NAGANA (*TRYPANOSOMA BRUCEI*): THE COURSE OF THE DISEASE IN LABORATORY ANIMALS WHEN INJECTED WITH CULTURES GROWN *IN VIVO* AND *IN VITRO*.

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Nagana, or Tsetse fly disease, as it is correctly called, is due to a specific trypanosomal parasite. It occurs naturally in many parts of Africa, and its causative agent, which was discovered by Bruce in 1894, bears the scientific name of *Tryanosoma brucei*.

Normally the parasite exists in the blood of certain animals which apparently have a marked tolerance for it, thus serving as its natural source of infection.

Most mammals are susceptible to Nagana with the exception, however, of man. The parasite can easily be perpetuated *in vivo* by resorting to the ordinary laboratory methods of inoculation. And also since the trypanosome can be cultivated *in vitro* upon blood agar medium, as Novy and MacNeal[2] first demonstrated in 1903, it lends itself ideally for experimental work. It is very interesting not only because it is a disease of distant Africa and due to a special type of parasite, but also because of the fact that it gives to a trypanosomiasis occurring in very distinct forms depending primarily upon the species of the animal and the virulence of the organism.

The source of the strain of *Trypanosoma brucei* used in our experiments is from an infected dog sent by Bruce to England in November, 1896, and it was this parasite which Novy and MacNeal used in their cultivation experiments.[3]

There are three distinct varieties of the disease:

First, the acute type which occurs in the rat, mouse, field mouse (Arvicola), marmot, hedgehog, dog, cat, squirrel and monkey.

Second, the subacute type which develops in the rabbit, guinea-pig, field mice (*Mus sylvaticus*, *Arvicola arvalis*), garden mouse (*Eliomys quercinus*), equine, and pig.

Third, the chronic variety which occurs in cattle, goats, sheep, geese and fowls.

Table I lists some of the animals susceptible to Nagana.

In an acute infection, the trypanosome makes its way into the blood in from one to several days. Following this infection the number of parasites usually increase constantly and regularly until the death of the host, which may occur in a few days, or a week, or even longer.

The subacute course of the disease is quite different for while the parasites do not appear in the blood as soon as in the acute form their development is not rapid and continuous for they may practically dis-

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[2] Jour. Amer. Med. Assn., 1903, 41, p. 1266; Jour. Infect. Dis., 1904, 1, p. 1.

[3] This work was made possible through the kindness of Dr. F. G. Novy, Professor of Bacteriology, University of Michigan, from whom trypanosomal blood was obtained.

"Proc. 38th Meeting, 1922 (1923)."

appear and reappear and repeat this activity several times before they may or may not become extremely numerous shortly before the death of the animal. The organism may be first seen in the blood stream in about two or more days and the extent of the disease may be from less than two to many weeks.

TABLE I. Showing the Results of Different Animals Infected with Nagana.

Animal	Method of Injection	Period of Incubation Days	Duration Days
Rat	S. C.	2	3½–5½
	I. P.	Less than 1	2½–3
Mouse	S. C.	2	3½–5½
	I. P.	Less than 1	2½–3
Field mouse (*Arvicola arvalis*)	S. C.	2–6	4–12
(*Mus sylvaticus*)	S. C.	8	30
Dog	S. C.	4–6	14–26
	I. P.	2–4	6–14
Cat	S. C.	4–5	19–44
	I. P.	2–4	14–28
Guinea-pig	S. C.	2–7	7–78
	I. P.	1–3	5–61
Rabbit	S. C.	6–8	13–89
	I. P.	2–3	10–75
Horse	S. C.	7–8	15–92
	I. P.	4–5	8–49
Ass.	S. C.	7–8	15–92
	I. P.	4–5	84
Cattle	S. C.	5	Months—may recover
Sheep	S. C.	3	6½ months—may recover
Monkey (Macacus)	S. C.	4	15
Hedgehog	S. C.	4	13–17

S. C.—Subcutaneous inoculation.
I. P.—Intraperitoneal.

In the chronic infection the trypanosome appears as a rule several days after the inoculation and then after running a very irregular course for months, during which time only very few organisms are usually observed, they may disappear entirely and the animal recovers.

Technique of Studying Trypanosomes in Living Condition.—Since the parasites are usually present in the blood stream the most convenient method of obtaining blood is employed. When the organisms are numerous in the blood a very small amount will suffice to reveal their presence.

In case of the rat and mouse the tail is clipped while in that of the dog, rabbit and guinea-pig a blood vessel in the ear may be pricked or a small incision made in the ear. A drop of the fluid is placed on a slide or cover-slip and by gentle pressure a one layer preparation, which is usually desirable, is obtained. At times, however, when the parasites are very scarce a heavy preparation will aid in identifying their presence by the movement of the red blood cells.

This simple preparation is excellent for making a hasty examination but currents are soon set up in the liquid due to desiccation, thus rendering further examination quite impossible. If longer observations are desired the preparation may be rimed with paraffin or a hanging-drop or the Ranvier slide preparation resorted to.

When, however, the trypanosomes are extremely scanty in numbers diagnosis cannot be made by this simple procedure, although repeated examinations are continued on successive days. In such cases the animals are bled for a larger amount of blood which, after defibrinating or chemically treating to prevent coagulation, is centrifuged at high speed. The parasites, if present, will be found just above the red blood cells.

The blood of the suspected animal may be inoculated into very susceptible animals and in turn their blood may be examined for trypanosomes. Stained blood smears may aid in establishing the development of trypanosomiasis.

At times the examination of the oedematous fluid or serous exudate from the lesions will reveal the presence of the parasite.

Inoculation of Animals with Cultures Grown in vivo.—Since in this work the common laboratory animals were used, as the rat, mouse, dog, rabbit and guinea-pig, the course of the disease will be described as it occurred in these animals.

Experiments with rats.—When a very susceptible animal as the rat or mouse are injected with the trypanosomal blood they, of course, succumb from the acute variety of Nagana. The period of incubation, that is the first appearance of the trypanosome in the blood stream after the inoculation, is usually 18 to 24 hours. From this time on the number of parasites increase at an enormous rate until death, which occurs in two and a half to five days, depending largely upon the virulence of the organisms, numbers injected and their avenue of introduction into the animal. Just before the fatality which is always certain, the organisms are as numerous as and even greater than the number of red blood cells.

If rats or mice are inoculated with trypanosomal blood from other animals such as the guinea-pig or rabbit, the virulence of the parasite for the former animals is somewhat attenuated as is evidenced by a slightly longer period of incubation, usually two to three days, and death does not occur until six or eight days. However, After the organism makes its appearance in the blood stream its development is about the same as that in a rat or mouse which had received the trypanosome from the blood of an animal of the same species.

In a period extending over nine years in which over 1,200 rats were used, the minimum period of incubation was about ten hours and

the maximum 74 hours, while the minimum duration of the disease was 20 hours and the maximum about 140 hours. The average period of incubation was about 23 hours and the average duration 80 hours.

During the course of the disease these animals seem to be normal except possibly during the last day when they may be more or less drowsy. There are no morbid symptoms or lesions and both rats and mice may or may not die with convulsions. They are, however, excitable and if excessively annoyed convulsions usually occur just previous to death.

Experiments with dogs.—Probably the dog is the most susceptible animal, excepting the rat and mouse. If the duration of trypanosomiasis is short, only a week or two, the development of the parasites is rapid and regular. On the other hand, if the disease runs along for three or more weeks there may be an increase in the number of trypanosomes at first, followed by an abrupt decrease, but at no time does the parasite entirely disappear from the blood stream. In this respect it is unlike the subacute or chronic variety of the disease. There may be several such relapses followed usually by a marked increase before death.

In the four dogs used in the experiment (table II) the minimum period of incubation was two to four days, the maximum seven to nine days; the minimum duration of the disease six days and the maximum duration 26 days. The average period of incubation was 4.3 to 6.3 days while the average duration was 16.3 days. The animals seem to become extremely weak and there is a loss in weight. There is marked hypertrophy of the inguinal glands, the vision appears to be disturbed and there is a rise in temperature.

Inoculation with Cultures Grown in vitro.

The medium employed to obtain this strain of *Trypanosoma brucei* was a modification of the original Novy and MacNeal blood agar. The isolation was obtained by using a pea and bean blood agar or a serum agar as described by the author in 1916.[4] The parasite after becoming well established outside of the animal was perpetuated upon blood agar medium.

TABLE II. Inoculation of Dogs with Cultures Grown *in vivo*.

No. of Dog	Weight, Gm.	Period of Incubation, Days	Death, Days	Weight After Death Gm.
1	5500	7–9	26	5000
2	3210	4–6	20	2970
3	3050	4–6	13	2950
4	3090	2–6	6	3090
Average	3712.5	4.3–6.3	16.3	3502.5

[4] Proceedings of the Ind. Acad. of Sci. 1916. pp. 264-271.

Experiments with rats.—From the foregoing it will be noted that the rat always succumbs from the acute form of the trypanosomiasis when inoculated with the blood strain of the parasite. When, however, it is injected with cultures grown *in vitro* the disease may be drawn out to the subacute and in some cases to the chronic variety of the disease. This is apparently due to the generation and age of the culture, temperature at which it was incubated and the number of trypanosomes introduced into the animal.

Rats inoculated with generation one of *Trypanosoma brucei* show variable results primarily due to the age of the culture. The possibility of the original blood survival being present is not taken into consideration although this may be actually the case. They have been observed but proven to be avirulent.

Infection can usually be produced when cultures of that generation are injected that are 6 to 11 days old. Cultures older than that are non-infective.

The period of incubation, as well as the duration of the disease varies. The former lies between four to eight days and the latter may be from 8 to 14 days. Whereas, if generation two, seven days old, which is the time of its maximum growth, is injected the period of incubation is three to seven days and the time of death is seven to ten days. The variations in the period of incubation and in the duration of the disease that have been noted when rats are injected with generation two are even more irregular when inoculations with subsequent generations are made. The higher the generation the greater the variations. Thus, the average period of incubation in rats receiving generation two is 4.6 days and death occurs in an average of 8.9 days.

Rats inoculated with subsequent generations gradually show a longer period of incubation and the time of death is deferred. With generation 40, the former was 12 to 13 days and the latter was 21 days, although with generations 50 to 57 and 61 the organism seems to have become temporarily exalted. The rats receiving the culture of these generations showed periods of incubation varying from five to nine days. In the rat receiving a seven day culture of generation 89 the parasites were present in the blood in six days and the animal did not die until the 94th day. Again, in the case of generation 154 the period of incubation was eight to ten days and death did not occur until on the 126th day. With generation 190 the rat became infected on the sixth to seventh day and died on the 78th day.

This variation seemed especially more pronounced if the cultures were incubated 14 or even 21 days before inoculations were made. This is nicely shown with generation 85. The period of incubation when a seven day old culture was used was six days, death occurred on the 41st day. If the age of the culture is 14 days the trypanosomes appear in the blood of the rat at about the same time (seven days) but the animal did not die until on the 84th day. And if a 21 day old culture of the same generation was used the period of incubation was 8 to 11 days with death coming on the 51st day. In one exceptional case, that of a 21 day old culture of generation 93, the rat became infected be-

tween the 11th and 14th day and after having the disease for 250 days the animal died on the 265th day.

In all cases where the cultures were set aside for 28 days the trypanosomes became so attenuated and scarce that no infection followed their injection.

These results lead one to conclude that the higher the generation of the organism the greater its attenuation and consequently the longer the duration of the disease. However, like in all experiments of this kind the individual susceptibility of the animal plays an important rôle. Thus, of two rats inoculated with a seven day old culture of generation 154 the duration of the disease in one case was 116 days and in the other it was only ten days. A rat receiving a seven day old culture of generation 85 became infected on the sixth day and died 35 days later. A single inoculation with a 14 day old culture gave a period of incubation of seven days and death occurred on the 84th day, while a rat which suffered from the infection with a 21 day old culture of the same generation (85) showed an 8 to 11 day period of incubation and death resulted on the 51st day.

As in the case of rats becoming infected with the blood strain of the parasite, those that succumb from the blood agar strain, no matter how long or short the duration of the disease, seemed invariably not to develop the common symptoms of Nagana, such as fever, oedema and anaemia.

Experiments with mice.—It has been shown that mice, like rats, when inoculated with trypanosomal blood develop the same type of the disease and as would be expected they respond in the same manner when injected with the test-tube strain. Of 14 mice inoculated intraperitoneally with seven day cultures of generations 143, 144, and 145, the minimum period of incubation was four to six days and a maximum of 8 to 12 days, an average of about 6.4 to 8.6. The minimum time of death was nine days, the maximum 23 and the average about 13.7 days, as will be observed in table III. And, again, like the rat, the mouse shows none of the characteristic symptoms of the disease.

TABLE III. Inoculation of Mice with Cultures of *Trypanosoma brucei* Grown *in vitro*.

Generation	Period of Incubation, Days	Death, Days	Generation	Period of Incubation, Days	Death, Days
143	5–6	18	144	8–10	14
143	5–6	13	144	8–10	12
143	7–8	17	145	6–8	14
144	4–6	9	145	6–8	14
144	4–6	15	145	8–12	14
144	6–8	12	145	8–12	14
144	6–8	23	145	8–12	13
Average				6.4	8.6

Note: Each mouse received an intra-peritoneal inoculation with varying amounts of a seven day old culture.

Experiments with dogs.—Of the four dogs inoculated with varying amounts of cultures of the trypanosome, which had been cultivated approximately three years *in vitro*, three died of Nagana.

One dog, after receiving five intra-peritoneal injections, each of ten tubes, of generations 140, 141, and 142 in 16 days became infected two days after the last inoculation, or 18 days after the first. After a rapid increase in the number of parasites the dog died on the 44th day.

The second dog received a single injection of ten cultural tubes of generation 144. Unlike the first and third dogs (table IV), this animal developed a typical chronic course of the disease after a period of incubation of only eight days. The parasites were not numerous but once (ten per field) and usually present to the extent of one or two per field with frequent intermissions. Death did not occur until on the 127th day.

The third dog received but one culture and showed trypanosomes ten days afterwards. He died nine days later of the acute infection following severe sickness.

The fourth dog received one-tenth of a culture and did not show parasites in its blood at the end of 39 days when he died.

TABLE IV. Dogs Inoculated with Culture Grown *in vitro*.

No. of Dog	Weight, Gms.	Generation	Amount Injected, Tubes	Period of Incubation, Days	Death, Days
1.....	8700	140–141	5x10	2–18	44
2.....	2950	142–144	10	8	127
3.....	2900	147	1	7–10	19
4.....	2600	147	1/10	No infection	39

Inoculation of Rabbits with Cultures Grown in vivo.—Rabbits inoculated with this strain of the trypanosome usually show a period of incubation of from two to five days but sometimes the organisms are not seen by the ordinary methods of examination until on the seventh to ninth day, or in very refractive animals as late as the tenth day or even much longer (31 to 49 days in a few of the exceptional cases where the animals received very small amounts of the infective material).

Also, depending upon the susceptibility of the rabbit, the duration of the disease varies greatly. Death may occur as early as ten days or it may be deferred as long as two and a half to three months. It will be seen in table V that the average period of incubation is 8.4 to 11.9 days, while the average duration of the disease is about 40 days. The number of trypanosomes to be found in the blood of these animals is usually very few and remains so even up to the time of death. The animals run an irregular course of fever and usually show oedematous swellings which may be marked, blepharo-conjunctiva, coryza, ulceration of the skin and alopecia.

TABLE V. Rabbits Inoculated with Cultures of *Trypanosoma brucei* Grown *in vivo*.

No. of Rabbit	Period of Incubation, Days	Death, Days	No. of Rabbit	Period of Incubation, Days	Death, Days
2.7	9–10	31	1.104	24–31	75
16.11	7–9	29	6.112	10–17	60
17.11	9–10	37	1.119	9–13	44
2.20	5–7	29	10.123	7–9	35
10.28	7–9	41	2.128	7–9	42
1.36	4–7	37	5.129	5–7	21
13.44	6–7	52	2.130	9–13	58
2.46	2–5	17	1.141	13–17	49
2.47	7–9	21	16.150	19–23	64
1.54	5–7	20	1.152	42–49	90
6.64	2–3	10	8.165	7–9	32
17.64	4–5	31	8.168	7–9	39
15.65	4–5	34			
Average.				8.4–11.9	39.9

TABLE VI. Rabbits Inoculated with Single and Multiple Doses of Ten Tubes Cultures.

No. of Rabbit	Generation of Culture	Amount Injected, Tubes	Period of Incubation, Days	Death, Days	Remarks
1	144	10	.	. .	Alive 98 days
2	145	10	. . .		Alive 95 days
3	145	10	. . .	. .	Alive 122 days
4	145	10	. . .	. .	Alive 111 days
5	146	10	. .	13	. . . . .
6	147	10	.	. .	Alive 203 days
7	148	10	66–70	. .	Alive 308 days
8	145–149	10x10	. . . . .	. .	Alive 95 days
9	145–149	10x10	17–49	58	. . . . .
10	145–147	4x10	1–12		Alive 518 days
11	146–151	10x10	. . .		Alive 110 days
12	146–151	10x10	. . . .		Alive 205 days
13	146–151	10x10	. .		Alive 205 days

Inoculation of Rabbits with Cultures Grown in vitro.—While rabbits suffer from the subacute type of Nagana when infected with the blood strain of the parasite the same does not hold true when the organism has been cultivated upon blood agar for many months.

Rabbits were not inoculated with early isolations of the trypanosomes. Seven rabbits were injected intraperitoneally with the contents of ten tubes of generations 144 to 148, inclusive, and of this set only one showed the presence of trypanosomes in its blood on the 70th day and again ten days later at which time further examinations were negative.

Out of six rabbits, each receiving multiple ten tube doses, two developed trypanosomiasis of which one proved fatal as table VI shows. Rabbit number ten, which received four bi-weekly injections of ten tubes each, revealed the presence of a small number (one per field) of trypanosomes in its blood 12 days after its first inoculation. The number of parasites increased to four per field on the 14th day and then disappeared until on the 27th, 37th, 44th and again on the 126th day when only a single individual was noted in each examination. This animal showed no symptoms of Nagana more than 500 days after its inoculation and was pronounced as recovered.

Rabbit number nine after receiving ten bi-weekly doses of ten tubes each also developed Nagana on the 49th day after the first injection or 17 days after the last injection. At this time but one trypanosome was found but 7 days later ten parasites per field were observed and two days later death occurred. This animal developed the characteristic symptoms of the disease.

Inoculation of Guinea-pigs with Cultures Grown in vivo.—The variation of the disease which has been noted in the rabbit is also markedly borne out in the case of Nagana in guinea-pigs. The parasites do not increase in numbers regularly or rapidly but they may disappear and reappear several times and finally, usually just before death, may be numerous in the blood stream. The disease, generally speaking, runs a more regular course and the organisms are more plentiful than in the case of the rabbit. Nagana is always fatal for guinea-pigs with a period of incubation from two to four or even as high as eight days, the average being a little less than four days when intraperitoneal injections are made and about five days when subcutaneously inoculated. The average duration of the infection is approximately 40 days.

Out of some 600 guinea-pigs examined the shortest period of incubation was 24 hours and the longest eight days. The duration was variable being from seven to 66 days. In the typical course of the disease and also when the infection borders the chronic variety, the guinea-pigs have more or less constant fever and develop conjunctiva which may be purulent and always alopecia which is usually marked especially along the back, around the anus and eyes. Some of the animals become very anaemic.

Inoculation of Guinea-pigs with Cultures Grown in vitro.—Guinea-pigs, like rabbits, because of their refractoriness, develop at best a chronic infection if the parasite has been out of the animal body for several years or the organism has entirely lost its virulence for these

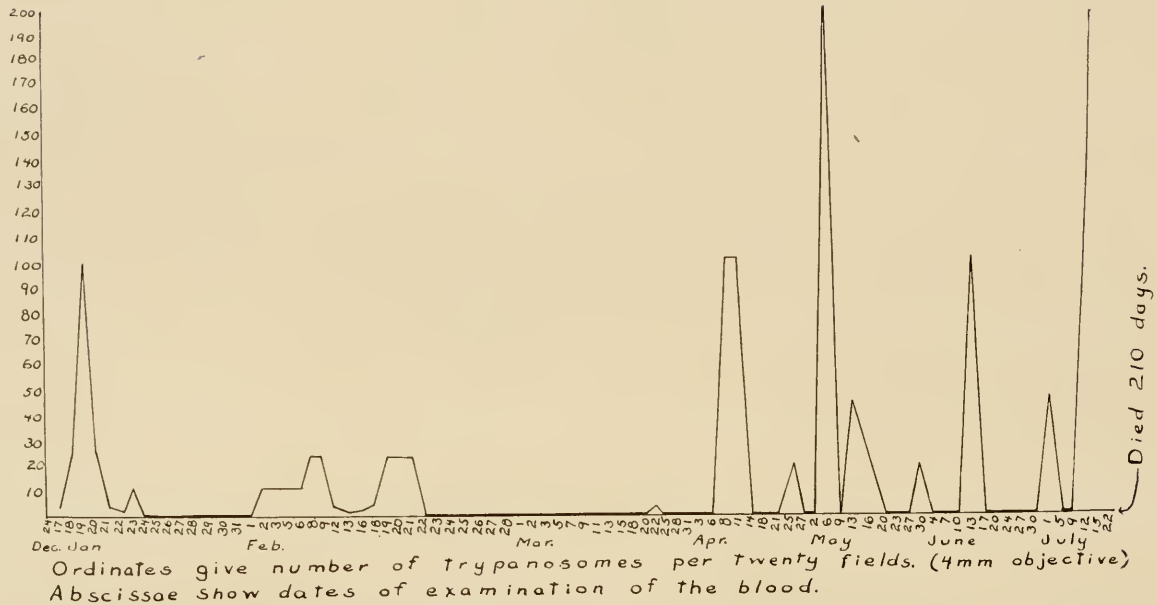

Fig. 1. Graph showing variation in the number of trypanosomes in guinea pig number 5.190 (taken from table VIII), from date of inoculation till death. Animal was inoculated with ten tubes of generation 140.

animals but may be still pathogenic for rats, mice and dogs. *Trypanosoma brucei* if not under artificial cultivation for too long a period will infect guinea-pigs although the period of incubation and the duration of the disease is prolonged, as shown by guinea-pig 16.94 (Table VII). In this case the period of incubation was 14 to 15 days and death did not occur until on the 59th day. On the other hand of 21 animals inoculated each with one tube of generations 133 to 153, inclusive, failed to contract the disease.

It was only when the contents of ten or a multiple of one or ten tube doses were injected that a small per cent of the animals became infected, and in each case the period of incubation and the duration were markedly prolonged as shown in table VII. Thus, also demonstrating that the parasite was not wholly avirulent for the guinea-pig. Since no infection followed by the inoculation of a single cultural tube of the trypanosome in its 105th or later generation, the effect of much larger amounts of the parasites were tested out. Animals were inoculated with ten, 100, ten multiple injection of one, and ten multiple of ten tube doses of the cultural material with surprisingly few infections resulting.

Ten Tube Inoculation.—Only four out of 60 guinea-pigs or six and two-thirds per cent became infected after inoculation with ten culture tubes of generation 133 to 153, inclusive. The minimum period of incubation and duration of the disease was 18 and 50 days, respectively, as compared to the maximum of 40 and 210 days. The average period of incubation for the four diseased animals was 28½ and the average duration 117¾ days.

Course of the Infection.—The courses of the disease in these animals was interesting and since they were more or less alike and were very characteristic of the typical chronic variety of Nagana, the description of the disease of one of these animals, that of number 5.190, will be taken up in detail.

TABLE VII. Showing the Positive Results Obtained in Guinea-pigs by Inoculations with Ten or More Cultures.

No. of Guinea-pig	Generation	No. of Tubes Injected	Period of Inoculation, Days	Death, Days
16.90	40	1	14–15	59
4.190	140	10	39–40	50
5.190	140	10	21–24	210
5.147	146	10	14–18	53
6.147	146	10	28–32	158
6.68	133–139	10x1	51–92	268
5.105	136–144	10x10	7–70	104

The parasite was first seen (one twentieth per field) in the blood stream of the guinea-pig 24 days after it had received a ten culture tube dose. During the next six days trypanosomes were present although for the greater part very few in numbers. The organisms then disappeared and were not again noted until on the ninth examination after which the following 12 examinations were positive. The next 23 examinations did not reveal the presence of the parasite except in one instance when but a single individual was noted. After being present fairly numerous (five per field) in the blood stream for two days, the next three consecutive examinations were negative only to be followed by a positive observation and again by an intermission. The next examination showed the organism in greater numbers (ten per field) than in any other previous examination, however, upon the following examination the parasite had again disappeared. This was followed by five positive findings and four intermissions and the animal died on the 210th day.

Out of the 82 examinations made after the trypanosome was first observed, 32 were positive and 50 were negative during which time there were 11 positive periods and ten relapses. At no time during the course of the trypanosomiasis did the parasite exceed 10 per field which was noted but twice. Their scanty numbers, however, were characteristic as will be noted in figure 1.

One Hundred Tubes Inoculations.—Of four guinea-pigs receiving such injections with generations 141 and 142 no infections followed. One would naturally conclude that if a small percentage of the animals became infected when inoculated with a 10 tube dose that surely at least in a few instances infection would follow if the material inoculated was tenfold.

Ten Inoculations of One Tube.—Six animals were used for this test and received generations 133 to 139 out of which one revealed the presence of the trypanosome in its blood at the end of 92 days after its first inoculation or 51 days after its last. It lived for 268 days never showing more than 10 parasites per field and that but once. During the greater part of its existence the blood stream was free from organisms and, as a matter of fact, although examined twice a week the trypanosomes were observed but five times.

Ten Inoculations of Ten Tubes.—Animals thus inoculated again showed marked variation as to infectivity with the parasite. One of the eight guinea-pigs which received a multiple of ten culture tube dose developed a very chronic form of Nagana. Trypanosomes were first seen in its blood seven days after the last or 70 days after the first injection and as in the case of the previous guinea-pig the organisms were only observed six times during the trypanosomiasis which lasted for ten days.

SUMMARY.

Nagana manifests itself in three distinct types: the acute; the subacute and the chronic.

The virulence of the trypanosome; its avenue of entrance into

the animal body; the number of parasites injected and the susceptibility of the animal plays an important rôle as to the variety of disease that ensues.

The behavior of the parasite in the animal body is noted by examinations of the blood.

The trypanosome is slightly attenuated when it passes through heterogeneous species.

Cultures of generation one usually infect when incubated from 6 to 11 days while longer incubation renders the culture avirulent.

Cultures of higher generations will infect after 21 days of incubation but they are avirulent after 28 days.

The greater the generation the more marked is the variation in the duration of the disease.

Rats, mice and dogs succumb from the acute type of Nagana when inoculated with trypanosomal blood.

The trypanosome multiplies with marked rapidity and regularity in rats and mice while this is not true in dogs, rabbits and guinea-pigs when infected with the blood type of the parasite.

Rats, mice and dogs suffer from the subacute variety of the disease when inoculated with the *in vitro* strain of the trypanosome.

Rabbits and guinea-pigs develop sub-acute and chronic Nagana when infected with the artificially cultivated parasite.

Rats and mice never develop symptoms of Nagana while dogs, rabbits and guinea-pigs usually do.

Nagana is always fatal to rats, mice, dogs and guinea-pigs while in some cases rabbits may recover, apparently spontaneously.

Rats, mice and dogs are still susceptible to the *in vitro* culture while only a small percentage of the rabbits and guinea-pigs become infected after receiving enormous doses of this parasite.