Further Observations on the Effects produced on Rats by the Trypanosomata of Gambia Fever and of Sleeping Sickness.

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(Communicated by Dr. C. J. Martin, F.R.S. Received November 21,—Read December 13, 1906.)

[Plate 1.]

In January, 1905, I published* a note of some experiments with the above-named strains of trypanosomata, and I have continued the experiments up to the present time, and have embodied the total results in this paper.

1. *Gambia Fever.*

The number of rats inoculated with this strain of trypanosomata was altogether 129 in 90 successive inoculations. In all of these rats, with the exception of the five mentioned below, trypanosomata were present in the blood from two to three weeks after inoculation (average time 18 days) until death; generally in good numbers, and increasing to large numbers for some days before death. The spleen was, in these cases, very much enlarged, and there was considerable blood destruction, with large numbers of poly-chromatophile corpuscles, these being the most obvious gross signs.

In the first 14 of these rats, as noted in the paper referred to above, no paralytic symptoms were present, but out of the total number inoculated, five became paraplegic, and the course of the disease in these animals differed from that in the remaining 124.

In the series, the numbers of the paraplegic rats were 35, 42, 68, 101, 102, and there were no perceptible differences in their conditions and those of the other rats used.

The first rat which became paralysed (No. 35, of the 25th inoculation) was inoculated on March 17, 1905, and on the 31st of the same month both hind legs became paralysed. No trypanosomata were found in the blood until April 18, and from that time till death, which took place on April 28, only a few were to be found in ordinary blood films. The second rat (No. 42, of the 28th inoculation) was inoculated on April 13, 1905, from the blood of another rat which had been 20 hours in the stomach of a leech, in which some of the trypanosomata will live for at least 36 hours. The rat became paraplegic on June 15, at which time no trypanosomata could be found in ordinary blood films. The rat was killed on June 16, and a few trypano-

* 'Roy. Soc. Proc.,' vol. 74.

somata were found both in the centrifugalised blood and in smears made from the spinal cord. The third rat (No. 68, of the 42nd inoculation) was inoculated on September 26, 1905, and became paraplegic on December 13. No trypanosomata could be found in ordinary blood films in 17 examinations made during the disease, nor at the time of death. The animal died on January 3, 1906, when a few trypanosomata were found in the centrifugalised blood, and in smears from the spinal cord. The fourth and fifth rats (Nos. 101 and 102, of the 70th inoculation) were inoculated on May 26, 1906, and on July 17 No. 101 became paralysed in the right hind leg, and on July 18 No. 102 also became paralysed in the right hind leg. Both of these rats had a good number of trypanosomata in their blood of the ordinary long, very motile kind. On July 19 No. 101 became completely paraplegic, and died on the 22nd. No. 102 died on July 23, without the other leg becoming paralysed.

The following table gives a comparative outline of these five experiments:—

Rat.	Duration of disease.	Appearance of paralytic symptoms.	Trypanosomata in blood during life.
35	41 days	14th day	Very scanty.
42	64 days, rat killed...	63rd day	Not found; few found *post mortem* in centrifugalised blood.
68	102 days	78th day	Ditto.
101	57 days	52nd day	Good number.
102	58 days	53rd day	Ditto.

It will be noted that, in Rats Nos. 101 and 102, trypanosomata were present in good numbers in the blood, but in those cases (Nos. 42 and 68) in which the paralysis was most marked, and which, to all appearances, were exactly like rats paralysed after inoculation with the Sleeping Sickness strain of trypanosomata, they were absent, or at any rate so few in number that they could only be found in centrifugalised blood after death. From all of these rats inoculations were made, with the constant result that the disease in the inoculated animals reverted to the generalised form mentioned above as characteristic of this strain of Gambia Fever.

This strain of trypanosomata was also inoculated into a monkey, which lived 33 days after inoculation, this being three days longer than the average time of the 14 monkeys I have inoculated with the Sleeping Sickness strain. Trypanosomata appeared in the blood 10 days after inoculation, and were of the type of the Sleeping Sickness strain. Rats inoculated from this monkey did not become paralysed; trypanosomata of the Gambia Fever

type were plentiful in their blood, and the disease ran the course of that produced by this strain.

2. *Sleeping Sickness.*

In all, 82 rats have been inoculated with this strain of trypanosomata, in 59 direct series of inoculations, and, of these, 22 have been paralysed. In these cases trypanosomata could be found only at intervals and rarely, and sometimes not at all, in ordinary blood films, and in some cases none could be found in the centrifugalised blood *post mortem*; but in cases where prolonged search was made a few were always found. The paralysis occurred most generally in those rats which were inoculated direct from monkeys, and it occurred less and less frequently as the passages got more remote, firstly from the monkeys and secondly from the original monkey. I have succeeded in producing paralysis once as far as the fourth passage from the monkey; generally after the second, in five instances after the first, passage the trypanosomata have become generalised in the blood, and the symptoms and course of the disease have been those of the Gambia Fever strain.

The following table gives an outline of the instance mentioned above, in which paralysis occurred in four successive passages:—

Rats.	Inoculated from	Duration of disease.	Appearance of paralysis after inoculation.	Trypanosomata in blood.	Spleen.
		mths. days.	mths. days.		
7*a*	Monkey which was inoculated from the monkey brought home by Col. Bruce	6 7	5 15	None seen	Not enlarged.
7*b*		3 14	2 2	None seen	Not enlarged.
8	Rat 7*a*	6 0	5 2	None seen	Not enlarged.
10*a*	Rat 8	3 23	3 1	Few	Enlarged.
10*b*		4 6	3 0	Few	Enlarged.
13*a*	Rat 10*a*	3 3	Not paralysed	Few in every field	Large.
13*b*		2 0	1 20	Few	Large.
16	Rat 13*b*	2 15	Not paralysed	Plentiful	Very large.

The rats Nos. 7*a* and 7*b* at the head of the above series were inoculated from a monkey which had been inoculated from the monkey Colonel Bruce, F.R.S., brought home from Uganda, this monkey having been inoculated on the spot with cerebro-spinal fluid from a case of advanced Sleeping Sickness.

In rats inoculated from the 10th monkey done in direct succession from Colonel Bruce's monkey, the paralysis occurred only in those inoculated directly from the monkey, the next passage producing trypanosomata and symptoms of the Gambia Fever type, as set forth in the following table:—

Rat.	Inoculated from	Duration of disease.	Appearance of paralysis after inoculation.	Trypanosomata in blood.	Spleen.
66*a*	Tenth monkey in direct succession from Col. Bruce's monkey	Killed at 3 mths. 25 days	2 mths. 22 days	None seen	Not enlarged.
66*b*		4 mths. 2 days	3 mths. 11 days	None seen	Not enlarged.
66*c*		4 mths. 6 days	3 mths. 22 days	None seen	Not enlarged.
68	Rat 66*a*	2 mths. 26 days	None	Few from 15th day after inoculation, increasing till death	Large.
69	Rat 66*b*	2 mths. 1 day	None	Plentiful	Very large.
70	Rat 66*c*	Killed at 1 mth. 20 days	None	Fair number	Large.
71	Rat 69	2 mths. 18 days	None	Plentiful	Very large.

3. *Paralysis after Inoculation with Trypanosomata.*

With regard to the question of paralysis occurring in the rat after inoculation with the above-mentioned particular strains of trypanosomata, it does not appear to have come within the experience of all those who have worked with these particular organisms. For instance, neither Dr. Thomas, of the Liverpool School of Tropical Medicine, nor Dr. Laveran, of Paris, appear to have encountered it in their work with these trypanosomata.

But Lieutenants Tulloch and Gray, in Uganda, have made a number of experiments to test the statement made in my Preliminary Note, and have recorded the same results in a paper published in No. VIII of the Sleeping Sickness Reports of the Royal Society.

In four rats inoculated with the blood of a monkey which had been previously inoculated with cerebro-spinal fluid from Sleeping Sickness patients, paralysis of the hind limbs occurred 10 or more days before death; and in three out of the four rats no trypanosomata could be found in the peripheral blood at the time of death. They also found that rats did not show any signs of infection after inoculation with the blood of Sleeping Sickness patients, which has also been my experience. I inoculated two rats with blood from a case of Sleeping Sickness, in University College Hospital, a year before death, and two more shortly before death, on both of which occasions trypanosomata were present in the patients' blood; the results in both series were negative.

The site of the inoculation apparently determines the locality of the paralysis. In all my experiments, with the exception of four, inoculation was made at the inner side of the hind leg, and the paralysis occurred in the lower limbs. Only in one rat, which lived nearly 11 months, did any weakness of the forelegs show itself; and this was due to extensive degeneration

ascending the spinal cord from the lumbar region. The lesions in the spinal cord were also confined more or less to the lumbar region, depending for their extent upon the duration of the disease. In two cases I inoculated the animals on the inner side of the foreleg, and both these animals became paralysed in the front legs, and the degenerative lesions were most marked in the corresponding parts of the spinal cord.

I inoculated two rats in the peritoneum, but in neither did any paralysis occur.

4. *The morphological characters of these two strains of Trypanosomata.*

It is impossible at present to insist that any differentiation based solely upon microscopical observations should be sufficient in the case of organisms so much alike as the trypanosomata. Our methods and observations are neither uniform nor good enough to enable us to make, at the present time by the microscopical method alone, a sufficient differentiation. Until all observers use the finer zoological methods of fixation, etc.,* instead of the barbarous method of drying blood films at present almost exclusively in use, we cannot look for much certainty in the microscopical differentiation of very similar organisms. This must be supplemented by observing the differences in their pathogenic action.

We must not forget, either, the variability of organisms in the same species of animals in the same country, and their still greater variability in different animals in the same, and in other countries; and we must remember the great differences in the physical conditions of an organism which are due to environment. So I would not insist, with our present knowledge, too strongly upon the differences in form in the trypanosomata mentioned above, under the different conditions which have been described. But if preparations are made under the same conditions, and with the same care, it can be seen that the trypanosomata from the cases in which paralysis has occurred (in which very few are found in the blood, the greater number being in the central nervous system) are short and thick, with the flagellum extending very little beyond the body of the organism; they contain a varying number of vacuoles, and sometimes many granules; they move slowly, even when the smear of cord or brain is diluted with normal citrate solution (fig. 3). On the other hand, those found in the generalised cases (the Gambia Fever type) are long, with a long flagellum, and are not vacuolated, and move quickly (fig. 4). I have not found the other form in any of these cases, even when the animal has been long dying. This short, badly staining, vacuolated form is probably due to some condition of environment about

* See Note on methods.

which we know nothing, which produces not only changes in the morphology of the organism, but also in its pathogenic effects, for, from the above experiments, it can be seen that the inoculation of these short forms may, in the first or subsequent passage, give rise to the long form in the inoculated animal, with quite different symptoms (*cf.* figs. 3 and 4: fig. 3 photographed from a smear of spinal cord of Rat 66 *b*, and fig. 4 from a blood film of Rat 69, which was inoculated from Rat 66 *b*).

5. *Pathological Anatomy.*

The examination of these two series of rats has shown that the pathological effects are fairly uniform in each of the two types of cases.

In those animals in which paralysis occurred, the principal lesions were found in the spinal cord. There was invariably congestion of the vessels, and in eight cases well marked hæmorrhages around and in the substance of the cord. There was the characteristic exudation around the blood-vessels of mononuclear cells, and of plasma cells, such as Dr. Mott has described in the brain in cases of Sleeping Sickness; and in all the cases a varying amount of glia proliferation (*vide* figs. 1 and 2). The nerve-cells in the affected areas of the cord were degenerated in varying degrees up to the disappearance of the processes, and even of the nucleus with vacuolation of the protoplasm.

The brain showed much less change, and only in the chronic cases was any exudation found around the vessels. Small hæmorrhages in the meninges were seen in five cases.

Trypanosomata, mostly in stages of degeneration, were found scattered through the substance of the cord and brain in varying numbers; none were found in the vessels, which were generally distended with blood.

The other organs showed very little change; they were anæmic and firm; the spleen was not enlarged in the chronic cases with marked paralysis, and was only slightly enlarged in the transitional cases. There was no enlargement of the superficial or deep lymphatic glands, and the eyes and genitals were normal.

In the cases in which the trypanosomata were plenteous in the blood, and which had no paralytic symptoms (the Gambia Fever type), the brains and cords were very congested, and the blood in the distended vessels contained large numbers of trypanosomata. None were seen in the substance of the brain or cord. In six cases small hæmorrhages were found on the meninges. There was no degeneration of the brains or cords.

The superficial lymphatic glands were often enlarged. The abdominal glands were always enlarged, and some of both superficial and deep glands were reddish to brown in colour. The enlarged glands showed changes of a

hyperplastic nature, and the reddish ones showed sinus formation, extreme congestion, and often hæmorrhages.

The lungs were always very congested, with sometimes subserous hæmorrhages in the lower lobes. Small hæmorrhages were found in 61 cases in the pleuræ.

The livers were always extremely congested, and were very soft in texture, and showed extensive parenchymatous degeneration, and often fatty infiltration and degeneration. The vessels were very dilated and distended, and often showed proliferation of their endothelium.

The stomach in 13 cases was ulcerated; generally one small ulcer filled with clot was found, but sometimes as many as three or four. They were always found on the greater curvature. In two cases death occurred from perforation.

There was generally a diffused congestion of the lining membrane of the intestines, and on the peritoneal surface ecchymoses dotted about, either singly or in groups, were often found. The kidneys were congested, and had always macro- or micro-scopical hæmorrhages.

The spleen in every case was enormously enlarged, with great congestion of the pulp, and considerable hyperplasia of the follicles. There was generally proliferation of the endothelium of the distended vessels, and a great deal of phagocytosis of red blood corpuscles and trypanosomata.

The bone marrow (femur) was profoundly altered, having the appearance of red marrow. It was very cellular, with scarcely any fat, and there was always considerable phagocytosis, and many nucleated red corpuscles.

6. *Conclusions.*

From the above summary of 211 experiments, extending over a period of nearly three years, it will be obvious that the tentative deductions which I made in my Preliminary Note from the few experiments therein recorded, that Gambia Fever and Sleeping Sickness are two distinct diseases, cannot be maintained.

This extended series of experiments and observations go to show that each of these two strains of trypanosomata has produced two different effects in the same class of animals, under conditions of which we at present know nothing; and that these effects are alike for the two organisms; and that the trypanosomata found in these two types of disease are one and the same organism, modified by passage from man through monkeys to rats, and perhaps in the strains I have used, by transplantation into animals of, and in, another country.

NOTE ON METHODS.

With regard to the mention made in Section 4 of methods, the following, which has been used by the writer for some years, gives uniformly good and, as he believes, accurate results. The specimen is never allowed to dry, and there is no shrinkage of the cells, and the finest cytological details can be observed.

1. Expose a cover-glass to the vapour of

Osmic acid, 1 per cent.	1 c.c.
Glacial acetic acid	3—5 drops

for 2 minutes.

(This can be most conveniently effected by using a block-dish covered by a thin glass having a hole in it rather smaller than the cover-glass used.)

2. Place a drop of fresh blood in one corner of the cover-glass, and expose again to the vapour for 30 seconds.

3. Spread the film carefully, and expose again for 15 to 30 seconds to the vapour until the surface appears no longer moist. (The film will not be really dry, and can be easily smeared off the glass with the finger: a really dry film will be much lighter in colour, and cannot be rubbed off with the finger.)

4. Place the cover-glass in absolute alcohol for 10 minutes.

5. Place the cover-glass in a faintly rose-coloured solution of permanganate of potash for 1 minute. (Two or three drops of a 1-per-cent. solution to 50 c.c. of water.)

6. Wash in water for 5 minutes.

7. Stain in a modified Romanowsky's stain, made by mixing just before use—

Azur I, 1 per cent.	1 c.c.
Eosin, B.A., 1—1000	2 „
Water.....	8 „

for 15—30 minutes.

8. Wash.

9. Differentiate in orange-tannin, 30 seconds.

10. Wash well and drain.

11. Absolute alcohol for a few seconds.

12. Alcohol—xylol (in proportion of 2 : 3), two or three changes.

13. Xylol, and mount.

Instead of 7—13, any other method of staining can be used, according to what structures it is desired particularly to show.

DESCRIPTION OF PLATE.

Fig. 1.—Section of half of spinal cord of rat which had been paralysed for 2 months before death. ×40. Shows meningitis, the exudation around the vessels, and a large area of degeneration.

Fig. 2.—Longitudinal section of vessel in spinal cord of rat which had been paralysed for 6 weeks before death. ×220. Shows the cellular exudation around the vessel, and on the right an area of degeneration.

Fig. 3.—Trypanosomata from a mash of spinal cord of rat, from which cord fig. 1 was photographed. ×1000. Stumpy forms, with very short flagella, staining badly, with very little differentiation.

Fig. 4.—Trypanosomata in blood of rat which was inoculated with the mash of spinal cord from which figs. 1 and 3 were photographed. Long, well-differentiated, easily staining forms. This rat was not paralysed.

FIG. 1. ×40.

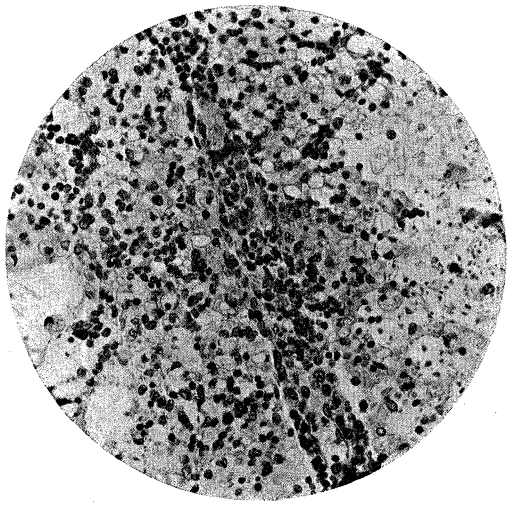

FIG. 2. ×220.

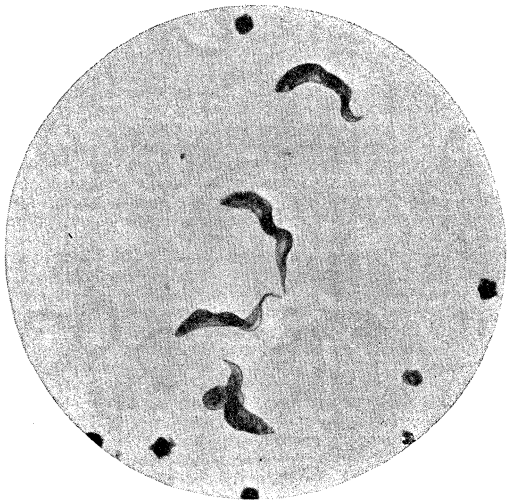

FIG. 3. ×1000.

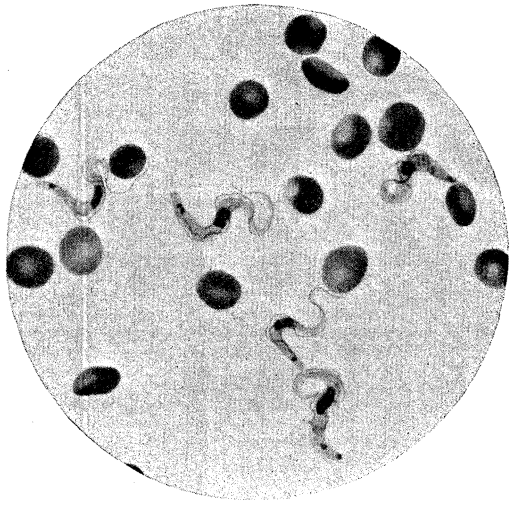

FIG. 4. ×1000.

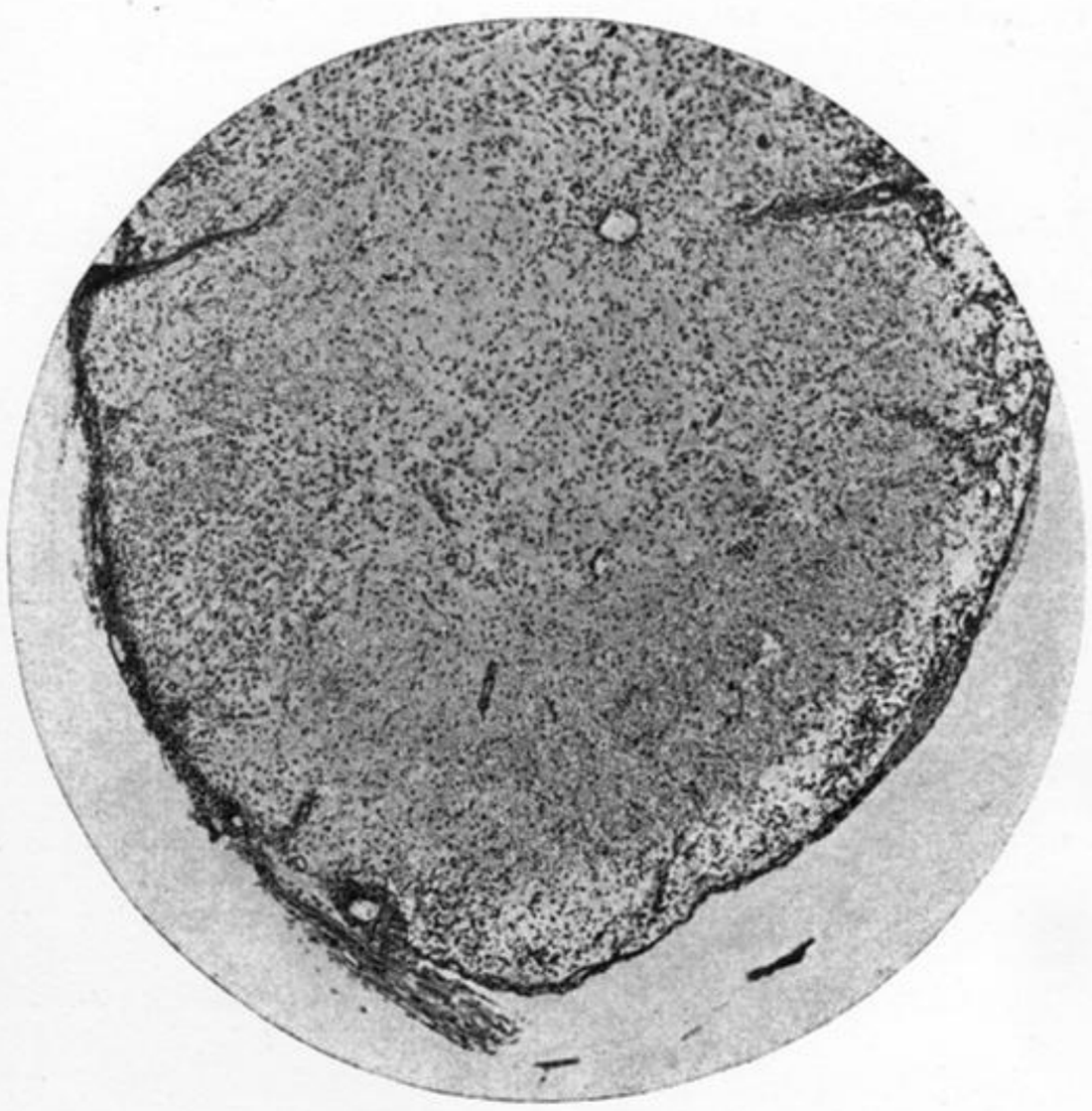

FIG. 1. ×40.

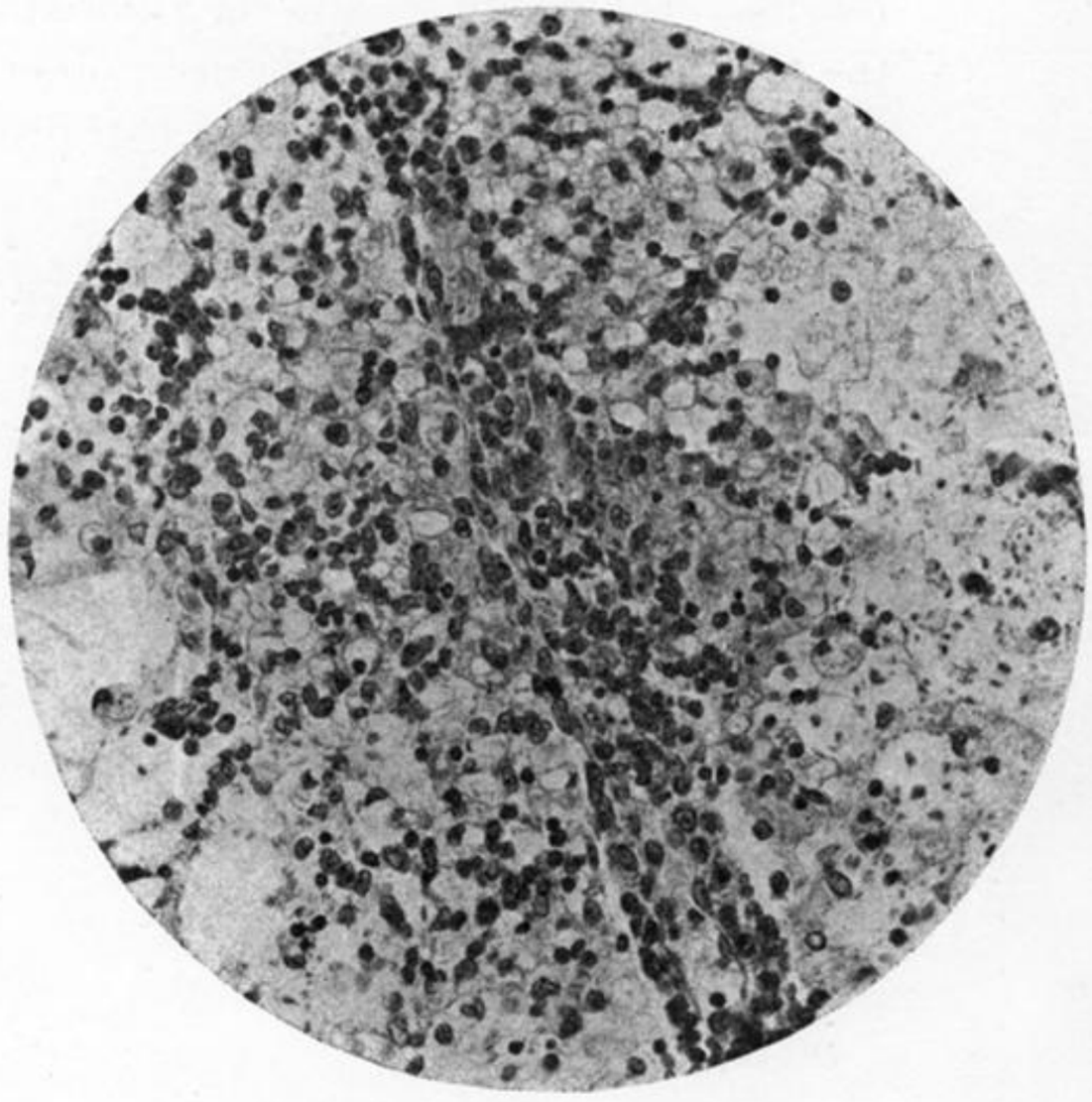

FIG. 2. ×220.

FIG. 3. ×1000.

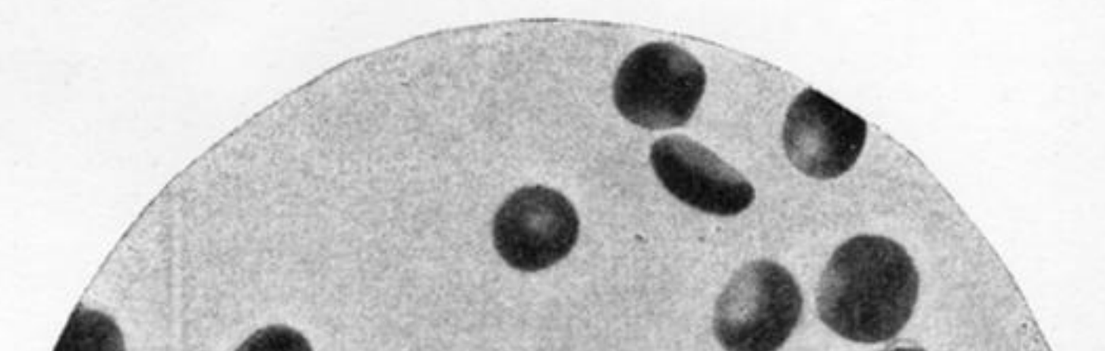

FIG. 4. ×1000.