South African Horse-sickness.

its pathology and methods of protective inoculation

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This disease, so far as is known, is peculiar to Africa.

It has been known since the year 1780 as affecting Southern Africa but probably exists also in more northern parts of that continent.

In my Annual Report to the Colonial Government for 1894, as Director of the Bacteriological Institute, I have dealt, at considerable length, with the history of this remarkable disease & it is, therefore, not requisite to enter with much detail into that side of the enquiry at this moment.

While affecting the Transvaal and Rhodesia every year - beginning about the end of October & continuing until the following May, or even later - it only affects the Cape Colony in an epizootic form in certain years, although sporadic cases occur annually in certain localities.

It

It affects horses, asses, & mules & it has been asserted - although I have never seen such cases - that quaggas have also been killed by it.

I have recently identified a disease, which occurs to a limited extent among cattle, as being, probably, of the same nature, while a disease which occurs widely throughout the Eastern Province among high bred goats and sheep, is closely related in its pathology with this malady.

Horsesickness occurs mostly in low-lying parts of the country, independent of the fact that the general locality may be considerably elevated above the sea-level; thus Johannesburg which is about 6000 feet above the sea-level is frequently subject to its baneful influence.

Certain atmospheric conditions seem to favour the production of this disease, for it is commonly observed to occur during periods when the air has become humid & has been associated with a high daily temperature.

Deep kloofs or gullies where vegetation is abundant and the ground, below the undergrowth, moist, are especially dangerous to susceptible animals.

During a season of sickness it has been commonly observed that animals, which are not allowed to graze after sundown nor before the sun has dried up the dew
from

from the herbage, do not so commonly become affected, but that where this routine is not carried out mortality commonly occurs.

Horses which are kept in the stable during the night are, as a rule, safe but during last year 60% of the stabled horses in Eshowe, Zululand, died of this sickness. Veterinary Lieutenant Coley A.V.D. who kindly made the observations for me, stated that these horses were mainly fed on "Guinea" or Ubaaba grass mixed with forage or Indian corn. This grass was usually cut in the evenings & made into bundles till next day. Those who took particular care to have the grass thoroughly dried in the sun before using it did not lose their horses while those who neglected this precaution lost heavily.

It is only directly contagious; for, while horses may be inoculated with virulent blood & be permitted to die among clean animals, I have never found, during observations extending over seven years, a single case of infection from such a source.

The Boers have been in the habit of sending their horses to elevated tablelands previous to the onset of the season of sickness; such areas being usually free from the disease. The onset of frost commonly puts an

end

end to the ravages of the disease and the first frost in a season has been usually regarded by the Boers as a sign that their horses might be recalled from their sanatoria.

The season of sickness usually begins about the beginning of November and lasts till the following May; the worst period being from the end of December until the end of February.

The mortality from this malady is terribly great in countries such as Rhodesia & the low-lying parts of the Transvaal, in which it makes its appearance annually. In Rhodesia the annual losses amount to about 90%.

Animals which have been affected and subsequently recover are spoken of as "salted". They are believed to be protected against the disease for the future & owing to this belief the value of a "salted" animal is from six to ten times what it would be in an "unsalted" condition.

As, however, the sickness does not always present symptoms during life which admit of a satisfactory diagnosis being made, it happens that most animals which become ill during the season, from whatever cause, are believed by the farmers to have horse-sickness & should they recover are immediately said to be "salted". It will therefore be evident that many animals sold as "salted"

are not really so & thus the report finds favour that even "salted" horses die of the sickness.

I have, however, had ample opportunities of ascertaining that some "salted" horses have actually died, notably animals which, after "salting" in one area, have been removed to a much more unhealthy one. My experiments, among other things, go to explain these occurrences.

Secondary fever

Animals which are really "salted" are subject at much later and irregular periods to attacks of fever. Such attacks have no relation to fresh infection as I have found them to occur in all "salted" horses which I have kept under close observation in the stable. The attacks vary greatly both in degree and duration; they may last for one day or may extend over as many as six days.

Symptoms of Horse-sickness

In the variety of the disease styled by the Dutch Dun-paardziekte, the symptoms are, for the most part, conspicuously absent until just before the onset of death. The animal may be seemingly in perfect health and vigour yet, within an hour, it may suddenly begin to show that its breathing is actively interfered with; it will heave violently at the flanks, stagger about for a few minutes and, falling down, eject a huge cloud of white foam from the mouth and nostrils, after which death is almost

immediate

immediate. In the other variety, known as Dikkop-ziekte, the neck, head, and lips swell up enormously; the latter commonly to such an extent as to fall back and expose the teeth of the lower jaw. A subvariety of this kind is called Blautong, owing to the swelling being mostly confined to the tongue which, then, presents a bluish colour owing to the venous engorgement of the organ.

Owing to the apparent absence of symptoms until just before the onset of death, it was commonly believed that the whole course of the disease, from the moment of infection until death, occupied only a short period. As, however, it has been found possible to carry infection from one animal to another by inoculation, the onset of fever has been found to follow the operation only after a prolonged period of incubation.

Post-mortem phenomena

Whichever variety of the sickness the animal may have suffered from it is usual, although not an invariable rule, to find a huge cloud of white foam around the mouth and nostrils of the dead animal. This usually remains unchanged, to any great degree, for a long time even during exposure to a hot sun.

If this exudation begins during life, one notices that it arises from a fluid which the animal is coughing

up and if one stands close to an affected horse during this stage the breath sounds ^{are} ~~as~~ clearly audible as if passing through some fluid material which is present in the air passages. As the animal stands, with head hanging down, this fluid falls from the mouth and nose spasmodically, especially during coughing. I have caught quantities of this material in glass vessels when it appears as a straw coloured fluid but is spontaneously coagulable, in the presence of minute traces of blood.

It is coagulated by heat, and has been found to consist almost entirely of the blood plasma.

On removing the skin of a horse affected with the ordinary form of the disease one finds among the fasciae in various situations, but particularly along the course of the jugular vein, lines of yellow, almost transparent, exudation, portions of which, when removed, present an appearance like gelatine.

The pericardium invariably contains an excess of fluid. As a general rule the amount present is such as to render the membrane so tense that when it is pricked with an knife the fluid, contained within it, is ejected with some little force. I have removed as much as 140 fluid ounces from one case but as a rule the amount is usually under 100 ounces.

In colour it is usually of a brilliant yellow, but, in some cases, it is slightly, or very deeply, tinged with blood, and in such cases, when examined microscopically, blood corpuscles are to be found in it.

Even when of a brilliant yellow colour it is liable to become coagulated very shortly after its removal from the sac, and indeed it is not uncommon to find that portions have already undergone coagulation previous to the opening of the pericardium. The parietal layer of the pericardium is usually injected and slightly opaque but I have never recognised any true inflammatory action to be indicated.

Extending from the base of the heart along the course of the great vessels and involving the surrounding connective tissue, there is frequently to be found considerable amount of a characteristic yellow gelatinous exudation.

Towards the base, on the outer surface of the heart and along the interventricular groove, there are distributed smaller ~~and~~ or larger extravasations of blood. In size these may vary from a pin point up to one eighth of an inch but, as a blending of individual points commonly occurs, the discrete areas of extravasation may measure half an inch or more in diameter. This condition is not at all characteristic or peculiar to Horse-sickness

as it also occurs in cases of Texas fever and Rinderpest among cattle.

The muscular substance of the heart frequently appears perfectly normal but in some cases it is paler and somewhat cloudy.

The endocardium is most usually somewhat opaque and in the subserous tissue, especially near the attachments of the Columnae carnae extravasations of blood are very commonly found.

In some rare cases anti-mortem clots are found in the cavities of the ventricles.

On examining the muscular tissue microscopically, it is usually seen that the striation of the muscular fibres is obscured owing to cloudy swelling; and among the fibres in numerous directions, near to an area of extravasation, there are to be found smaller or larger extravasations of blood, which are commonly found to be undergoing degenerative changes and may include masses of blood pigment and blood crystals.

The pleural cavity usually contains much fluid which, like that seen in the pericardium, is either of a clear amber colour or more or less blood stained.

The lungs, in horses which have been stabled and not worked during their illness, appear pale in colour or may shew patches of congestion. Close examination usually

reveals

reveals the presence of a diffuse pigmentation.

The most characteristic feature however is an exudation of clear yellow fluid into the interlobular tissue, which dissects the lung up in every direction but more particularly towards the free edges. At the latter situation the clear bands of exudation may measure over half an inch in diameter and if the lung is held up between the observer and the light the exudation bands appear transparent and of a yellow colour.

In other cases, where the animal has been ridden during its illness, the lungs may appear engorged with blood to such an extent as to simulate croupous pneumonia. On microscopic examination of the lung tissue one finds that exudation of lymph has taken place in all directions.

The endothelium of the acini is swollen and the nuclei are frequently seen to be undergoing proliferation. Among the interstitial tissue the cells are also found to be swollen and proliferating while smaller or larger portions of blood pigment are to be seen either among, or actually within the proliferating cells.

The veins are usually distended with blood corpuscles and, in cases of the congested variety, enormous and innumerable areas of extravasated blood are to be found.

The spleen, as a rule, is enlarged, deeply congested and over the whole surface may be seen small haemorrhagic

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points. On section the capsule immediately retracts and the substance is seen to be very dark in colour owing to venous engorgement.

On microscopic examination the pulp and sinuses are distended with blood corpuscles while innumerable extravasations of blood, now more or less altered, have led to the formation of innumerable and frequently enormous masses of pigment and blood crystals, the presence of which causes sections to be cut with great difficulty.

The kidneys are usually slightly enlarged and on section one may find in the pelvis more or less of a clear gelatinous exudation. The capsule is readily separable. In some cases the medulla appears only slightly congested and the cortex is deeper in colour than normal.

On microscopic examination I have found evidence of irritation throughout the whole organ : the nuclei of the epithelial lining of the tubules are increased in number as are also those of the glomeruli. In various situations the swollen epithelial cells suggests an approach to true catarrhal nephritis but this is not a general condition.

Judging from the condition which I have found in the lungs one might expect to find venous congestion

well-marked in the kidney but it is for the most part absent.

It is difficult to define intertubular serous exudation in hardened preparations, but there is evidence in the form of granular debris involving the intertubular tissue which goes to show that during life this tissue must have been the seat of an albuminous infiltration.

The gastro-intestinal tract does not usually present any appreciable abnormality, but during the epizootic of 1892 several cases, which occurred in the veld, were found to have varying degrees of enteritis present. As however the farmers of this Colony have a considerable liking for dosing sick animals with paraffin oil, sheep-dip, and other remedies, it is more than probable that the unusual symptoms could be referred to the influence of treatment. The cerebro-spinal system to some extent also suffers from this serous exudation, but the only symptoms which have come under my own observation have consisted in slight flattening of the convolutions of the brain and some increase in the amount of fluid found in the fifth ventricle.

The conjunctivae are always injected and the cornea is of a dusky hue, the buccal mucous membrane is not as a rule, but the nasal mucous membrane is frequently injected.

Infiltration.

Infiltration of the same serous material also involves the epiglottis and the larynx; in one case the swollen condition of the vocal cords which arose from this exudation gave rise to a very great difficulty in breathing.

Pathological phenomena are therefore for the most part to be ascribed to the marvellous exudation of blood plasma, which, while seen more or less throughout the serous and subcutaneous tissues, is best marked within the thoracic cavity. The enormous exudation into the pericardium must exert some very definite interference with cardiac action, while the pleural exudation combined with infiltration into the interlobular pulmonary tissue and eventually into the acini themselves furnishes physical impediments to the function of respiration.

Throughout the course of numerous post-mortem examinations one negative feature stands out in remarkable prominence, namely, the almost total absence of any true inflammatory phenomena while the conditions obtaining might be, for the most part, ascribed to acute venous congestion with exudation of blood plasma. This however cannot be considered as the primary cause of death for, as I shall shew, there is a definite toxic property associated with the contagium of this disease which seems to me to exert an action on the vaso-motor system.

system.

Inoculation Experiments.

For the purpose of conveying to healthy animals the infection from those already sick, three materials have been made use of, namely, the yellow fluid from the trachea, the fluid of the pericardium and the blood, of infected animals.

The operations have been carried out in three ways;-

- (a) By subcutaneous injection.
- (b) By the insertion of a seton, impregnated with the infective material, under the skin.
- (c) By drenching e.g. giving a dose by the mouth.

The sites selected for inoculation have been;-

- (a) directly into the lung tissue, by hypodermic syringe, operating through the skin over an intercostal space.
- (b) into the subcutaneous tissue of the neck.
- (c) into the subcutaneous tissue of the flank, immediately anterior to the ilium.
- (d) intravenously.

Previous to describing the effects produced in each case I may detail the general symptoms which are usually produced.

Pericardial fluid

In two cases the injection of pericardial fluid in
doses

doses of three cubic centimetres, failed to produce the disease; in several other cases it succeeded in initiating the malady.

Horse-sickness blood

The blood of virulent Horse-sickness, when used by subcutaneous injection or by seton or by intravenous injection, has invariably been followed by the production of the disease in susceptible horses.

When an injection of from one to five cubic centimetres of virulent blood is made into the subcutaneous tissue of the neck in a horse, the following train of symptoms usually appear; -I may add that I possess records of more than two hundred inoculations.- In a few cases, where fresh blood is used which has been taken from an animal that has been dead for some hours, a slight elevation of the temperature of the inoculated horse may occur on the evening of the day of inoculation. Where however the blood used for inoculation has been drawn from an animal during life, and immediately thereafter used for the inoculation of a clean horse, or where the blood used has been preserved for some time previously, it is unusual to find such an elevation occurring. Where it occurs I am disposed to consider it due to some slight septic infection acting in the immediate site of the inoculation.

inoculation.

Injection of pericardial fluid

Horse No. 4 was inoculated on the 9th. February with 5 c.c. of pericardial fluid taken from a horse which had died during the night. The fluid was clear and of a pale straw colour. It was injected directly into the lung tissue by means of a hypodermic syringeneedle, three inches in length, which was inserted through the skin over an intercostal space and pushed down into the lung tissue beneath.

Nothing unusual was noticed till the morning of the 15th. February.

Temperature $101^{\circ}.4$ at 7 a.m. and $103^{\circ}.4$ in the afternoon. 16th. Feby. temperature $103^{\circ}.0$ in the early morning and $104^{\circ}.0$ later. The conjunctival mucous membranes were dusky in colour and shewed a number of injected points.

February 18th. - Temperature $104^{\circ}.0$ at 7 a.m.; fell to $103^{\circ}.4$ at 10 a.m. rose to 105° at 1 p.m.

At this time the animal was found to be breathing irregularly and the rate was much above the normal. The temperature was now falling and the animal began to heave at the flanks while each effort of inspiration was accompanied by an indrawing of the skin over the
intercostal

intercostal spaces. It would lie down occasionally for a few minutes and then get up again. At 4 p.m. it lay down, stretched itself out and giving a few spasmodic kicks, ejected a huge cloud of white foam from the mouth and nose and died.

The post-mortem examination revealed the usual lesions; much pericardial effusion and pleural effusion, with interlobular infiltration of serum into the spaces between the lung tissue.

Chart No. 1 gives the recorded temperatures of this case.

Inoculation of preserved blood.

Horse No. 15 was inoculated by subcutaneous injection with 5 c.c. of preserved blood. This blood was obtained from a case of Horse-sickness at Pietermaritzburg in Natal. It was preserved by mixing it with an equal volume of the following preservative and kept for about one month previous to being used.

Glycerine 1000 c.c.

Water 10000 c.c.

Carbolic Acid 5 grammes.

The inoculation was made on my return to the Cape Colony on the 6th. of March. The site chosen was the subcutaneous tissue of the shoulder.

March 12th. temperature rising in the evening to 103.4F.

March 13th.

March 13th. still rising

" 14th. still rising ; conjunctival membranes injected.

" 15th. Temperature 106°0 in the afternoon. No other symptoms observable. Animal ate and drank freely.

" 16th. Condition unchanged. Breathing normal.

" 17th. Temperature falling rapidly; animal not feeding.

Breathing accelerated and towards three p.m. heaving at the flanks was noticed. Death occurred at 8 p.m. Froth was found in great amount around the mouth and nose.

A post-mortem examination revealed lesions commonly found; pleural and pericardial effusion; haemorrhagic extravasations in the interior of the heart and infiltration of serum into the spaces between the substance of the lungs.

The accompanying chart No. 2 gives the temperatures which were recorded in this case.

Inoculation by fluid taken from the trachea of an infected animal after death

Horse No. 7 was inoculated with fluid taken from the trachea of an animal which had died of the disease.

2 c.c. of this was injected subcutaneously into each side of the neck on March 14th.

During the subsequent three days the sites of inoculation were swollen, very tense, and evidently painful on the slightest pressure.

March 17th. I opened both swellings, evacuated much pus, and washed out the cavities with weak carbolic lotion.

The temperature which had been elevated during the past three days now fell almost at once.

March 21st. The temperature began to rise suddenly and during six following days was very high with slight remissions.

March 26th. The lips and neck were seen to be swollen

March 27th. Swelling of lips and neck very pronounced.

The supraorbital fossae seem to be filling up

March 28th. Swelling of head and neck very great;

Supraorbital fossae bulging. Animal trying to rest head on sides of stall. Breathing interfered with owing to infiltration of

larynx. Later on in the day heaving at the flanks became evident and it died suddenly.

At the post-mortem examination, the subcutaneous tissue of the head and neck, and slightly also that of the anterior portion of the thorax, was infiltrated with brilliant yellow coagulated lymph.

The mucous membrane of the larynx was greatly congested and so oedematous that the passage through the Rima glottidis was very greatly reduced.

The pericardial effusion was present in very great quantity and was deeply bloodstained. Interlobular infiltration of serum into the lung tissue occurred but sparingly and was almost entirely limited to the free edge of the right lung. Chart No. 3 gives the recorded temperatures of this case.

Inoculation into flank.

Horse No. 15 was inoculated by subcutaneous injection of 5 c.c. of preserved Natal Horse-sickness blood into the left flank immediately anterior to the external angle of the Ilium on the 29th. March.

April 4th. Temperature rising.

" 5th. Temperature rising, after remission during night.

" 6th. Fever had remitted during night; temperature was 100°·8 at 7 a.m. but rose steadily and rapidly during the day to 106°·4 at night.

" 7th. Temperature seemed to be falling and animal did not evince a single bad symptom; indeed no signs of illness except the elevated temperature were to be observed.

" 8th. Animal found lying dead in the stall. No froth was found either at ^{nose}~~nose~~ or mouth.

At the post-mortem examination a very small amount of

yellow

yellow fluid serum was found in the trachea. The pleurae contained much fluid. The lungs were pale while only very slight evidence of interlobular infiltration ^{was} ~~were~~ to be seen at the free edges of the lungs. The pericardium contained much fluid. Th abdominal cavity contained very much fluid which consisted almost entirely of blood-stained serum. The mesentery was much thickened and opaque, from the presence of infiltrated serum which had become solidified. The lymphatic glands throughout the abdomen were enlarged and hard. The intestines were much injected throughout almost their entire length but there was no true peritonitis nor enteritis. The liver was deeply congested.

In this case the abdominal pathological changes were quite unique. I have never, in any other case, observed an abdominal exudation which could be described as other than ~~an~~ ~~slight~~ excess of normal peritoneal fluid. On the other hand I have never seen a case in which the lungs were so remarkably free from infiltration of serum. It seems reasonable to conclude that the site of the inoculation was concerned with the changes produced.

Production of the "Dikop" form of the disease.

A horse having died from typical Horse-sickness in the commonage during the night, was handed over to me for

examination

examination. The post-mortem examination made it clear that the disease was the ordinary form of horse-sickness from which animals were dying in all parts of the district.

The blood was coagulated in all the veins and in the ventricles of the heart. As I was at the moment in need of material for inoculation, I emptied out the clots from the jugular veins and breaking them up I separated a quantity of bloody serum which smelt of putrefaction. To this I added an equal volume of the preservative and after keeping the mixture for seven days I inoculated Horse No. 16 by injecting 5 c.c. subcutaneously in the neck on the 24th. May.

As ~~no~~ immediate reaction occurred ^{nor} during the subsequent seventeen days, I was about to conclude that the virus had not been virulent.

On the 18th day however (10th June) a slight elevation of the animals temperature occurred reaching to 101°F. at midday.

June 11th. Temperature in afternoon and evening 103°F.

" 12th. Temperature remitted during night but at 10a.m. reached 102°.4 and was 103°.6 at night.

" 13th. Temperature had again remitted during night, but rose steadily during the day until it reached 106°.6 at night.

June 14th.

- June 14th. Temperature had again remitted during night.
Animal seemed to be swollen around the thorax
and abdomen.
- " 15th. Temperature had remitted during the night;
the maximum for the day was less than
yesterday. Swelling more definite.
- " 16th. Remission of temperature during the night.
The maximum for the day less than yesterday.
Swelling more definite.
- " 17th. Temperature normal. Swelling very great.
- " 18th. Temperature rose during the day to 102°F.
Swelling stationary.
- " 19th. Temperature almost normal.
- " 21st. Temperature normal. Swelling slightly less.

As absolutely no symptoms of discomfort, accelerated breathing or pain had been manifested at any period of the fever I determined to shoot it and examine into the pathological changes which might have occurred. This was accordingly done; a bullet was fired into the brain toward the base, death being instantaneous.

On making a post-mortem examination the following very interesting changes were found. The subcutaneous tissue, in all directions, was infiltrated with a golden yellow exudation which was transparent in character. This exudation occurred in greatest amount toward the lower parts of the subcutaneous tissue of the thorax and abdomen, mostly in the latter situation. At this part it was

found to occupy a depth of from one to two and a half inches and fully accounted for the swelling which had been recognised during life. The heart shewed extensive patches of ecchymoses on its pericardial and endocardial surfaces. The endocardial surface of the left ventricle had very numerous patches of these ecchymoses, especially in relation to the attachments of the columnae carnaeae. No clots were found in the ventricles but the blood found there in small quantity coagulated immediately on removal. The pericardial sac contained a very large amount of yellow fluid which coagulated on removal to a clean vessel. The amount removed was slightly over eighty fluid ounces. A considerable amount of a similar fluid was found in the abdominal cavity.

The lungs presented on their surfaces large patches of a white fibrinous exudate and the subpleural lymphatics were distended with a clear transparent yellow fluid which was partly coagulated. The substance of the lung was quite normal in appearance. The pleural cavity contained a large amount of yellow transparent fluid. The peritoneal cavity, as already stated, contained a considerable amount of the yellow fluid but it was difficult to estimate the amount, as during the examination one of the intestines was opened accidentally. The stomach and intestines presented a normal appearance

with

with the exception of a few petechiae on the peritoneal surface of the large colon. The liver, spleen, pancreas and kidneys presented no abnormal feature.

In the ordinary "Dikkop" form the infiltration is found to occur mostly in connection with the head and neck but it is also found, though in much lesser degree, in the subcutaneous tissue of other parts of the body.

This case is quite unique in my experience.

Infection by gastro-intestinal tract

Horse No. 30 was given by mouth eight fluid ounces of fresh defibrinated blood which was taken from Horse No. 32 that was dying of Horse-sickness. The dose was administered on the 3rd. of September.

- Sept. 11th. Temperature 101° at night.
- " 12th. Temperature registered $106^{\circ}.6$ in the afternoon.
- " 13th. No great remission occurred during night, temperature being 104° at 7 a.m. and $107^{\circ}.2$ at night.
- " 14th. Remitted during night to 105° but was again $107^{\circ}.2$ during afternoon.
- " 15th. Remitted to $105^{\circ}.1$ during night and remained nearly the same all day.
- " 16th. Temperature much as yesterday.
- " 17th. Had died at a later hour the previous night.

There was very slight congestion of the vessels
of the

of the intestinal tract but no change of greater character was found in the abdominal cavity. The pleural and pericardial cavities contained much yellow fluid and the pulmonary pleurae were covered with yellow coagulated lymph. Frothing at the mouth and nose had occurred at the moment of death. This case presented no symptom other than those found in ordinary spontaneous cases which are for the most part identical with those produced by subcutaneous inoculation. Chart No. 6 shews the temperatures which were registered in this case.

From the observations I have made I have found that the subcutaneous injection of fresh or properly preserved horse-sickness blood produces symptoms during life and shews pathological changes after death which are not to be distinguished from those found in the spontaneously occurring cases of the lung form of the disease. It is somewhat remarkable that the only cases in which I have succeeded in producing the "Dikkop" form are those already described and in which the virus used was somewhat septic. Where, however, I have inoculated virulent preserved blood into partially protected animals I have in a number of cases, where the virus used has been sufficiently attenuated as not to produce death, but a longer febrile period than is found in the fatal

cases

cases, I have also seen the "Dikkop" form produced.

Properties of the virus

Preservation. For the purpose of preserving the virus, the following procedure is now adopted. An animal in the last stages of the disease is bled from the jugular vein by means of a long plated cannula, having an incurved point. This cannula has attached to its distal end a piece of india rubber tubing, which in turn carries a short glass nozzle. Bottles, each capable of holding about 300 c.c. of fluid, are previously prepared by placing in each 50 c.c. of a 10% solution of neutral citrate of potash. The mouths are plugged with cotton wool after which they are sterilized in an autoclave. The cannula, rubber and glass mouthpiece are sterilized first in the autoclave and then boiled in a 1 in 40 solution of carbolic acid in which solution they are brought into the operating room. The jugular vein of the horse being exposed by a small incision, the cannula is inserted and the blood permitted to flow sufficiently to drive out any lotion lying in the tube. The bottles in turn are then opened and the blood received. After each has been filled they are replugged and set aside in an ice safe. Within twelve hours the red corpuscles will have become deposited at the lower part, and more than half of the original bulk will be seen to

consist

consist of a citron yellow fluid. This fluid will be slightly turbid in character owing to the white corpuscles which do not settle for a considerable time later. I draw off by a sterile aspirator sufficient of this yellow fluid to equal more than half of the original bulk of the blood. The remaining corpuscular matter is then measured and sufficient of the aspirated fluid is added, if necessary, to make up the bulk to exactly half its original amount. An equal amount of preservative is then added so that the mixed fluids when completed are exactly equivalent to the original volume of blood.

Virus thus preserved retains its virulence quite unimpaired for an exceedingly long period of time. I append a chart No. 7 of Horse No. 175 which was inoculated by subcutaneous injection with 1 c.c. of the preserved virus taken from Horse No. 120. The virus had been preserved during a period of 235 days, when it was used, and the result was to produce a very virulent attack of horsesickness after an incubation period of eight days. I may add that since this experiment I have used the same virus for the inoculation of numerous horses the last inoculation being made one year and nine months after the date of the preservation. Up till this moment I have not observed the very slightest diminution

diminution of virulence, despite the fact that the stock is kept only in a dark chamber at ordinary temperatures, varying from 32 deg. F. during the winter to 109 deg. F. in the ^{shade in the} summer.

Effect of desiccation on the virus.

I bled from the jugular vein two affected horses into citrate solution, and the blood thus collected was afterwards dried. When thoroughly dry it was reduced to powder and placed in a drying chamber for forty eight hours. The dried blood was thereafter mixed with sterile salt solution and, when dissolved (dried citrated blood dissolves completely), one horse was then inoculated with each sample of blood by subcutaneous injection, the material injected containing exactly two grammes of the dried blood. In neither case was any change produced excepting a slight elevation of temperature which occurred within two hours of the inoculation, subsided almost at once, and was probably due to local irritation.

I now determined to experiment with blood dried in an exceedingly thin layer, and to this end some of the blood of horse No. 92, collected in citrate solution, was dried on clean sheets of glass. The layers were each so thin as scarcely to admit of the blood running off when the glass was inclined to ⁿoe side. They were dried in the shade of a verandah and the process was complete within a few minutes time. Afterwards the blood was

scraped off, powdered, and kept in a drying chamber during twenty four hours. The blood, in doses of two grammes was thereafter dissolved in sterile salt solution and used to inoculate two horses Nos. 98 and 99. of which I append the charts Nos. 8 and 9.

In each of these cases febrile changes occurred which in my opinion were not due to local reaction but to the virus of horsesickness, and although a later inoculation with virulent blood shewed that the animals were not fully protected, the subsequent experiments I shall detail will demonstrate that protection is not always quickly produced and only where one very severe attack or numerous ~~small~~ mild ones have been borne by the animals which it is sought to protect.

The action of Sulphuric acid.

I have mixed virulent preserved virus with an equal volume of a 50% solution of glycerine and water containing 1 in 800 of Sulphuric acid so that the mixture of blood water and glycerine contained 1 in 1600 of Sulphuric acid. Immediately after mixing I inoculated 19 horses with doses of 2 c.c. each. In no case was the disease induced but when subsequent inoculation was made of an equal dose of virus containing 1 in 800 of acid in the mixture, the disease was induced in one animal, only after the lapse of a period of twenty three days.

Experiments made in regard to protective inoculation.

The yellow fluid removed from the pericardium of an animal which had died from the disease was filtered through a Pasteur filter. Of this, 100 c.c. was injected subcutaneously into Horse No. 100 on the 18th of September. An immediate reaction followed which was accompanied by swelling and pain in the site of the inoculation. After a few days these symptoms subsided and the temperature became normal. Eleven days after inoculation it was reinoculated with 3 c.c. of virulent horse-sickness blood.

The result was seen in the production of virulent horse-sickness and death. There was no evidence of any protective influence having been exerted by the injection of the exudate. See Chart No. 10.

Effect of large doses of Calomel upon the disease in an inoculated animal.

On the 30th January Horse No. 35 was inoculated by subcutaneous injection in the shoulder with 5 c.c. of virulent preserved blood.

7th. February	temperature is suspicious; 102° 8 at night. Gave 30 grains of calomel by mouth.
8th. "	temperature rising; 104° 6 at night. Gave 30 grns. calomel both in morning and at night.
9th. "	temperature 102° 2 in the morning but 105° at night. Gave 30 grns. calomel in morning

and at night.

- 10th. February temperature 103°.6 in morning but 106°.8 at night. Had same doses of calomel morning and night.
- 11th. " temperature 103°.2 in the morning but 107° during afternoon. Conjunctival membranes somewhat congested. Gave same dosea calomel morning and night.
- 12th. " Temperature 103° in the morning and 106° during afternoon. Same doses of calomel given.
- 13th. " Temperature 104°.8 2 in morning and 105° at night. No calomel given.
- 14th. " Temperature 103°.6 in morning and 104° at night.
- 15th. " Temperature normal all day but 102°.4 at night.
- 16th. " Temperature normal and remained so.

About a month later, on the 15th. May, it was reinoculated with 5 c.c. of virulent blood without the slightest effect being noticed. Chart No. 11 shews the temperatures which were registered in this case.

On the 6th. March, Horse No. 26 was inoculated in the flank with the same dose of the same virus as in the preceding case.

March 14th.

- March 14th. Temperature rising.
- " 15th. Temperature $103^{\circ}.1$ in morning and $105^{\circ}.2$ at night; Gave 30 grns. calomel morning and evening.
- " 16th. Temperature $103^{\circ}.1$ in morning $105^{\circ}.3$ at evening. Same doses of calomel given.
- " 17th. Temperature 104° in morning and $105^{\circ}.2$ during afternoon. Same doses of calomel given.
- " 18th. Temperature 104° in morning and $105^{\circ}.2$ during afternoon. Only one dose of calomel given in morning.
- " 19th. Temperature $103^{\circ}.2$ in morning and $104^{\circ}.4$ at night. Only one dose of calomel given.
- " 20th. Temperature $102^{\circ}.2$ in morning and $103^{\circ}.1$ at night. No calomel given.
- " 21st. Temperature $101^{\circ}.4$ in morning and 102° at night. No calomel given.
- " 22nd. Temperature almost normal.
- " 23rd. Sudden rise of temperature to $103^{\circ}.6$ in morning and $104^{\circ}.4$ at night.
- " 24th. Temperature $104^{\circ}.4$ in morning and $102^{\circ}.4$ at night. No other symptoms.
- " 25th. Temperature $105^{\circ}.4$ in early morning.

Animal now breathing irregularly, quickly and heaving violently at flanks. Died at 10 a.m. with high

temperature

temperature (106 F). At death foam was ejected from the nose and mouth.

A post-mortem revealed the ordinary pathological conditions; lungs being considerably affected with the interlobular infiltration. Chart No. 12 shews the temperatures which were observed in this case.

Immediately after death the jugular vein was exposed and cut open and from it a large quantity of semicoagulated blood was obtained which was defibrinated and thereafter preserved. This blood proved afterwards, in the cases of three horses which were each inoculated with 5 c.c. to be so modified in type that it produced an attack of the disease from which they all recovered, but when it was used still later to inoculate two horses, each in good condition, both died after passing through severe and rather long attacks of the fever.

At that time I believed that the absorption of the calomel had had an attenuating effect upon the virus. Since then however I have found that whenever and by whatever cause the animal can be so assisted that the febrile period is extended, the virus, taken at a time beyond the average death period, is, slightly, but most distinctly, reduced in virulence. I have therefore concluded to ascribe to calomel a useful degree of specific curative action and it has surprised me to find that its use in such quantity exerts little or no

injurious effect. It has however to be borne in mind that the animals made use of in these experiments were ordinary hardy animals, and I should certainly hesitate to use such treatment in the case of well-bred animals.

Transference of the disease from horses to other animals

It has been reported to me that donkeys, while rarely affected by horse-sickness, are, in some cases, severely attacked and actually die. I have therefore inoculated twelve donkeys at different periods of time and by different samples of virus under varying doses. The following cases are set forth as exemplifying the results which are obtained by such inoculations.

On the 12th. January, Donkey No. 203 was inoculated by subcutaneous injection in the shoulder with 10 c.c. of fresh virulent blood from Horse No. 188.

Four days later the temperature rose and registered 104°3 during the afternoon. It fell to 104° at night and within four days had entirely regained the normal.

See Chart No. 13.

On the 18th. March Donkey No. 220 was inoculated by subcutaneous injection in the shoulder with 10 c.c. of fresh virulent blood from Horse No. 212.

Only on two days, the 21st. and 26th. March respectively, did the temperature shew any definite elevation; on the former occasion it reached only to 101°3 and on the

latter

latter to 102°F. See Chart No 14.

On the 29th of August, Donkey No. 255 was inoculated by subcutaneous injection in the shoulder with 15 c.c. of preserved virulent horsesickness blood from Horse No. 120. On the 30th and 31st. there was a slight febrile elevation, probably due to local action of the glycerinated material. On the 4th 5th and 6th of September there were slight elevations reaching, respectively, to 102.4 102° and 102.4 after which the normal was ~~obtained~~ maintained, with the exception of a rise to 102° on the evening of the 9th September. See Chart No. 15.

The following two dinkeys were each inoculated from the same fresh blood but with different doses.

Donkey No. 258 was inoculated by subcutaneous injection in the shoulder with one c.c. of fresh virulent virus from H Horse No. 256 on the 6th. December.

December 9th. Temperature rose during the afternoon to 102.2

" 10th. Temperature 101.3 in the morning and fell during day.

During the subsequent four days the temperature shewed elevations during afternoon and evening, 102.2 being the maximum.

During the 15th. and 16th the normal was nearly maintained but

but, on the subsequent three days, the temperature ascended each day after remitting during night and was in each case over 103° deg.; 103° 8 being the maximum.. From this date the temperature was normal. See Chart No. 16.

On the same date as foregoing, Donkey No. 259 was inoculated by subcutaneous injection in the shoulder with 50 c.c. of fresh virulent blood from Horse No. 256.

On the third day Dec. 8th. the temperature rose to 104° 2. On the 9th. December it reached 105° from which period it gradually fell to the normal which was reached on Dec. 20th. See Chart No. 17.

These cases are quite types of the results which I have obtained from the inoculation of donkeys with virulent horsesickness blood. They reveal a most interesting fact namely, that, independently of the doses of the virus, donkeys shew a most surprising irregularity in susceptibility. In some cases a huge dose of as much as 50 c.c. has almost entirely failed to raise the animals temperature, while in other cases a comparatively small dose has produced a definite febrile ~~reactive~~ period. Some years ago I endeavoured to transmit the disease to two young cattle but, having failed, I hastily concluded that they were totally insusceptible. While, however, I was engaged in investigating Rinderpest I had 300 head of cattle, which had recovered from rinderpest, running in

my camp. One day I was hastily summoned to see a cow which had seemed to be in perfect health in the morning but had suddenly started to heave at the flanks, lain down, ejected a white froth from the mouth and nose and then died. The post-mortem examination revealed conditions which, had it been a horse, would in my opinion have justified an emphatic diagnosis of horsesickness. I therefore withdrew some blood and inoculated a clean ox with 5 c.c. but, excepting a slight irregularity of temperature, during the second week subsequent to inoculation, no result followed.

I therefore ~~attempted~~--- determined to again attempt the transmission of horsesickness from horses to cattle.

On the 4th February I inoculated a pure white young
(No 1)
ox by intravenous inoculation with 30 c.c. of preserved horsesickness blood from Horse No. 120.

An immediate~~d~~ reaction followed and between the sixth and tenth of February the temperature was never lower than 105° and reached as high as 107°.

From the 11th February the febrile curve changed in character being high at night and low in the morning after remitting during the night. The difference between the night and morning temperatures was excessively great; while as low as 100°.6 in the morning it reached 106° or

more at night.

It died suddenly on the 24th February.

At the post-mortem no froth was found at the nose or mouth but a considerable amount was found in the trachea. The pleural and pericardial cavities were filled with yellow serous fluid. The lungs had much yellow exudation under the pulmonary pleura while the interlobular spaces were greatly distended by exudation.

The case presented every characteristic feature of horse-sickness as seen in the horse, except for the absence of froth around the mouth and nose, and which I may add does not always occur even in that animal.

Chart No. 18 shews the temperatures which were registered in this case.

On the 8th February this ox was bled and, with 10 c.c. received in citrate solution, Horse No. 122 was inoculated by subcutaneous injection in the shoulder. On the 14th. February a slight elevation of temperature occurred (101°4) which was repeated on the following day and from this period the temperature kept rising reaching a maximum of 104°3. It died of characteristic horse-sickness on the 21st. instant. See Chart No. 19. In this way the proof was completed that the fever induced in the ox was due to the contagium of horse-sickness.

I have inoculated, in all, 21 head of cattle. Definite febrile

febrile reaction was produced in 7 cases; 4 died.

It is thus seen that in cattle an irregular susceptibility also exists. I may add that the spontaneous disease, from which the cow died, at my camp, is known among the natives by the name Imapunga, and I have, since then, obtained other cases from the blood of which two animals were inoculated. In neither case, however, was death produced, although a febrile reaction set in, of the same type as that produced by the inoculation of oxen with horsesickness blood. I am therefore justified in stating that the disease induced in cattle by the injection of virulent horsesickness blood is indistinguishable from the disease known as Imapunga, and that they are probably identical.

On the 9th February I inoculated Goat No. 7 by intravenous injection with 10 c.c. of preserved virulent blood from horse No. 91. Five days later a definite elevation of temperature occurred which reached to $104^{\circ}.8$. It then fell, but on the 16th. February. it suddenly rose to 107° . From this period it gradually subsided to the normal. See Chart No. 20.

On the 13th. February (10 days after inoculation) the goat was bled and of this blood 50 c.c. was used by intravenous injection to inoculate ox No. 4. A febrile period ensued of the same type as that seen in the ox, No. 1, inoculated

inoculated with horse-sickness blood. See Chart No. 21. It died on the 6th. March, and at the post-mortem examination conditions were revealed which were quite identical with that found in the former ox. The febrile condition together with the results found on post-mortem examination were so definitely in agreement with the former case, that I considered it unnecessary to sacrifice a horse by inoculating it with the blood of the ox. I have inoculated 17 goats with horse-sickness blood. In no case was death produced but in 10 cases was a definite febrile reaction set up.

I have also inoculated 10 sheep of which 6 shewed a febrile reaction but none died.

In sheep and goats there is also to be recognised an ~~xx~~ irregular susceptibility.

Having in this way ascertained that the disease could be transferred to other animals, I set myself to determine whether, and, if so in what manner, the transference could be utilized for the purpose of giving protection to horses.

As the amount of blood procurable from goats or sheep is small in amount I proceeded in the first instance to deal with cattle.

As already shewn the blood of the ox, which had been inoculated with horse-sickness blood, proved virulent

when

when used in the fresh state to inoculate a clean horse.

As however the very large dose of 10 c c. which was used in that case, produced the fever only after a period of nine days incubation, it seemed to me that a certain measure of attenuation had been produced, as 1 c.c. of virulent horse blood usually produces fever after eight days incubation.

At the period when the ox was bled for the purpose of inoculating the horse I took a considerable quantity of the blood and mixed it with an equal quantity of the glycerine preservative and after keeping it for a month I used portion of it, in doses of 10 c.c. to inoculate six horses by subcutaneous injection in the shoulder.

The following is the history of the six horses.

Horse No. 127

9th.	April	- Inoculated
16th.	"	- Temperature rose to 103°.4 in afternoon.
17th.	"	- Temperature falling.
18th.	"	- Temperature normal.
19th.	"	- Reinoculated with same material and dose.
28th.	"	- Temperature rose to 102°.4 and remitted during night.
29th.	"	- Temperature rose again and remitted.
30th.	"	- Temperature rose to 104°.

An irregular febrile period was maintained until the 7th.

May when it registered 104°F. The normal temperature was regained on the following day.

May 12th. 2 c.c. of virulent preserved blood from No. 135 was inoculated subcutaneously without effect.

June 4th. 10 c.c. of preserved virulent blood No. 120 was injected without effect.

This horse was subsequently sent to Rhodesia was exposed in an unhealthy region and is still alive.

Horse No. 123.

April 9th. Inoculated, no result followed.

" 19th. Reinoculated

" 26th. The temperature rose suddenly to 103°F.2 and remained high during six days.

May 1st. Died of horse-sickness.

Horse No. 129.

April 9th. Inoculated. No result followed.

" 19th. Reinoculated. Was left untouched until the 22nd. May. During this period slight evening rises occurred from time to time.

May 22nd. Inoculated with 1 c.c. of preserved virulent blood from No. 135. No result followed.

June 1st. Inoculated with 1 c.c. of preserved virulent blood from No. 120. During the subsequent ten days no result followed.

June 11th

June 11th. Temperature rose suddenly to 103°6, remained high during four days when it regained the normal. The animal ate and drank well and shewed no evidence of malaise. I cannot be certain whether this febrile period was caused by the inoculation or was simply secondary fever. I incline to the latter view.

At later periods this animal received several enormous doses of fresh virulent blood, the maximum single dose being 200 c.c., without any reaction being produced.

Horse No. 130

April 9th. Inoculated, had erratic temperatures during six days.

" 19th. Reinoculated, had erratic temperatures during 10 days.

May 24th. Inoculated with 2 c.c. preserved virulent blood No. 135.

" 31st. Temperature rose to 103.1

June 1st. Temperature 105 and remained high until

" 6th. when it became normal.

This animal received several doses of serum which may have assisted it but the evidence which I shall shew later will suggest that this assistance could have been of little value. This horse has been subsequently sent to

Rhodesia, exposed in an unhealthy area and is still alive.

Horse No. 131

April 9th. Inoculated without any obvious result.

" 19th. Reinoculated. During the 14 days which followed there was practically no variation of the temperature from the normal but from May 4th. till May 13th. an erratic condition of the temperature was seen running up almost daily to about 102 and on the 13th. May to 104.

May 14th. Temperature quite normal.

" 22nd. Inoculated with 1 c.c. of preserved virulent blood of Horse No.120. The temperature immediately rose to 102.4

" 23rd. Temperature reached 103.2

" 24th. Temperature reached 103.3. 200 c.c. of serum given.

" 25th. Temperature reached 106.6

" 30th. Died of Horse-sickness with haemoglobinuria which latter I attribute to having given a dose of serum.

Horse No. 132.

April 9th. Inoculated. Erratic febrile curve followed.

" 18th. Reinoculated.

" 22nd. Inoculated with a dose consisting of 0.5 c.c. preserved virulent blood from No. 120 and

1.5 c.c. of preserved blood taken from the ox which died after inoculation from the goat which had been inoculated with horsesickness.

- April 27th. Temperature rose to 103.6
- " 28th. Temperature rose, after remitting during night, to 103.4 in the evening.
- " 29th. Temperature rose after a slight remission to 106.2
- " 30th. Animal died of Horsesickness.

In the last two cases it is to be noted that, subsequent to the inoculation of the attenuated virus and when the temperature was quite normal, the injection of virulent blood was followed in both cases by the onset of the virulent fever after a much shortened period of incubation. In the former case the onset was almost immediate. These cases are examples of a considerable number which I do not now detail, but which indicate that an attenuated virus, though giving rise to no visible sign, may yet be producing important changes in the body.

Of the six cases just recorded the best result was obtained in No. 127 which gave the most distinct reaction after the inoculation with the attenuated virus.

In the case of No. 129 the virulent inoculation was made only after a period of 33 days had elapsed after the

date of the second inoculation with attenuated virus. In the case of No. 130, 34 days were allowed to elapse after the second inoculation with attenuated virus before the virulent blood was injected.

In both these cases definite protection has clearly been given although during the period of action of the attenuated virus very little reaction had been observed. The death of No. 131 I attribute to the use of serum. When this material comes to be dealt with the reason for my opinion will be abundantly evident.

Thus out of six animals, three certainly, and probably four, had received a definite amount of protection from the use of the attenuated virus while one, No. 132, which died, was inoculated with virulent blood only four days subsequent to being reinoculated with attenuated virus.

The results therefore go to shew that the attenuated virus did give actual protection, which was the better evinced when a considerable ~~portion~~ period of time was permitted to elapse after the use of the attenuated virus, before reinoculation.

The main difficulty, however, in the preparation of this virus lies in the irregular susceptibility to horsesickness which is evinced by cattle and on this account the experimentation with cattle blood was not
carried

carried much further.

In regard to irregular susceptibility, it is also noteworthy that, while five of the horses had but slight febrile reaction as a result of the use of the attenuated virus and that this action varied in the different animals, yet one animal actually died from horsesickness as a result of the use of the attenuated virus itself.

Having established the fact that the fever could be transmitted to donkeys from the horse, it became necessary to determine whether the blood of infected donkeys could be used, in turn, to infect horses and, if so, the amount of attenuation or modification which had been produced thereby.

Donkey No. 203 (See Chart No. 13) was inoculated on January 12th. with 10 c.c. of fresh blood from Horse No. 188. As already shewn a reaction up to 104.4 occurred four days subsequently and gradually fell to the normal which it reached on the 21st. It was bled on two occasions the first being on the 18th, while the temperature was still high, and again on the 21st. when the temperature was normal.

At the first bleeding, three horses, Nos. 204, 205, and 206 and one mule, No. 207, were inoculated by subcutaneous injection with 5 c.c. of fresh blood. The following is the histories of the four animals.

No. 204.

Jan. 18th. Inoculated.
 " 23rd. Temperature $101^{\circ}.6$ at 4 p.m.
 " 24th. " $101^{\circ}.6$ " 7 p.m.
 Feby. 5th. " $102^{\circ}.4$ " 7 p.m.

Irregularity of temperature followed for eight days during which the highest temperature was $102^{\circ}.6$

No. 205

Jan 18th. Inoculated. Temperature began to rise next day and kept steadily rising until
 " 24th. when it registered $103^{\circ}.8$ at 7.p.m.
 gradually falling to the normal which was reached on
 " 28th.
 Jan 30th. Temperature rose to $103^{\circ}.6$ at 4 p.m. and remained irregular during 7 days being rarely above $102^{\circ}.0$

No. 206.

Jan. 18th. Inoculated.
 " 27th. Temperature rose suddenly to $102^{\circ}.4$ and almost immediately regained the normal.

No. 207. Mule.

Jan. 18th. Inoculated. No reaction followed.

Nos. 206 and 207 were each inoculated with 1 c.c. of virulent preserved blood from No. 120 on the 2nd. Feby.

In the case of No. 206 the temperature rose on the 12th. day after this inoculation and it died on the 16th.

No. 207. The temperature rose on the eighth day after this inoculation to 105°2 and remained high for six days after which it regained the normal.

No. 183. was inoculated with donkey blood at a little later period than the former ones and at a time when the febrile reaction in the donkey, had passed off.

Jan. 21st. Inoculated.

" 27th. Temperature rose to 102°2 and kept steadily rising with remissions during the night until

" 30th. Temperature 106°.

The temperature remained nearly constant at 106° for three days and then descended to normal.

It is therefore to be recognised that the blood of the first bleeding taken during a febrile period produced absolutely no reaction on the mule and affected the three horses in very varying degrees.

The blood of the second bleeding, at a period subsequent to the febrile reaction, produced in the inoculated animal a severe degree of fever.

Horse No. 221. was inoculated on the 27th. March by subcutaneous injection with 5 c.c. of fresh blood from Donkey No. 220 (See Chart No. 14)

March 27th. Inoculated.
 April 11th. Temperature 102°6
 " 14th. " 103°0
 " 17th. " 103°0
 " 25th. Up till now (30 days after inoculation) no real reaction had occurred although the temperature had been slightly erratic. On this day however it reached 105°6 at night.

April 26th. Temperature 106°0 in the morning and 106°4 during the afternoon, from this date it gradually decline to normal on

May 2 nd. and was then irregular during seven days

May 8th. Shot.

During the whole period no illness had been manifested.

(See Chart No. 22) It was therefore shot in order to determine the amount and character of the pathological changes.

At the post-mortem examination there was seen much yellow fluid in the pleural and pericardial cavities while under the posterior costal pleurae, in the thorax, and under the peritoneum, in the abdominal cavity, in near proximity to the dorsal vertebrae a considerable

amount

amount of solidified yellow exudation was seen.

Horse No. 222. was inoculated by subcutaneous injection with 5 c.c. of fresh blood from Donkey No. 220 on the 27th. March.

A slight irregular temperature ensued from the 1st. of April till the 13th. of the same month, never, however, reaching as high as 103° .

April 20th. Temperature rose suddenly to $104^{\circ}.8$ in the evening but regained the normal on the following day.

May 1st. Temperature 103° at night.

" 2nd. Temperature $103^{\circ}.6$ at midday. A very slight irregular temperature now set in until the 15th. May.

May 15th. Temperature 104° in the afternoon but immediately regained the normal.

(See chart No.23.)

Numerous other experiments of the same nature have been made all of which result in shewing

(a) That donkeys possess a very irregular susceptibility in themselves.

(b) That the blood of donkeys which have evinced moderate action may produce intense reaction in some horses and practically none in others.

- (c) That the blood of donkeys, which do not react, may produce no effect when inoculated into the horse.
- (d) That a mild reaction in the donkey furnishes no definite assurance as regards the reaction which its blood may set up in horses.

In a horse which is imperfectly protected, the subsequent inoculation of virulent blood produces a febrile reaction and if blood is drawn during this condition and used to inoculate a clean horse it may produce a modified reaction. In other words if the protection given to a horse is of a grade similar to that which normally obtains in the donkey, an inoculation of virulent blood acts upon it similarly to the donkey and its blood during the second febrile stage acts like donkeys blood.

The following case is an example in point.

Horse No. 130 was inoculated on the 24th. May by subcutaneous injection with 2 c.c. blood from Horse No. 135. This blood was slightly less virulent than that which I term "virulent" blood.

May 31st. Temperature began to be elevated at 10 a.m. (102°6) and kept rising until the maximum of June 2nd. 106°2 was reached. Temperature remained high during seven days when it fell to normal on " 7th. this date (June 7th.)
 " 28th. A slight secondary elevation of the temperature occurred.
 " 29th.

June 29th. Temperature was 103°.6 and from this period fell to the normal.

July 4th. Inoculated subsequently with 10 c.c. of virulent preserved blood from No. 120.

" 15th. Temperature started to rise and was very irregular during sixteen days, the maximum being 105°.6 See Chart No. 25.

It was bled on the 4th day of this febrile period and with this blood a horse was inoculated.

Horse No. 157.

July 18th. Was inoculated with 5 c.c. of fresh blood of No. 150 by subcutaneous injection, ~~of~~

" 26th. Temperature rose to 103° in the evening.

" 27th. " " 103°.6 in the evening.

" 28th. " became normal.

" 29th. " was 102° in the evening.

" 30th. " 105° in the evening and

remained irregular until the 8th August.

(On the 6th August it was reinoculated with 2 c.c. of very virulent blood and died of horsesickness on the 18th August thus shewing that the reaction produced was inadequate to protect against this large dose of virus.)

Having experienced this irregularity of reaction which infected donkeys-blood produces I determined upon

preserving

preserving such blood and testing its action some time after preservation. I anticipated, by this means, being able to determine whether the modified contagion^{um} would withstand preservation and if so what samples could be made use of to produce a febrile reaction.

In these experiments the blood which was drawn from the donkey was preserved by the same means as I am accustomed to use for horse blood.

Horse No. 243. was inoculated by the subcutaneous injection of 2 c.c. of preserved donkey blood from No. 252. On the ninth day the temperature became erratic, frequently being 102°8 in the evening. This irregularity was maintained for fifty days. It was inoculated on the 22nd. November with virulent preserved blood from Horse No. 259.

The temperature shewed no change for 12 days (an extremely extended incubation period) and after five days it died of horsesickness, its temperature, at the moment of death being 107°0

Horse No. 254.

Sept. 7th. Inoculated with 3 c.c. subcutaneously, preserved donkey blood No. 253. No result followed.

" 23rd. Reinoculated with 4 c.c., by intravenous inoculation, of preserved donkey blood from No. 255.

The temperature rose on the 7th. day and it died on the 12th. day after the last inoculation. The post-mortem examination revealed the ordinary sharacteristic lesions of horsesickness. In this case, therefore, the horse died from the inoculation of preserved donkey blood, and it is of interest to note that another horse, which was inoculated with the same donkeys blood, had very little reaction

These two cases suffice to shew that preserved donkeys blood may produce very little reaction or may, on the other hand, be dangerous. It is however established that the virus in its attenuated condition might be preserved for indefinite periods of time, and by a proper selection of virus that a means might be afforded of producing some slight degree of protection.

From my experience, I had come to note that the blood of animals, whose febrile period had been extended beyond the normal, was usually slightly less virulent than that of those which had died on or about the 12th. day subsequent to inoculation. It seemed therefore possible that what occurred in the animal body might possibly be attempted experimentally "in vitreo".

I therefore prepared a large number of bottles, ^{capable of} each holding 250 c.c. by adding to each one 10 c.c. of a 5% watery solution of neutral citrate of potash. Each

bottle

bottle was then plugged by cotton wool and was subsequently sterilized on three successive days in the autoclave.

I selected a horse which was suffering from inoculated horsesickness and was still alive on the 12th. day subsequent to its inoculation.

A straight cannula was prepared by having a piece of rubber tubing, one foot in length, attached to the distal end, to which, in turn, there was affixed a short glass nozzle. The cannula, rubber, and glass nozzle was well sterilized by boiling, first in water and subsequently in a $2\frac{1}{2}\%$ watery solution of phenol. The horse was shaved over the course of the jugular vein in the neck and the part thoroughly cleaned, first with soap and warm water and subsequently by hot carbolic lotion. A small incision was made through the skin; the cannula was then made to pierce the vein and enter its lumen and the blood was then permitted to pass from the nozzle. The room in which the operation was conducted had the floor moistened with carbolic lotion to prevent any dust arising.

The blood was received, in one experiment, into fourteen bottles each of which after plugging was placed in the incubator and maintained at a temperature of 102° Fahr. for 10 days. During this period the bottles were examined daily to determine whether any putrefactive changes were in process; but of the number only one became affected by

extraneous microorganisms; and on the 10th day thirteen bottles appeared, even when examined microscopically to be quite aseptic. In some other experiments I was not so fortunate, and it seemed to me in one case that the organism which developed to an enormous extent in every bottle had been already present in the blood when it was drawn, as it agreed in form and size with an organism which I had recognised in the blood. Having received the bottles of aseptic blood from the incubator I next proceeded to remove the supernatant plasma and after measuring the remaining corpuscular material and debris of altered blood I added to it an equal volume of the preservative to which I have already alluded.

The following four ^{records} ~~animals~~ may be taken as examples of results produced by the intravenous injection of 1 c.c. of this material into the jugular veins of each of the horses.

Horse No. 227.

April	8th.	Inoculated with 1 c.c. No. 193 intravenously.
"	10th.	Temperature 102°.2
"	12th.	" " 102°.8
"	26th.	" " 103°.4
May	11th.	" " 102°.4 ; was then hypernormal
		for seven days.

May 15th.

May 15th. Temperature 105°4

In these notes the principal elevations only are shewn but, as chart No 27 will shew, an irregular temperature was maintained during nearly the whole period.

Horse No. 226.

April 8th. Inoculated with 1 c.c. No. 193 intravenously.

" 11th. Temperature 103°4 From this date it steadily rose till its maximum was attained of 106°4

" 16th. on April 16th.

It then declined and reached on

" 21st. the normal.

It is to be noted that this febrile period was as severe in reaction as the usual cases of virulent horsesickness, yet when tested with 0.5 c.c. of virulent preserved blood on the 23rd. May (about 30 days after the febrile reaction had declined) it died of horsesickness. See Chart No. 28.

It is to be noted however and may be recognised in chart No. 28 that after inoculation with the virulent blood the temperature began to rise on the following day; in other words the incubation period was almost entirely wanting. Moreover if the height of the temperature is observed it will be recognised that the maximum in both the former and the latter febrile periods were identical,

thus

thus suggesting, what abundant observations confirm, that the cause of death cannot be held as being, in any case, directly attributable to hyperpyrexia. I might add that in several cases animals have recovered after having experienced temperatures exceeding 107° degrees Fahr.

Horse No. 239.

June 9th. Inoculated with 1 c.c. No. 193. intravenously
 " 13th. Temperature $102^{\circ}.6$
 " 22nd. Reinoculated with 2 c.c. of a similar virus
 but of a different sample (No. 221.)
 " 30th. Temperature 103° in the evening
 July 5th. Attained maximum $106^{\circ}.0$ F.
 " 10th. Died of modified horsesickness (See chart No. 29)

Note. Numerous animals have been inoculated from No. 221. Of these some have reacted while a number have scarcely shewn any change.

Horse No. 230 /

April 8th. Inoculated with 1 c.c. No. 193. intravenously.
 " 12th. Temperature rose to 102.2
 " 14th. " $104^{\circ}.2$
 " 17th. " normal
 " 18th. " "
 " 19th. " $102^{\circ}.6$

April 20th.

April 20th. Temperature 104°.4

" 21st. " 106°.2

It then died of characteristic horsesickness.

These cases will serve to shew, what I have abundantly observed during a number of inoculations made with similar virus, amounting in all to nearly 300 animals, that when an attenuated virus is used the reactions produced are of the most variable description. The reactions are subject to variations in two directions, firstly in point of the period of onset, which may vary from a few days to a month, and secondly in the degree of severity as shewn by the febrile temperature.

For the purpose of producing "protection" in a number of horses, I have made use of a system of consecutive inoculations and in the following records, numbers to which the capital letter "D" is affixed, indicates that the virus was preserved donkeys blood, while the letter "I" indicates a horse virus which has been preserved after being incubated.

Horse No. 250.

April 8th. Inoculated with lc.c. No. 193.I. by
intravenous inoculation.

" 12th. Temperature 102°.2 in the evening

" 13th. " 102°.6 " " "

April 14th.

April 14th. Temperature 104°.2 in the evening.

" 15th. " 103°.8 " " "

" 16th. " 102°.0 " " "

Temperature was then normal for two days.

" 19th. " 102°.6 in the evening.

" 20th. " 104°.4. " " "

" 21st. " 105°.0 " " "

" 22nd. " 106°.2 at 4 p.m. when it died of characteristic horsesickness.

This case is noteworthy inasmuch as it died from the inoculation of this virus which has been used for the inoculation of 19 horses. In addition to this case two others died after being inoculated for the second time with this virus while a fourth died from one inoculation of double the amount used for No. 230. Two were protected by one inoculation against a subsequent ~~one~~ one of virulent preserved blood, while of the remainder some had slight reactions while others seemed to be unaffected.

This series of experiments seems to demonstrate the irregular susceptibility of horses when a weakened or attenuated virus is used for inoculation.

No. 236.

June 9th. 1c.c. No. 193.I. Intravenously. Only slight reaction followed.

" 22nd. 1 c.c. No. 221.I. intravenously. No reaction.

July 8th. 2 c.c. No. 221.I. subcutaneously.
 " 17th. Evening temperature 102°6 and continued to
 be irregular during three days.
 August 3rd. Temperature 102°3 at 4 p.m.
 " 4th. " 103°4 at 7 p.m.
 Temperature continued to be irregular never
 being higher than 103°6
 " 21st. Died.

At the post-mortem the only indications of
 horsesickness consisted in the presence of yellow
 solidified serum, in many situations, particularly the
 intermuscular fasciae of the neck, and the presence of a
 small quantity of exudation in the pericardium and
 between the lobules of the lungs.

No. 237.

June 9th. 1 c.c. of 193.I. intravenously.
 " 22nd. Temperature rose in the evening to 102.0
 " 23rd. Evening temperature 103°4
 " 24th. " " 103°6
 " 25th. " " 103°0
 " 26th. " " 102°4
 " 27th. Temperature normal.
 " 30th. 1 c.c. No. 221.I intravenously. Little reaction
 July 14th. 2 c.c. No. 193.I. subcutaneously

August 7th.

August 7th. 2 c.c. No. 242.D. subcutaneously.
 slight reaction.

Sept. 5th. 2 c.c. No. 242 D. subcutaneously

Nov. 22nd. 0.25 c.c. No. 239. (virulent preserved blood)

Dec. 6th. 0.5 c.c. No. 120 sub. (virulent preserved blood)

" 14th. Temperature rose to 101°.0 in evening.

" 15th. " " " 105°.0 " "

" 16th. " " " 101°.6 " "

" 17th. Died, While temperature was 100°.6, of
 horsesickness.

No. 239.

June 9th. 1 c.c. No. 193.I. intravenously.

" 13th. Temperature 102°.6 in the evening.

" 22nd. 1 c.c. No. 221.I. intravenously.

" 26th. Temperature 102° in the evening and was
 subsequently normal during three days.

" 30th. Temperature began to be elevated. (103° in evening)

July 1st. Temperature 103° in the evening.

" 2nd. " 103°.6 " " "

~~6th~~ 3rd. " 105°.0 in the evening

July 4th. " 105°.4 " " "

" 5th. " 106°.0 " " "

" 6th. " 105°.4 " " "

" 7th. " 105°.0 " " "

July 8th.

July 8th. Temperature 103°6 in the evening
 " 9th. " 101°4 " " "
 " 10th. Died extremely suddenly from heart failure.

At no time during its illness had any visible symptoms been manifested. At the post-mortem examination 4 litres of clear yellow exudation was found in the pericardium. The surface of the heart shewed some congestion and yellow coagulated lymph was found along the interventricular furrow. The inner surface of the heart was ecchymosed and the mitral valve was very much thickened and oedematous. The surfaces of both lungs ^{were} ~~was~~ covered with solid yellow exudation and some interlobular infiltration occurred at the free edges of the lungs.

No. 231.

April 8th. 1 c.c. No. 193.I. intravenously. Temperature was irregular during 15 days.

" 24th. Temperature 105°0 in the evening

" 25th. " 103°8 " " "

" 26th. " 104°0 " " "

The temperature now gradually fell reaching

" 29th. the normal on the 29th.

June 1st. Slight irregularity of temperature during 2 days

" 22nd. 1 c.c. No. 221.I. intravenously.

" 27th. Temperature rose to 102°6 in the evening.

" 28th. " " " 103°4 " " "

June 29th.

June	29th.	Temperature rose to 102°3	in the evening.		
"	30th.	"	" " 102°0	"	" "
July	4th.	2 c.c. No. 221.I. sub.	No reaction.		
"	14th.	2 c.c. No. 193.I. subcut.	No reaction		
"	24th.	2 c.c. No. 242.D.	subcutaneously.		
Aug	1st.	Temperature 101°6	in the evening.		
"	3rd.	"	103°0	"	" "
		Fell to normal. She was bled on the 4th. and the blood tested by inoculation into a clean horse. No reaction followed.			
"	10th.	0.5 c.c. of No. 120 subcutaneously.			
		(<u>virulent</u> preserved blood)			
"	21st.	Temperature 101°6	in the evening		
"	22nd.	"	102°6	"	" "
"	23rd.	"	103°4	"	" "
"	24th.	"	105°0	"	" "
"	25th.	"	105°4	"	" "
"	26th.	"	105°6	"	" "
"	27th.	"	104°0	"	" "
"	28th.	"	102°4	"	" "
"	29th.	"	101°6	"	" "
"	30th.	"	100°8	"	" "

This horse has since been tested by inoculation of a large dose (5 c.c.) of most virulent blood and has shewn no reaction. She is therefore fully protected.

No. 232.

April 8th. 1 c.c. No. 193.I. intravenously.
 " 13th. Temperature 105°0 in the evening.
 " 14th. " 104°4 " " "
 " 15th. " 104°6 " " "
 " 22nd. Temperature continued to be irregular until
 the 22nd.

May 4th. Had a period of irregular temperature until
 " 7th. the 7th.

June 8th. 2 c.c. No. 193.I. intravenously
 " 24th to 27th. Irregular temperature.
 " 30th. 2 c.c. No. 221.I. intravenously. No reaction.

July 14th. 2 c.c. No. 193.I. subcut. No reaction.

Aug. 19th. 2 c.c. No. 242.D. subcut. No reaction.

Nov. 22nd. 0.25 c.c. No. 239 (Virulent preserved blood)
 subcutaneously. No reaction.

Dec. 6th. 0.5 c.c. No. 120 (virulent preserved blood)
 subcutaneously. No reaction.

" 26th. 1 c.c. No. 120 (virulent preserved blood)
 subcutaneously. No reaction.

She is therefore fully protected.

No. 233.

June 9th. 1 c.c. No. 193.I. intravenously.
 " 27th. Temperature 103.0 in the evening.

June 28th.

June	28th.	Temperature	102°6	in the evening.
"	29th.	"	102°0	" " "
"	30th.	1 c.c. No. 221.I.	Intravenously.	No reaction.
Aug.	11th.	2 c.c. No. 242.D.	Subcut.	No reaction.
Sept	23rd.	3 c.c. No. 253.D.	Intra.	No reaction.
Nov.	22nd.	2 c.c. No. 255.D.	Subcut.	No reaction.
Dec.	6th.	0.5 c.c. No. 239	No reaction.	
"	19th.	0.25 c.c. No. 245	(virulent preserved blood)	
"	26th.	Temperature	103°4	in the evening.
"	27th.	"	104°0	" " "
"	28th.	"	105°2	" " "
"	29th.	"	103°0	" " "
"	30th.	"	103°0	" " "
"	31st.	"	101°0	" " "
Jan.	1st.	"	101°8	" " "

Was normal until

" 8th. when "secondary" fever set in lasting six days during which the maximum was 104°6.

This animal is now fully protected.

No. 185.

November 19th. 2.c.c. No.172 I. Subcutaneously.

No reaction.

December 6th. 2.c.c. No. 181.I. Subcutaneously

" 14th. Temperature 102°6 in the evening.

" 15th. " 102°6 " " "

December	16th.	Temperature	103°.2	in the evening.
"	17th.	"	104°.8	" " "
"	18th.	"	107°.2	" " "
"	19th.	"	105°.2	" " "
"	20th.	"	103°.6	" " "
"	21st.	"	Normal.	

At a subsequent date 27th. December "secondary" fever set in lasting seven days during which the maximum registered was 107°. He was now allowed to run in the camp for twelve months and then brought in to be tested. 10 c.c. of virulent preserved blood No. 256 was inoculated by subcutaneous injection. No reaction followed, thus proving the animal to be fully protected.

No. 209.

Jan	18th.	1 c.c. No. 193.I.	intravenously.
"	28th.	Temperature	102°.2 in the evening.
"	29th.	"	104° in the evening
"	30th.	"	105°.2 in the evening.
"	31st.	"	105°.0 " " "
Feb.	1st.	"	103°.0 " " "
"	2nd.	"	Normal.
"	28th.	0.5 c.c. No. 120	<u>virulent</u> preserved blood.
March	10th.	Temperature	102°.0 in the evening.

March 11th.

March	11th.	Temperature	103°.4	in the evening.		
"	12th.	"	103°.2	"	"	"
"	13th.	"	103°.8	"	"	"
"	14th.	"	104°.4	"	"	"
"	15th.	"	104°.8	"	"	"
"	16th.	"	104°.1	"	"	"
"	17th.	"	102°.4	"	"	"
"	18th.	"	104°.2	"	"	"
"	19th.	"	Normal.			

While therefore these experiments demonstrate that it is possible to protect horses by repeated inoculations of an attenuated virus, they equally demonstrate the irregularity of action, owing to the varying susceptibility to the disease, in its attenuated form, which obtains among horses.

Several important facts however are deserving of careful consideration namely:-

- 1.- Death in cases of horsesickness cannot be directly ascribed to hyperpyrexia inasmuch as several horses have recovered after having experienced temperatures of over 107° while others which have died and in which characteristic lesions have been found, have not had a temperature exceeding 105°.
- 2.- Protection can be arrived at without the production of very great reaction, provided that a number of

inoculations

inoculations are made into the animal and that these have been so arranged as to proceed very gradually to the highest degree of virulence.

3.- It is exceedingly difficult to determine the exact degree of ~~virulence~~-- attenuation in any particular attenuated virus; I have usually attempted this by the inoculation of the virus into one or at most two horses but if the susceptibility of such animals happens to be of a low grade the result is very little reaction. As one cannot determine the special susceptibility of any animal it follows that, in the latter case, the slight reaction-- which would indicate a mild virus-- may be misleading.

4.- The indication for future experimentation has thus been to call for the discovery of some method by which a virus of standard virulence might be, at will, reduced to any required degree of attenuation.

5.- "Secondary fever". By this term I mean to imply a febrile state which comes on spontaneously in animals which have been fully protected against horsesickness. The periods of its onset are irregular, it may appear in an animal a few weeks after "salting" or many months later. It has no relation to infection from without, as it occurs equally in animals which have simply been left after "salting", and in others which, after "salting", have received progressively increasing doses of the most

virulent

virulent fresh blood (maximum dose being 1000 c.c.)

6.- During "secondary" fever in ^{two}~~three~~ animals, and while the temperature has been as high as 106°, I have drawn their blood and immediately inoculated ^{clean}~~color~~ horses with doses of 5 and 10 c.c. In no case has any reaction followed and I therefore conclude that the blood of horses suffering from "secondary" fever is non-virulent.

7.- It became necessary to determine whether, after "salting" a residual infection remained for any length of time. Accordingly I took an animal (No. 106) which after "salting" and subsequent exposure in a deadly region for a year, was reinoculated with the following progressively increased doses of virulent blood, 5 c.c. 50, 200, 500, 500, 400, and 500 c.c. One month after the last inoculation I bled it and inoculated six horses with 100 c.c. each of its fresh blood. No reaction followed and no protection was afforded, hence I conclude that no residual infection was present in the circulating blood. I had previous to the time that these experiments with attenuated virus were begun, been engaged in experimenting with the serum of recovered animals, but the result of its use under the methods which I then used were so disastrous that I withdrew from that line of enquiry. Since, however, I have carried out the experiments in the direction of attenuation I have received indications

which

which have led me to return to the investigations of serum but on different lines.

Experiments with serum and defibrinated blood of animals which have recovered from horsesickness.

The experiments made have included serum derived from:-

- 1.- Animals formerly "salted"
- 2.- Animals formerly "salted" and subsequently inoculated by periodic injections of gradually increasing doses of virulent blood; the maximum dose being 1000 c.c.
- 3.- Animals treated as in clause No. 2 but subsequently permitted to rest for several months and then reinoculated with a small dose (5 c.c.) of virulent blood.

Accordingly, an animal, which had been inoculated two years previously, was inoculated with 5 c.c. preserved virulent blood. After a period of twelve days, during which no fever had occurred, it was bled and after separation of the serum had occurred, the latter was passed through a Berkenfeld filter.

Horse No. 95 was inoculated with 100 c.c. of this serum by subcutaneous injection in the shoulder. It was immediately thereafter inoculated on the opposite side with 5 c.c. of virulent preserved blood while a control

animal

animal No. 96 was at the same time inoculated with 5 c.c. of virulent preserved blood.

Nine days later the temperature of each animal began to rise. On the eleventh day No. 95 received 100 c.c. of serum without any obvious effect. It died on the twelfth day while the control died on the day following. See Chart No. 25.

There was no evidence of the serum having exerted any effect.

Horse No. 79. was inoculated on September 3rd. with 100 c.c. of serum and immediately thereafter with 2 c.c. virulent preserved blood.

Sept. 3rd. Inoculated
" 7th. 100 c.c. serum injected.
" 11th. Temperature now beginning to rise. 100 c.c. serum injected.
" 12th. 100 c.c. serum injected.
" 13th. " " " "
" 14th. " " " "
" 15th. Temperature falling; no serum injected.
" 16th. " rising 100 c.c. " "

Animal died of characteristic horsesickness

See Chart No. 26.

Horse No. 101.

September 18th. Inoculated 2.5 c.c. virulent preserved blood.

Septem.	21st.	100 c.c. serum injected.
"	22nd.	" " " "
"	23rd.	" " " "
"	24th.	" " " "
"	25th.	" " " "
"	26th.	" " " "
"	27th.	" " " "
"	28th.	" " " "
"	29th.	" " " "
"	30th.	" " " "

October 1st. Died of characteristic horsesickness.

I now determined upon testing the value of serum from "salted" horses which subsequent to "salting" had been inoculated with progressively increasing doses of virulent horsesickness blood.

Two horses which had been "salted" and subsequently exposed in the veld of Bechuanaland during a season of severe sickness, were brought back to the Institute. After their arrival they were each inoculated by subcutaneous injection with 5 c.c. of virulent blood on November 1st. After 8 days had elapsed, during which no elevation of their temperature had been observed, they were each inoculated with 50 c.c. of fresh virulent blood. On the 20th each received 200 c.c. fresh virulent blood. Nine days later, during which no reaction had occurred.

occurred, one animal~~x~~ was bled and the serum from the blood collected.

On December 1st. two horses Nos. 110 and 111 were each inoculated with 2 c.c. virulent preserved blood and 50 c.c. of this serum.

<u>No. 110.</u>		<u>No. 111.</u>
Dec. 1st.	50c.c. & 2.c.c. virus	50c.c. & 2c.c. virus.
" 3rd.	50 c.c.	
" 4th.		50 c.c.
" 7th.	50 c.c.	50 c.c.
" 9th.	50 c.c.	
" 10th.		50 .c.c

In both cases on the twelfth day after the first inoculation and as no elevation of temperature had yet occurred, each was inoculated with 5 c.c. of virulent blood; but as, on the second day following, the temperature began to rise I considered that it had been~~y~~ due to the former inoculation, the effect of which had been delayed by the serum or by reason of the virus being old.

(the particular virus used was over a year old)

No.110 now received the following injection of serum.

December	17th.	200 c.c.
"	18th.	100 c.c.
"	19th.	200 c.c.
"	20th.	200 c.c.

Dec. 21st.

December 21st. 200 c.c.
" 23rd. Died of horsesickness.

No 111 received no further injections of serum and died on the 21st. December.

No. 110 had in all about 1300 c.c. of serum while No. 111 received only 200.

There was therefore but slight evidence, if any, of curative action in regard to this serum.

In regard to the serum which was used, it was derived, as I have already stated, from two horses which after being "salted" and exposed in the veld were inoculated on the 1st. November with 5 c.c. of virulent preserved blood, ~~and~~ again on the 9th. November with 200 c.c. of virulent blood.

On the 13th. December each of the serum animals received 500 c.c. of virulent blood and eight days later, during which no reaction had occurred, one animal (No. 106) was bled and the serum collected from its blood. On December 22nd. two horses Nos. 113 and 114 were each inoculated with 2 c.c. of preserved virus and immediately thereafter in a different situation, with 50 c.c. of the same serum as had been used in the former experiment.

Dec 27th. Temperature rising.

" 28th. 100 c.c. fresh serum in the morning and 200 c.c. in the evening. Urine highly charged with blood.

" 29th. 100 c.c. serum given.
"

Dec 31st. 200 c.c. given in the morning and 100 c.c.
given intravenously in the evening.

Jan 1st. Temperatures falling.

" 2nd. No. 114 died.

" 5th. No. 115 died.

The post-mortem examinations were in these cases of a most interesting character and disclosed conditions which are not characteristic of horsesickness.

The subcutaneous tissue was of a brilliant yellow colour; the liver was congested and ~~the~~ bile-stained. The kidneys were large and pale and the bladder was filled with a deeply blood stained urine. The blood was very peculiar in appearance, being of a pale colour, watery in density and uncoagulable. The veins contained large post-mortem clots which were quite translucent in appearance. Two other horses were now inoculated, one, No. 119, with virus and injection of serum, the other No. 120 with virus only.

No. 119.

January 24th. 50 c.c. serum given

" 25th. 2 c.c. virus.

" 30th. Temperature rising. 100 c.c. serum given.

" 31st. 100 c.c. serum.

February 1st. 100 c.c. serum.

" 2nd. 150 c.c. serum

Feb. 3rd.

February	3rd.	150 c.c. serum
"	4th.	200 c.c. serum.
"	5th.	100 c.c. serum.
"	6th.	100 c.c. serum.

The animal made a good recovery but with great difficulty. It is more than probable that serum was of assistance in this case but considering the amount used, 1050 c.c. it is clear that from the point of view of curative action no great reliance can be placed on the use of serum. See Chart No. 27.

No. 120 Inoculated with virus only. Died nine days after inoculation, of horsesickness.

No. 116 was inoculated on January 31st. with 50 c.c. serum and on the following day had one c.c. of preserved virulent blood from No. 91. thus the initial dose of serum being the same as in the last experiment, the dose of virus was diminished to one half.

January	31st.	50 c.c. serum.
February	1st.	1 c.c. virus.
"	4th.	50 c.c. serum.
"	6th.	Temperature rising. 200 c.c. serum given.
"	7th.	Urine seen to be of a dark colour.
"	8th.	Condition of urine still more marked.
"	9th.	Urine deeply blood stained.

February 10th.

February 10th. Animal died. (See chart No. 28).

The occurrence of this haemoglobinuria which eventually became a true haematuria was somewhat difficult to understand. When it was first recognised I feared that some epizootic had established itself in the stable and accordingly all animals were turned out and isolated while the stables were cleaned and thoroughly disinfected. After this had been accomplished clean animals were obtained and experiments carried out when it was found that animals inoculated, with virulent blood only, died of ordinary horsesickness but where serum was given to infected animals this condition usually made its appearance. I may recount a few more examples.

No. 117.

January 19th.	50 c.c. serum.	
" 20th.	2 c.c. virus.	
" 22nd.	50 .c.c serum.	
" 24th.	Temperature rising.	
" 25th.	50 c.c. serum.	
" 26th.	50 c.c. serum.	
" 27th.	100 c.c. serum.	Haemoglobinuria started.
" 28th.	100 c.c. serum	
" 29th.	100 c.c. serum.	
" 30th.	100 c.c. serum.	
	Died.	

No. 123.

March 9th. 1 c.c. virus.
" 16th. Temperature rising
" 17th. 200 c.c. serum intravenously.
" 19th. 100 c.c. serum intravenously.
Haemoglobinuria started.
" 20th. Haematuria.
" 21st. See chart No. 29.

No. 144.

July 3rd. 1 c.c. virus.
" 10th. Temperature rising. 100 c.c. serum.
" 12th. 100 c.c. serum. Haemoglobinuria at 4 p.m.
Died during the evening.

No. 145.

July 10th. 1 c.c. virus.
" 13th. Temperature rising. 100 c.c. serum given.
" 20th. 100 c.c. serum.
" 21st. 100 c.c. serum. Haemoglobinuria.
" 22nd. 100 c.c. serum. Haematuria.
Died at night.

No. 147.

June 24th. 1 c.c. virus.
" 29th. Temperature rising.

June 30th. 100 c.c. serum.
 July 3rd. Haemoglobinuria started.
 " 4th. Died See chart No. 30.

No. 159.

August 11th. 2 c.c. No. 130 preserved attenuated virus.I.
 " 16th. Temperature rising.
 " 19th. 100 c.c. serum.
 " 22nd. Haemoglobinuria started though in slight amount
 Made a good recovery. See Chart No. 31

No. 162.

August 24th. 3 c.c. No. 130 preserved attenuated virus I.
 No reaction.
 Sept. 2nd. 1 c.c. preserved virulent blood.
 " 5th. Temperature rising.
 " 6th. 50 c.c. serum.
 " 7th. 200 c.c. serum.
 " 8th. 100 c.c. serum intravenously.
 " 9th. Haemoglobinuria started.
 " 10th. 50 c.c. serum.
 " 11th. 50 c.c. serum.
 Died.

It is to be noted that this animal was inoculated
 with

with the same attenuated virus as in the former case but as no result followed it was reinoculated with 1 c.c. of virulent virus and as a result the temperature began to rise three days later. I have frequently found that where an attenuated virus seemed to produce no reaction the subsequent inoculation with virulent blood set up a reaction after an extremely short period of incubation; thus suggesting that a latent infection had already existed.

While in Buluwayo I inoculated 180 horses by the intravenous injection of attenuated virus. At the time in question no horsesickness was known in Buluwayo and a certain number of the horses had been kept in the military stables for some weeks previously. So far as was reported to me no animals had been found to have a suspicious temperature. Four days after the inoculation had been made three of the animals which had been stabled were found to be in the last stages of horsesickness, e.g. heaving violently at the flanks and discharging the yellow fluid and foam from the mouth and nose. These animals were, therefore, at a stage of the disease which is usually reached about the 12th or 14th. day subsequent to inoculation with virulent blood and consequently the cause could not possibly be ascribed to the inoculation only. It is reasonable to conclude that they had possessed a

latent

infection which was brought to activity by the reinforcement of the intravenous inoculation. It is interesting to note that all of these animals recovered.

In regard to the haemoglobinuria I was desirous of determining whether it arose, under some irritative action, in the kidney or had its origin in the circulating blood. Where the blood of a healthy animal, or of one affected by uncomplicated horsesickness, is bled into citrate solution, the precipitation of the corpuscular elements very soon occurs, leaving a large volume of supernatant plasma as a pale yellow fluid. In the case however of animals which, at a later date developed haemoglobinuria, I found, commonly about 24 hours previous to the appearance of the latter, that, if the animal was bled, the supernatant plasma was deeply blood stained; thus shewing that the ~~ly~~ liberation of haemoglobin had its origin in the blood itself.

The haemoglobinuria commonly passes into a true haematuria probably due to increased tension in the renal vessels.

In my experiments it was clearly demonstrated that this condition did not follow the use of simple recovered serum in an unaffected animal but was only induced in cases where the serum which was used had been taken from animals which, after "salting" had received repeated and constantly increasing doses of virulent blood. The cases
invariably

invariably went on to a fatal issue where this serum was used in the presence of virulent infection. Where, however, in three cases, the infection was arrived at by the use of an attenuated virus, the haematuria, which ensued went on to recovery with the subsidence of the fever.

In order to determine whether this serum was of itself productive of mischief and secondly whether its use produced any practical degree of immunity, I carried out the following experiment:-

Horse No. 195. was inoculated by subcutaneous injection on the 9th December with 500 c.c. of serum.

The serum was taken from an animal which, after "salting", had been reinoculated as follows:-

November 1st.	5 c.c.	fresh virulent blood	subcutaneously
" 9th.	50 c.c.	" "	" "
" 20th.	200 c.c.	" "	" "
December 13th.	500 c.c.	" "	" "
" 30th.	500 c.c.	" "	" "

It was then permitted to rest until the following February when further inoculations were made.

Feb'y 5th. 400 c.c. fresh virulent blood subcutaneously

June 4th. 300 c.c. " " " intravenously

and 200 c.c. subcutaneously.

On the 24th. of August the horse was bled and the

serum

serum after being filtered through a Berkenfeld filter was bottled asseptically and thereafter kept in the refrigerator.

After the horse No. 195 had been inoculated by subcutaneous injection with 500 c.c. of the serum, no change in the condition of the animal was apparent during 12 days. After this time the temperature rose to over 103 and remained thus with scarcely any remission for four days after which the normal was regained. Thirty days after the date of this inoculation I injected the animal subcutaneously with 3 c.c. of preserved virulent blood (No. 140) and 1 c.c. of the same, by intravenous injection. The temperature rose on the sixth day following and maintained a steady rise during four days when the animal died with a temperature of 106°.8 If, therefore, the serum possessed any protective influence it is clear that it must have entirely disappeared previous to the moment of the inoculation with the virulent preserved blood.

There was not however any indication of toxic action on the part of the serum thus suggesting that the long period of rest after the final inoculation of the serum produces had admitted of the toxicity being lost.

In order to determine whether, and if so, during what time, the living virus of horsesickness can live in the body of a well "salted" animal I made the following experiment:-

Horse No. 118. which after "salting" had been reinoculated at the following periods:-

March 29th 1898. 10 c.c. virulent blood.

April 13th. " 20 c.c. virulent blood.

June 4th. " 50 c.c. " "

July 4th. " 150 c.c. virulent blood.

was again inoculated on the 8th December of the following year with 50 c.c. of fresh virulent blood. On the 16th December, no elevation of temperature having occurred meanwhile, I bled it and with 5 c.c. of its blood I inoculated a horse, by subcutaneous injection. On the 6th. and 8th days following, the latter animal had distinct elevations of temperature, which, occurring on and about the usual incubation period and in an animal whose temperature was usually of the most regular type, suggested that the contagium had not been destroyed after its injection into a thoroughly "salted" animal although very evidently weakened in its virulence.

As former experiments had suggested that the serum of "salted" animals, which had been inoculated with smaller doses of virulent blood and subsequently permitted to rest, possessed a slight inhibitory action on the virus of horsesickness. I determined upon testing the value of serum taken from animals which, after prolonged

periodic

periodic inoculations with virulent blood, had been permitted to rest for at least a month.

For the carrying out of this line of experiment certain important indications had already been elicited by means of former experiments.

- 1.- A definite high reaction lasting as long as the fatal fever - if produced by a modified virus - does not always produce such protection as is sufficient to safeguard the animal against a subsequent inoculation of virulent blood.
- 2.- Inoculation with a modified virus, even if it fail to induce a high degree of fever, has very definite effect in inducing infection. This has been accurately determined. (a) by killing animals at a period of fifty days subsequent to inoculation, when extensive evidence was found of a subacute or chronic form of the disease. (b) by inoculating such cases with virulent blood, when the virulent disease was usually induced after a much shortened period of incubation.
- 3.- After the inoculation of a partially protected animal with a virus which it could resist, it was ^{proved} ~~found~~, nine days later, that the contagium was still existant in the circulating blood.

determine

I concluded therefore to ~~determine~~ determine the amount of serum which, when mixed with a definite amount of virulent blood, would serve, acting in concert with the natural

protective bodies in the system of the average horse, to ensure the production of the modified disease. After 14 days should elapse subsequent to this inoculation provided a severe reaction was not set up, I intended to reihoculate with the same dose of virulent blood but with a much reduced quantity of serum. Again after 14 days the proceedure should be repeated; the dose of virulent blood remaining a constant quantity but the serum being still further reduced. I hoped, in this way, gradually to diminish the serum until pure virulent blood could be used by itself. Former experiments made both in the Institute and in Rhodesia and in the veld of Bechuanaland proved that an animal, which, by whatever means protected, had withstood 0.5 c.c. of pure preserved virulent blood, was thoroughly protected against exposure to the virulent disease in the most sickly areas and against subsequent inoculations with a much larger dose of virulent blood.

Horse No. 261 was inoculated with 1 c.c. of preserved virulent blood (No. 256) and 100 c.c. serum, by subcutaneous injection, on the 19th December.

This mixture of serum and blood had been allowed to stand for twenty four hours in the ice chest after being mixed.

On the 7th. day following, a slight elevation of

temperature

temperature occurred which immediately remitted, but on the tenth day the temperature rose to 105° and remained high but with slight nocturnal remissions for six days. During a further twenty days the temperature was irregular. Thirty nine days after inoculation the horse was reinoculated with 1 c.c. of preserved virulent blood No. 120. Very slight irregularity followed and twenty three days later it received three c.c. virulent blood which it entirely withstood.

The severe febrile reaction which followed the 1% mixture of preserved virulent blood and serum is in my opinion, to be ascribed to special susceptibility on the part of the animal inasmuch as, in a large number of cases where a similar mixture was used, little or no reaction was observed.

No. 263 was inoculated with 1 c.c. of preserved virulent blood mixed with 200 c.c. of serum which had been treated in the same manner as in the former experiment. A slight reaction (102°) occurred on the 8th, 11th, and 13th, days. On the 14th day it was reinoculated with 1 c.c. of virulent blood and 20 c.c. of serum. A sharp reaction occurred three days after this inoculation and subsided after two days. Eight days later it was inoculated with 1 c.c. of pure virulent preserved blood (No 256) without obvious effect. Since this date it has been three times reinoculated

with

with doses of virulent blood; 1 c.c. No. 120; 2 c.c. No. 120 and 2 c.c. of fresh blood No. 276. The results shewed the animal to be perfectly protected.

On the 5th. January, three animals Nos. 245, 260, and 262 were each inoculated with 1 c.c. of preserved virulent blood and 100 c.c. of serum. In these and the experiments which follow the serum and blood was injected immediately after being mixed.

No. 245

Jan. 5th. 100 c.c. serum & 1 c.c. blood (No. 256)

No reaction followed.

" 16th. (12th day) 50 c.c. serum and 0.5 c.c. blood.

No reaction followed.

" 26th. (11th day) 30 c.c. serum & 0.5c.c. blood.

A slight reaction occurred on the 6th day and lasted during 5 days.

Feb. 6th. (12th day) 15 c.c. serum & 0.5 c.c. blood.

No reaction followed.

" 17th. (12th. day) 0.5 c.c. pure virulent blood.

No reaction followed.

" 28th. (12th day) 2 c.c. pure virulent blood.

No action. Animal fully protected.

No. 260.

Jan 5th. 100 c.c. serum & 1 c.c. virulent blood(No.120)

No reaction followed.

" 16th. (12th day) 40 c.c. serum & 0.5 c.c. virulent blood.

No reaction.

" 27th. (12th day) 25 c.c. serum & 0.5 c.c. vir. blood.

No reaction.

Feb. 6th. (11th day) 0.5 c.c. preserved vir. blood (No.256)

No reaction

" 16th. (11th day) 1 c.c. prserved vir. blood (No 120) (

No reaction. Animal fully protected.

No 262

Jan. 5th. 100 c.c. serum & 1 c.c. virulent blood.

No reaction.

" 16th. (12th day) 40 c.c. serum & 0.5 c.c. vir. blood

No reaction.

" 27th. (12th day) 25 c.c. serum & 0.5c.c. vir. blood

Slight oscillation on 8th day.

Feb. 10th. (15th day) 0.5 c.c. virulent blood.

No reaction.

" 20th. (11th. day) 2 c.c. preserved virulent blood.

No reaction.

Of these three animals it will be noted that two were perfectly protected without evincing any reaction at all during the period of treatment while one had a mild
febrile

febrile period which followed upon the third inoculation of serum and blood.

In the next series of experiments involving twelve animals, I determined to make the first inoculation with virulent blood at an earlier period than that of 15 days.

I had formerly found that the influence of the former inoculation was still apparent as late as nine days afterward. While therefore I assumed that the protection would not be so great as to withstand the virulent blood as early as the ninth day, I expected that if it were used ~~on~~ on the tenth day (five days earlier than in the former experiment) I might obtain a more severe reaction and thus so to speak accelerate the serum producing condition of the animal. As will be seen this expectation was fully realized.

Nos. 264, 265, 266, 267, and 268, and 269, were each inoculated with 100 c.c. serum and 1 .c. virulent blood.

	264	265	266.
February 3rd.	100S, 1B. No reaction	100S, 1B. Slight reaction	100S, 1B. No reaction
February 14th. (12th. day)	30 S, .5B. No reaction	30S, .5B. No reaction	30S, .5B. No reaction.
February 24th. (11th day)	15S, 0.5B. No reaction	15S, 0.5B. No reaction	15S, 0.5B No reaction

March 5th.

	264	265	266.
March 5th. (10th. day)	.5 c.c.B. severe reaction Died	.5 c.c. B. modified reaction Died	.5 c.c. B. very slight.
	267	268	269
Feb. 3rd.	100S, 1B. No reaction	100S, 1B. No reaction	100S, 1B. No reaction.
Feb. 14th. (12th day)	30S, 5B. No reaction	30S, 5B. No reaction	30S, 5B. Slight on 7th. day.
Feb. 24th. (11th. day)	15S, 5B. No reaction	15S, 5B. A slight reaction	15S, 5B. No reaction.
March 5th. (10th day)	.5 c.c.B. severe reaction which subsided.	.5 c.c.B. very slight on 5th day	.5 c.c.B. slight for 7 days.

No 264 After virulent blood had been used alone a severe reaction set in on the evening of the eighth day and it died on the 16th day.

No. 265. After virulent blood had been used alone a severe slight reaction began on the eighth day and after remaining thus for four days eventually broke into a severe reaction from which it died on the 16th day.

No. 266. After virulent blood had been used alone a severe very slight reaction occurred on the 4th and 5th days after which it subsided and the animal subsequently

withstood

withstood larger doses of blood.

No. 267. After virulent blood had been used alone, a severe reaction followed which subsided after four days.

No. 268. After virulent blood had been used alone, a very slight reaction followed on the fifth day and immediately subsided. It withstood subsequent inoculations of virulent blood.

No. 269. After virulent blood had been used alone, a mild reaction followed on the 15th. day and, lasting seven days, subsided. It withstood subsequent inoculation with virulent blood.

Nos. 270, 271, 272, 273, 274, & 275 were primarily inoculated with various doses of serum and blood but in the subsequent stages were treated exactly as in the case of the former six.

	270	271	272
Feby. 5th.	50S, .5B. No reaction	50S, .5B. Slight reaction	90S, 1B. No reaction
Feby. 17th. (13th day)	30S, .5B. No reaction	30S, .5B. No reaction	30S, .5B. No reaction
Feby. 26th. (10th day)	15S, .5B. No reaction	15S, .5B. No reaction	15S, .5B. No reaction.
March 7th. (10th day)	.5B. severe reaction	.5B. No reaction	.5B. No reaction

	273	274	275
Feby. 5th.	90S, 1B. No reaction	90S, 1B. No reaction	90S, 1B. No reaction.
Feby 17th. (13th day)	30S, .5B. No reaction	30S, .5B. Slight reaction	30S, .5B. No reaction
Feby 26th. (10th day)	15S, .5B. slight for 3 days	15S, .5B. No reaction	15S, .5B. No reaction.
March 7th. (10th day)	.5B No reaction	.5B severe reaction <i>Died</i>	.5B. No reaction.

No. 270 After inoculation with virulent blood, a severe reaction followed which subsided.

No 271 After inoculation with virulent blood, no reaction followed. It subsequently withstood virulent blood.

No 272 After inoculation with virulent blood, no reaction followed. It subsequently withstood virulent blood.

No. 273 After inoculation with virulent blood, no reaction followed. It subsequently withstood virulent blood.

No 274 After inoculation with virulent blood, ^a ~~no~~ reaction set in on the sixth day and after remaining high for seven days it died.

No 275. After inoculation with virulent blood, no reaction followed. It withstood a further inoculation of virulent blood.

Thus it will be noted that four animals were fully protected without evincing any reaction after the use of virulent blood alone; three shewed slight reaction which subsided; five had severe reactions of which three died.

Of these three which died only one shewed the characteristic symptoms of horsesickness, the other two, on post-mortem examination, shewed conditions which resembled the cases in which haemoglobinuria had been produced in former experiments.

The result goes to shew that it is unsafe to use the virulent blood so early as the tenth day subsequent to the former inoculation as, owing to the effect of the latter being frequently delayed, its explosion, when it occurs, is reinforced by the virulent blood; and the partial protection, hitherto set up, may be in this manner overturned.

As regards the efficiency of the method it is unquestionable that, tested, as it has been, in this severe manner, the results (thirteen protected out of a total of sixteen) are extremely good and offer a satisfactory solution of ~~the solution of~~ the great problem of protecting the horses which have to live in unhealthy districts.

Out of the mass of established facts which have accumulated in my hands as a result of experiment and observation

observation, there arises the important question as to the nature of the "protection" which a "salted" horse possesses.

At the first glance the occurrence of "secondary fever" in such animals would incline one to ~~think~~ the supposition that a "residual" infection remained, long after the moment when the animal had returned to health. In support of such a hypothesis I have found that the living virus, when injected into a horse which has been already protected to a much higher degree than the simple "recovered" animal, is not destroyed after a period of eight days and I feel therefore justified in arriving at the conclusion that a true immunity, in horses, against this disease does not exist.

Neither the serum taken from the blood of simple "recovered" animals (simple recovered serum), nor that obtained from the blood of horses after a prolonged series of periodic inoculations with constantly increasing doses of virulent blood (fortified serum) seems to possess any very appreciable degree of curative action nor any true antitoxic quality.

Virulent blood when mixed with fortified serum in the proportion of 1 in 100 and kept thereafter for twenty four hours, produces quite as much reaction as when inoculated into a susceptible horse as does the same

material when used directly after being mixed. If this serum possessed any great germicidal power one would expect that the virulent blood, if kept in serum for twenty four hours, would have less virulence than that which had only remained in the presence of the serum for a few minutes. Experiment, however, shewed that practically no difference in the action of the two was observable.

On the other hand this serum, when mixed with a dose of such virulent blood as suffices to invariably kill any unprotected horse, at once deprives it of its fatal quality i.e. the virus immediately assumes the character of a modified virus. This result seems unquestionably to indicate that fortified serum exerts a peculiar and definite action on the virus.

While, however, 100 c.c. of fortified serum suffices to prevent 1 c.c. of virulent blood producing any great elevation of temperature in a susceptible animal, I have shewn a case where a definite and prolonged reaction followed. Since, in another case, 200 c.c. of the same serum, with an equal amount of virulent blood, was followed by a reaction and a definite amount of protection, it must be evident that the difference in susceptibility between the two animals was equal to 100 c.c. of "fortified" serum. Moreover, as already shewn, when the virus is attenuated by its passage through less

susceptible

susceptible animals such as the donkey or cow, its effect, when used in the same dose, either by subcutaneous or intravenous injection, varies very greatly in different animals; in some producing no evident reaction; in others setting up some fever; while again, in other animals, its use was followed by the onset of the virulent disease resulting in death. If therefore the admixture of serum with virulent blood is followed, on inoculation, merely by a modified form of the disease it must be concluded that the serum cannot of itself be credited with this result but that a peculiar quality, existing in the animal body, and varying in amount from animal to animal, must play an important part. Whether this principle is a simple body, or is a combination of several, cannot at this moment be determined but for convenience sake I would suggest the name "Antagonese" should be applied to it. The term need not be taken to imply either an antitoxic or germicidal body but merely to denote "defensive" properties which are already existent to a greater or less degree in all animals, or are produced or increased under special stimulation.

It seems from a careful consideration of the foregoing experiments, that the protection, which already exists to a high degree in donkeys and can be induced in horses by the methods I have explained, must consist

largely

largely of a "tolerance". This is indicated fully by the fact that the contagium can live in the blood of a well "salted" horse for at least nine days and also by the fact that the inoculation with serum taken from ~~of~~ recently fortified, animals, if carried out in the presence of active virulent infection, produces a "toxic" action.

This is further borne out by observations which I ~~have~~ have made to test the immunity, produced by the use of the method which has been described. In one case, a mare, which was in foal, had been already inoculated with serum and virulent blood. Towards the end of the inoculation periods it aborted and cast a foal which shewed characteristic appearances of horsesickness. Up to this period the mare had not shewn any reaction at all. A few days later, however, its temperature started to rise and after passing through a severe reaction the animal again shewed a normal temperature. Unfortunately very soon after this the mare died from pyaemia which evidently began from a uterine infection. The uterus shewed gangrenous patches; the lungs had small collections of pus towards the surface of the pulmonary pleurae while similar conditions were found in the kidney (surgical kidney).

Of the two animals which passed through the serum and blood inoculations without shewing any reaction,

one succumbed after a second larger dose of virulent blood. The other No. 262 was still more remarkable; it was first inoculated with 0.5 c.c. of virulent blood, which it withstood. Eleven days later it was inoculated with 2 c.c. of virulent blood which it likewise withstood. In none of these cases had any reaction been produced although control experiments had shewn that the blood was of the highest standard of virulence. Twenty three days later it was again inoculated with 2 c.c. of virulent blood and strange to say, it died on the 14th day afterward, of horsesickness. On the other hand I have carried the fortification of an animal up to 100 c.c. without any reaction being produced.

These observations shew that while some animals may be fully protected without evincing any reaction, yet, in others, the protection comes on very slowly and may be at any time overturned. Where a reaction had been produced, protection is usually very complete, unless the animal had been a peculiarly susceptible one and has had its reaction under the operation of a very mild attenuated virus which, in the average horse, would be productive of no evident change. As an illustration of this I may mention the case of six animals which were each inoculated intravenously with attenuated virus;

five

five shewed practically no reaction but one had a severe fever reaching up to 105°F. Inoculation of this latter animal at a later date with 0.5 c.c. of virulent blood was followed by the onset of the disease in its ordinary form and although the animal eventually recovered there had been but little evidence of protection afforded by the previous inoculation.

In connection with the use of serum it is most remarkable the effect which it has, ^{in the presence of active infection} if taken from animals that have been recently fortified. I have already shewn that a liberation of haemoglobin occurs in the blood, which is followed by haemoglobinuria and that the post-mortem examination reveals conditions of enormous congestion in organs, which, in uncomplicated horsesickness, are practically free from such changes. Moreover in one experiment it has been noted that, where death followed the injection of virulent blood, in partially protected animals, similar conditions were produced.

As however such serum loses its toxic properties in the living body at a date subsequent to that at which fortification is stopped and, as the contagium, which, injected into a "salted" animal lives for at least nine days, ~~and~~ disappears at a later date, it is probable that the "tolerant" power enables "phagocytosis" to come into play

play and to act destructively on the specific virus.

Thus if F be made to represent infection induced by the injection of the virus, TS to represent toxic serum and BW. to represent Blackwater or haemoglobinuria then:-

1st.- In the first case the use of "toxic" serum in the presence of active infection gave rise to Blackwater or haemoglobinuria.

$$F + TS = BW$$

2nd.- In the case of recently partially protected animals the inoculation of actively virulent blood gave rise to pathological changes similar to those occurring in Clause 1.

Now in this case it is fair to assume, as a result of recent partial protection, that toxic serum had been induced in the bodies of the animals.

$$TS + F = BW$$

I would emphasise the fact that a very clear distinction is to be drawn between the primary fever - the result of inoculation or ordinarily natural infection - and that which I style "secondary fever". The latter fever occurs at irregular periods subsequent to recovery from the primary fever and, at different times and in different animals, varies very greatly both in point of intensity

intensity of fever and duration .

The blood in primary fever is always virulent , but in two cases of secondary fever - one case being specially selected owing to the fever being as severe (fever lasting eight days; maximum being 105.8) as that which follows infection and usually ends in death, inoculation with 5 c.c. of fresh blood into clean animals produced no elevation of temperature. I therefore feel justified in concluding that the circulating blood in "secondary fever" does not contain the microbe of the disease.

During my recent sojourn and travels in Rhodesia I learned that the so-called blackwater malarial fever of man rarely, if ever, occurred primarily but was found to appear, as a rule, in persons who had formerly suffered from one or more mild attacks of ordinary malarial fever.

It is therefore possible that the condition which I have produced experimentally in horses, may be analogous to the blackwater fever of man. In any case the observations are most suggestive and ought not to be lost sight of in the determination of the nature of the pathology of human malaria.

Alexander Leitch M.B., C.M.

March 30. 1900

Disease

HorsesicknessThesis on Horsesickness
by Alexander Leighton M.D.Chart 1

No.

6Infecting Material 5.c.c. Subcutaneously, for Pericardial fluid from Horse No. 4.

1892. Feb. 7.

Date

9. 10. 11. 12. 13. 14. 15. 16. 17. 18.

Day

Disease Horsesickness.

No. 13

Infesting Material 5.c.c. Subcutaneous. Natal virus.

Chart 2.

1894 March

Date

Day

6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17.

Disease Horsickness.

No. 7

Infesting Material Inoc. 2. c.c. Subcutaneously on each side quack milk tracheal fluid from Horse No 8.

1892. March.

Date

Day

Disease

Horsesickness.

No.

16

Infecting Material

5 c.c. glycerinated Horsesickness blood. Subcutaneous.*The blood had been taken after death
(Smell of putrefaction) was then glycerinated*

1898. May.

Disease

No. 16

Infecting Material

Chart 5. contd.

Date 18. 19. 20. 21

Day

Disease

Horsesickness.

No.

30

Infecting Material

Drenched with 8. ozs. blood from Horse No 32

Chart 6

1894 Sept.

Date

Day

Disease Horsesickness.

No. 173

Infecting Material 1 c.c. subcutaneously: preserved Horsesick virus from Horse No 120.

(Preserved 233 days.)

1898.

September.

October.

Date

Day

24. 25. 26. 27. 28. 29. 30. 1. 2. 3. 4. 5. 6.

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13.

Disease Horsesickness.

No. 98

Infecting Material 2 grammes dried blood 8th day. No 92.

1897.

September

Date

Day

Disease Horsesickness.

No. 99

ho. 9 Chart 9

Infecting Material 2. grammes - 2nd day dried blood from N° 92.

1897. September

October

Disease

Horsesickness

No.

100Chart 10Infecting Material 100. c.c. Filtered Pericardial Exudate.

October

Day

Date

18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12.

Disease

HorsesicknessNo. 35Chart 11

Infecting Material S.c.c. subcutaneously Horsesickness blood, from № 17. - in shoulder.

1895. Jan.Feb.

Date

Day

No. 26

Infesting Material i.c.c. Subcutaneously, Horse sickness virus No. 17. in flank.

1894 March

* = 2. grammes Calomel.

MEAT SCOT

Disease Horse sickness.

13
Chart 13

No. 203 DONKEY.

Infecting Material 10. c. c. Sub. N° 188.

Feb. 7.

January

Date
Day

Disease

Horsesickness.

No.

220

DONKEY.

Chart 14

Infecting Material

March.

Date

Day

Disease

Horsesickness.

No. 253

DONKEY.

Chart 15

Infecting Material 15. c.c. Sub. Horsesickness virus No 120.

1899. Aug^t

Sept^r

Date

Day

Disease Horsesickness.

No. 258 DONKEY.

Infesting Material l.c.c. subcutaneously: fresh blood from № 256.

1899.

December.

Date

Day

Disease

Horsesickness.

No.

259

DONKEY.

17 Chart 17

Infecting Material 50. c.c. Subcutaneously: fresh blood from N^o 256.

1899.

December.

Date

Day

Disease

Horsesickness.

No. Ox. 1

Chart 18

Infecting Material 30. c. c. Horsesick blood - in jugular. from No 120.

1898. Feb.

Date

Day

Disease

Horsesickness

Chart 19

No.

122

HORSE.

Infecting Material

10. c.c. citrated blood from Horsesickness Ox N^o 1. Subcutaneous.

808. 26?

Date

Day

Disease

Horse sickness.

No. GOAT, 7

Infecting Material 10.c.c. blood from Horse No. 91.

898. July.

Date

Day

Disease

Horsesickness (from Goat No. 7.)

No.

Ox. 4

Chart 21

Infecting Material 30. c.c. citrated blood - in Jugular - from Goat No. 7.

898. Feb 7

March

Date

Day

Disease

Horse sickness.

No. 221

Chart 22

Infecting Material S.c.c. sub. N° 220 Donkey.

1899. March

April.

Date

Day

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

Disease

Horse sickness

No. 221

Chart 22 contd

Infesting Material

1899. April

May

Date

Day

Disease

Horse sickness

Chart 23

No. 222

Infecting Material S.C.C. Sub. 220. Donkey.

1899. March

April

Date

Day

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

27. 28. 29. 30. 31. 1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20.

Disease

Horsesickness.

Chart 23 cont^d

No. 222

Infecting Material

1899. April.

May.

Date

Day

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15.

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

Disease

Horsesickness

No.

254

Chart 24

Infecting Material 3. c. c. subcutaneous from 253. Donkey (fresh.)

1899. Sept^r

Rect.

Date

Day

Disease

Horsesickness

No.

254

Chart 24 cont¹

Infecting Material

29. Oct^r

Date

Day

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26.

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

4.c.c. Sub. N° 255 Monkey fresh.
(12 day)

Disease

Horsesickness.

No.

254

Chart 24. contd

Infecting Material

1899. Oct^r

Nov.

Date

Day

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

27. 28. 29. 30. 31. 1. 2. 3. 4.

110°
109°
108°
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
95°

Disease

Horsesickness.

No. 95

Chart 25

Infecting Material 5.c.c. preserved virus & 100.c.c. Serum, on different sides

17. Aug.

Date

Day

Disease

Horsesickness

No. 79

26
Chart 26

Infecting Material 2. c.c. preserved virus & 100. c.c. Serum, on different sides.

1897. Sept.

Date

Day

Chart 27

Infected Material 2 c.c. Sub. *Homesickness virus*, (25-1-98.)

Feb 9

1898.

January

Date _____

Day

Disease

Horn sickness

No. 116

Chart 28

Infesting Material

1918 Jan 7

2167

Date

21. 1. 2. 3. 4. 5. 6. 7. 8. 9. 10 11.

Day

Disease

Horse sickness

No.

123

Chart 29

Infecting Material

March.

Date

Day

Disease

Horseshoe

No. 147

Chart 30

Infecting Material

June

Date

Day

24. 26. 26. 27. 28. 29. 30. 1. 2. 3. 4.

Disease

Horsesickness

No.

159

Chart 31

Infecting Material

August.

Date

Day