Different species of trypanosomata observed in bovines in India / by A. Lingard.

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with Alfred Lingard's compliments

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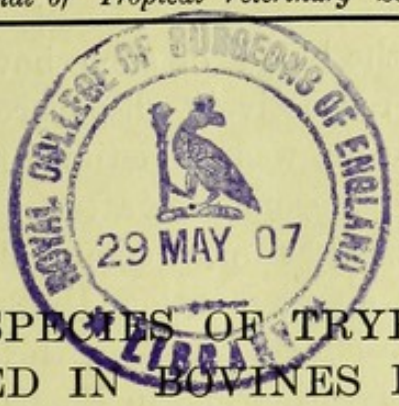

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INTRODUCTION.

For some years past an uncertain percentage of bovines indigenous to this country have been known to harbour trypanosomata of one or more species in their systems; but as these breeds of animals, as a rule, exhibit a high degree of immunity against these organisms, it was but seldom that opportunities offered for observing such cases, namely, those in which flagellates could be found on microscopical examination of the peripheral blood.

It was further recognized at an early period of our investigations (1893), that unless cattle were the subjects of a second form of disease, either of spontaneous origin or inoculated, which tended to lower the vitality of the body and so render the system more susceptible to disease, trypanosomata were but occasionally encountered in the peripheral circulation.

Nevertheless the blood of such bovines, in which no flagellates could be respectively discovered, when inoculated into susceptible animals, was sometimes found to be capable of reproducing trypanosomiasis after varying periods of incubation. The diseases most commonly found at a later date to produce the above-mentioned effect were rinderpest, anthrax, piroplasmosis, etc., yet in the primary instances encountered in our investigations in Poona and elsewhere, the *T. Evansi* from an equine source was almost always the secondary causal agent which brought to light the species of trypanosomata, when such were lying dormant in the cattle under observation.

In the present paper it is my intention to recount the various species of trypanosomata discovered in bovines indigenous to the Deccan, United Provinces, South Punjab and sub-montane tracts in Kumaon, by myself and by other observers; later to record the results of certain investigations followed up during the past two years in animals which have been submitted to inoculation with one or other form of virus, and those utilized in the preparation of anti-rinderpest serum.

PREVIOUS OBSERVATIONS.

During our long series of experiments conducted in Poona, between 1890—95, into the intimate etiology and treatment of spontaneous equine trypanosomiasis (Surra), bovines, in addition to many other species of animals, were submitted to inoculation with the *T. Evansi*. In several instances, the cattle under experiment exhibited in their peripheral blood, on one or more occasions, flagellates of a totally different species to those which were severally injected in the first instance. The points of interest in the three cases observed were as follows:—

A plains bull brought to the Laboratory on the 5th March 1891, was kept under observation until the 24th, during which period the animal appeared to be in health, and on daily microscopical examination of the blood nothing abnormal was observed. On the latter date it was inoculated subcutaneously with 1·75 c. c. of blood containing numerous *T. Evansi*, drawn from the jugular vein of Horse No. XIX. The flagellates appeared in the peripheral circulation for a period of twenty-four hours, after a period of

incubation lasting five days and then disappeared for twenty-four hours, to be again present for forty-eight hours. Then followed an intermission lasting 507 days, during which interval daily microscopical examination of one or more specimens of blood failed to disclose the presence of the Surra parasite. On August 22nd, 1892, seventeen months from the date of the primary inoculation of the animal, it was determined to re-inoculate it with what was previously proved to be a more virulent type of *T. Evansi*, obtained by passing the trypanosoma, derived in this instance from the blood of a rat (*m. d.*), through a horse and a donkey respectively, by subcutaneous inoculation. One cubic centimetre of blood, containing numerous and active flagellates, was injected subcutaneously into the post-scapular region of the bull. After an incubation period of five days, instead of the *T. Evansi* making its appearance in the blood, an exceedingly large form of trypanosoma was observed. The length of the organism was equal to the diameter of fourteen red blood corpuscles and its width, at the widest part, was equal to 2·5 to 3 red corpuscles. A round nucleus was readily discernible in the protoplasm of the dilated portion of the body of the organism. The body remained almost motionless, while the long flagellum twisted and twirled, lashing the corpuscles to right and left; at a later period the undulating membrane became very distinct.

Microscopical examination of the blood of this bull was continued twice daily for a further period of three hundred and six days (October 7th, 1893), but no trypanosoma of the large or small variety was again observed.

The second animal, which exhibited a large form of trypanosoma in its blood, was submitted to inoculation on the 28th August 1892. After a period of six days, during which nothing abnormal was noted on microscopical examination of the blood, the animal received subcutaneously 1·0 c. c. of blood containing numerous *T. Evansi* taken from the jugular vein of a donkey, the subject of Surra. The small form of flagellate appeared in the blood on the sixth day following inoculation. On the 7th November 1892, between the second and third paroxysms, that is, on the sixth day of the second intermission of the disease, and during the temporary absence of the

T. Evansi from the peripheral circulation of the animal, a large form of trypansoma was observed, which exhibited a length many times that of the smaller species of flagellate. It exhibited a somewhat similar outline to that observed in the bull, previously mentioned, and the body movements were the same, but no undulating membrane could be made out. Although the blood was carefully examined microscopically twice a day up to the 9th February 1893, no further example of the giant form of flagellate was discovered; the small variety, however, made its appearance twelve days after the above observation was made. On the 9th February 1893, the animal under experiment was re-inoculated subcutaneously with 2·0 c. c. of blood containing numerous trypanosomata drawn immediately after death from the jugular vein of a horse, a case of spontaneous equine Surra. The *T. Evansi* appeared in the circulation after an incubation period lasting ten days. Six paroxysms of the disease followed of 1, 1, 2, 1, 1 and 2 days, and five intermissions of 10, 29, 70, 9 and 36 days respectively. From the date of the last paroxysm, October 28th, 1893, observations were maintained daily for a further period of 203 days, *viz.*, to the 19th March 1894, but the small variety of trypanosoma did not reappear in the blood.

The second occasion on which a single example of the giant trypanosoma was observed in the specimens of blood taken from this animal, was on 15th June 1893, 127 days after re-inoculation. Two guinea-pigs, each inoculated with 0·5 c. c. of blood direct from the cow, contracted trypanosomiasis (Surra) after two days' incubation, and death followed in nine and seven days respectively, but no examples of the large flagellates were discovered in their circulations, although carefully searched for in the blood and tissues of the organs and marrow of the bones. The third observation of the giant trypanosoma was made on the 3rd July 1893, when again a single example was discovered. The fourth observation occurred on the 2nd September 1893, during the prolonged absence of the small variety. Microscopical observations with regard to the condition of the blood were continued daily for a further period of 271 days, but neither the large, nor the small forms of trypanosomata, were again encountered.

During the presence of the giant trypanosoma in the circulation of the affected animal, there was no oscillation in the temperature from the normal, and in fact no symptom of any kind to show that its presence acted as a foreign body. The blood corpuscles exhibited a perfectly normal appearance and no symptoms of anæmia were observable. The blood of animals inoculated with the blood of the bovines drawn at the time when the large form of parasite was present, including equines, monkeys, dogs, rabbits and guinea-pigs, although kept under observation for long periods, never revealed on daily microscopical examination the presence of this large variety of hæmatazoon. In addition to the several bovines subcutaneously inoculated with the blood of rats (*m.d.*) containing large number of *T. Lewisi*, the above-mentioned bovines alone exhibited the giant trypanosoma.

Both the bull and cow showed varying degrees of immunity to the first inoculation with the *T. Evansi*, but especially the former; when respectively re-inoculated, at a later date, with the same species of organisms, they both exhibited complete protection. The *T. Gigantium*, or its immature form, which most probably had been lying dormant for long periods in the spleen and bone-marrow, was lighted up owing to certain changes usually designated, loss of power of resistance, but which was more probably brought about in the blood and tissues, subsequent to the elaboration of a second form of toxin by the *T. Evansi*.

The second form of trypanosoma discovered in the peripheral blood of cattle in this country was also observed in Poona. Unfortunately, this species was only met with on one occasion, and no opportunity was afforded of making a detailed examination of it in stained specimens. Bull No. IV, a small plains or Deccani animal, was inoculated on the 27th July 1892 with 2·0 c. c. of blood containing large numbers of the *T. Lewisi* taken from the heart of the rat (*m.d.*) immediately after death. A considerable amount of swelling and œdema followed at and around the seat of the injection of blood, which, however, gradually decreased, and finally disappeared by about the 10th August 1892. From the 27th July until the 10th October, the blood examined from the peripheral circulation of the animal remained free from any

form of flagellate. On the evening of 9th September, an urticarial eruption appeared on each side of the neck, chest and scapular regions, but had entirely disappeared by the following morning. Soon after 6 A.M., on the 3rd October, a similar eruption to the former made its appearance, and again faded on the evening of the same day. On microscopical examination of the peripheral blood of the animal on the morning of the 11th October, the 76th day following inoculation, a long and active form of trypanosoma was discovered, the undulating membrane being particularly well developed. From the latter date up to the 9th February 1893, the 121st day subsequent to the appearance of the flagellate, no further organisms were observed, although several specimens of blood were subjected to microscopical examination daily.

In December 1895, I received the orders of Government to proceed to the Remount Depôt, Karnal, S. Punjab, in order to investigate an epizootic which had broken out amongst horses and which caused a loss of some twenty animals. During my sojourn, I took the opportunity of making enquiries with regard to the bovines on the estate used for agricultural purposes, and discovered that the authorities had lost some twenty-six animals during the preceding eleven months, due to what was thought to be anthrax, and that twenty-five were still under observation in hospital. On the 14th December, in company with Vety.-Lieut. F. Eassie, A. V. D., I visited the bullock hospital and selected two animals Nos. 13 and 152, as being likely subjects of trypanosomiasis. These animals were somewhat thin and ill-conditioned, but otherwise their temperatures were within normal limits, and they presented no symptoms to attract attention to them particularly. On the morning of the 15th, specimens of blood from both these animals were submitted to microscopical examination in the paddock, and a small variety of flagellate was found to be present in each. Bullock No. 13 succumbed at 2-45 P.M. on the 16th, and an autopsy was conducted after a short interval. The usual pathological conditions of the organs were noted, more especially the increased size of the spleen, and in addition, microscopical examination of a specimen of blood taken directly

from the heart, exhibited not only a small form of trypanosoma (*T. Evansi*), but also a second species of flagellate, which appeared to be about twice the length of the small variety, and which has since been considered to have been an example of the *T. Himalayanum*. A dog was therefore inoculated with 1·25 c. c. of blood collected from the heart. This animal succumbed after exhibiting all the usual symptoms, in a period of 94 days, but only the small species of flagellate was observed in this animal's circulation during the numerous paroxysms of the disease. Bullock No. 152 presented no symptoms, and I had on subsequent occasions, within the next eighteen months, further opportunities of making microscopical examination of its blood, but never observed either filaria embryos or trypanosomata present. Of the 23 bullocks in the hospital, the blood of twelve was selected for microscopical examination, but in no instance was a trypanosoma detected.

A trypanosoma was discovered by me at Muktesar, on the 6th June 1902, in the blood of a hill bull, just before death from inoculated anthrax. This animal was brought in the first instance from a valley bordering on the foot of the snow mountains. This species of flagellate, since described as the *T. Himalayanum*, was found concurrently with numerous anthrax bacilli in the same specimen of blood. The length of this parasite was equal to thirteen times the diameter of a red corpuscle, and the width of the widest part to 0·8 of the same. The flagellum was fairly short, the posterior extremity prolonged as in *T. Lewisi*. The undulating membrane appeared to be only moderately developed, and the blepharoplast and nutritive nucleus were undergoing division.

Durrant and *Holmes* described in 1904, a trypanosoma which they found at Muktesar in a hill bull, aged 5 years, body weight 250 lbs., which had been used for testing the potency of a bulk of anti-rinderpest serum, a week after the rinderpest reaction had passed over, and during which period the animal fed well. Later, other symptoms appeared, diarrhœa, intermittent fever, loss of appetite, staring coat, anæmia. The bull died six days later. After staining some specimens taken from the spleen with double stain, it

was seen that this trypanosoma varied in many respects from that of horse Surra. It is 2 to 4 μ shorter, the body is much thicker, and the posterior extremity blunter. The centrosome is situated much nearer to the nucleus and not close to the posterior end as in the *T. Evansi.* The nucleus and flagellum are more difficult to stain.

Holmes in a later communication with regard to this species of flagellate observed: "The parasites which were first noticed, and which were taken from the spleen, were evidently immature forms, and that the description then given differs very considerably from that of the mature flagellate forms found in the blood-stream. The full-grown trypanosomes are much longer than the Surra parasites, and are probably the longest yet described. The largest measured 91 μ in length. The average length is from 60 to 80 μ. The breadth is from 2 μ to 4 μ, and in some cases as much as 6 μ. The latter forms were not typical, and seemed to be undergoing change. The posterior extremity is very fine and elongated, measuring from centrosome to end 10 to 17 μ. The flagellum is well developed, and measures from 15 μ to 25 μ. In the blood taken from two infected bulls, I found peculiar developing forms bearing a striking resemblance to the crescents of human tertian fever."

The disease was found in each instance in hill cattle injected with rinderpest blood, and never in the plains breed, or in untreated hill cattle. Plains cattle respectively injected with a large dose of infective blood did not become infected with the trypanosoma. Only bovines in a debilitated condition succumbed to the disease. The flagellates were observed for several days in the blood, seldom above five in a field. During a period of five or six weeks, the blood was examined, but when once the trypanosoma had disappeared from the peripheral circulation of the respective animals, the flagellates were not again observed. Equines, bovines, plains and hill breeds, and rabbits proved refractory to subcutaneous or intravenous inoculation.

I.—(T. Evansi).

The first form of trypanosomiasis to be described is of interest, more especially to countries outside of India, which

may find it necessary at any time to import cattle for economic purposes.

It does not appear to be of serious importance to this country, as I have only discovered plains cattle suffering from the malady on a few previous occasions, and then the mortality was insignificant as compared with the similar form of enzootic in South Africa, which is said to carry off some 40 per cent. of the animals attacked. In India, plains animals exhibit a high degree of immunity, as a consequence, no doubt, of the ravages of the disease in former times, and to the present stock being the progeny of the survival of the fittest. It has long been recognized that cattle indigenous to this country, when inoculated with the *T. Evansi* from a spontaneous equine source, usually pass through one or more marked paroxysms of the disease, and later at long intervals extending to twelve or eighteen months or perhaps longer, may periodically show a slight rise of body temperature and exhibit the flagellates in the peripheral blood for a day or two, or may be only a few hours. Further, susceptible animals inoculated with blood during the above-mentioned period, in the majority of instances, sooner or later, present the trypanosoma in their blood, although neither mature nor immature forms of the organisms could be demonstrated in the fluid injected. It may now be recognized how very serious it might prove to a second country totally free from any such disease, if bovines, the subjects of such an insidious and smouldering form of malady, should be imported from India and allowed to mix with the indigenous animals of the same and allied species, for, the biting diptera would accomplish the problem of disseminating the disease.

According to French observers, such a calamity occurred amongst the horses, mules and cattle in the Island of Mauritius in April 1902, when an estimated mortality of from 70 to 80 per cent. took place amongst the indigenous breeds of animals. It is stated "that the disease was imported from India; ordinarily Mauritius is provisioned from Madagascar as regards cattle, but owing to the Transvaal war most of the animals were bought up for S. Africa, and hence they had to be got from India; infected beasts were introduced and the disease thus propagated in the island."

I will now place on record the facts with regard to the cases recently met with in hill cattle in Kumaon, together with a description of the flagellate discovered in this breed of bovines, the effects produced by inoculation of susceptible animals, and then contrast the characteristics of the parasites with those met with in spontaneous equine Surra (*T. Evansi*) and after passage of the latter organisms through hill cattle.

Hill Bull No. 4880, æt. six years, body weight, 231 lbs., was immunized on the 24th April 1906, by the simultaneous method, against rinderpest. The animal exhibited a slight thermal reaction, but no symptoms of the disease were manifested. On the 9th May, a dose of 2,000 c. c. of virulent rinderpest blood was injected subcutaneously, the material being collected from two control hill bulls, 1,300 c. c. from No. 4664 and 700 c.c. from No. 4669. A further reaction, maximum temperature 40·2°C., followed, but no symptoms. An interval of seven days was allowed to elapse after the temperature had returned to normal limits, before a second dose of 2,500 c. c. was injected. This latter quantity was made up of 2,300 c. c. from No. 4970 and 200 c. c. from No. 4981. A third thermal reaction was the result, which however subsided in a few days and between the 4th and 9th June, normal records were registered. Under the usual conditions, the animal would not exhibit any further rise of body temperature and would have been bled for the first time for serum fifteen days after the termination of the last reaction. Instead of the above-mentioned procedure taking place, on the evening of the 9th June, the temperature of the animal began to rise, and a maximum of 41°C. was recorded on the evening of the 12th. The animal fed well during this latter period and showed no symptoms of disease.

The blood on microscopical examination was found to contain a small form of trypanosoma.

Hill Bull No. 4879, æt. 3 years, body weight 242 lbs., was immunized on the 24th April 1906, by the simultaneous method, against rinderpest, the animal passing through a slight reaction. On the 8th May it was injected subcutaneously with 2,000 c. c., (1,200 c. c. from No. 4,669, 800 c. c. from No. 4655,) maximum temperature 40·5°C. on the evening of the eleventh day.

On the 31st May after an interval of nearly three weeks, an increased amount of virulent rinderpest blood, 2,500 c. c. was injected, of which 2,200 c. c. was collected from Hill Bull No. 4969 and 300 c. c. from No. 4,968. A thermal reaction followed. On the evening of the 10th June the body temperature began to rise (39·2°C.) and on the following evening registered 40·6°C., when trypanosomata were discovered in smears of blood taken from the peripheral circulation of the animal.

It will be observed that on the first injection, each of the Hill Bulls, Nos. 4879 and 4880, received a quantity of the total blood (2,000 c. c.) from one and the same animal, No. 4669, but no trypanosomata were diagnosed. On the second occasion the 2,500 c. c. in each instance was collected from different animals, and still no symptoms of a secondary infection were exhibited.

It was only after a normal temperature had been recorded for some days in each case respectively, that a fourth thermal reaction unaccompanied by any symptoms of disease was observed. As this could only be accounted for on the supposition that the animals were suffering from a previous or an inter-current infection, and as reactions were noted in the two animals concurrently, which had been subjected to similar treatment, it was considered to be more than probable that the presence of a trypanosoma was the cause of the rise in temperature.

It would appear, therefore, that in each instance, either the dose of 2,500 c. c. of virulent rinderpest blood derived from two distinct sources must have contained the trypanosoma and been injected into the affected animals, or else the two bovines must have been primarily infected before immunization, and the organisms were lying dormant in their systems, only to be lighted up after three thermal reactions had been passed through. Had the trypanosoma been present in the blood injected on the 8th May, it should have made its appearance in the course of a few days, for our subsequent experiments on Hill cattle have shown that when quantities of from 1 c. c. to 7 c. c. of blood containing the flagellate have been inoculated, the period of incubation only extended over six and a half days and in two instances occupied but five and a half days. Death following quickly in one instance.

EXPERIMENTAL RESULTS.

The following animals were inoculated with infective blood either when the organism of the disease was present in the circulation or during the period of intermission which followed :

Hill Bull No. 5045, æt. $2\frac{3}{4}$ years, body weight 160 lbs., was inoculated intravenously with 7 c. c. of blood direct from the jugular vein of No. 4880, on the 14th June 1906. The period of incubation occupied five and a half days. The trypanosoma was observed during two paroxysms lasting four and six days respectively with an intermission of four days between. The organisms have not been visible in the blood for a period of four and a half months and the animal presents a perfectly healthy condition.

Hill Bull No. 5044, æt. 2 years, body weight 144 lbs., was inoculated at the same time and in a similar manner to the former. The period of incubation occupied five and a half days. The trypanosoma gradually increased during the first three days until 100 in a field were counted and a similar number on the fourth day ; a decrease to eleven in a field took place on the fifth morning. A few hours later, this animal which presented no symptoms of illness, suddenly fell dead. The autopsy, which was conducted after an interval of one hour, disclosed the fact that death was due to a cerebral hæmorrhage and that a considerable amount of blood was extravasated over the superior and external surfaces of the right cerebral hemisphere. A third Hill Bull No. 4979 was inoculated subcutaneously with 1·0 c. c. of blood from the jugular vein of No. 4880 on the 27th June, at a time when no flagellates were present in the peripheral blood of the host. In this instance the period of incubation lasted for six and a half days. Three paroxysms lasting 4, 11 and 1 day respectively followed and at the time of writing the flagellate has not been discovered for a period of over three and a half months, the animal to all appearances being in perfect health and condition.

Plains Bull.—A bull No. 3917, aged $5\frac{3}{4}$ years, body weight 358 lbs., was inoculated intravenously with 5·0 c. c. blood from the jugular vein of Hill Bull No. 4880. Period of incubation six and a half days. Two paroxysms of 6 and 2 days followed. No flagellates have been discovered for a

period of over four months, and the animal exhibits a condition of perfect health.

Buffalo, æt. 5 years, body weight 304 lbs., was inoculated intravenously on the 5th July 1906 with 5·0 c. c. of blood from No. 4880 at a time when the mature flagellate was absent from the peripheral circulation. Period of incubation occupied four and a half days. At the time of writing nine paroxysms and eight intermissions lasting 132 days have occurred. The animal, however, has gained in weight since the date of inoculation, for it now scales 332 lbs. instead of 304 lbs.

Equines.—A country-bred pony entire, æt. 10 years, body weight 472 lbs., was inoculated intravenously on 14th June 1906 with 5 c. c. of blood collected from No. 4880. Period of incubation five and a half days. One long paroxysm lasting 57 days took place, the animal succumbed on the 15th August. The action of the trypanosoma approximated more to the *T. Brucei* rather than to the *T. Evansi*, in that the course of the disease was marked by one paroxysm only, as is sometimes observed in the mountain districts in this country when Surra is passed from equine to equine. Loss in body weight, 182 lbs.

An Australian gelding, æt. 12 years, body weight 931 lbs., was inoculated subcutaneously on the 22nd June 1906 with 5·0 c. c. of blood from the jugular vein of Hill Bull No. 4880. Period of incubation, six and a half days. Two paroxysms lasting 30 and 10 days respectively, with an intermission of five days, amounting to forty-five days in all, occurred, when death took place.

A country-bred donkey,* æt. 2 years, body weight 204 lbs., received 5·0 c. c. of blood intravenously from Bull No. 4880 on the 14th June 1906. Period of incubation five and a half days. During five months subsequent to inoculation, fifteen paroxysms and fourteen intermissions of disease have been passed through, yet the animal has not decreased in weight (218 lbs.).

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* This animal succumbed on the 26th November 1906. It suddenly refused all food; with forelegs wide apart and head hanging, it remained in a deep somnolent condition for a period of 5 days; temperature meanwhile gradually fell to 32·8°C. It then assumed a recumbent position, and remained comatosed until death. Course of disease 160 days.

Two monkeys were inoculated subcutaneously on the 27th June, each with 5·0 c. c. of blood from the bull during the absence of the mature trypanosoma from the peripheral circulation. Periods of incubation occupied four and a half and five and a half days respectively. After exhibiting numerous paroxysms and intermissions of the disease, both animals succumbed in 43 and 45 days respectively.

Two pariah dogs each received subcutaneously 5·0 c. c. of blood from No. 4880 on the 14th June 1906. Period of incubation six and a half days. Death on the 12th and 14th day of the disease respectively.

Tuo rabbits, each inoculated subcutaneously with 1·0 c. c., presented periods of incubation of seven and a half and ten and a half days, followed by death on the twelfth and fifty-ninth day of the disease respectively.

Two guinea-pigs received each 1·0 c. c. subcutaneously. The periods of incubation occupied seven and a half and nine and a half days respectively. One lived for a period of seventy-nine days, while the other succumbed on the one-hundreth day of the disease.

In addition, two rats were inoculated with blood from No. 4880 and two from No. 4879, with the result that the former showed an incubation period of $8\frac{1}{2}$ and $7\frac{1}{2}$ days, and succumbed in seven and two days respectively; while the latter both exhibited incubation periods of $6\frac{1}{2}$ days, and succumbed in each instance on the second day of the disease. Of four mice, each inoculated on 14th June, with 0·25 c. c. of blood taken from Nos. 4880 and 4879, two from each bull, the incubation periods of the former occupied 7·5 and 6·5 days, and death ensued on the fourth day of disease in each instance. Of the two mice inoculated from the latter bull one exhibited flagellates after a period of 10·5 days, and it succumbed on the fourth day of disease. While the second mouse only developed the trypanosoma in its blood on the 21st July, the 38th day following inoculation. The parasites were present in the blood for three days, but during the interval up to the date of death, 6th November, no further organisms were observed. A white rat inoculated from this latter mouse at the time of death, developed organisms after an incubation period of thirteen days.

As a result of these experiments, we find that of twenty-five animals of various species inoculated with this form of trypanosoma, 84 per cent. succumbed to its effects within a period of 5·5 months, the majority in less than three months. Of four bovines, one fell a victim to cerebral hæmorrhage, the result of the flagellate, for the animal was but two years of age, and its vessels were not diseased. The blood of the three remaining has not again exhibited parasites. A buffalo and a donkey which have now survived inoculation 4 and 4·5 months respectively, have not lost in body weight during those periods, although the paroxysms and intermissions continue their course uninterruptedly. There is however no reason to be too sanguine of a non-fatal result, for both these animals may eventually and perhaps suddenly prove victims to the disease.

With regard to the dimensions of this trypanosoma, the causal factor of this form of spontaneous disease in hill cattle, but little could be ascertained in the original hosts as the paroxysms of disease had proceeded several days before the organisms were recognized. For this reason careful observations were conducted in the inoculated animals especially in No. 5044 which received 7 c. c. of blood intravenously from No. 4880 with a period of incubation lasting four and a half days, and a paroxysm of five days which terminated suddenly in death. DIMENSIONS, &c.

The blood, on each of the five days of the paroxysm alluded to, was examined in stained specimens after the various modifications of the Romanowsky method, and drawings made of the various forms discovered as may be observed on reference to Plate I. On looking over the coloured illustration, a point to draw attention is that on the first day of the paroxysm the endoplasm of the trypanosomata stains a blue or a violet-blue colour with the exception of the nuclei and flagellum. That on the second day the organisms depicted exhibit a violet-blue with the exception of two parasites, the only ones discovered in the whole specimen, which were probably dead and therefore have taken a red stain. On the third and fourth day the flagellates have taken a reddish colour in the majority of instances, although several have been depicted, which presented a bluish colour.

As the paroxysm did not end by crisis as frequently happens in equines, an opportunity was observed of examining trypanosomata which exhibited islands in their endoplasm, mostly towards their posterior extremities which took a bright red colour.

Until the appearance of Prozawek's paper but little was actually known with regard to the intimate modes of development of trypanosomata in säugetiere, and the different forms they assume in the internal organs, although much time has been expended over those forms commonly met with in the peripheral circulation of both man and some animals.

The most obvious method of reproduction of this form of trypanosoma in the peripheral blood is by longitudinal division. It is also not difficult to recognize organisms of different ages, according to their size and development. Even on the first day of the paroxysm a number of the flagellates exhibit a considerable number of dark-stained micro-granules in their plasma, anterior to the nutritive nucleus, although many more are demonstrable towards the end of a paroxysm when the organisms are swarming. These micro-granules, according to Schaudinn, probably represent reserve material.

The flagellates possessing much reserve material should, in this instance, if the changes which take place are according to a scheme similar to that observed in the organisms in the *Athena noctuæ*, be female trypanosomata, whilst the clear flagellates, large and small, as they appear to possess no reserve material, should represent the males and indifferent parasites respectively.

The length of the posterior extremity extending between the blepharoplast and the tip in this species of trypanosoma varies considerably in various single organisms, previous to any signs of division taking place.

They were found to vary in length between $0{\cdot}82\mu$ and $2{\cdot}13\mu$ on the first day of the paroxysm, but during the second and third days they were observed in considerable numbers measuring from $4{\cdot}59$ to $4{\cdot}92\mu$.

The mean measurement of a considerable number of trypanosomata taken on each of the five days, gave a mean length of the posterior extremity respectively, first day, $1{\cdot}38\mu$; second, $2{\cdot}72\mu$; third, $2{\cdot}87\mu$; fourth, $1{\cdot}76\mu$; fifth, $1{\cdot}38\mu$.

So it would appear as if the flagellates in this instance lived for at least three days, and were then destroyed by the presence of the increasing toxin, which appears to act first on the more mature parasites present in the peripheral blood.

It has been found that during a paroxysm lasting five days in all, when the mean measurements of a considerable number of flagellates on each day of the period in question have been made, the trypanosomata gradually increase in length, until the parasites had been swarming in the peripheral blood for a period of twenty-four hours. During the following two days they decreased in length. The mean length on each of the five days were as follows :—

	μ
1st day	26·77
2nd „	29·35
3rd „	30·36
4th „	28·81
5th „	27·35

The maximum, minimum and the mean measurements of eighty trypanosomata are given below, together with the percentage of the mean measurements.

	Length from posterior extremity of trypanosome to the centre of the blepharoplast.	Length from centre of blepharoplast to posterior edge of nutritive nucleus.	Postero-anterior length of nutritive nucleus.	Length from anterior edge of nutritive nucleus to termination of body protoplasm anteriorly.	Length of free flagellum.	Total length of trypanosoma.	Maximum width of body.
	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Maximum ...	3·28	9·02	3·28	16·40	3·28	35·26	1·64
Minimum ...	0·00	4·92	2·95	8·20	3·28	19·35	1·31
Mean of 80 trypanosomata ...	2·03	7·30	2·55	11·64	4·29	27·85	1·64
Percentage of the mean ...	7·38	26·24	9·16	41·80	15·20	100·00	5·93

Now if we compare the percentage of the mean just given, with that obtained from the mean measurements of

one hundred and twenty-six trypanosomata (*T. Evansi*) in spontaneous equine trypanosomiasis we find the following :—

	I	II	III	IV	V	VI	VII
Percentage of the mean of 126 trypanosomata	6·82	28·31	11·87	39·85	13·15	100·00	6·14

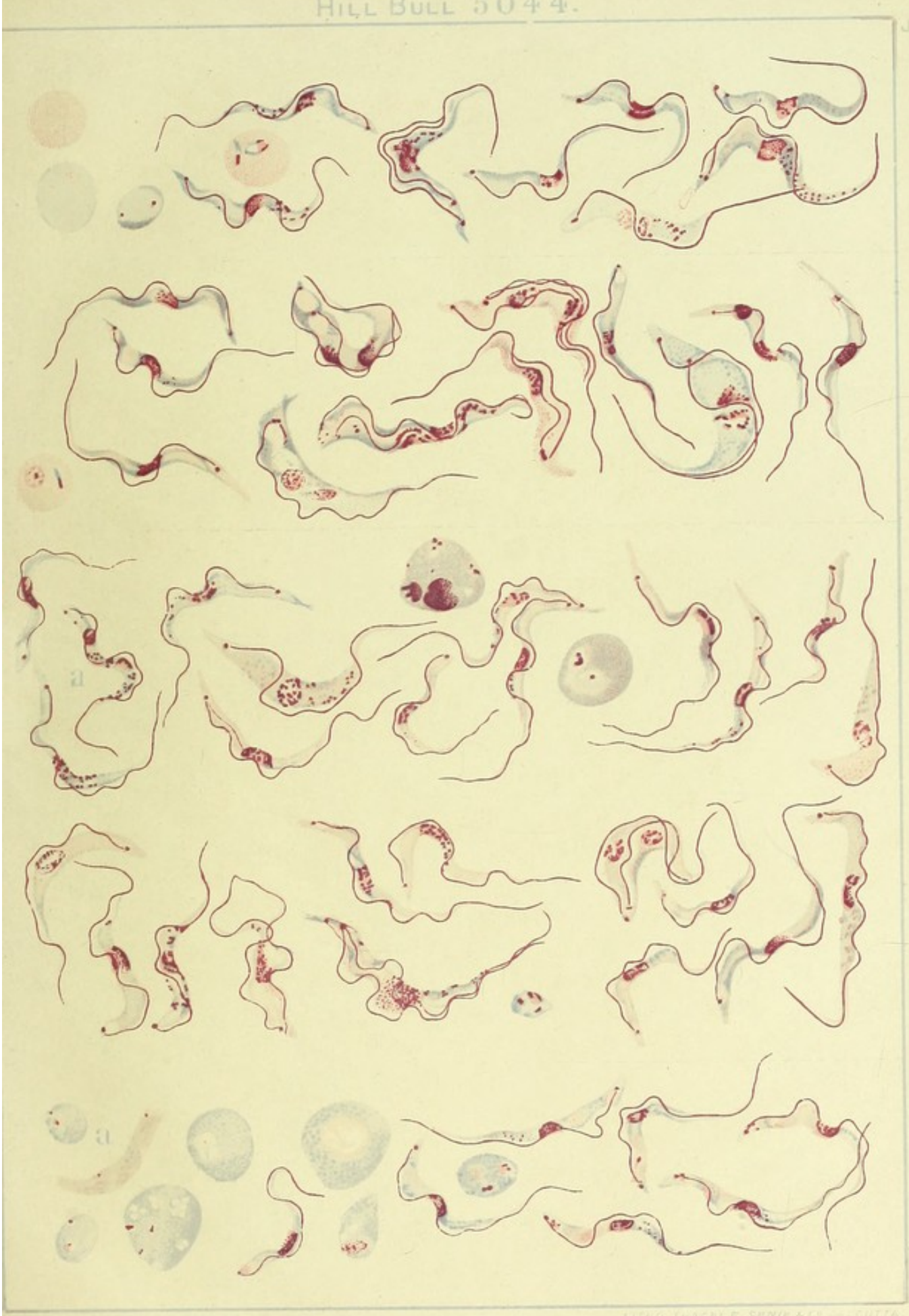
These latter figures, however, are those found in the horse and may not coincide with those as observed in spontaneous bovine trypanosomiasis. It is, therefore, necessary for the sake of comparison that the measurements of the *T. Evansi* should only be made use of after having been passed through the hill breed of cattle. Let us, therefore, take the figures representing the mean percentage under these latter conditions :—

T. Evansi passed through Hill Bull.

	I	II	III	IV	V	VI	VII
Percentage of the mean of 85 trypanosomata ...	7·48	26·39	8·58	42·83	14·72	100·00	5·50

On comparison of the percentage of the mean measurements of the trypanosomata from bull No. 4880, with those obtained from a large number of observations after passage of the *T. Evansi*, through a hill bull, we find that the figures are almost identical in each case. There is strong evidence, therefore, that the trypanosoma discovered in the spontaneous form of disease which attacked hill bulls Nos. 4879 and 4880, and the *T. Evansi* of spontaneous equine disease belong to one and the same species. This probability is again emphasized when we bear in mind that other species of trypanosomata affecting cattle in this country are only with difficulty inoculated into others of the same breed, whereas the great majority of animals of different species succumbed within a few weeks to the flagellate under experiment, with the exception of the buffalo, donkey and one mouse, in the first two of which the disease may yet prove fatal. Hill and plains cattle

Hill Bull 5044.



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seldom or ever succumb to uncomplicated Surra from a spontaneous equine source.

II.—(T. Himalayanum.)

The second species of flagellate met with for the first time in Karnal, but in stained specimens in June 1902 at Muktesar, and on several occasions during the past two years, has of late only been met with in the peripheral blood of hill cattle after they have been inoculated with the causal agent of a second form of disease, be it anthrax, spontaneous equine Surra (*T. Evansi*), or virulent rinderpest blood. I will give a few notes of two hill bulls, which were being utilized in one or other of these lines of investigation when the *T. Himalayanum* was discovered on microscopical examination of their blood.

Hill Bull No. 3951, aged 4 years, body weight, 232 lbs., was inoculated intravenously with 4 c. c. of blood collected from a case of spontaneous equine trypanosomiasis, on the 7th July 1905. The period of incubation occupied three and a half days. The first paroxysm occurred on the 16th July and lasted for six days, during which period the *T. Evansi* in the peripheral blood increased for the first four days, from two in a specimen to eighty in a field, and on the two following days decreased to thirteen in a field, and one in a specimen. On the 14th July, the fourth day, in addition to the *T. Evansi*, a large form of organism was discovered. The various forms observed, to be described later, were only found on the one day, although the blood of the animals was submitted daily to careful examination for a further period of several months.

Hill Bull No. 5078, aged 5 years, body weight, 197 lbs., was subcutaneously inoculated by the simultaneous method on the 21st June 1906, with 17·73 c. c. anti-rinderpest serum and 0·5 c. c. virulent blood. A thermal reaction followed and occupied from the 26th June until the 3rd July 1906. On the 10th July, as the temperature had been normal for some days, the animal received its first dose of 2,000 c. c. virulent blood towards hyperimmunization. A slight rise in temperature took place, maximum 39·3° C. Then followed an interval during which a normal condition was exhibited.

A secondary reaction commenced on the 16th July when an evening temperature of 40·3° C. was registered, and this was recorded for several days. The trypanosoma was discovered on the 19th. In this case also microscopical examination of the blood was continued for a considerable period, but no more trypanosomata were discovered, although a daily search was made for them.

The *T. Himalayanum* assumes considerable proportions, and although in fresh specimens some difficulty may be experienced in coming to a decision as to the species, nevertheless in stained specimens, coloured after any of the modifications of the Romanowsky method, good results may be obtained and little difficulty encountered in coming to a correct diagnosis. The blepharoplast, nutritive nucleus, margin of the undulating membrane and free flagellum, stain a deep red, the two latter portions of the parasite being particularly well marked, while the endoplasm takes a blue colour. It frequently happens that the body protoplasm is studded with microgranules, somewhat coarse in structure. These may stain any tint from a dark-red to a deep violet. The posterior extremity of the organism is long and gradually tapers to a point, the blepharoplast of medium size, nearly circular in form, lies close to one edge of the organism, occupying one-third to one-fourth of its breadth, and at a distance from the posterior extremity, which varies between 6·56 to 10·66μ in different specimens. The nutritive nucleus, as a rule, stains a lighter shade of red than any other portion of the parasite which assumes the same colour. Its size, in single forms of flagellates, varies almost as much as its situation, and in many instances is ovate in form, the wider extremity being in proximity with the undulating membrane. The distance observed between the smaller and larger nuclei, in the great majority of organisms, varies from 5·74 to 13·12μ, but a minority exhibit the two nuclei in close apposition, the larger having emigrated to the situation of the smaller one. The undulating membrane in mature organisms frequently presents an appearance of having been artificially gauffered, and the free flagellum in a large majority of parasites in the peripheral blood attains to exactly the same length, namely, 13·12μ.

The following are the maximum, minimum, and mean measurements, together with the percentage values of twenty-two single examples of the *T. Himalayanum*, as observed in the peripheral blood of a hill bull :—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Maximum ...	13·12	13·12	2·46	27·88	13·12	69·70	2·46
Minimum ...	6·56	5·74	2·46	18·04	11·84	44·28	2·46
Mean of 21 trypanosomata ...	9·30	10·19	2·29	23·13	12·34	57·25	2·91
Percentage of mean ...	**16·23**	**17·86**	**3·99**	**40·36**	**21·56**	**100·00**	**5·06**

Several interesting forms of this trypanosoma have been met with in the peripheral blood. (*a*) The most numerous are those which exhibit a considerable number of darkly stained micro-granules in the endoplasm, which latter takes a blue colour and following Schaudinn's work in the trypanosoma of the *Athena noctuæ* we may be justified in recognizing as females. (*b*) Other trypanosomata in small numbers of about the same dimensions as the former, were observed, in which the absence or nearly so of micro-granules was at once noticeable. This form of parasite takes a fainter shade of blue stain than the former. These then, according to the dictum of the late savant, we may designate as males.

As neither of the affected bovines succumbed to the disease or as a result of complications, I have been unable to follow up the further changes, which would almost certainly have been observed in the spleen and internal organs, in the so-called male forms. Interesting observations concerning the parasites which contain large quantities of micro-granules (pigment) were made. In some trypanosomata this material occupies only the anterior three-quarters of the body protoplasm ; in others also the portion between the blepharoplast and posterior extremity is free, while in a third variety the pigment extends into the posterior portion also. The first sign of an involutionary change taking place is observed in the posterior portion of the parasite. This consists of swelling

and loss of contour which gradually extends until the whole body is involved. The endoplasm then loses its power of absorbing stain, and the body of the organism becomes flattened and considerably increased in width, assuming more or less the outline of a lizard. These forms are particularly fragile and are more than likely to be injured during the process of making smears of blood, which contain them, if too much pressure be used. The micro-granules become scattered throughout the endoplasm, and in some instances the nutritive nucleus also breaks up and the fragments are widely disseminated. The margin of the undulating membrane, always well developed in this species of trypanosoma, appears to take a deeper red stain, and become somewhat broader, during the period the organism is undergoing the process of enlargement and flattening. The length from the blepharoplast to the end of the free flagellum in the two specimens illustrated (Pl. II, Figs. 25-26) measure 67·24 and 66·42μ and the breadth 13·12 and 11·48μ respectively, but these measurements do not indicate the full length of the parasites, as the posterior portion is not included.

Occasionally one meets with a parasite in which the blepharoplast is enlarged, but no definite re-duplication or dumb-bell formation could be made out. The nutritive nucleus has not unfrequently been observed to move posteriorly, so that it takes up a position close to or in apposition with the smaller nucleus. I have not up to the present encountered any trypanosomata which exhibited any marked indications of division in their longitudinal axes, and am inclined to look upon this mode of reproduction as being uncommon in the peripheral blood, at all events shortly before the death of the host.

As I shall have to go into some detail with regard to the results of my observations as to a mode of reproduction not yet described in trypanosomata further on in this paper, when describing the changes encountered in a third form of trypanosoma which in all probability are similar to the changes observed in this parasite, I will avoid recapitulation, and now pass on to contrast the characteristics of the *T. Himalayanum* with a species of flagellate discovered by Theiler in 1903 in the blood of cattle in South Africa.

This trypanosoma, designated the *T. Theileri* by Laveran, was met with during the course of immunization of cattle against rinderpest and was found to be very generally distributed. Two forms of parasites were observed, the distinguishing feature being the position of the centrosome.

In the ordinary form the centrosoma lies at one end (the posterior) of the body, a considerable distance from the nucleus ; in the other rarer form it is quite near the nucleus, and is occasionally seen attached to it, or lying actually on it.

The length varies from 20 to 70μ and the breadth from 2 to 6μ . The longest are usually found amongst the ordinary forms, the broadest under the rare forms.

Laveran, to whom specimens were submitted, considers the trypanosomata of two distinct species and has described them under the names of *T. Theileri* and the rarer one as *T. Transvaalensi*, but Theiler injected blood containing the rare form and produced the ordinary form, hence this observer interprets his results as proving that the trypanosoma is polymorphic. The mode of reproduction of the ordinary form is longitudinal ; the protoplasmic body of the rarer forms appears to have a spherical or oval shape in which posterior and anterior ends are hardly discernible. It is impossible to discover whether such forms represent a longitudinal or a transverse division. Abnormal forms are commonly met with which must be explained either as degenerate or mutilated parasites seeming to be of very fragile structure.

These trypanosomata are only infective for cattle. Mortality, 8 out of 40 inoculated, or 12·5 per cent. The incubative period varies according to the number of trypanosomata which the infective blood contains, average four to six days ; when 1 c. c. was injected, the period was 18 days ; when 50 to 100 c. c., five to six days. The length of time the flagellates are present in the blood varies considerably, and is dependent upon the refractory condition the animal presents against the trypanosoma. The longest period was thirteen days, the average nine days.

Through the kindness of Mr. H. E. Cross, the second Assistant Bacteriologist, who has placed at my disposal, for examination, several of his microscopical specimens of blood

containing the *T. Theileri*, I have been enabled to make a careful study of these parasites, and compare them with the *T. Himalayanum*, as found in this country. As a result, I may mention that three forms of flagellates have been observed in these specimens :—

(i) A form corresponding to the *T. Theileri*.

(ii) A smaller variety, which may correspond to the rarer form of Theiler.

(iii) A long narrow form with a long flagellum, differing essentially from either form of the *T. Theileri*.

The measurements, etc., of the *T. Theileri*, as noted by me in the S. African specimens, were as follows :—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Maximum ...	10·66	13·12	2·62	27·88	13·12	67·40	2·46
Minimum ...	6·56	3·28	3·28	16·40	11·48	41·00	1·64
Mean of 24 trypanosomata ...	9·38	7·86	2·55	23·19	12·68	55·78	2·34
Percentage of the mean ...	16·82	14·28	4·58	41·58	22·74	100·00	4·21

In the same specimens of blood a considerable number of trypanosomata were observed, corresponding to the broad involution forms of *T. Himalayanum*, the endoplasm of which were full of micro-granules (Pl. II, Figs. 25—26), which have been described as females. The maximum length was found to be 59·04 to 60·68μ, and in addition many blepharoplasts with attached flagella were observed lying loose in the plasma, varying in length from 45·92 to 62·32μ, thus indicating that the longer ones originally belonged to parasites exceeding in dimensions the ones already described.

The smaller variety of *T. Theileri* encountered, measured from 30·34 to 39·36μ in length and from 1·96 to 2·46μ in breadth. This flagellate was found in much smaller numbers than the larger form. A third variety encountered in the same specimens of blood, not described by *Theiler*, was a narrow form of flagellate, extreme length 45·10μ, which exhibited a posterior extremity tapering to a fine point, a large blepharoplast, long oval nutritive-nucleus occupying the

whole width of the body, and a free flagellum, twice the length of that possessed by the *T. Theileri*. The mean measurements and percentage of the mean measurements of the latter parasites were as follows :—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Mean measurements ...	6·56	4·10	3·28	11·48	19·68	45·10	1·14
Percentage of mean ...	14·54	9·09	7·27	25·45	43·65	100·00	2·53

DIAGNOSIS BETWEEN *T. Himalayanum* AND *T. Theileri*.

On comparing these two forms of trypanosomata one with the other, one observes there is a marked similarity in their contour and general characteristics. The *T. Theileri* more frequently, however, assumes complete or open circles when at rest, and appears somewhat shorter and narrower than the *T. Himalayanum*. Another marked characteristic present in the S. African variety, but absent in the Indian, is the alveolar structure of the endoplasm ; this was especially well marked in the posterior portion of the original organism depicted (Pl. III, Fig. 7). The so-called females assume the same swollen forms in the peripheral blood as those discovered in bull No. 3951, but in the same specimens of S. African bovine blood I have been unable to discover flagellates devoid of micro-granules, pointed out previously as male forms.

On reference to the individual arbitrary measurement (percentage of mean measurements) of the different parts of the body, the greatest difference is observed to be in that lying between the blepharoplast and the posterior edge of the nutritive nucleus :—

Percentage of mean measurements of the T. Himalayanum and the T. Theileri.

	I	II	III	IV	V	VI	VII
T. Himalayanum	16·39	17·54	3·97	40·32	21·72	100·00	5·13
T. Theileri ...	16·82	14·28	4·58	41·58	22·74	100·00	4·21

The percentage of the mean showing a difference of three and a half per cent. in favour of the Indian trypanosoma, and about one per cent. in each of the three anterior measurements, otherwise it is extraordinary that the figures should approximate so closely one with another. One is necessarily obliged to come to the conclusion that these two flagellates, the hosts of which are the bovines of India and S. Africa respectively, are simply varieties of one and the same, or nearly allied species of trypanosomata.

A further fact connecting the two varieties of trypanosomiasis, occurring in the blood of cattle indigenous to countries so widely separated is the presence of large ovoid cells (Plate III, 5 a, b, and 10 e) 8·20 to 9·0μ in length which exhibit a hyaline structure and contain a considerable number of darkly staining bodies (dark-purplish red after Romanowsky) single or united in couples resembling large micro-cocci and diplo-cocci which seem to multiply by gemmation. The circular forms measure from 0·6 to 1·0μ. The cells burst and discharge their contents, and when the bodies become free, they appear in some instances to increase in size somewhat, before respectively coming to rest in the red corpuscles, where they undergo further changes. In a future paper, to be published shortly, it is my intention to bring forward the results of a considerable number of observations concerning these forms of cells, but in the meantime I may mention that I have not found any evidence pointing to the fact that these bodies form any link in the chain of developmental changes of the *T. Himalayanum* or *T. Theileri* respectively.

III.—(T. Indicum.)

It will have been noted that Durrant and Holmes discovered the species of mature trypanosoma described by them in hill bulls at Muktesar. During the present year we were fortunate enough to come across the same species of flagellate in a plains bull, which was immunized against rinderpest. As the parasite just previously described has up to the present only been observed in the blood of *hill* cattle, and has received the name *T. Himalayanum*, I propose to give the name of *T. Indicum* to the one described by the

above observers, as it has now been recognized in the blood of bovines of both the hills and the plains. In this paper I shall therefore refer to this species of flagellate as the *T. Indicum*.

Plains Bull No. 4428, aged 3½ years, body weight, 288 lbs., was brought up to Muktesar from the Terai, on the 13th November 1905. After a period of segregation, the animal was immunized against rinderpest by the so-called simultaneous method; it received 6·7 c.c. anti-rinderpest serum, equivalent to 14 c. c. per 600 lbs. body weight, and 1·0 c. c. of defibrinated virulent blood taken from the jugular vein of a control hill bull on the third day after the initial rise of temperature. A good reaction followed, max. temp. 41·1° C., but no symptoms of the disease were noted. After recovery had taken place, the animal was detained in a segregation kraal for some days before being returned to the herd, and preparatory to hyperimmunization.

Death occurred on the 26th April 1906, without the animal exhibiting any premonitory symptoms. Trypanosomiasis was diagnosed by Mr. G. Dunlop Martin, Assistant Bacteriologist, on microscopical examination of specimens of the blood and spleen. Unfortunately the carcass of the animal was destroyed before the diagnosis had been arrived at; consequently the following observations have been entirely made from the two blood and two spleen smears, which are usually taken for reference at the time of each autopsy, unless previous attention has been drawn to any particular case. The animal in question had been dead for several hours, when the abovementioned specimens for examination were made.

IN THE PERIPHERAL BLOOD.

The flagellate forms met with in the peripheral blood will first be described, and later those discovered in the spleen will be recorded. These particular specimens were stained by the Leishmann-Romanowsky method.

Three forms of trypanosomata were observed in the peripheral blood.

(*a*) With long, somewhat narrow bodies. (Plate II, Figs. 1, 2, 3.)

(*b*) Parasites with swollen bodies or portions of their bodies distended, together with various involution forms. (Plate II, Figs. 17, 18, 19.)

(*c*) Very long, narrow serpentine form. (Plate II, Fig. 15.)

(*a*). This species of trypanosoma is readily recognizable by their total length, as also by the length of their flagella, and shape of the posterior extremity which in the mature forms from being fairly wide, rapidly diminish in width to a point. The blepharoplast in such flagellates is large, oval, occupying one-half of the width of the body at that situation. The nutritive nucleus is situated at about the junction of the anterior two-thirds with the posterior third of the body protoplasm, and the undulating membrane is not gauffered. Three different varieties of these species of flagellate can be made out in the peripheral blood.

(i) One in which the width of the posterior extremity is narrow and the space entirely occupied by a large round form of blepharoplast, the nutritive nucleus is compact and the endoplasm stains blue. Micro-granules are in the majority of instances absent, or only one or two of fairly large size are present in the anterior portion of the body plasma.

(ii) In the second form the posterior extremity is broad and quickly attains to a point, the blepharoplast oval and only occupies a portion of the width of the body cavity where it is situated; between the posterior nucleus and the nutritive nucleus several deeply stained granules are visible, while in the anterior portion of the organism a single large micro-granule is observed. The endoplasm throughout stains blue.

(iii) The third form is the most noticeable, for the endoplasm in the majority of instances takes only a faint blue colour, being dappled throughout with white areas of considerable size. The anterior portion of the flagellate body is more or less dotted throughout with micro-granules, while the posterior portion exhibits but very few. In this variety of trypanosoma the blepharoplast also is oval in form, but only occupies half the width of the body cavity; the undulating membrane being curved but not gauffered. These latter forms would appear to answer to females.

The following measurements include the maximum, minimum, and mean measurements of twenty-seven trypano-

somata together with the percentages of the mean measurements :—

	I μ	II μ	III μ	IV μ	V μ	VI μ	VII μ
Maximum ...	6·56	8·20	1·96	21·32	13·12	51·16	1·64
Minimum ...	1·64	6·56	1·64	14·76	11·48	36·08	1·31
Mean of 27 ...	4·51	7·57	1·91	17·18	12·68	43·85	1·64
Percentage of mean ...	10 29	17·26	4·36	39·20	28·89	100·00	3·73

(*b*). Mixed up with the forms just described are numerous parasites which present swollen or distended bodies, either partially or wholly involved. The posterior extremity, instead of being acutely pointed, has become completely rounded off. There is usually a large number of micro-granules scattered through the endoplasm, but for some distance round the situation of the blepharoplast, the plasma is devoid of these bodies. In some parasites the nutritive nucleus is observed to break up and apparently becomes scattered, for it is difficult in certain instances to make out its exact position. It has been frequently noticed that the deformed condition primarily commences at the posterior extremity of the trypanosoma and gradually extends forward until the whole body has become involved. Later the undulating membrane undergoes changes which lead to its disappearance, for it no longer can be stained by the usual reagents.

The following measurements refer only to the so-called female forms, which contain many micro-granules, the bodies of which have become swollen and consequently considerably altered in their contour :—

	I μ	II μ	III μ	IV μ	V μ	VI μ	VII μ
Maximum ...	4·92	8·20	3·28	31·16	13·12	60·68	4·92
Minimum ...	4·92	7·38	1·64	16·40	9·84	40·18	3·28
Mean of 20 tryp.	5·09	7·50	2·13	21·56	11·64	47·92	3·43
Percentage of mean ...	10·62	15·65	4·43	45·00	24·30	100·00	7·16

(*c*). Only one example of this attenuated form of parasite was observed in the blood. It measured 54·76μ in length. Unfortunately in the preparation of this blood smear the parasite was injured, its posterior extremity being broken, so that the dimensions given do not represent its full measurement. The endoplasm is devoid of micro-granules, but the blepharoplast and nutritive nucleus both appear to be undergoing division. A number of somewhat similar but intact forms were discovered in the parenchyma of the spleen ; these will be described subsequently in this paper.

In the Spleen. The trypanosomic forms discovered in the spleen comprised—

(*a*) A broad form of trypanosoma with well-marked flagellum. (Pl. II, Fig. 5.)

(*b*) A large form of parasite, without any flagellum. (Plate II, Fig. 14.)

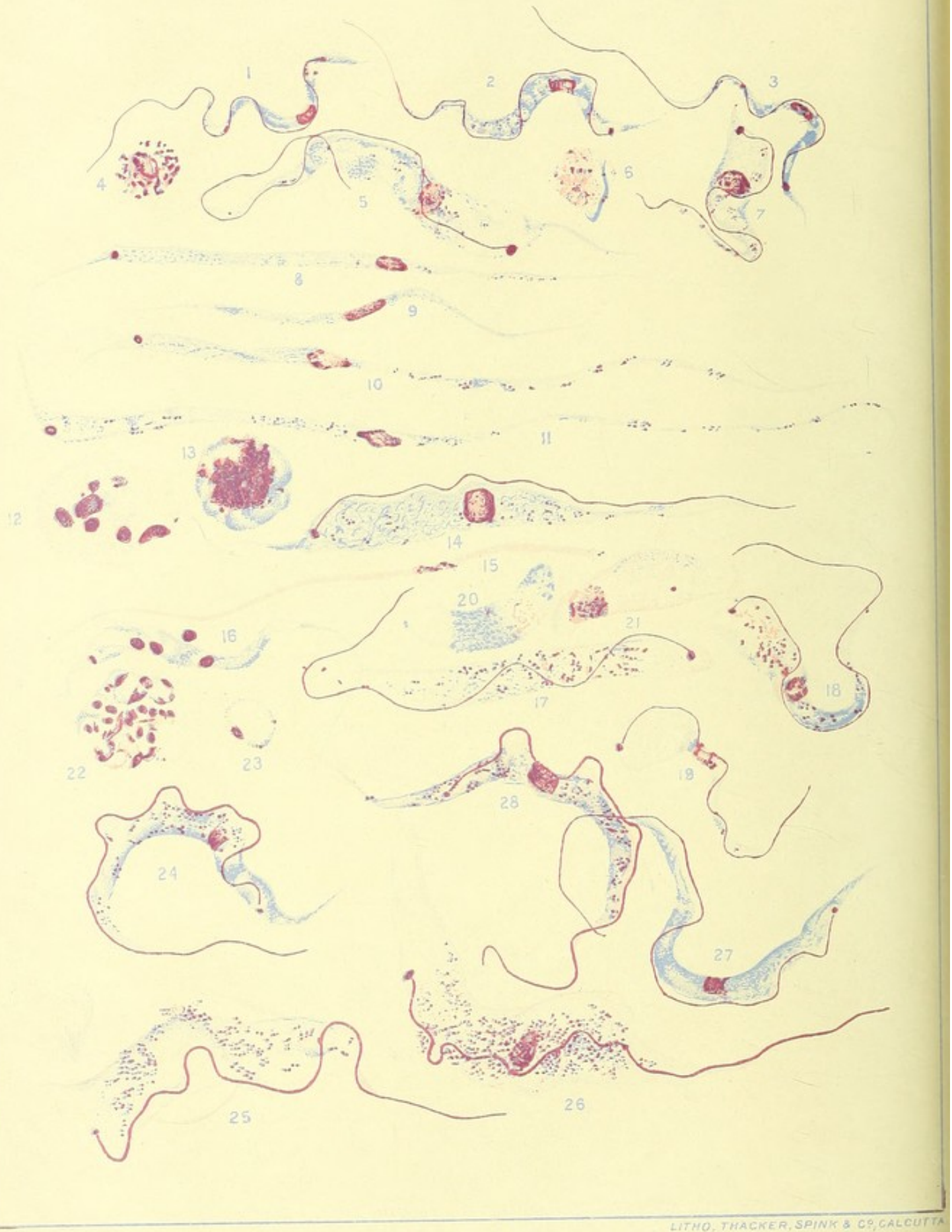
(*c*) Involution forms of trypanosomata.

(*d*) Elongated or attenuated forms, exhibiting micro-granules. (Pl. II, Figs. 8, 9, 10, 11.)

(*a*) A trypanosoma with fairly long posterior extremity which tapers to a point, the body is broadest, just anterior to the situation of the blepharoplast, slightly narrowing until the junction of the anterior quarter with the posterior three-quarters is reached. It then quickly becomes narrower, until it terminates where the free flagellum commences. The blepharoplast which is circular is situated close to the peri-plast and only occupies about $\frac{1}{5}$th of the diameter of the body at this situation. The nutritive nucleus situated a little posterior to the centre of the parasite is oval and lies in the transverse axis of the body. A few micro-granules are dotted through the endoplasm, especially anteriorly. The length of the free flagellum is about one-fifth of the total length of the organism.

A smaller example than the former (Pl. II, Fig. 7), exhibits a much larger nutritive nucleus for its size, and a short and stumpy free flagellum.

(*b*) A flagellate very much resembling (*a*) is represented (Fig. 14). This, however, possesses a shorter posterior extremity and a more massive nutritive nucleus, but no free flagellum, for the body protoplasm extended in the original,



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the whole length anteriorly. Its measurements were, total length, 58·86μ and breadth 5·4μ.

A single trypanosoma (Pl. II, Fig. 16) was also observed in the spleen, whose protoplasm stained entirely blue, with the exception of the nuclei. This is particularly mentioned as it presented four nutritive nuclei, one dislocated, but only one blepharoplast undergoing division. The measurements of twenty-one parasites described under (*a*) were as follows :—

	I μ	II μ	III μ	IV μ	V μ	VI μ	VII μ
Maximum ...	4·92	9·84	2·46	29·52	13·12	29·86	3·28
Minimum ...	3·28	8·20	2·46	18·04	3·28	35·26	2·46
Mean of 21 tryp.	3·98	8·86	2·31	22·63	8·32	46·10	3·25
Percentage of mean ...	8·63	19·22	5·01	49·10	18·04	100·00	6·54

(*c*) A few involution forms of female trypanosomata were observed, as figured (Pl. II, Fig. 2), and described above in the peripheral circulation, but these were shorter, maximum length 28·60μ, the breadth varying from 1·1μ anteriorly to 2·0μ posteriorly. The shape these forms assume, resembled somewhat the appearance of scimitar. The blepharoplast in one parasite had undergone division into two distinct nuclei, and occupied a clear space near to the posterior extremity, while the larger and compact nucleus, oval in form and 2·0μ in length, lying in the long axis of the organism, occupied a position at the junction of the posterior third with the anterior two-thirds. The rest of the body of the parasite was occupied by the endoplasm, which stained a faint blue colour, and contained a thickly set mass of micro-granules.

(*d*) These long or attenuated forms (Pl. II, Figs. 8—11) observed by me for the first time, lying free in the parenchyma of the spleen, presented a weird appearance, resembling snakes, threading their way through the corpuscles and cells. I was able to trace in a few instances the gradual suppression of the undulating membranes by their respective stained margins, proceeding from the smaller nuclei for a very short distance anteriorly. They were, however, but faintly marked

and appeared as fine red lines, possibly on account of the tension they had been subjected to. The majority of these attenuated trypanosomata exhibit both kinds of nuclei, but they are widely separated. In one form depicted (Fig. 9), which was found lying in close proximity to the others, only a long nutritive nucleus could be observed, the smaller one being wanting. The length of this one was 54μ, breadth 1·26μ. In some specimens of the attenuated form there appeared to be a fine line, stained red, running from the blepharoplast to the nutritive nucleus, and a well marked line, but less distinct, running from the same nucleus anteriorly. Several circular or ball forms of this parasite were noted, which respectively exhibited the posterior extremity of the flagellate protruding from one side and the fine tapering extremity on the other. It will be seen on reference to the detailed measurements of these parasites given below, that the maximum length of this form was 118μ, while the minimum was only 48·06μ. I was unable to satisfy myself whether these attenuated forms, as observed in the spleen smears, and one example in the peripheral blood represent a distinct species of trypanosoma or are an involution form of the *T. Indicum.*

More probably the latter, for the suppression of the undulating membrane and free flagellum has constantly been observed by Schaudinn in certain changes in the *T. Noctuæ.*

Measurements of the elongated trypanosomic forms observed lying free in the parenchyma of the spleen.

	I μ	II μ	III μ	IV and V μ	VI μ	VII μ
(i)	10·80	25·20	3·60	57·60	97·20	2·16
(ii)	9·00	18·00	5·40	39·60	72·00	2·16
(iii)	14·40	34·20	4·50	65·70	118.80	2·16
(iv)	4·50	15·30	3·60	39·60	63·00	1·44
(v)	4·50	16·56	3·60	23·40	48·06	1·08
(vi)	3·60	14·40	3·60	39·60	61·20	1·26
Mean of 6 tryp. ...	7·80	20·61	4·07	44·25	76·71	1·71
Percentage of mean	10·17	26·87	5·28	57·68	100·00	2·23

IV—(T. Muktesari).

The trypanosoma to be now described was discovered in a Hill Bull under exactly similiar circumstances to those previously described under No. II.

Short notes of the case are as follows :—

Hill Bull, No. 5112, aged 4½ years, body weight 224lbs., was immunized by the simultaneous method with 60 c. c. of A. R. Serum and 0·5 c. c. of virulent rinderpest blood. A reaction followed, max. temp. 40·3°C. On the sixteenth day the animal was subcutaneously inoculated with 2,000 c. c. of virulent blood, reaction, max. temp. 40·5°C. on the twentieth day. On the twenty-ninth day a further inoculation of 2,500 c. c. of virulent blood was made, and a third time a reaction resulted, but on this occasion, the temperature did not register higher than 39·8°C., namely, on the evening of the thirty-second day.

On the morning of the thirty-fifth day the temperature was normal, but later in the day hæmoglobinuria was observed for the first time, and the blood on microscopical examination exhibited the *P. bigeminum*.

Twenty-four hours later trypanosomata were discovered in the peripheral blood, and the animal succumbed to the combined diseases on the morning of the thirty-eighth day after the primary inoculation.

Provisionally, the large form of trypanosoma depicted in Plate III, Fig. 8c, morphological forms of which are illustrated, *a* and *b* will be given the name of T. Muktesari, in order to distinguish it from the others severally described.

In the peripheral blood of this animal several forms of flagellates were met with, which did not conform to those previously described.

(*a*) A large trypanosoma, of considerable breadth (Plate III, Fig. 8*c*), with very short flagellum.

(*b*) A long narrow form with short flagellum.

(*a*) This parasite presents a long posterior extremity and broad body. The blepharoplast is very small for the size of the organism, whereas the nutritive nucleus is large and resembles that figured in the *T. Himalayanum*. The undulating membrane is fairly developed, but its margin is not nearly as well marked as in the above-mentioned flagellate, and does not stain so deeply, while the free

flagellum does not average one-twentieth of the total length of the organism. The following are the measurements and percentage of the mean measurements of 20 of this species :—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Maximum ..	13·12	10·66	2·46	31·16	2·46	59·86	4·92
Minimum ...	4·92	4·10	2·46	11·84	1·64	24·96	1·64
Mean ...	7·13	6·97	2·34	19·00	2·05	37·94	2·45
Percentage of mean ..	19·02	18·59	6·24	50·68	5·46	100·00	6·53

Three examples of trypanosomata were observed (Fig. 8*b*), which respectively exhibited no free flagellum ; the body protoplasm being continuous up to the anterior extremity. The total length of these varied between 38·54 and 46·24μ only. The nutritive nucleus had left its usual position and assumed one in close proximity to the blepharoplast, and in one parasite the smaller nucleus had undergone division, and a second flagellum floated free in the blood plasma. In addition, one example of a small form of flagellate (Fig. 8*a*) was met with, its two nuclei being in apposition, but with a free flagellum measuring one-third of its total length, 36·90μ.

(*b*) This long narrow form of trypanosoma, which somewhat resembles the *T. Himalayanum* in form, but is much smaller, presents other differences also ; the blepharoplast is small and centrally placed instead of close to the periplast, the nutritive nucleus which is oblong is situated midway between the blepharoplast and termination of the body protoplasm anteriorly, the margin of the undulating membrane does not stain deeply, and the free flagellum is only just noticeable. The percentage of the mean measurements of a number of these trypanosomata, which were found to be as follows, also materially differ from those of the above-mentioned species :—

	I	II	III	IV	V	VI	VII
Percentage of mean ...	17·55	35·09	5·26	38·60	3·50	100·00	4·56

It will be observed that none of the parasites found in the peripheral blood of this host exhibit any micro-granules or pigment in their endoplasm, and on comparison with those figured in Plate II, Figs. 5 and 14, the former has a free flagellum equal to nearly one-fifth of its total length, while it is absent altogether in the latter.

V.—(T. Himalayanum and T. Indicum.)

The chief point of interest to be recorded under this heading is the fact that both the species of flagellates, the *T. Himalayanum* and *T. Indicum*, were found in the peripheral circulation of a Hill Bull at one and the same time. The following are short notes of the case:—

Hill Bull, aged 2 years, body weight, 150lbs., was immunized by the simultaneous method with 27·0 c. c. of serum and 0·5 c. c. of virulent rinderpest blood. Eight days later vesicles appeared on the mucous membrane of the tongue, followed by ulcers, which subsequently healed. There was no thermal reaction. On the nineteenth day the animal was inoculated with 2,000 c. c. of virulent blood, a thermal reaction followed, maximum temperature 40·6°C., but by the morning of the twenty-sixth day a normal temperature was again registered. Two days later, however, there was again a rise to 40·0°C. in the evening, and intermittent temperatures were recorded until the twenty-fourth morning when the animal appeared dull, and diarrhœa supervened. Death occurred on the thirty-fifth day. Trypanosomiasis was diagnosed shortly before death.

In the peripheral circulation, as also in the spleen, liver and kidneys, two species of trypanosomata were observed. The various forms were as follows:—

T. Himalayanum—

With, without, and with short flagellum.

T. Indicum—

With a long and with a short flagellum.

As these two species of trypanosomata have been previously described, it will only be necessary to place on record certain points of interest particularly noticeable in the parasites found in this animal.

(*a*) *T. Himalayanum.*

(i) The majority of flagellates observed belonged to this species. A large number were measured, and the following details, with regard to them, may be recorded:—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Maximum ...	13·12	11·48	2·46	26·24	13·12	66·42	2·95
Minimum ...	6·56	9·02	2·13	21·32	9·84	48·87	2·46
Mean ...	9·84	9·92	2·34	24·02	12·79	58·91	3·02
Percentage of mean ...	16·71	16·84	3·97	40·77	21·71	100·00	5·13

(ii) A variety of this species was found in small numbers. They are shorter than the aforementioned parasites, and are distinctive in that they have no free flagellum. Their measurements were found to be as follows:—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Mean ...	8·20	8·47	3·01	26·79	0·00	46·47	2·73
Percentage of mean ...	17·65	18·23	6·47	57·65	0·00	100·00	5·87

(iii) A second variety found in small numbers, were specially noticeable on account of the extremely short free flagella they exhibited. Their total length was almost equal to the mean of the large variety, and they may simply be trypanosomata which present the results of a stunted growth.

(*b*) *T. Indicum.*—Two forms of parasites were particularly noticeable, one with free flagellum with a mean length of 11·07μ, while the other was possessed of a stumpy short one about 1 to 2μ in length. But few swollen trypanosomata undergoing involution were observed in this animal, and those contained but a small amount of pigment. Quite a number of parasites showed reduplication of their smaller or posterior nucleus; these in every instance were lying side by side in the transverse axis of the organism. In several instances the nutritive nuclei were observed to be enlarged, and the chromatin arranged in definite order round the cell, like the numbers on the face of a clock. Other organisms were observed to have undergone considerable broadening in

the centre of the body, while the anterior and posterior extremities were bent at right angles. The blepharoplast which was compact and circular in contour was exceedingly large, occupying the whole breadth of the wide posterior extremity and exhibited a dark red colour. The nutritive nucleus had undergone division into two ; these were large and oval in form, lay side by side, one above the other, in the transverse axis, but only occupied respectively half the breadth of the body of the parasite.

In addition, several immature forms of flagellates, presenting the appearance of a shield, were noted; these exhibited a fair sized circular blepharoplast and a large nutritive nucleus in each instance situated a short distance apart, and in contact with the edge of the cell.

The following are the measurements of a large number of mature single forms of the *T. Indicum* :—

	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Maximum ...	4·92	8·20	2·95	21·32	13·12	50·51	2·46
Minimum ...	3·28	3·28	2·13	8·20	4·92	21·81	1·14
Mean ...	3·85	5·27	2·47	13·61	1	36·27	1·85
Percentage of mean ...	10·61	14·53	6·81	37·52	30 52	100·00	5·01

Modes of Reproduction.

The diversity of the forms of trypanosomata here observed, renders it a most difficult problem, especially in the absence of fresh specimens, to follow up the distinctive phases of the various flagellates in their metamorphoses in the blood and organs of the affected host. It brings to mind the words of Schaudinn, when describing the changes in the *T. Noctuæ* : "There are males and females more or less well nourished ; according to their origin, one may be old, the other young ; they may have originated, some asexually by simple fission, others by means of parthenogenesis ; in short, I am convinced that the cause of the variability of the germinal cells, and therewith the differentiation of the sexes in these seeming simple organisms, are represented by the combination of factors as complicated and manifold as is the case in the highest organisms.''

I may now describe the various modes of reproduction in the various forms of trypanosoma encountered in different species of trypanosomata.

A. *T. Evansi.*—The first form of trypanosomiasis in hill bovines described in this paper appears to have as a 'causal agent' the *T. Evansi* only, as previously mentioned. This hæmatozoon reproduces itself in the circulation of the host by longitudinal fission, for the most part into two, less frequently into three, and very rarely into four individuals. As a rule, the blepharoplast divides first, and later the nutritive nucleus.

In addition, as observed and described by Prozawek in *T. Lewisi* and *T. Brucei*, auto-synthesis of the karyosoma, reduction and parthenogenesis may be occasionally met with in different parasites.

B. *T. Himalayanum.*—Theiler pointed out that the usual mode of reproduction of the parasite called after him, was by longitudinal division. The centrosoma dividing first, followed by splitting of the flagellum. In other forms he states that it was impossible to discern whether they followed a longitudinal or a transversal division ; however, the flagellate may be distinguished at once from any small forms of trypanosomata by its larger size and polymorphic character.

During my observations, although I have met with trypanosomata in which the blepharoplast was in course of division, and the large nucleus had already divided into two, nevertheless, in no instance have I found in *T. Himalayanum*, either in the peripheral blood or in the organs, any parasite exhibiting longitudinal division in a more advanced stage than those above mentioned,—forms so frequently seen in the blood of animals, the subjects of *T. Evansi.* As the specimens of blood, etc., examined by me were collected just before or after death, an explanation may be forthcoming in the fact that schizogony might be more or less suppressed a short time previous to the death of the host.

C. T. Indicum.—In the peripheral blood of Plains Bull No. 4428, many most interesting forms were met with, which really require the knowledge of an expert proto-zoologist to do justice to their description and elucidation. In the first place, those trypanosomata (Pl. II, Figs. 17, 18, 19), designated females ? on account of their endoplasm containing a large

number of micro-granules and reserve material, become enlarged throughout their entire length, or as a preliminary step, the posterior portion of the body first increases in size, then gradually the whole body becomes similarly altered. The chromatin of the nutritive nucleus breaks up, and this is followed by suppression of the flagellate apparatus. Co-existent with these various forms, one observed—

(*a*) Numerous vermicule-like bodies, 14·76 to 21·50μ in length by 4·10 to 4·92μ in breadth. The endoplasm recedes from the posterior extremity (Pl. III, Fig. 1, *c* to *o*) for a distance of one-fourth to one-fifth of the length of the parasite, leaving only the ectoplast stained a faint pink colour. The coarse pigment granules, which were previously scattered through the endoplasm, now collect in a more or less compressed mass towards the anterior extremity of the gregarine-like form the organism assumes. The remaining four-fifths of the body cavity is occupied by protoplasm of a coarse granular character which stains either a dark or a fainter blue colour, except at the edge where only the ectoplasm is visible. A little posterior to the middle of the parasite the smaller nucleus may be observed as a circular dot surrounded by a halo, while in a similar situation in other organisms collections of chromatin possessing from 5 to 11 elements may be recognized.

(*b*) In addition circular bodies (Fig. III, *a-b*) 8·20μ in diameter for the most part, but measuring up to 9·84μ are come across, the most marked feature of which is a somewhat circular or oval mass of pigment granules lying in some instances near to the periphery of the enveloping membrane and overshadowing a collection of chromatin, while on the opposite side of the cell one or two small collections of chromatin granules separated from each other by a short interval may generally be discerned. In one circular body the chromatin occupied a position a short distance from the periphery of the cell, while directly in front of it, what appeared to be a well-marked cone of reception was visible.

(*c*) The bodies (Pl. III, Fig. 1, *e*) would appear to illustrate the oökinet-like forms, in process of formation of a special appendage for the purpose of getting rid of the pigment and a portion of the residual body, by constriction of the posterior

part of the body, as pointed out by McCallum in the *Halteridium* in birds, and by Schaudinn in the metamorphosis of male, female and indifferent oökinets in *T. Noctuæ*. In the same specimens and in addition to the forms just mentioned, crescent-shaped bodies were encountered 14·76 to 16·40μ in length by 4·10 to 4·92μ in breadth, almost symmetrical in contour, with rounded extremities, one being free from endoplasm, while a short distance from the other a large circular open nucleus 3·28 to 4·92μ in diameter, composed of a number of chromatin particles, was situated. This form of organism exhibited few, if any, micro-granules in its endoplasm, but the protoplasm was coarsely alveolar in structure.

(*d*) I have on several occasions observed crescent-shaped bodies, resembling those of *Laverania malariæ*, in the blood of cattle in Poona, in Surra Bull I during the year 1892, and also in Karnal bullock No. 13 in 1895, the contours of which resembled those depicted in Pl. II, Fig. 20 ; and in each instance the animal was the host of a large species of trypanosoma. *Holmes*, as previously noted, observed numerous large crescent forms in the blood taken from two infected bulls. These bodies stained blue and contained numerous chromatin granules. In each instance in the illustrations furnished, these forms are more or less rounded at one extremity, while the other is somewhat narrower and pointed.

Hunt, quoted by Minchin, "found crescents in the blood of cattle (Texas fever) and has observed their change into a spheroidal shape, but while comparing these bodies to the crescents of the malarial parasites, he at the same time regards them as a form of sporulating body producing spores endogenously having mistaken the coarse granules in them for minute spores." These crescent forms, above described, are frequently co-existent with the *Piroplasma bigeminum*, or the smaller form in the blood of Indian cattle. Possibly the presence of the crescents in Queensland bovines may point to the fact that these animals in some instances are also the hosts of a large form of trypanosoma, which up to the present has not been demonstrated in their blood. *Prowazek* remarks in connection with his work on the Trypanosoma Lewisi that in general these forms are not very frequently met with in Säugetiere. They are directly

comparable with Herpetomonas and bird-trypanosomata, and point to the fact that the cell possesses at one and the same time hermaphroditic characters, and sometimes one and sometimes the other of the two nuclei is prepotent. The same observer goes on to say, "Bei *Herpetomonas* wurde gelegentlich der Besprechung dieser Fälle, die dort sehr kompliziert sind und die Darstellung erschweren, darauf hingewiessen, dass dort gleichsam zwei Formationen des Protoplasmas vorkommen und zwar ein dunkles reservestoffrieches Protoplasma mit einem grossen Nährkern und ein blasses, reservestoffarmes Protoplasma,—auch hier wurden derartige Bilder beobachtet; die Involutions formen mit dem blassen, lichtblau sich färbenden Protoplasma und dem dunklen, massigen Kern (männlicher charakter) tragen zumeist mehr oder weniger deutlich ausgeprägte Degenerations-stigmata zu Schau und dürften bald zugrunde gehen." So that if the involution forms follow the same course as those observed in the Herpetomonas, the crescent-shaped bodies exhibiting characteristics of the male usually degenerate.

(*e*) In the same specimens of peripheral blood one found large cells (Pl. III, Fig. 4), two in close apposition, respectively exhibiting only a very fine limiting membrane. In the smaller of the two cells eight large oval nuclei may be observed fairly well differentiated, and in addition a much smaller sized one at the left upper border. They stain deeply by the Leishmann-Romanowsky method, while in the larger cell the nuclei are much less distinct, and their number cannot be made out with certainty, as there appears to be a certain amount of residual material covering them. It may be of importance to note that the number of nuclei, eight, coincides with the number of chromosomes in the nutritive nucleus of a trypanosoma found in the same blood and depicted (Fig. 3, *b*).

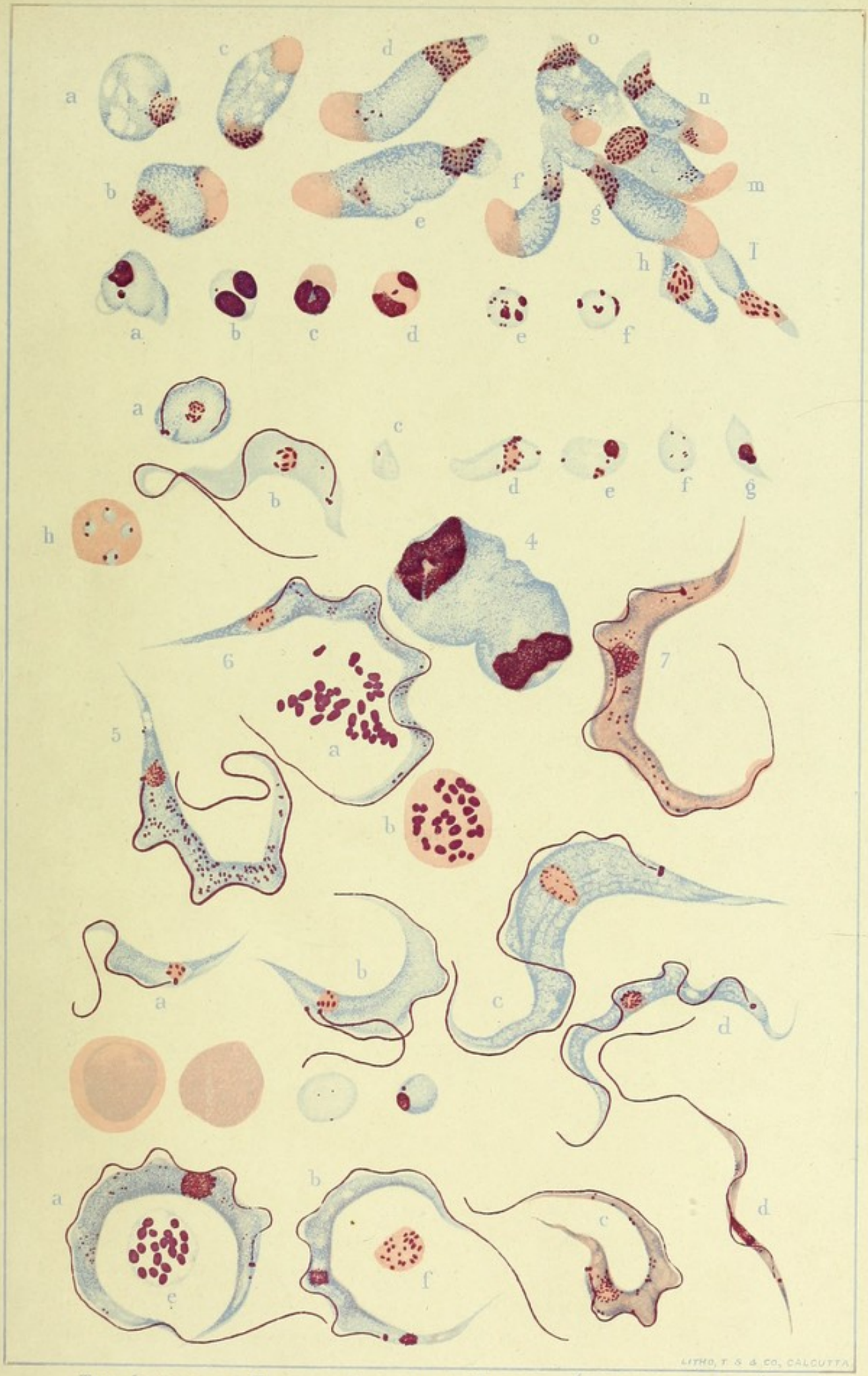
(*f*) Further, a large number of immature trypanosomic forms were encountered (Pl. III, Figs. 2 & 3), representing the pyriform, circular and irregular-shaped immature forms of flagellates, for the most part exhibiting two large nutritive nuclei and either one or two blepharoplasts in each cell. These latter found in the circulation would appear to have been set free in some instances, by the bursting of large circular bodies (Pl. II, Fig. 22), replete with immature forms found only in the spleen.

(*g*) Two other involution forms were also met with in the spleen (Pl. II, Figs 12, 13). The former somewhat reminds one of the stage of degeneration of the blepharoplast in parthenogenesis of Herpetomonas, as described by Prowazek.

The latter body surrounded by a fine limiting membrane, the contents of which are more or less obscured by a mass of residual material can, nevertheless, be made out to contain irregular-shaped bodies arranged around the periphery of the cell. Each contained unit respectively exhibits one or more circular nuclei.

(Pl II. 6) (*h*) Certain bodies were discovered lying free in the parenchyma of the spleen, circular in form, about 5μ in diameter surrounded by an exceedingly fine limiting membrane. No very definite structure could be made out with regard to the contents of such cells, but elongated bodies, exhibiting one or more nuclei, could be observed in some instances. In one cell met with, however, an organism exhibiting the contour and special characteristics of a small trypanosoma was clearly observed, a posterior extremity, with a clearly defined blepharoplast, a wide clear space, then a nutritive nucleus, which appeared to have undergone division. Anteriorly the minute parasite was curved upon itself and no undulating membrane or free flagellum could be made out. Some weeks after this observation had been made, May 7th, 1906, two officers of the Institute für Schiffs und Tropenkrankheiten, Hamburg, Dr. F. Füllerborn and Dr. Martin Mayer, visited Muktesar at the instigation of the late Dr. F. Schaudinn, and Dr. Mayer, after examining the microscopical specimen in question, confirmed my view that we had to do with a body in which a typical trypanosomic form was contained. If this form of cell be regarded as an oöcyst, then the thinness of its envelope is a marked feature, which permits it to absorb nutriment like a gregarine.

Now, according to the usual course of events, the zygote resulting from the fertilization should penetrate the cells or tissues and should come to rest, becoming encysted as an oöcyst. This mode of procedure has not been seen in the higher vertebrates up to the present, but Minchin observes that "in a great many instances amongst *Hæmosporidia* of the



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lower vertebrata, sporogony as well as schizogony occurs in the vertebrate host. In the case of Lankesterella of the frog Hentzl has shown that the motile zygote leaves the blood to encyst in an epithelial cell of the gut and that the resistant cyst so found passes out with the fæces.'' It still remains to be proved, but one can hardly imagine, that such a course would be followed in the higher vertebrates, but in such animals ''sporulation might be free, that is extracellular, especially in the spleen pulp.''

After examining the specimens, which unfortunately were few in number, one is led to the belief that the changes observed point to the fact that we have to do with forms representative of an endogenous and an exogenous mode of reproduction. It would appear that after a number of endogenous generations have been accomplished, a limit of propagation is reached, and shortly before death of the host, the parasite prepares itself in addition for sporogenous changes.

Nothing definite with regard to the presence of microgametes can be determined until an opportunity offers for examining fresh specimens, and following up in detail the various changes which each undermined trypanosomic form may undergo, when submitted to close observation.

I would particularly draw attention to the value of the metrical method of measurement for trypanosomata, as a means of diagnosis as to the known species of flagellates met with in any particular host. It must, however, be borne in mind that the measurements of a number of parasites should be made on each day of a paroxysm of the disease, and the mean measurements, calculated for the whole number dealt with, before anything like an accurate estimate for comparison can be obtained. Trypanosomata do not, as a rule, attain their mature proportions for a period of three days from the commencement of the paroxysm.

One field of research for biting flies is in the submontane tracts and valleys between the outer Himalayas and the snow mountains. All the small breed of cattle used at Muktesar are collected from Kumaon and Western Nepaul. A number of bulls have now been found to be the subjects of one or other forms of trypanosomiasis, and all such hosts have beer brought from the above-mentioned regions.

Note.—Just before going to press I have had my attention drawn, thanks to Dr. F. Mott, F.R.S., to the interesting paper of Dr. Antoine Pricolo, in a recent copy of the Centralblatt für Bakteriologie, in which this observer illustrates certain minute oöcysts found in the blood of mice (*Mus musculus*), the previous phases of development of which ought to have included conjugation of gametes. This observation in mice tends to confirm those made by me with regard to a sporogenous cycle of the *T. Indicum* in bovines.

Explanation of Plates.*

Plate I.

T. Evansi from peripheral blood of Hill Bull No. 5044, on each day of the primary and only paroxysm up to time of death.

June 20th. First day of paroxysm. Endoplasm blue in colour, posterior extremities of flagellates short. One corpuscle contains piroplasmata.

„ 21st. Second day of paroxysm. Endoplasm of majority of flagellates stained purple; individual parasites exhibit long posterior extremities although majority are short.

„ 22nd. Third day of paroxysm. About 100 in a field. Endoplasm of majority of organisms stained a reddish hue, although less mature forms exhibit a faint blue colour. (*a*) female forms.

„ 23rd. Fourth day of paroxysm. Over 100 in a field. Majority of parasites exhibit a red colouring of their endoplasm. Posterior extremities short.

„ 24th. Fifth day of paroxysm. Eleven in a field. Further differentiation of endoplasm observed. One or more areas stained red. All posterior extremities short. (*a*) gregarine or vermicule-like form.

Plate II.

Figs. 1, 2, 3. *T. Indicum* from peripheral circulation of Plains Bull No. 4428.

* All magnifications × 1500 diameters

Figs. 4 and 6. Observed in spleen, in some numbers.
Fig. 4. Chromosomes in free nutritive nucleus.
„ 6. Oöcyst, exhibiting sporozoite ?
„ 5. Large broad form of trypanosoma observed in spleen only, furnished with long flagellum.
„ 7. Small form of trypanosoma observed in spleen only.
Figs. 8, 9,10, 11. Elongated trypanosomic forms found in the spleen.
„ 12, 13. Involution forms from peripheral blood.
Fig. 14. Large broad form, absence of free flagellum. Protoplasm extends whole length anteriorly.
„ 15. Long and attenuated form, found in peripheral blood, posterior extremity injured. Both nuclei undergoing process of division.
„ 16. Trypanosoma observed in spleen, four nutritive nuclei, one unattached, dislocated, and lying free.
Figs. 17, 18, 19. Probably female forms from peripheral blood. Bodies swollen ; endoplasm contains more or less pigment and reserve material.
Fig. 20. Crescent or bean-shaped form, peripheral blood, replete with reserve material and large open nucleus.
„ 21. Gregarine or oökinet form, exhibiting massing of pigment granules at one extremity. Peripheral blood.
„ 22. Cell surrounded by limiting membrane, containing immature trypanosomic forms.
„ 23. Immature circular body, contains two blepharoplasts and one large nutritive nucleus.
„ 24. *T. Himalayanum* from peripheral blood of Hill Bull No. 3951. Female form.
Figs. 25 & 26. Swollen forms of *T. Himalayanum* from peripheral blood. Large number of microgranules.

Fig. 27. *T. Himalayanum.* Male form? Absence of micro-granules. Faint colouring.

„ 28. Ditto from peripheral blood of Hill Bull No. 5078.

Plate III.

Fig. 1. *T. Indicum. a-b*, circular forms, *c-o*, gregarine or oökinet forms from blood of No. 4428, peripheral blood.

„ 2. Immature forms, from peripheral blood.

„ 3. (*a*) Immature form, exhibiting two nuclei and flagellum.

(*b*) Trypanosoma with nutritive nucleus exhibiting eight chromosomes.

(*c-g*) Immature forms; (*h*) erythrocyte containing four piroplasmata, each undergoing division.

„ 4. Two large involution forms, one contains eight large nuclei; mass of residual material cloaks the contents.

„ 5. *T. Himalayanum*, from peripheral blood of Hill Bull No. 5078. Two nuclei in apposition posteriorly. (*a*) Group of deeply stained bodies which were discovered free in the plasma No. 5112. (*b*) Cell in peripheral blood of No. 5078, containing some 26 deeply stained bodies.

Figs. 6, 7, 10. *a, b, c, d. T. Theileri* observed in the peripheral blood of a S. African bull.

Fig. 6. Nuclei in close apposition.

„ 7. Nuclei distance apart; endoplasm closely alveolar.

„ 10. (*b*) A second mass of chromatin posterior to blepharoplast.

„ 10. (*d*) Long thin form in S. African blood, with long flagellum.

„ 8. Trypanosomata discovered in the peripheral blood of Hill Bull No. 5112.

„ 8. (*c*) Large trypanosoma of great width, and only very short free flagellum.

Fig. 8. (*a*) Small trypanosoma, young form.

8. (*b*) Small form, with two blepharoplasts absence of free flagellum.

8. (*d*) Long thin form of trypanosoma, blepharoplast single, nutritive nucleus exhibiting chromosomes.

„ 9. (*c*) (*d*) Immature forms of trypanosomata from Bull No. 5112.

„ 10. (*e*) Large cell containing some 19 deep-staining bodies from the peripheral blood of S. African bull. Similar to 5 (*a*) and 5 (*b*).

„ 10. (*f*) Oval form of cell, resembles free nutritive nucleus.

Bibliographical References.

Lingard, A. ... Report on Surra. Vols. I and II. (Parts i and ii). 1893–1899.

Lingard, A. ... Report on Surra, Filariasis, etc. Karnal, 1895.

Durrant and Holmes. A Trypanosoma found in blood of Cattle in India, *Journal of Comp. Pathol. and Therap.*, p. 209.

Holmes ... Trypanosomiasis among Cattle of India. Ibid, p. 322.

Theiler, A. ... A New Trypanosoma, and the disease caused by it. Ibid, p. 193, 1903.

Minchin, E. A. ... The Sporozoa. Part I. A Treatise on Zoology. Edited by Ray Lancaster.

Prozawek, S. ... The Development of Herpetomonas, a flagellate related to Trypanosoma. Arbeiten a. d. Kaiserlichen Gesundheitsamte. Heft III, Band Vol. XX, 1904.

Prozawek, S. ... Studien über Säugetier Trypanosomen. Ibid, Band XXII.

Schaudinn, F. ... Generations und Wirtwechsel bei Trypanosome und Spirochæte. Arbeiten a. d. Kaiserl Gesundheitsamte. Band. Vol. XX. Heft 3, 1904.

Lingard, A. ... A new species of Trypanosoma found in the blood of rats, together with a new metrical method of standardizing the measurements of Trypanosomata. *J. Trop. Vety. Science*, January, 1905.

PERCENTAGE OF MEAN MEASUREMENTS OF VARIOUS SPECIES OF TRYPANOSOMATA FOUND IN BOVINES, TOGETHER WITH THOSE OF SEVERAL OTHER SPECIES FOUND IN MAN AND ANIMALS FOR COMPARISON.

Description of Trypanosomata.	I.	II.	III.	IV.	V.	VI.	VII.	REMARKS.
T. Evansi (spontaneous in Hill Bull)	7·38	26·24	9·16	41·80	15·42	100·00	5·93	Pl. I, various days. Mean of 80 tryps.
Do. (spontaneous Equine Surra)	6·82	28·31	11·87	39·85	13·15	100·00	6·14	Mean of 126 tryps.
Do. (spontaneous Equine passed thro' Hill Bull)	7·48	26·39	8·58	42·83	14·72	100·00	5·50	Mean of 85 tryps.
* T. Brucei	6·36	29·56	9·56	41·25	13·27	100·00	4·78	
* T. Equinum	6·90	28·90	7·06	42·38	14·76	100·00	6·48	
* T. Gambiense	5·69	26·52	10·54	42·38	14·87	100·00	5·36	
T. Equiperdum (English Horse)	6·43	24·67	9·23	36·65	25·02	100·00	5·00	Plaques.
Do. (Arab do.)	8·18	28·38	8·75	42·00	11·68	100·00	7·74	,,
Do. (C. B. Mare)	7·09	32·25	13·33	28·96	18·36	100·00	6·22	,,
Do. (Mares)	6·10	29·02	11·23	34·79	18·86	100·00	6·75	Vaginal mucus.
T. Himalayanum (Hill Bull No. 3951)	16·23	17·86	3·99	40·36	21·56	100·00	5·06	Pl. II, Fig. 24.
Do. (do. No. 5078)	16·22	17·94	3·94	39·99	21·91	100·00	5·18	,, ,, 28, Pl. III, Fig. 5.
Do. (do. No. 5489)	16·71	16·84	3·97	40·77	21·71	100·00	5·13	
Do. (mean of above 3)	16·39	17·54	3·97	40·38	21·72	100·00	5·13	Mean of 61 tryps.
* T. Theileri (large tryp.)	16·82	14·28	4·58	41·58	22·74	100·00	4·21	
Do. (small do.)	21·17	4·71	8·24	42·35	23·53	100·00	6·34	
Do. (very long and narrow)	14·54	9·09	7·27	25·45	43·65	100·00	2·53	Pl. III, Fig. 10 (*d*).
T. Himalayanum (No. 5489 without flagellum) ...	17·65	18·23	6·47	57·65	0·00	100·00	5·87	
T. Indicum (Plains No. 4428)	10·29	17·26	4·36	39·20	28·89	100·00	3·73	Peripheral blood, Pl. II, Figs. 1, 2, 3.
Do. (swollen forms)	10·62	15·65	4·43	45·00	24·30	100·00	7·16	Peripheral blood, Pl. II, Figs. 17, 18, 19.
Do. (large flat, broad)	8·63	19·22	5·01	49·10	18·04	100·00	6·54	Spleen, Pl. II, Fig. 5.
Do. (attenuated forms)	10·17	26·87	5·28	57·68		100·00	2·23	,, ,, Figs. 8, 10, 11.
T. Muktesari (No. 5112, short flagellum)	19·02	18·59	6·24	50·68	5·46	100·00	6·53	Pl. III, Fig. 8 (*c*).
Do. (do. without flagellum)	16·19	22·01	4·81	56·99	0·00	100·00	5·42	,, ,, (*b*).
Do. (do. short do.)	17·55	35·09	5·26	38·59	3·51	100·00	4·56	,, ,, (*d*).

* Specimens kindly lent by H. E. Cross Esq., 2nd Asst. Bacteriologist.