*

The tumour in the trachea, the changes in the mucosa, the perichondrium and the cartilage offer most interest; the cartilage is absolutely normal, a few foci of leucocytes are remarked in the perichondrium; the peritracheal tissue is no longer recognisable, dense layers of a tissue containing very many cells surround the cartilage, between the layers are insulae of blood vessels and typical condensations of leucocytes and fibrine. All stages of inflammation such as described as above can be noted, likewise muscle atrophies and resorptions. Examined with a low power, large round deeply stained rings or reticulations become visible. The tumour, which half fills the trachea, consists of a stroma of connective tissue in which pleuro-pneumonia lesions with all their peculiarity are observed. The inner surface consists of a band composed of leucocytes, fibrine, blood vessels, and connective cells; within this there is a layer of transverse sections of vessels, connective tissue with reticulations of elastic fibres fills the interior. The mucosa has entirely disappeared; the connective elements appear to be derived from the perichondrium, as the connective trabecles originate on the perichondrium to proceed into the tumour, where they form reticulations. In the different layers of the oesophagus one finds similar lesions. As the tumour only represents a combination of all pleuro-pneumonia lesions on the connective elements of various organs, the illustrations will demonstrate all the changes in more eloquent manner than would a recapitulation of what has already been said.

Conclusions.—Pleuro-pneumonia virus has an absolutely specific effect on the tissues, it chiefly attacks the connective elements, the lesions which can be demonstrated histologically are characterised by the following phenomena :—

(1) The virus multiplies in the lymph vessels after having invaded them (lungs, muscles, subcutaneous connective tissue, lymph glands, intestines and mucosae and serosae generally). It causes an inflammatory process, which presents itself microscopically as *lymphangitis serofibrinosa*, *lymphothrombosis*, emigration of leucocytes, etc.

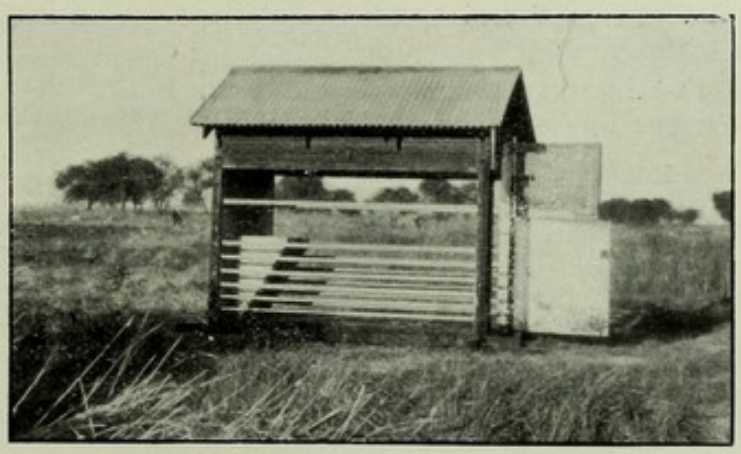
(2) The process proceeding only along the lymph vessels (*per continuitatem*) involves also the lymph spaces of the walls of blood vessels, a peri- and mesoarteriitis, with formation of thrombus results. Thick rings of leucocytes with deposits of fibrine surrounding the blood vessels at a short distance in circular symmetrical arrangement give quite a characteristic appearance to the microscopical lesions.

(3) The blocking of the blood vessels gives rise to ischaemic necrosis (Cohnheim), in which all stages of necrobiosis, demarcation, and of incipient regeneration can be demonstrated.

(4) The dead portion can undergo sequestration, a capsule of connective nature being formed by reactive inflammation (lungs, muscles), or the primary pleuro-pneumonia process comes to a standstill, and the tissue become transformed into a cicatrice by opposition of connective tissue (subcutaneous tissue).

(5) The lesions of pleuro-pneumonia having originated by metastasis present microscopically the image of a corpuscular infiltration with pronounced serofibrinous exudation, without there being any localisation of the process within definite portions of the tissue. It is in the nature of the virus to form metastases, especially in the joints, the age of the affected animal being apparently immaterial.

(6) The histological lesions around the vessels differ from those of chronic productive pneumonias, and can be used with certainty for diagnosis.



## Descriptions of Plates.

*Plate I.*

Intertracheal tumour of a calf, as a sequel of drenching. (Natural size.)

*Plate II.*

The incrustations of fibrine and leucocytes specific for pleuro-pneumonia. Kockel's fibrine stain, bordeaux red (1 : 62).

*Plate III.*

Fig. 1.—Interstitium of the lungs; lymph spaces with incrustations of leucocytes. Parenchym in different stages of croupous pneumonia (1 : 10).

Fig. 2 (Case 1).—Subcutaneous tissue with typical lymphangitis and periarteritis. Fibrine stain (1 : 10).

Fig. 3 (Case 4).—Progressive stage of lymphangitis and periarteritis, incipient cicatrisation. Van Gieson's stain (1 : 70).

*Plate IV.*

Fig. 4 (Case 7).—Traverse section through a piece of the tail. Wide lymph spaces, dissociation of muscle fasicles, and in places changes in the blood vessels. Haemalum and eosine (1 : 4).

Fig. 5 (Case 7).—Portion of same section enlarged, shows tangles of capillaries and incrustation of leucocytes (1 : 80).

Fig. 6 (Case 1).—Transverse section through a muscle. Shows incrustation of leucocytes in the perimysium internum and around the blood vessels. Van Gieson (1 : 60).

Fig. 7 (Case 1).—Portion of same section with muscle fibres enlarged (1 : 10).

Fig. 8 (Case 6).—Changes of the tissue around necrotic muscles. The incrustation of leucocytes around blood vessels is in concentric rings. May-Gruenwald-Fischer (1 : 4).

*Plate V.*

Fig. 9 (Case 6).—External lesions of articulations. The large swelling on the right fetlock joint is due to pleuro-pneumonia.

Fig. 10 (Case 1).—Lymphatic glands, shows thickening of capsule, thrombosis of blood vessels of hylus, and serose infiltration of cortex sinus. Van Gieson (1 : 10).

*Plate VI.*

Fig. 11 (Case 10).—Traverse section of rectum. (Reduced to 0·6 natural size.)

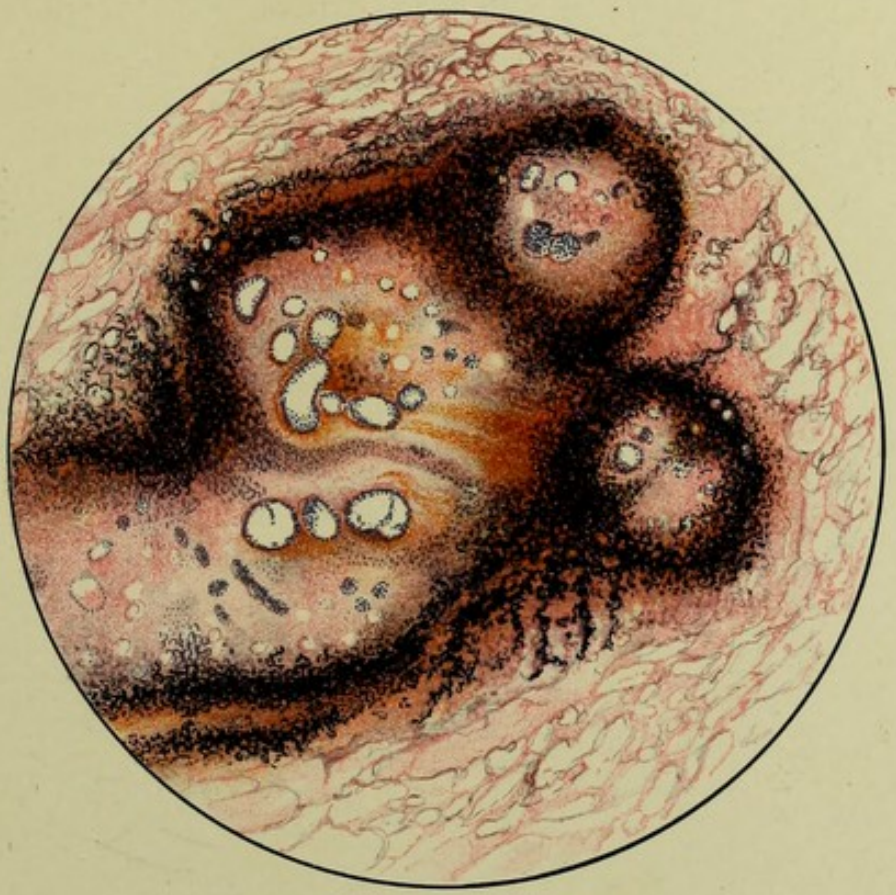
Fig. 12 (Case 10).—Rectum laid open longitudinally. (Reduced to 0·6 natural size.)

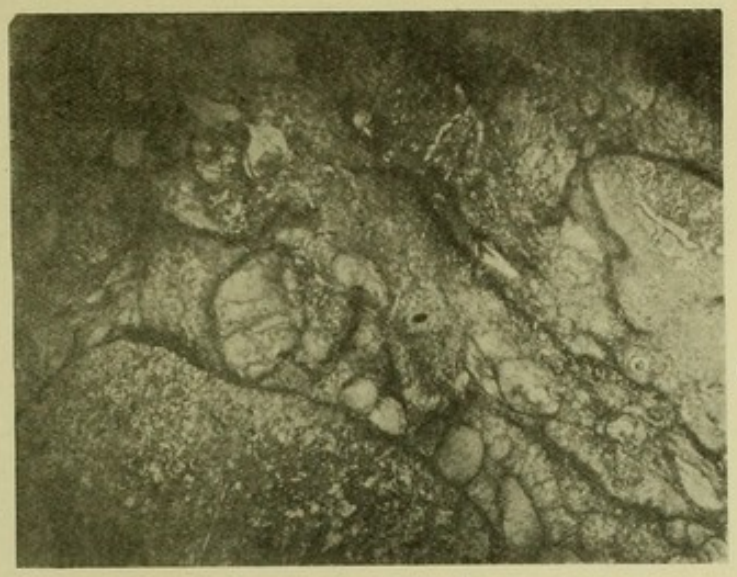
*Plate VII.*

Fig. 13 (Case 10).—Lesions in the rectum, mucosa, muscularis mucosae, submucosa and the beginning of the intermuscular plexus. Van Gieson (1 : 4).

Fig. 14 (Case 10).—Lesions in rectum (same section as Fig. 13). Intermuscular plexus, longitudinal muscles and serosa. Van Gieson (1 : 4).

Fig. 15 (same case as Plate I).—Pleuro-pneumonia tumour in section of trachea. Haemalum-eosine (1 : 4).





(*a*) (*c*)

Fig. 1.

Fig. 2.

*Plate* 3. Fig. 3.

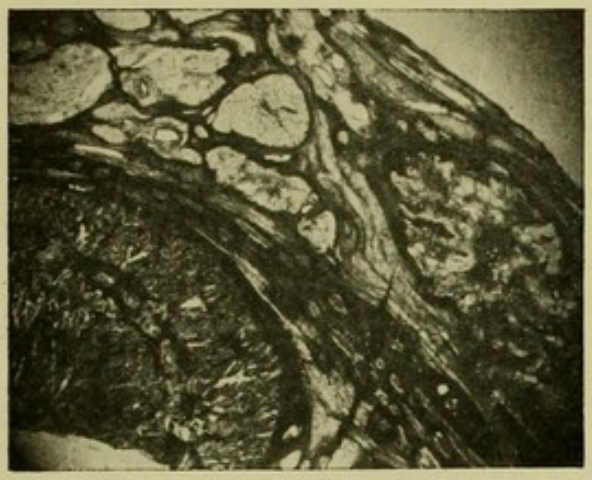


Fig. 4.

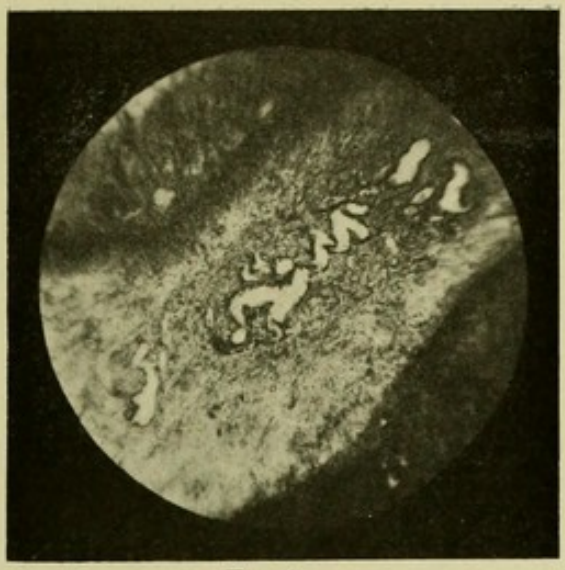


Fig. 5.

Fig. 6.

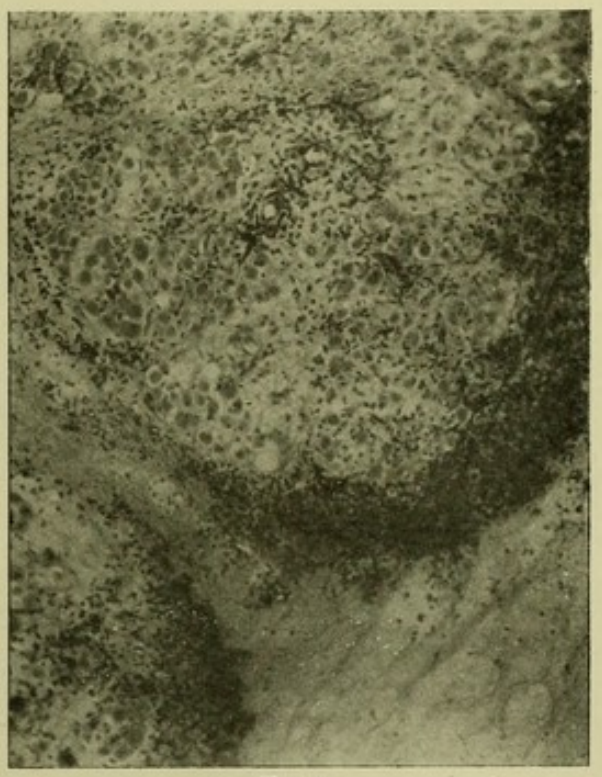


Fig. 7.

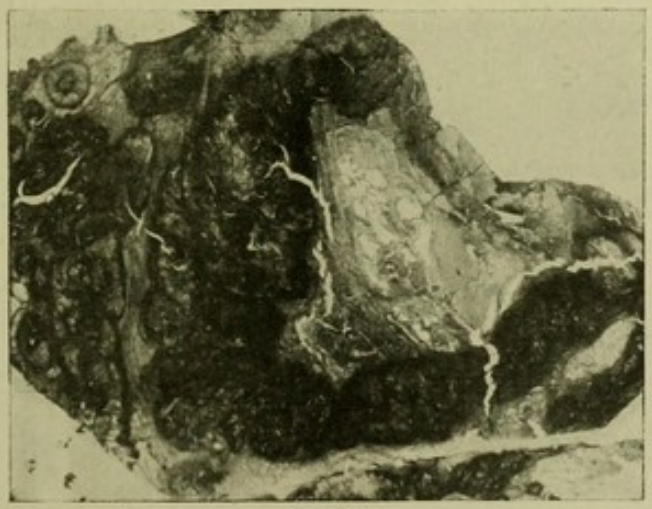


Fig. 8.

*Plate* 4.

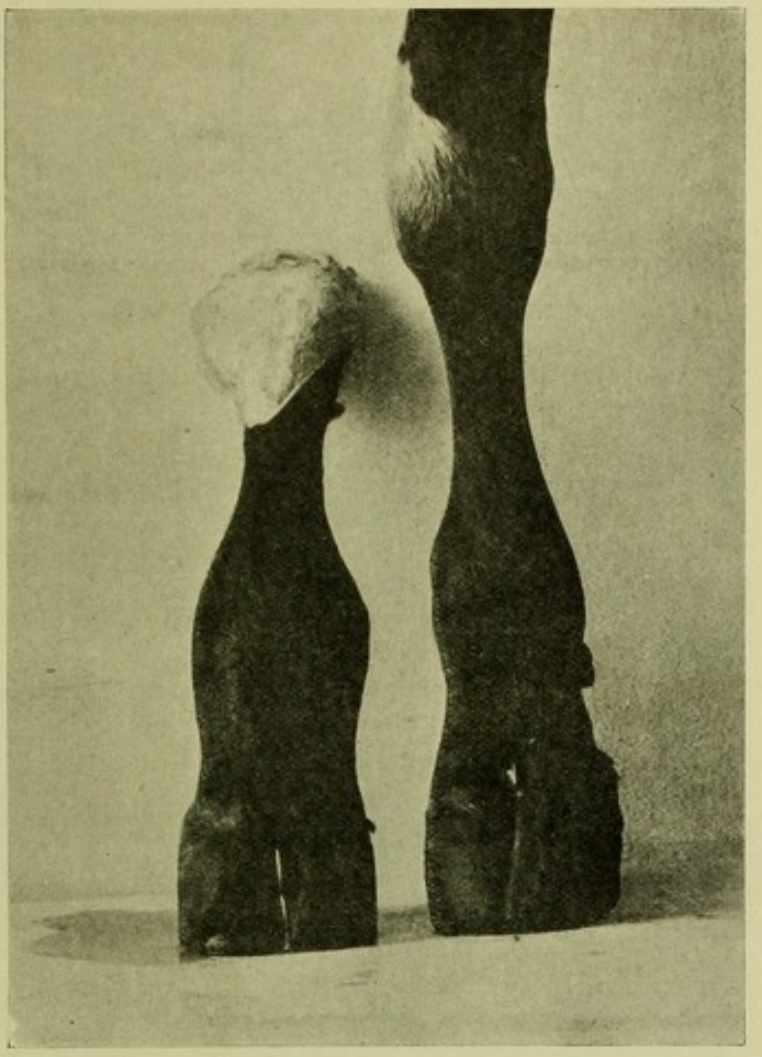


Fig. 9.

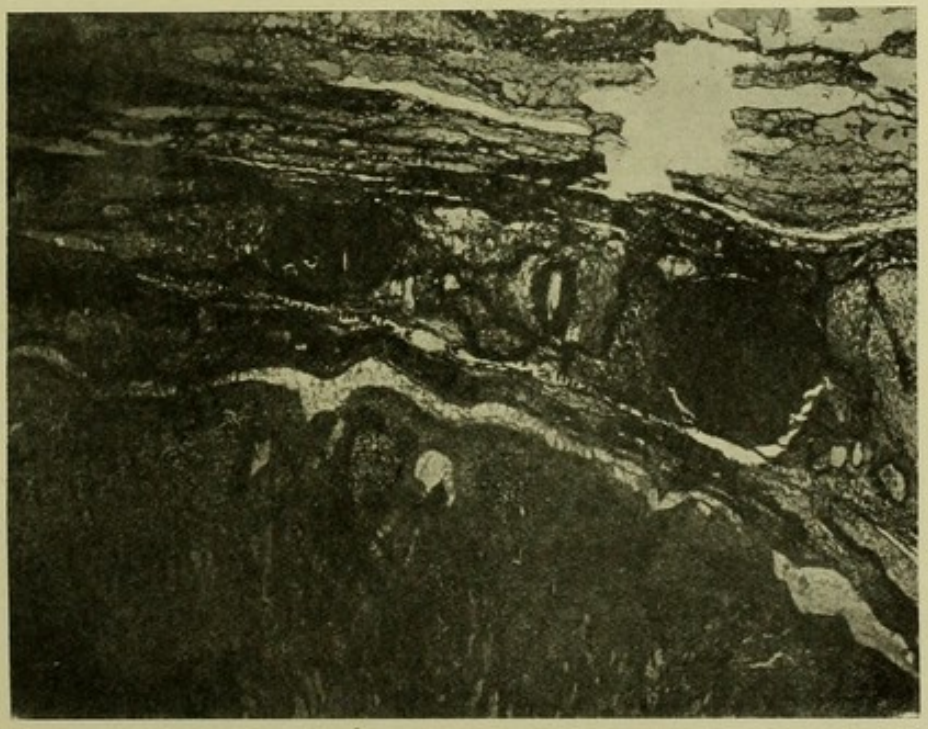


*Plate* 5.

Fig. 10.

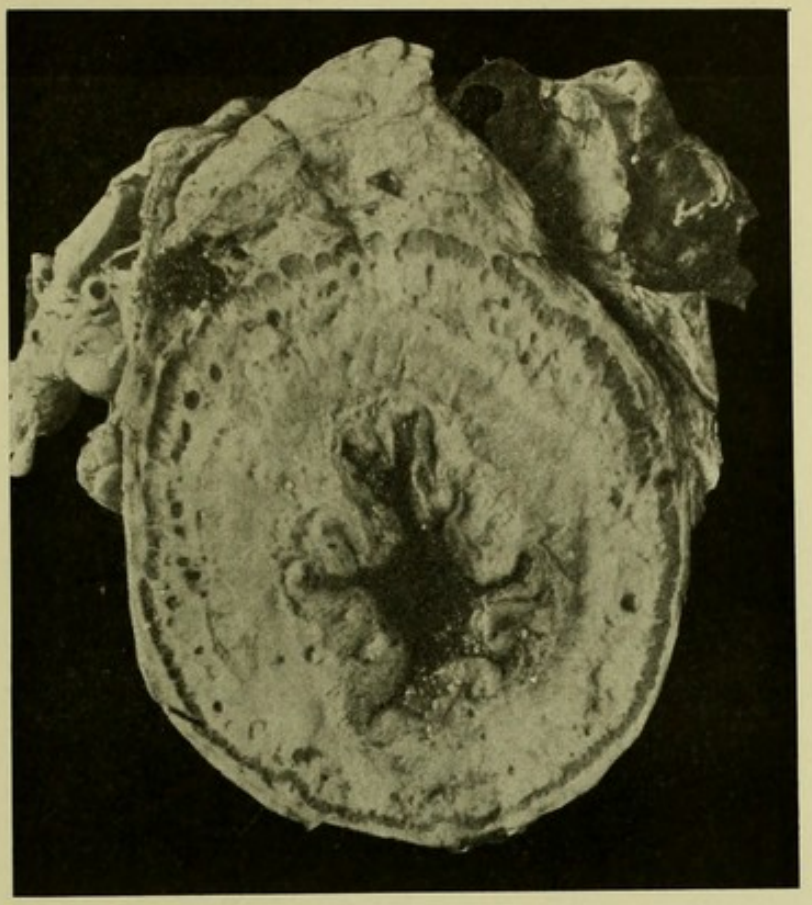


Fig. 11.

*Plate* 6.

Fig. 12.

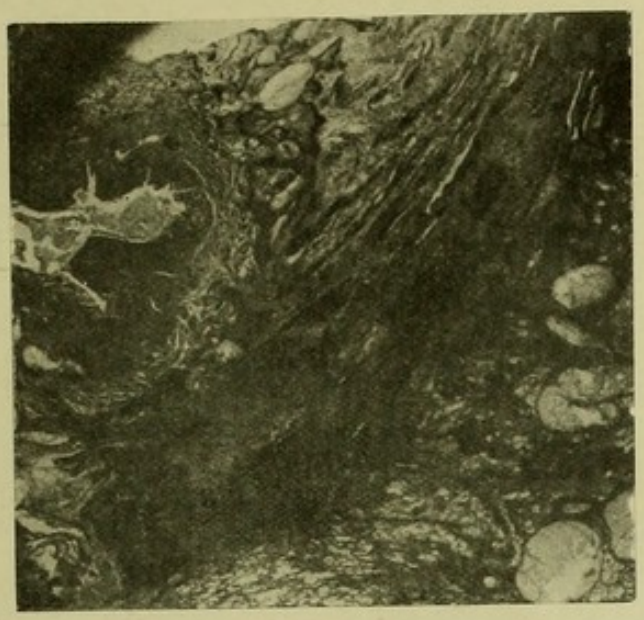


Fig. 13.

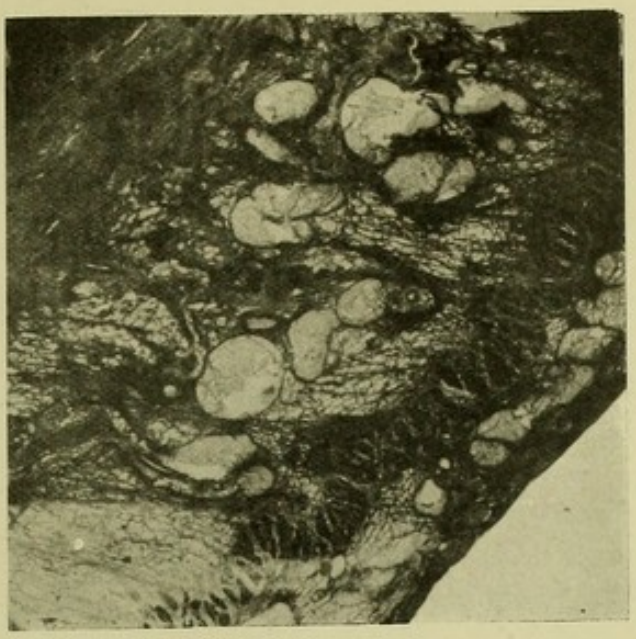


Fig. 14.

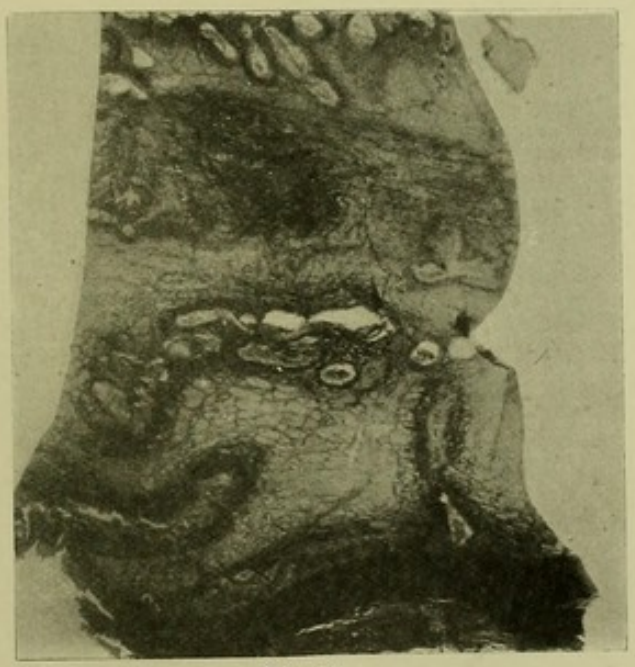
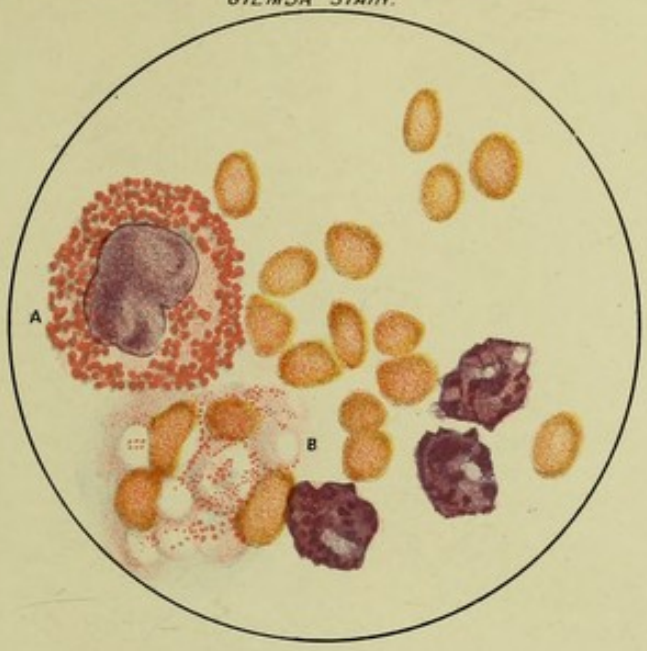


Fig. 15.

*Plate 7.*

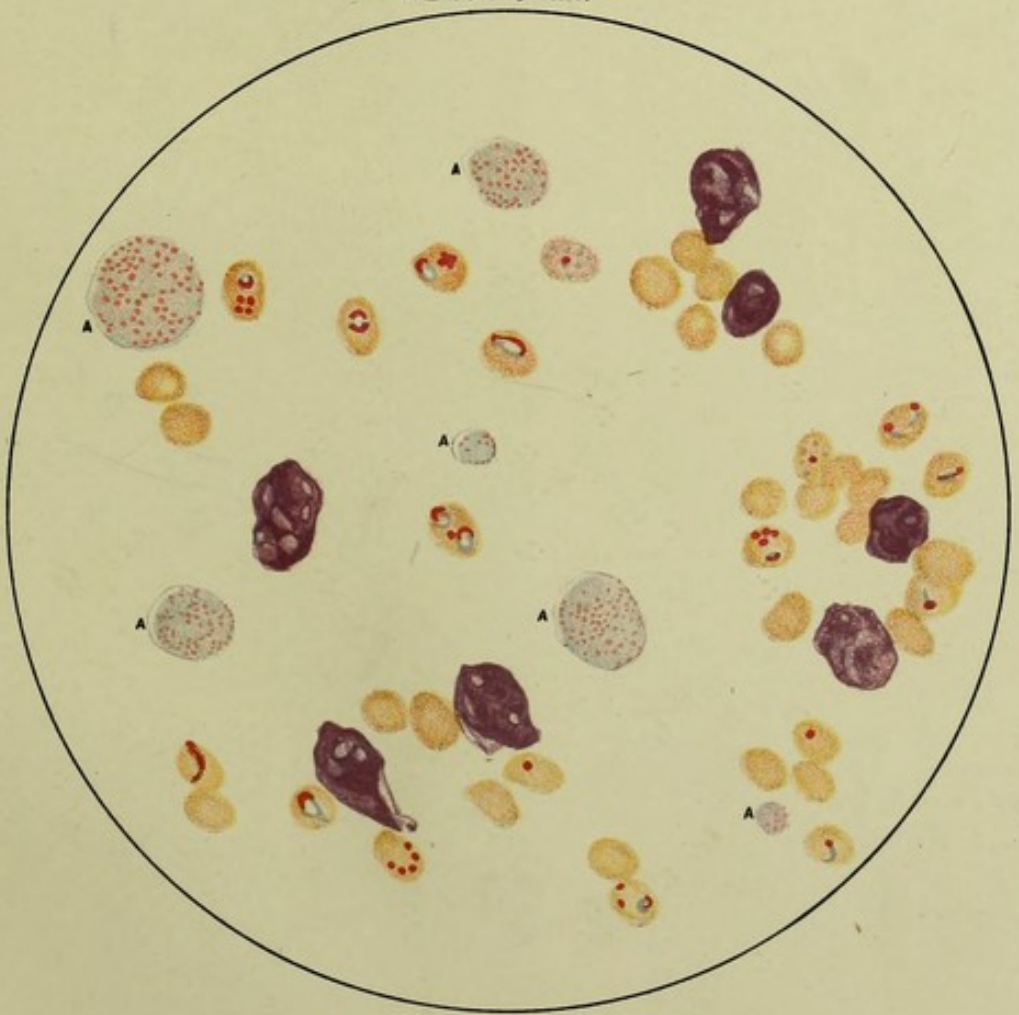
## NORMAL SPLEEN.

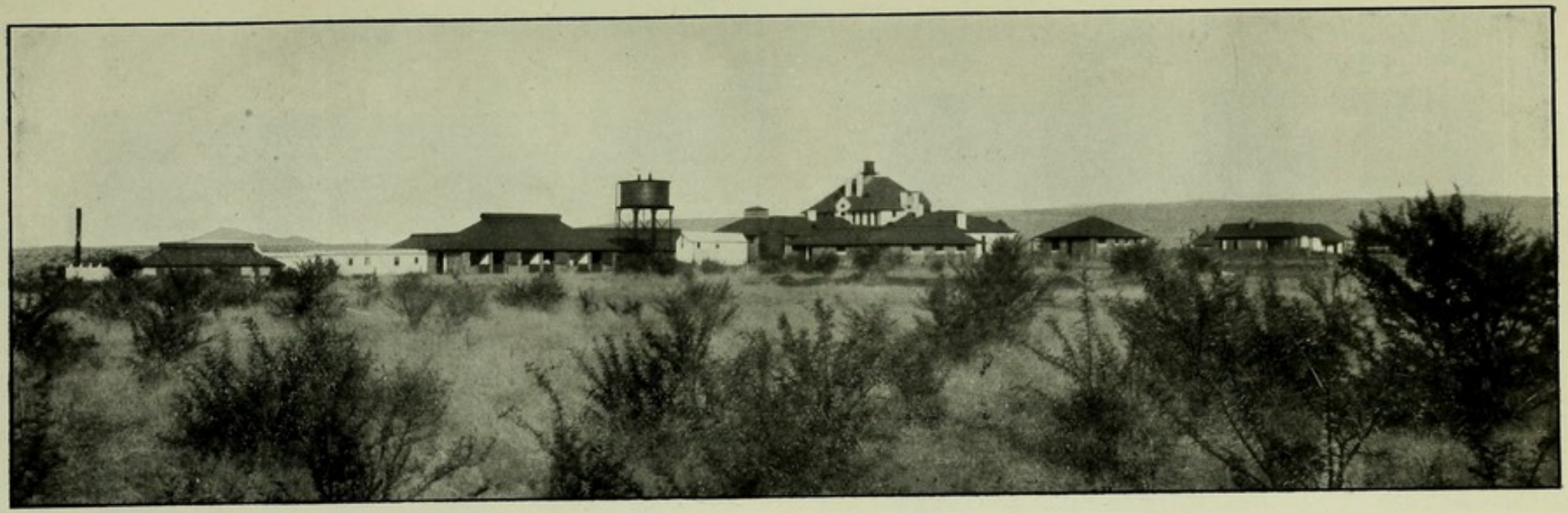
(A.) EOSINOPHILE. (B.) NEUTROPHILE GRANULATIONS.
GIEMSA STAIN.




## ABNORMAL SPLEEN.

(A.) KOCH'S GRANULES.
GIEMSA STAIN.

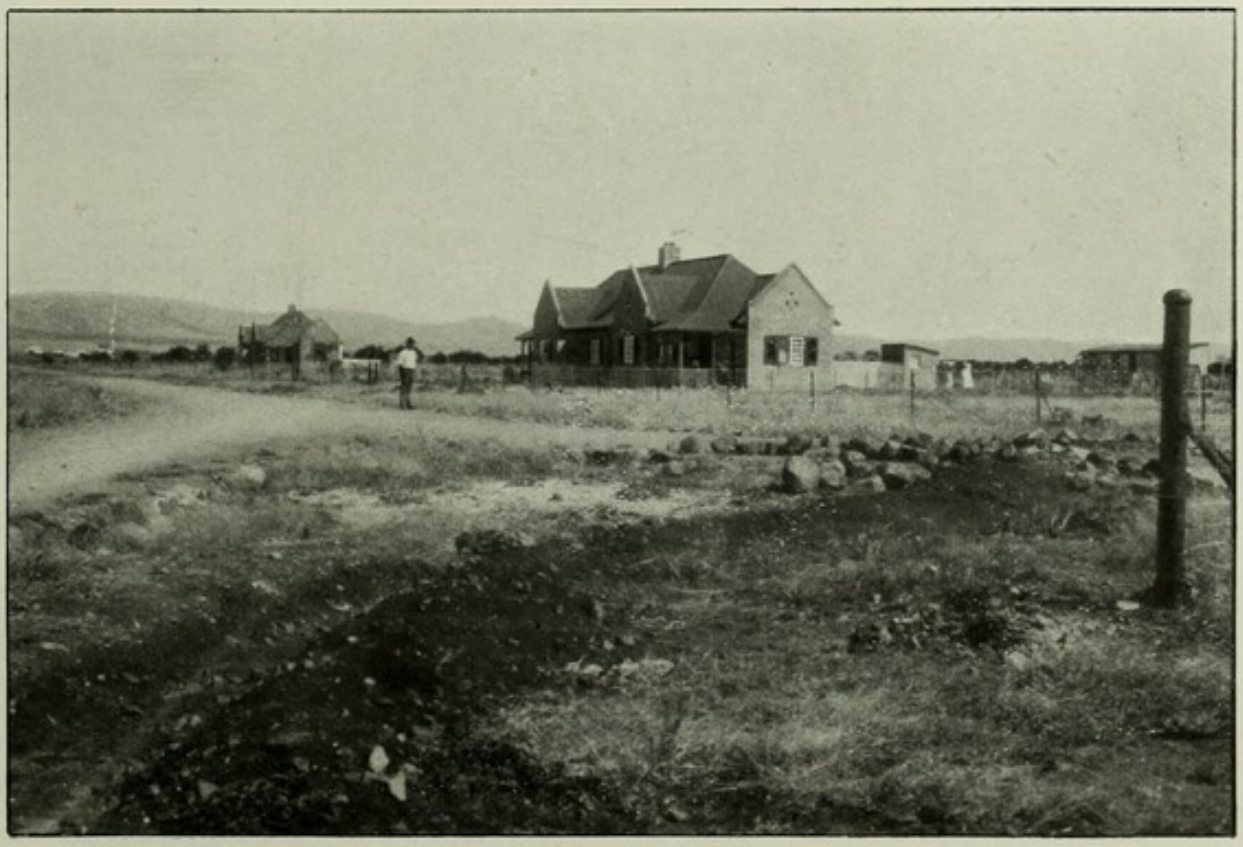




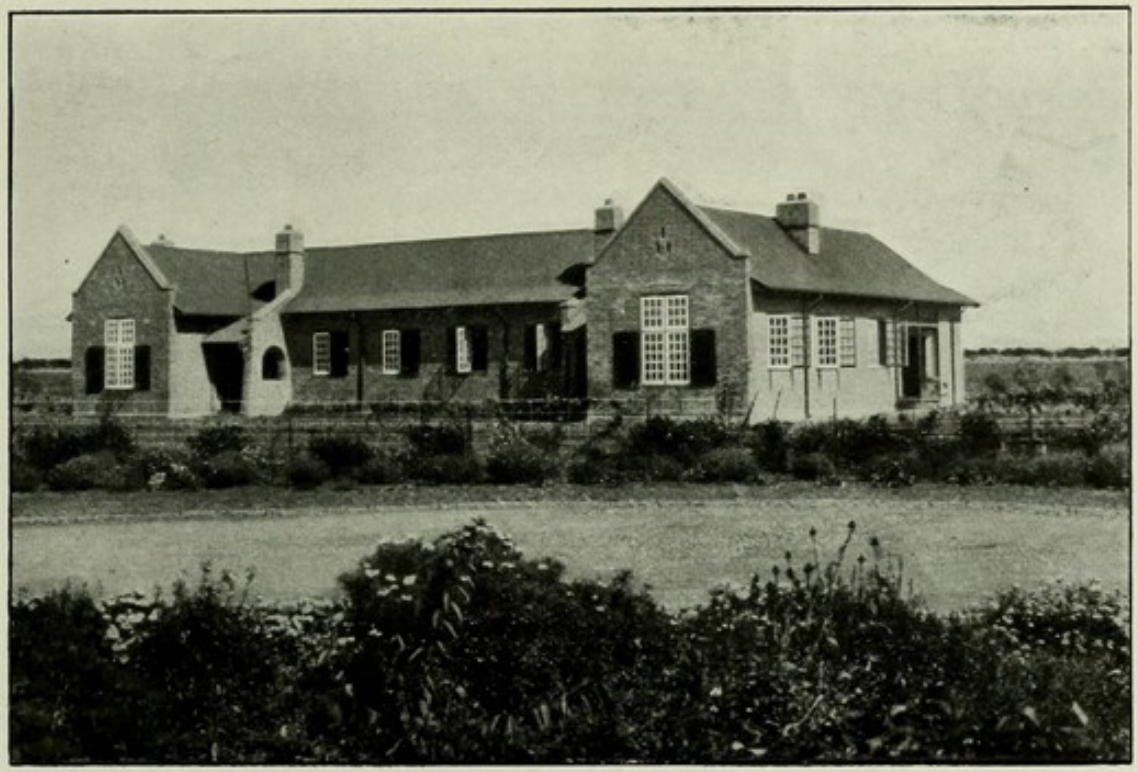
*General View from the West.*

*General View from the East.*

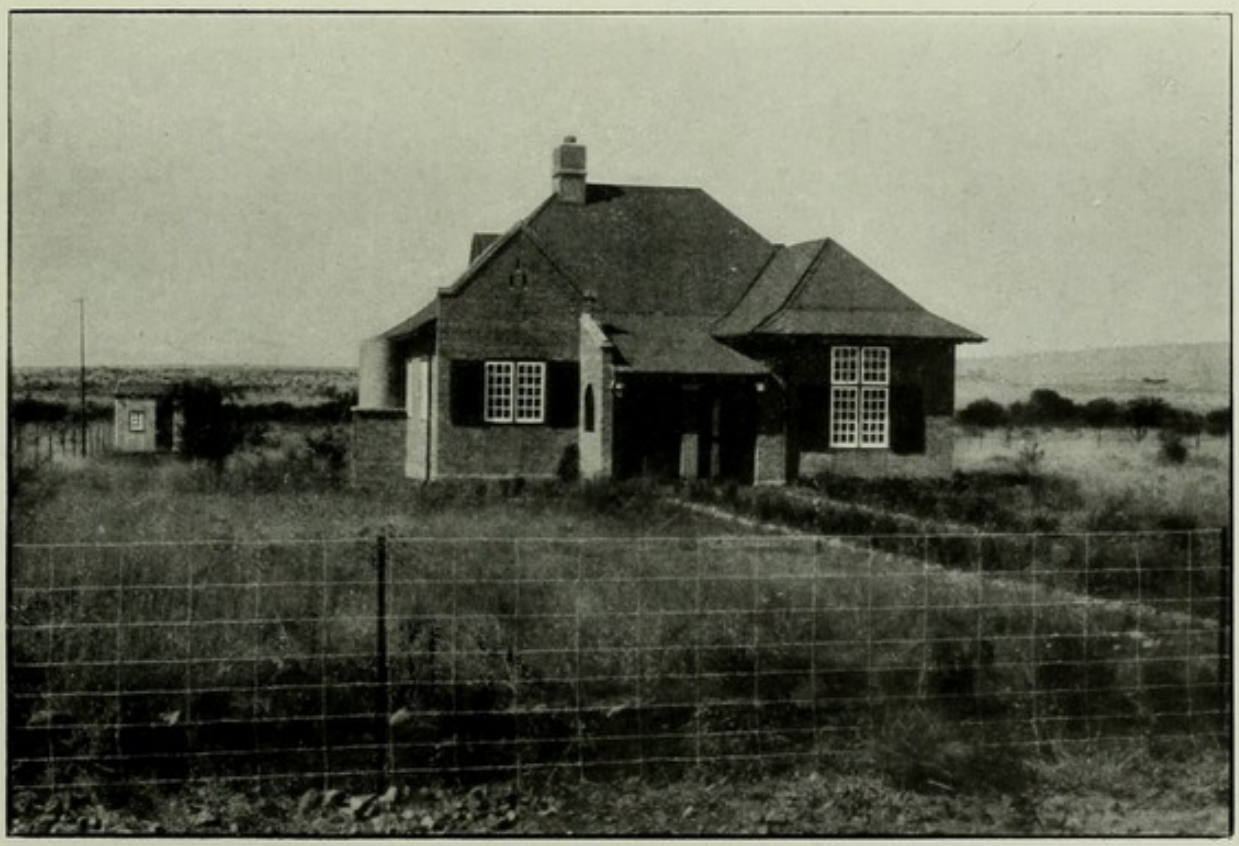
*Government Veterinary Bacteriologist's House.*



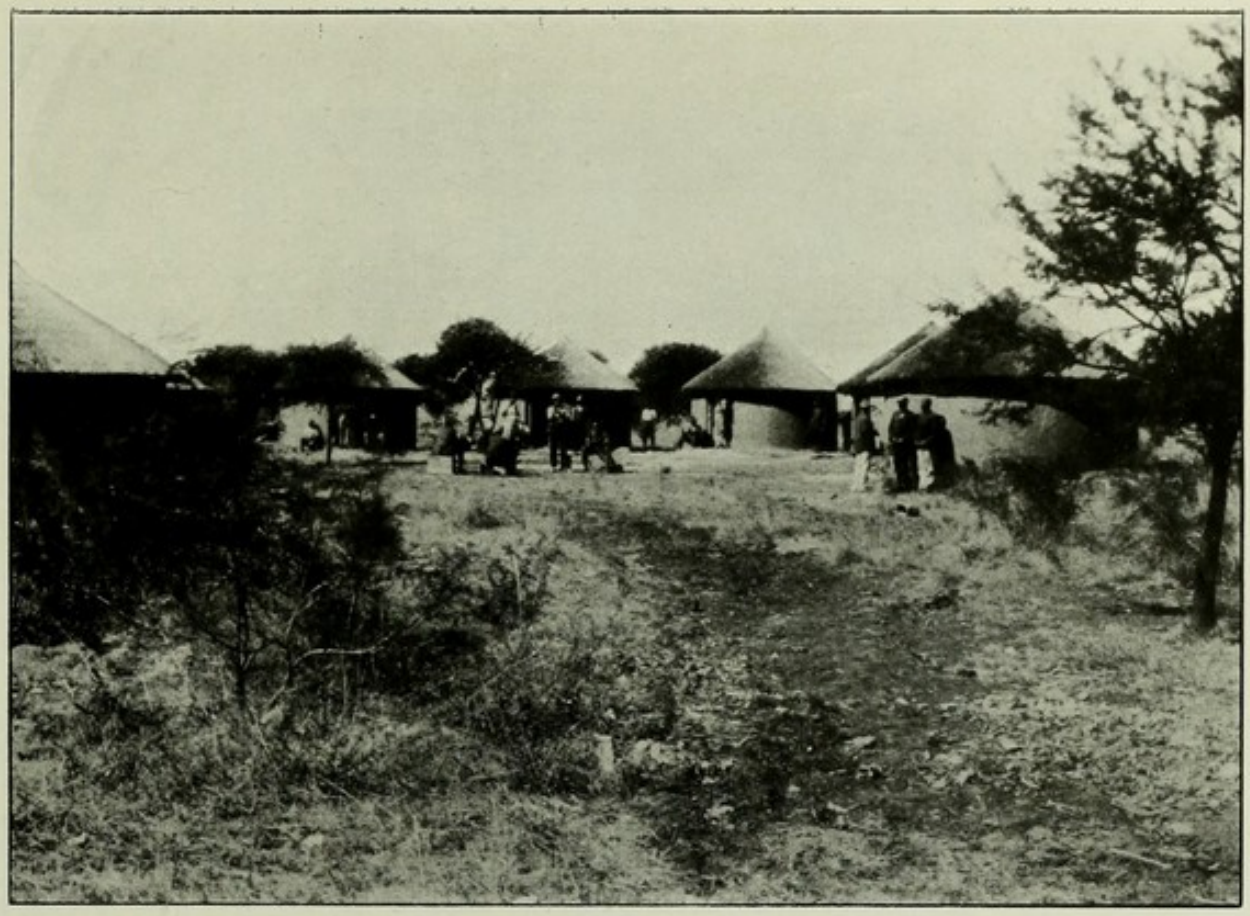
*The Quarters of the Staff.*



*The Hostel.*



*The Vaccine Lymph Laboratory.*

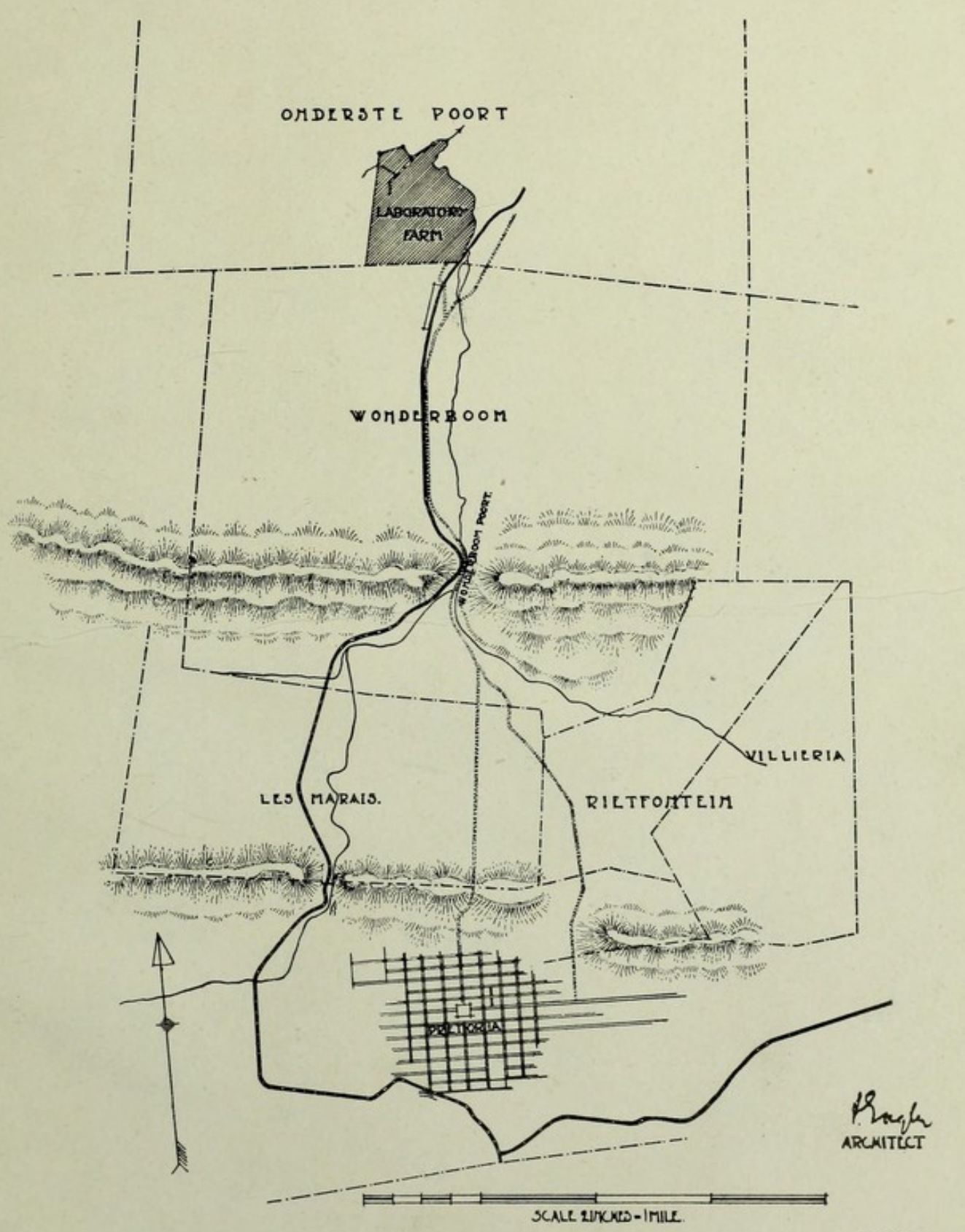


*The Native Quarters.*

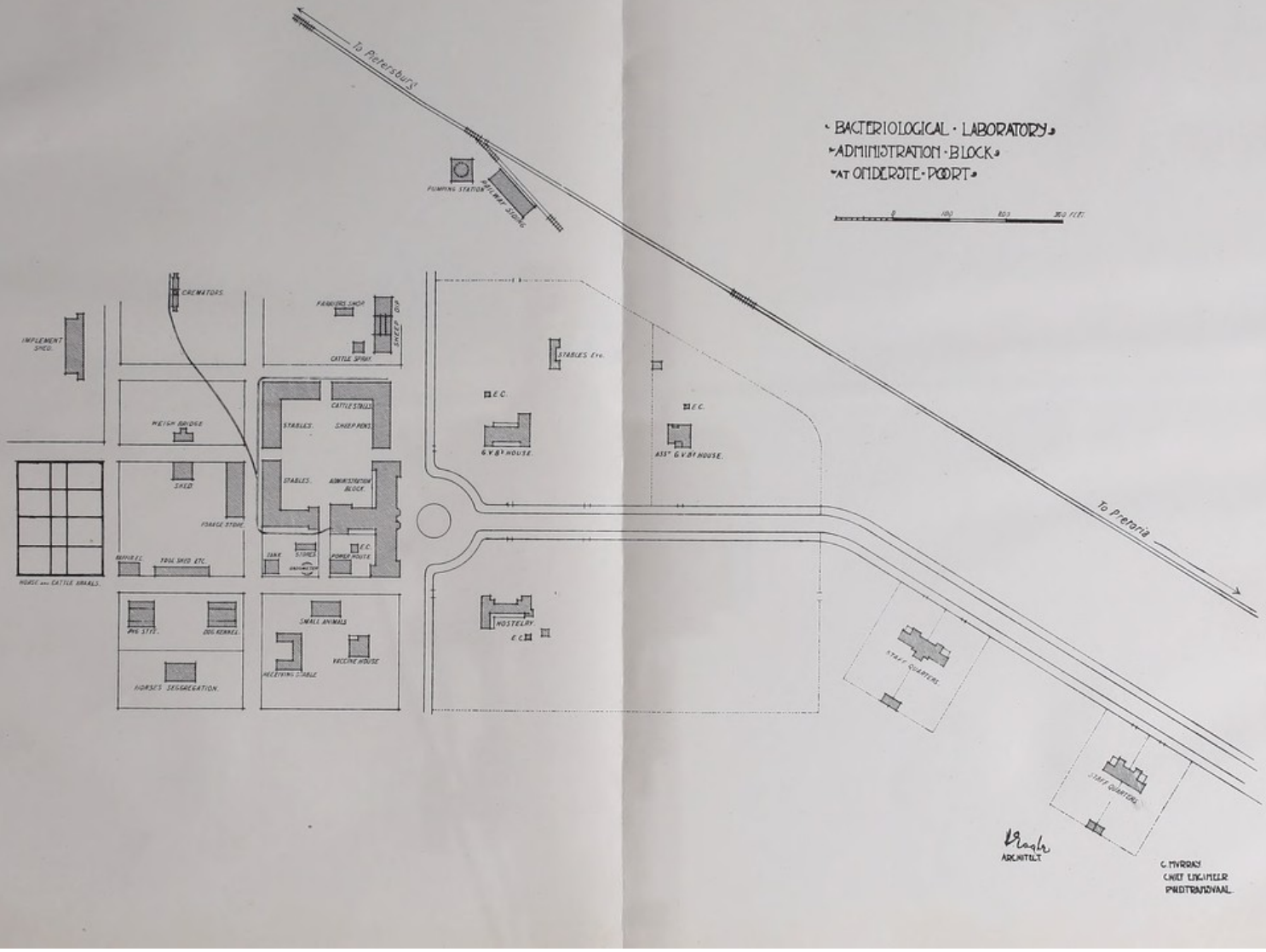
*The Native Staff.*


*Some Visitors to the Laboratory.*

# BACTERIOLOGICAL · LABORATORY
# ADMINISTRATION · BLOCK
# AT ONDERSTE · POORT




C. MURRAY,
CHIEF ENGINEER
PRETORIA


To Pietersburg
PUMPING STATION
RAILWAY SIDING
BACTERIOLOGICAL · LABORATORY
ADMINISTRATION · BLOCK
AT ONDERSTE · POORT
0 100 200 300 FEET
To Pretoria
CREMATORS
FARRIERS SHOP
SHEEP DIP
CATTLE SPINK
IMPLEMENT SHED
WEIGH BRIDGE
CATTLE STALLS
STABLES
SHEEP PENS
SHED
STABLES
ADMINISTRATION BLOCK
FORAGE STORE
W.C.
POWER HOUSE
SMITHY ETC
TOOL SHED ETC
TANK
STORES
ENGINE ROOM
HORSE and CATTLE KRAALS
PIG STYE
DOG KENNEL
SMALL ANIMALS
RECEIVING STABLE
VACCINE HOUSE
HORSES SEGGREGATION
STABLES Etc.
E.C.
G.V.&P. HOUSE
ASS.T G.V.&P. HOUSE
HOSTELRY
STAFF QUARTERS
STAFF QUARTERS
ARCHITECT
C. MURRAY
CHIEF ENGINEER
PRETORIA