

Notes on the Genus Toxoplasma, with a Description of Three New Species.

By H. G. PLIMMER, F.R.S., Professor of Comparative Pathology in the Imperial College of Science and Technology, London.

(Received February 25, 1916.)

[PLATES 9 AND 10.]

The genus *Toxoplasma* has not yet any definite systematic position. By some, the organisms which bear this name are regarded as being allied to the *Leishmania*, and by others to the yeasts. This uncertainty must exist so long as we do not know the complete life-history of these organisms; but, since any addition of new facts concerning them may help towards the acquisition of this knowledge, it has been thought worth while to record the finding of three new varieties, and to describe them.

The organisms were first seen by Splendore (1), and were described by him in July, 1908. He found them in the blood of a rabbit in Brazil, and the name *Toxoplasma* was given to them by Nicolle and Manceaux (2), who found them in a *gondi* in Tunis, shortly after Splendore's discovery, and described them in October, 1908. Since then, others have been found in the dog by Mello in Italy and by Yakimoff in Germany, in the mole by Prowazek in Japan, and in the pigeon by Carini in Brazil.

It seems probable, so far as our knowledge of these organisms extends at present, that they are more nearly related to the *Hæmogregarines* than to any other of the *Hæmosporidia*; they have no micronucleus, and therefore cannot belong to the *Leishmania*, as Nicolle and Manceaux thought, and the absence of a distinct capsule, and the fact that no budding process has been observed, should prevent them from being regarded as yeasts, as has been suggested.

The *Toxoplasmas* may occur free in the blood, but are generally found in the large mononuclear leucocytes; their distribution in the body of the affected animal is often peculiar. They give rise to very marked wasting and to considerable blood destruction, almost as marked as in the *Babesia* infections.

The *Toxoplasmas* are organisms of either a crescentic or bi-convex shape, sometimes with pointed and sometimes with rounded ends; occasionally, and under pressure, they may become nearly round. There is no capsule or definite membrane to be seen around them. The protoplasm is very fine

and delicate, and sometimes contains vacuoles. They are, on this account, difficult to fix satisfactorily. I have found that fixation by the vapour of iodine dissolved in chloroform, as described by me in 'Roy. Soc. Proc.' B, vol. 86, p. 389, gave the best results. The nucleus consists, in what may be regarded as a normal parasite, of a round dot or karyosome, which, before division of the cell, lengthens and becomes rod-shaped and then dumb-bell-shaped. Rarely, a ring form of nucleus may be found, as in Plate 9, fig. 6.

In many of the organisms the nucleus is either broken up into granules, or the cell gets filled with granules which take chromatin stains (hæmatoxylin, fuchsin, thionin, have been those used), so that they are indistinguishable from the nucleus. These granules may be the so-called "infective granules," such as have been described by the late Major W. B. Fry and Captain Ranken, V.C.(3), in Trypanosomes, which the late Prof. Minchin (4) acknowledged in the last paper written by him; for the amount of ordinary division seen does not seem to correspond with the enormous number of parasites found in individual leucocytes. They have none of them the reaction, with iodine fixation, of the reserve food granules seen in Trypanosomes.

The varieties of *Toxoplasma* I have found in a fossa, a fruit pigeon, and a Say's snake, showed no motility when examined fresh, even on the warm stage, neither did I observe any changes of shape.

As regards size, the organisms vary too much in the same animal for very approximate measurements to be of much use. In the fossa they were the smallest, and varied from 2μ to 8μ in straight length from end to end, and from 1.4μ to 2.5μ in breadth at middle. In the fruit pigeon the length varied from 3μ to 8μ , and the breadth from 2μ to 5μ . Those found in Say's snake were larger, and varied from 7μ to 10μ in length, and from 3μ to 6μ in breadth. These measurements were made on fresh, unfixed organisms.

In all these cases the Toxoplasmas were found free in the blood, but in very small numbers; they were generally found in the large mononuclear leucocytes from the affected organs, often occurring in enormous numbers. They were found principally in the lungs and pleural effusion and in the bone-marrow in the fossa; in the lungs and exudation from the lungs in the fruit pigeon, and in the liver in Say's snake. None were found in the bone-marrow of the bird or snake. The infected leucocytes show but little alteration in the early stages of the infection, when they contain only one or two parasites, but as these multiply the leucocyte enlarges enormously, and the protoplasm becomes extremely thin and frothy in texture, and there is always a marked hyperchromatosis of the nucleus; the cell eventually

breaks up. The parasites are sometimes found in the nucleus itself ; of this an example is shown in Plate 9, fig. 2. There is a tendency for these leucocytes to mass themselves together, but no true giant-cells are formed. Very few parasites were found in the endothelial cells of these three animals ; a few were found in the omentum of the fossa and in the mesentery of the fruit pigeon. This is quite contrary to the experience of Miss H. L. M. Pixell (5), who found cells containing large numbers of the parasites from these situations.

The multiplication of the *Toxoplasmas* is effected ordinarily by longitudinal division. The nucleus first enlarges, then becomes rod-shaped, and later of dumb-bell shape, and eventually the daughter nuclei are formed, the cell having already begun to divide. I have seen no example of transverse division in these cases. In the bone-marrow of the fossa several round cysts were found in enlarged leucocytes, which suggested schizogony (Plate 9, fig. 3), and these are somewhat like the schizont in the *Coccidia* when it is about to divide up into merozoites ; and in the Say's snake (Plate 10, fig. 9) there is a structure which is apparently a later stage, showing the formation of merozoites. No flagellated forms have been seen.

Many attempts at cultivation in various media were made, but none were successful.

The *Toxoplasma* has a very wide geographical as well as zoological distribution. The three cases here recorded came respectively from Madagascar, the Aru Islands, and Mexico, and those previously described by various observers were found in Brazil, Tunis, Italy, Japan, and Germany.

The varieties to be described were found in the course of the *post-mortem* examination of the animals which have died in the Zoological Gardens, and the following paragraphs will indicate the distribution of the parasites and the points of difference between them :—

I. *Fossa*, *Cryptoprocta ferox*, from *Madagascar*. Plate 9, figs. 1–3.

The animal was very wasted. Both pleural, peritoneal, and pericardial cavities contained a quantity of blood-stained fluid. The lungs and kidneys were very congested, and there was a layer of lymph on the under-surface of the diaphragm. The blood was extremely anæmic, and contained many poikilocytes and nucleated erythrocytes. A few *Toxoplasmas* were found in the blood ; many were found in the blood from the lung, and in the pleural and peritoneal exudation, and in the bone-marrow. Few were found free ; nearly all were contained in the large mononuclear leucocytes, often a great number, as many as 36, in a single leucocyte. The leucocytes were very much enlarged, and their protoplasm was extremely thin and delicate, many

being ruptured in the preparation of the film, even with the greatest care. The nucleus of the leucocyte invariably showed signs of hyperchromatosis, often very marked. The nucleus of the parasite was often broken up into granules, or chromidia, but many showed the single dot form with a clear area around. Sometimes the *Toxoplasma* was found in the nucleus itself (Plate 9, fig. 2). Schizonts were found in the bone-marrow in various stages up to the apparent breaking up into merozoites (Plate 9, fig. 3). Occasionally the parasites were found in the polynuclear cells in the bone-marrow: this was possibly a phagocytic process, as the shapes of the ingested parasites were much altered.

II. *Blue-tailed Fruit Pigeon*, *Carpophaga concinna*, from the *Aru Islands*.

Plate 9, figs. 4-6; Plate 10, fig. 10.

Died in an emaciated condition. The lungs were very congested, and contained a large quantity of exudation. There was some bloody fluid in the body cavity. Very few parasites were found in the blood, but large numbers were present in the blood and exudation from the lungs. Some were found free, but they were mostly contained in the large mononuclear leucocytes. These cells had undergone more destruction than was the case in the fassa, and the blood was extremely anæmic. The nucleus of the *Toxoplasma* was generally single and definite, and was not broken up into granules. A few were found in the bone-marrow, but none showing definite schizogony. The drawing, reproduced in Plate 10, fig. 10, was made to scale from an unfixed preparation, just tinted with 1-2000 methylene blue in 0.8 per cent. salt solution. At 1, there are ordinary forms of the *Toxoplasma*; at 2, a form with the nucleus in form of chromidia or granules; at 3, a large mononuclear leucocyte containing several parasites, some quite differentiated; and at 4, possibly an early stage of schizogony.

III. *Say's Snake*, *Coluber melanoleucus*, from *Mexico*. Plate 10, figs. 7-9.

The snake was very wasted, and its blood, which was very anæmic, contained a few hæmogregarines. The lung was pneumonic and full of exudation; the liver was small and pale. Toxoplasmas were found in small numbers in the exudation from the lung, and in enormous numbers in the liver; a few single ones in the blood.

The above is all that it has been possible to find in this material. A careful watch is being kept for new cases, from which it is hoped to obtain material which will enable the knowledge of this curious parasite to be carried further.

I am much indebted to Mr. A. T. Watson, the laboratory assistant at the Zoological Gardens, for very intelligent help in the preparation of the specimens, and to Dr. A. Norman for the photographs of a difficult object.

[*Note added May 1, 1916.*—Since the above was written I have found *Toxoplasmas* in another bird, a Pied Bush Chat (*Pratincola caprata*) from India. They were found in the blood and exudation from the lungs, which were inflamed and cedematous, in the mononuclear leucocytes as before. Further early stages of schizogony were found in the large endothelial cells in the lungs.]

REFERENCES.

1. Splendore, A. "Um novo Protozoo parasita de conigli," 'Rev. da Soc. Scientifica de S. Paulo,' Nos. 10-12, 1908.
2. Nicolle, C., and Manceaux, L. "Sur une infection à corps de Leishman du Gondi." 'Compt. Rend.,' October, 1908, and February, 1909.
3. Fry, W. B., and Ranken, H. S. "The Extrusion of Granules by Trypanosomes," 'Roy. Soc. Proc.,' B, vol. 84, p. 79, and vol. 86, p. 380.
4. Minchin, E. A. "On the So-called 'Infective Granules,'" 'Ann. de l'Institut Pasteur,' vol. 29, p. 537.
5. Pixell, H. L. M. "Notes on *Toxoplasma gondii*," 'Roy. Soc. Proc.,' B, vol. 87, p. 67.

EXPLANATION OF PLATES 9 AND 10.

FIGS. 1-3, FROM FOSSA.

The preparations were photographed under a magnification of 1000 diameters.

They were fixed wet in the vapour of iodine dissolved in chloroform, and were stained with Giemsa's stain, made alkaline, and followed by acetone and xylol.

FIG. 1.—Mononuclear leucocyte in blood from lung, showing enlargement of leucocyte, and the delicate, frothy protoplasm, with hyperchromatosis of the nucleus. The leucocyte contains two parasites, one of ordinary shape just to left of nucleus, and a larger one with undefined extremities at the upper part of the cell.

FIG. 2.—Two mononuclear leucocytes from the peritoneal exudation, both showing hyperchromatosis of the nuclei. The cells are filled with parasites, and in the cell on the left the nuclei of the parasites are broken up into granules. In this cell a parasite is seen in the nucleus.

FIG. 3.—A large mononuclear leucocyte from the bone-marrow, with considerable hyperchromatosis of the nucleus, containing a mass of parasites, apparently in process of schizogony, and of breaking up into merozoites. These were found in many stages.

FIGS. 4-6 AND 10, FROM FRUIT PIGEON.

Fig. 4 fixed by the method of Carnoy-Lebrun, and stained with Weigert's hæmatoxylin. Figs. 5 and 6 fixed and stained as Figs. 1-3. Photographed $\times 1000$.

- FIG. 4.—From exudation from lung, showing three free toxoplasmas to the right, and to the left a mononuclear leucocyte, with a hyperchromatic nucleus, containing parasites, two apparently in late stage of division.
- FIG. 5.—From same exudation, showing three free toxoplasmas at upper part, a large mononuclear leucocyte containing one parasite on right, and a similar cell containing several on the left.
- FIG. 6.—From blood from lung, showing a large mononuclear leucocyte containing several toxoplasmas. Below the hyperchromatic nucleus can be seen two parasites with ring-shaped nuclei.
- FIG. 10.—Drawing made from fresh unfixed specimen of exudation from Fruit Pigeon's lung. 1. Ordinary toxoplasmas. 2. Form with nucleus in form of granules. 3. Large mononuclear leucocyte containing parasites. 4. Probably an early stage of schizogony.

FIGS. 7-9, FROM SAY'S SNAKE.

Fig. 7 fixed in vapour of iodine dissolved in chloroform and stained with alkaline Giemsa's stain. Fig. 8 fixed by method of Carnoy-Lebrun and stained with Weigert's hæmatoxylin. Fig. 9 fixed as Fig. 8 and stained with alkaline Giemsa's stain. Photographed $\times 1000$.

- FIG. 7.—From liver, showing a fusion of three large mononuclear cells containing a number of parasites. It will be noticed that the toxoplasmas lie in vacuoles, and that they are larger than those in either the mammal or the bird.
- FIG. 8.—From liver, showing one very distended mononuclear leucocyte, with nucleus about to break up, and with very delicate frothy protoplasm, apparently about to give way, containing 14 parasites, each lying in a distinct vacuole.
- FIG. 9.—A section from the liver, showing several free toxoplasmata in lower part, and above what is probably a later stage of schizogony than that shown in Plate 9, fig 3, as the shape of the parasites is more defined.
-



FIG. 1.

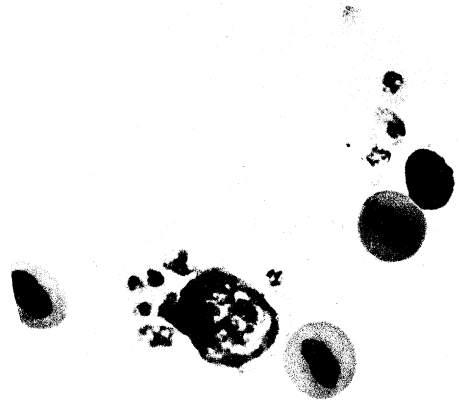


FIG. 4.

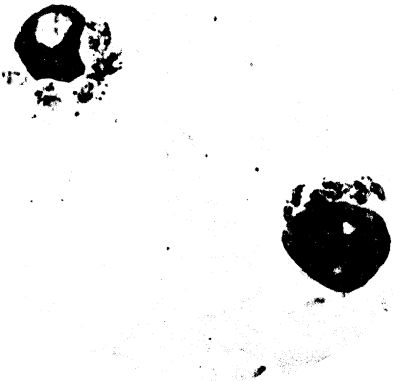


FIG. 2.



FIG. 5.

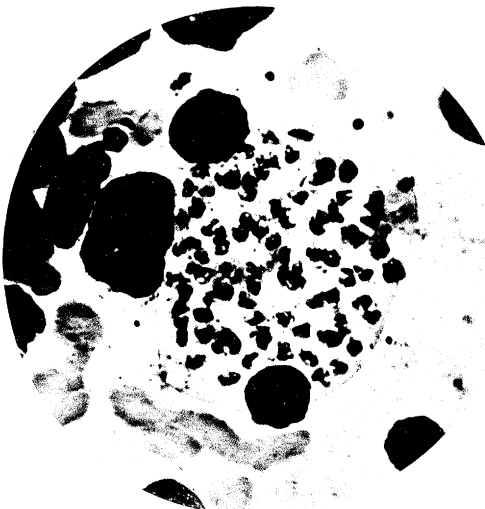


FIG. 3.



FIG. 6.



FIG. 7.

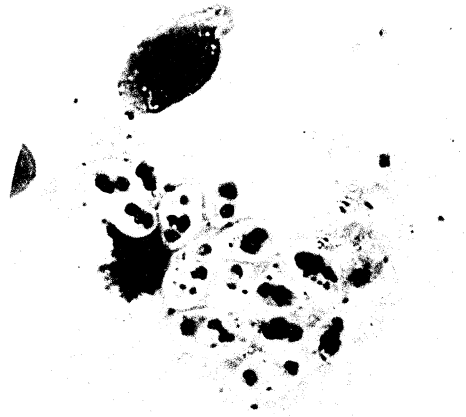


FIG. 8.



FIG. 9.

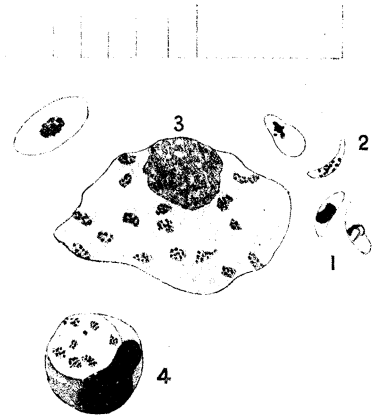


FIG. 10.

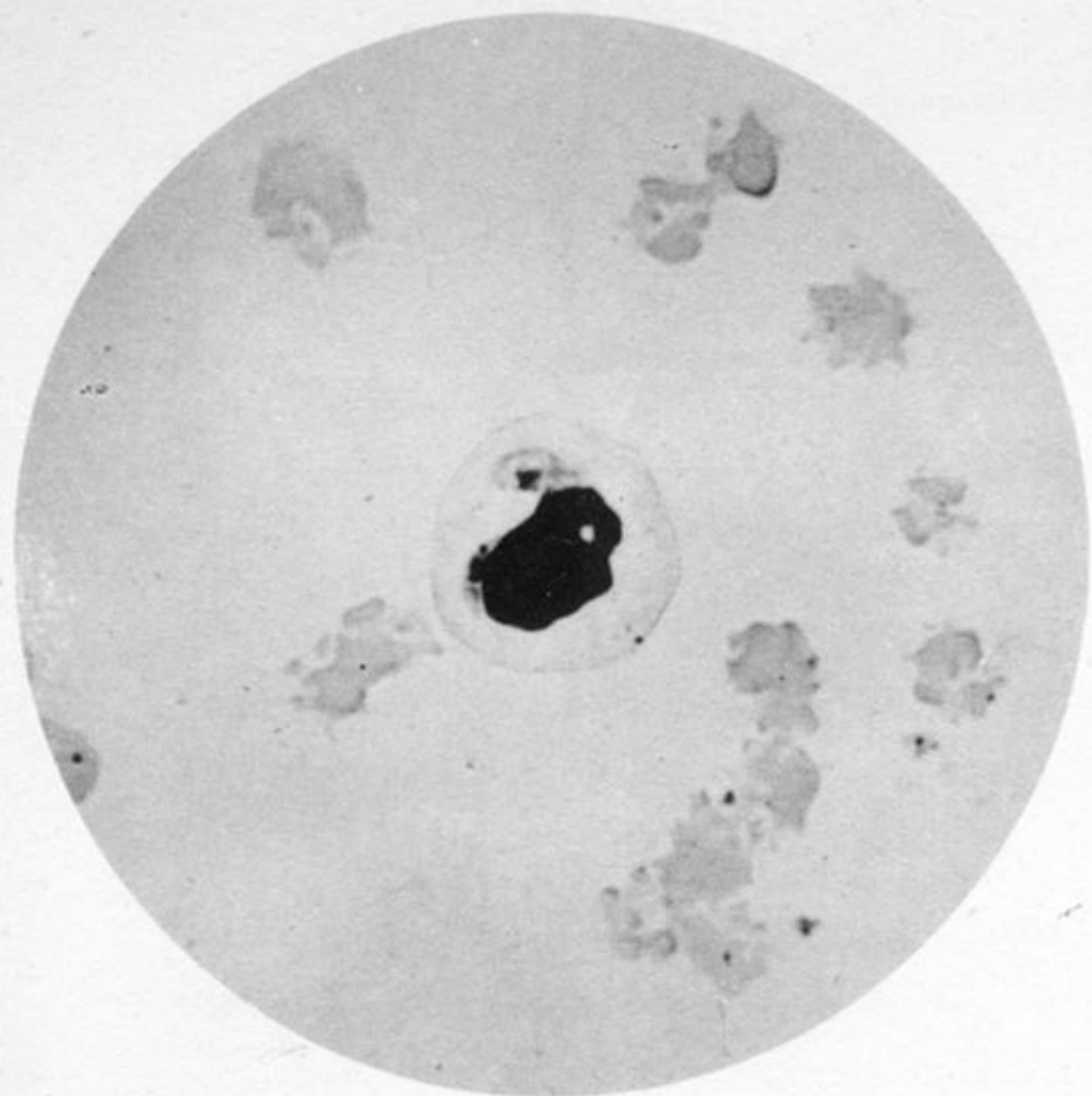


FIG. 1.

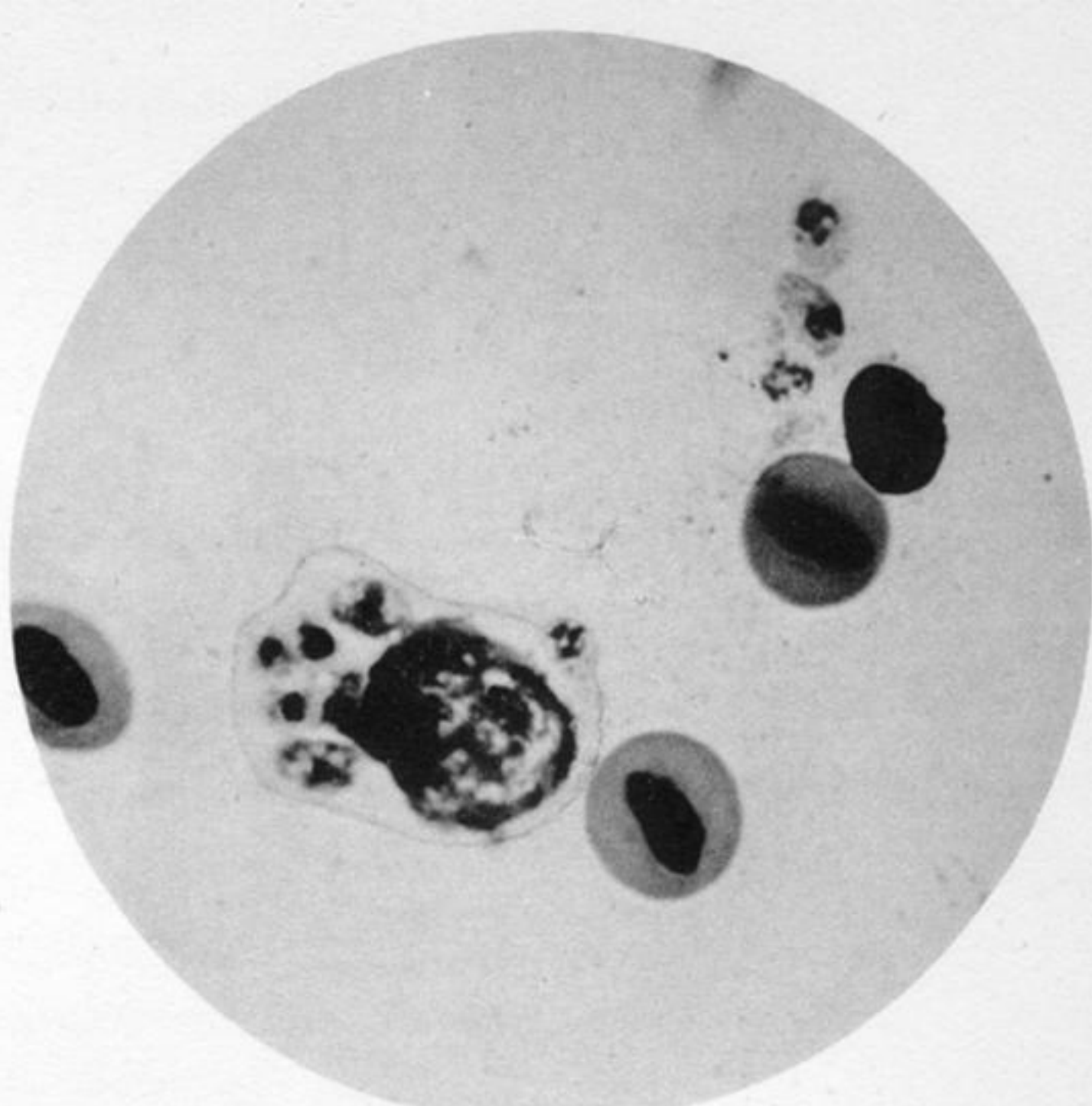


FIG. 4.

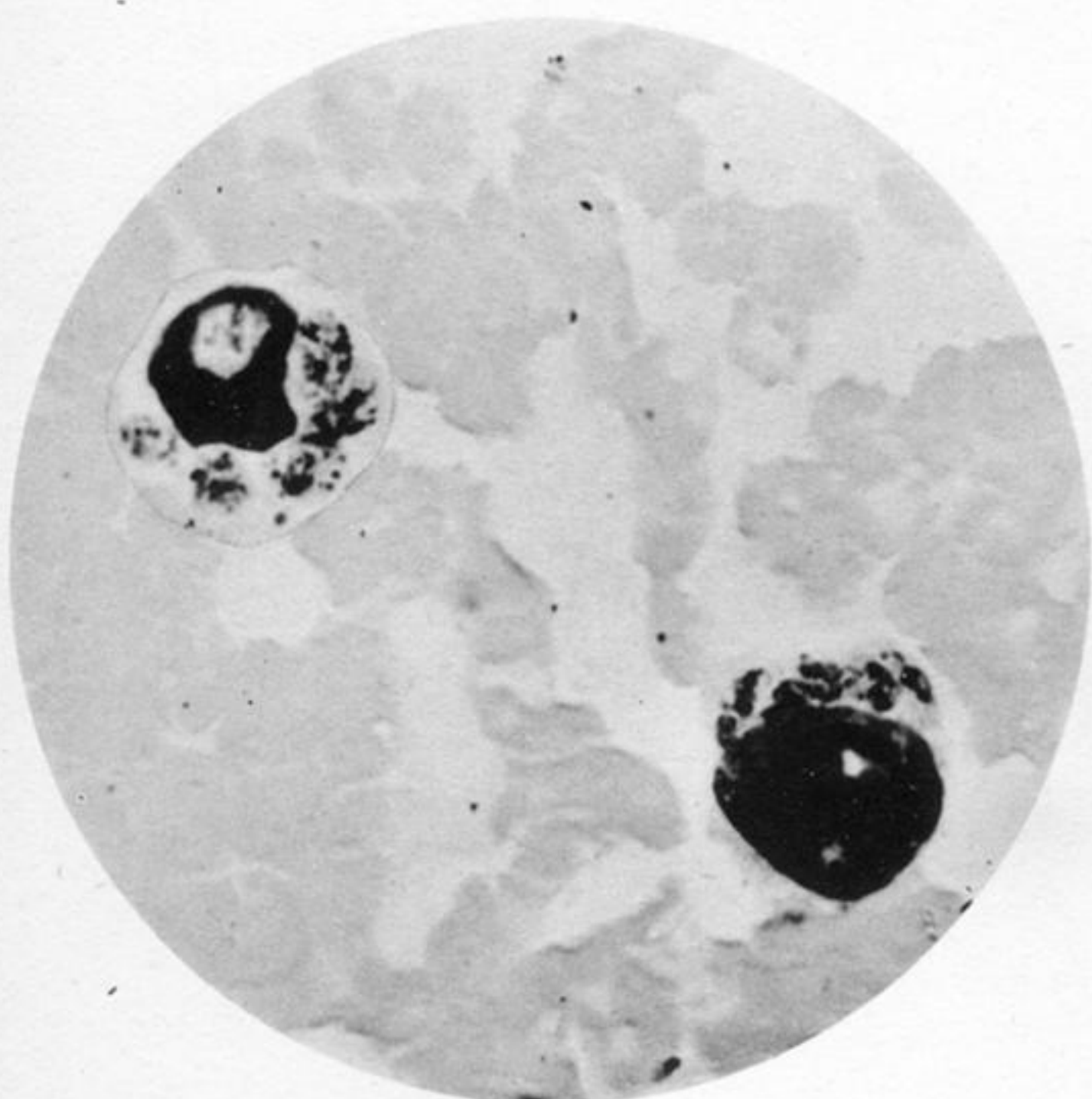


FIG. 2.

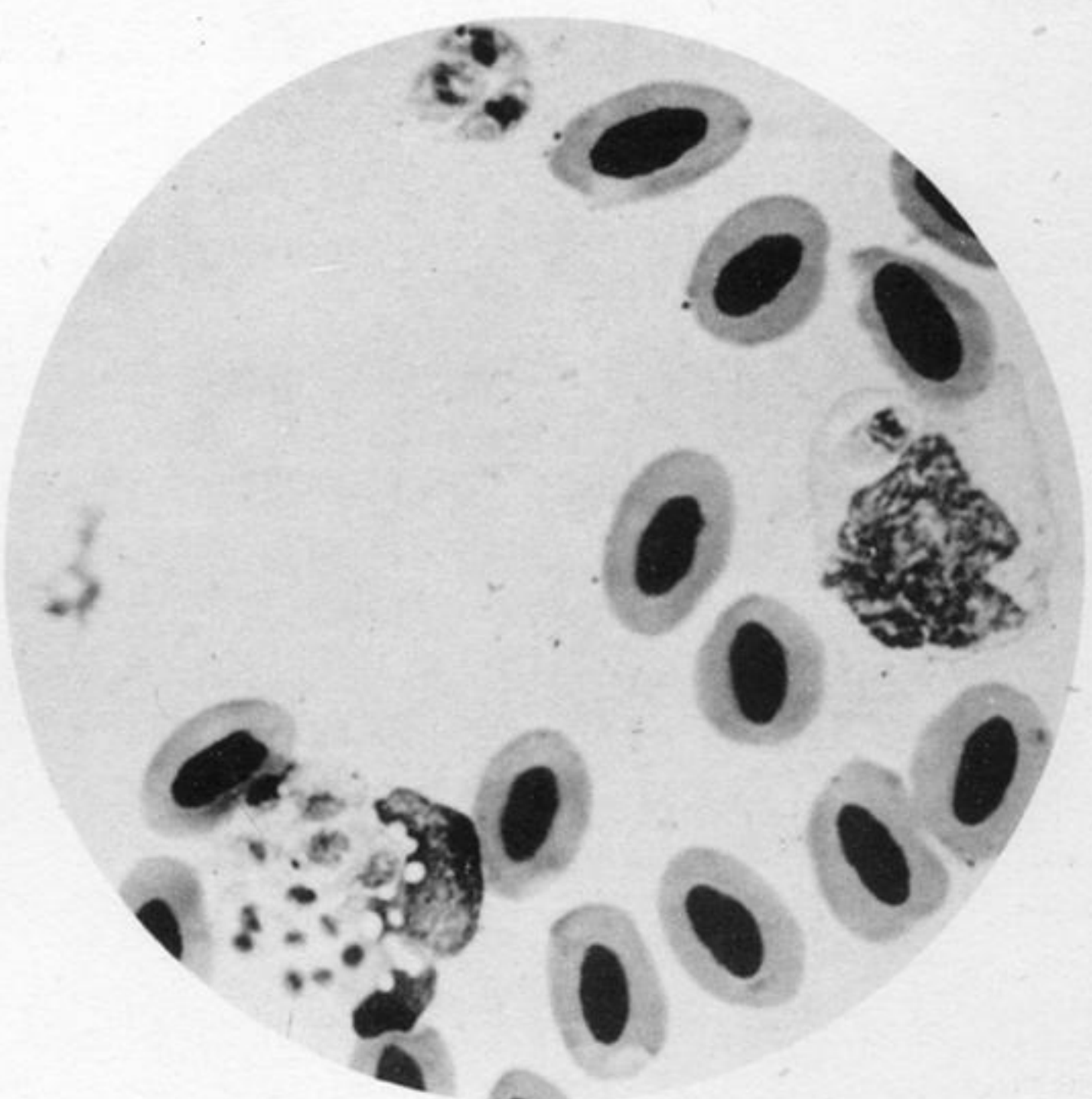


FIG. 5.

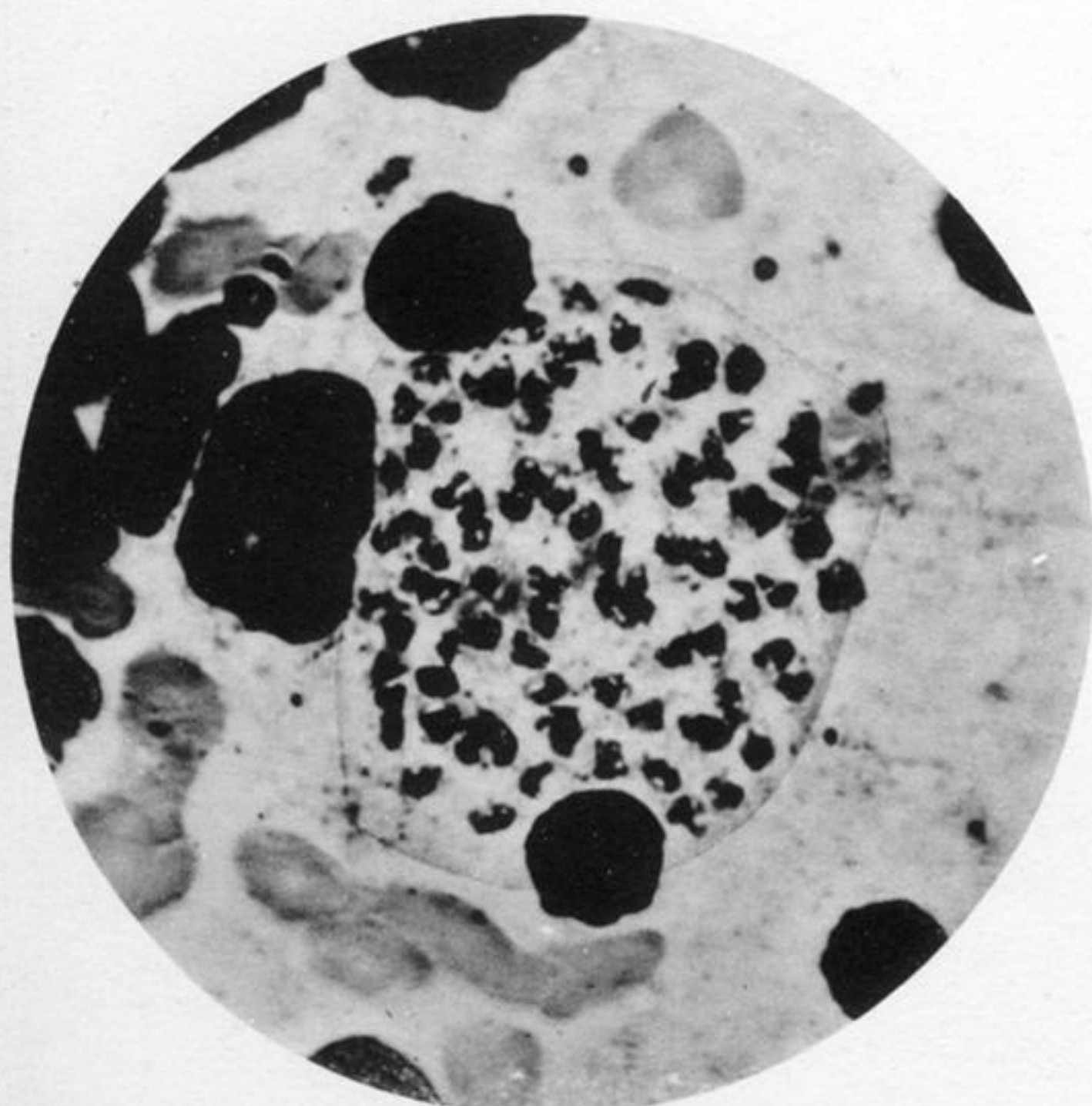


FIG. 3.

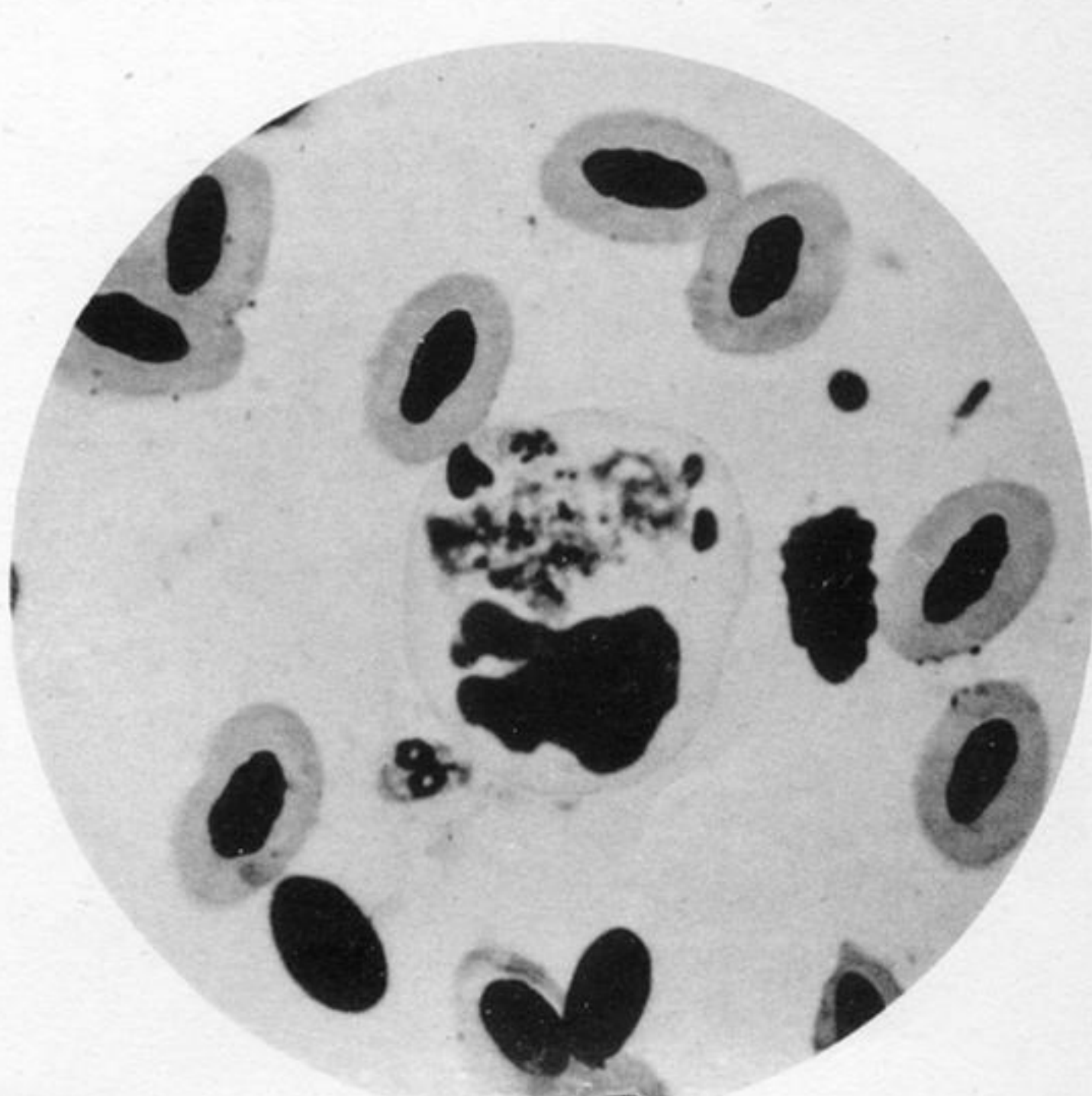


FIG. 6.

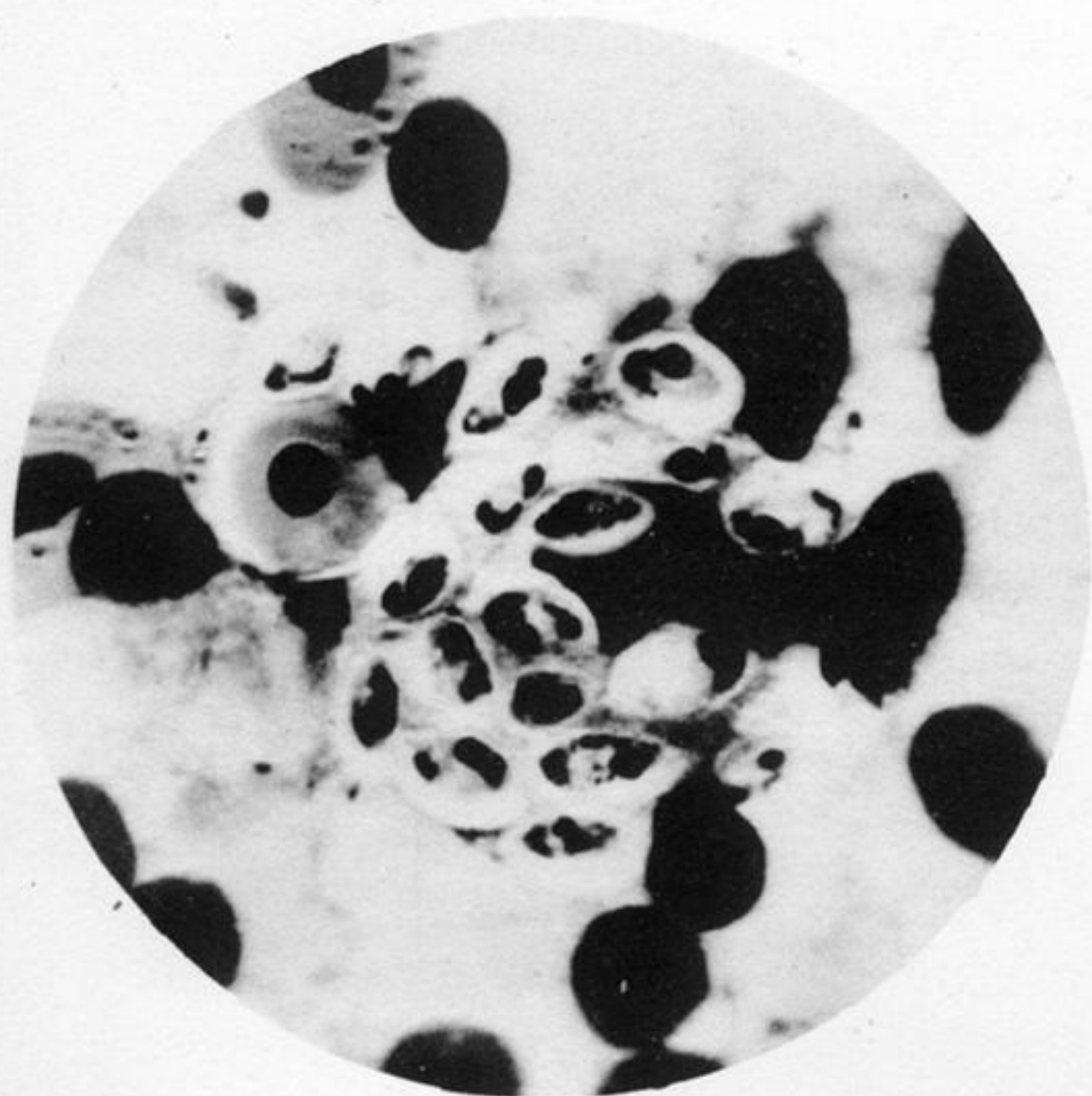


FIG. 7.

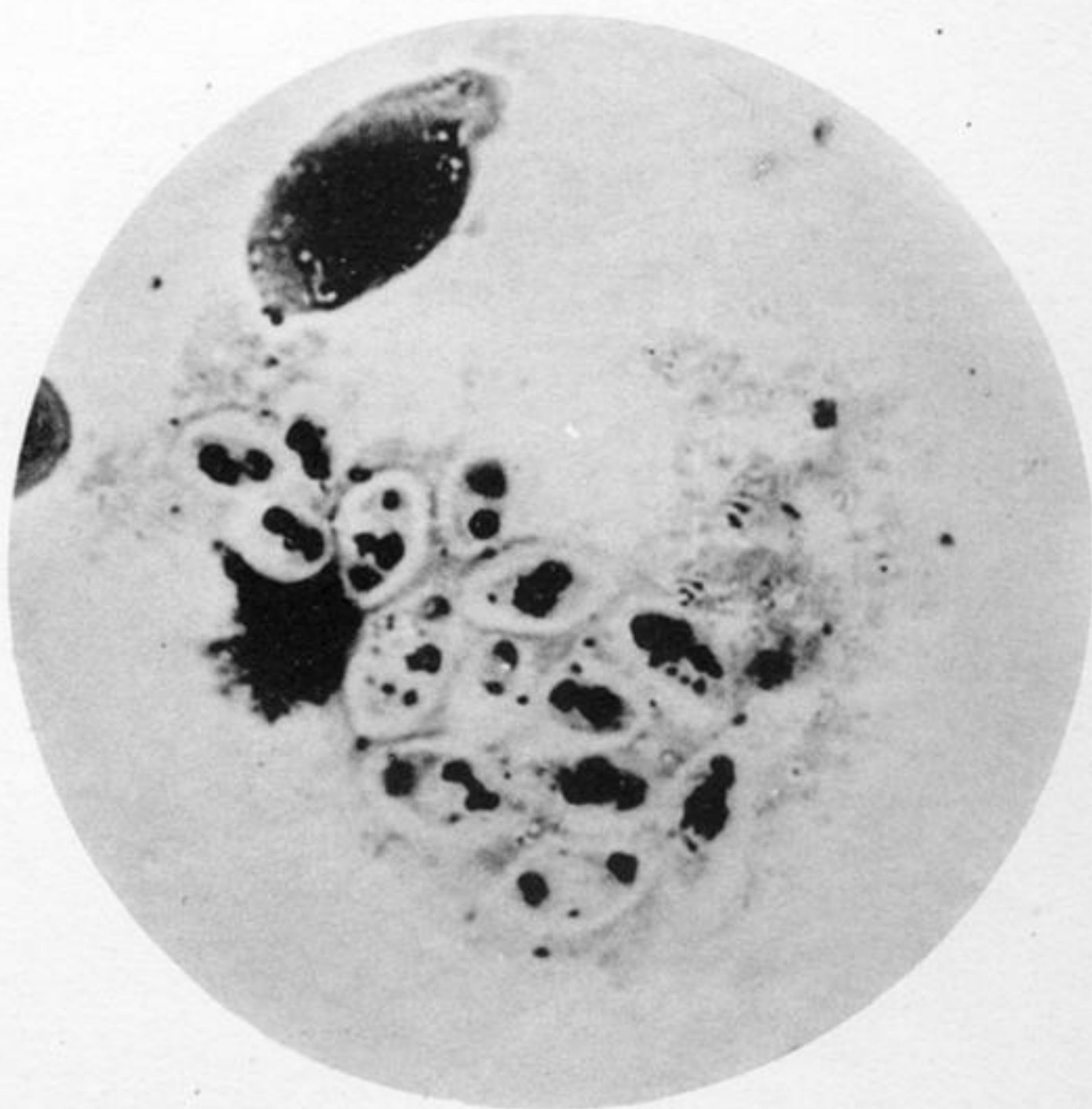


FIG. 8.

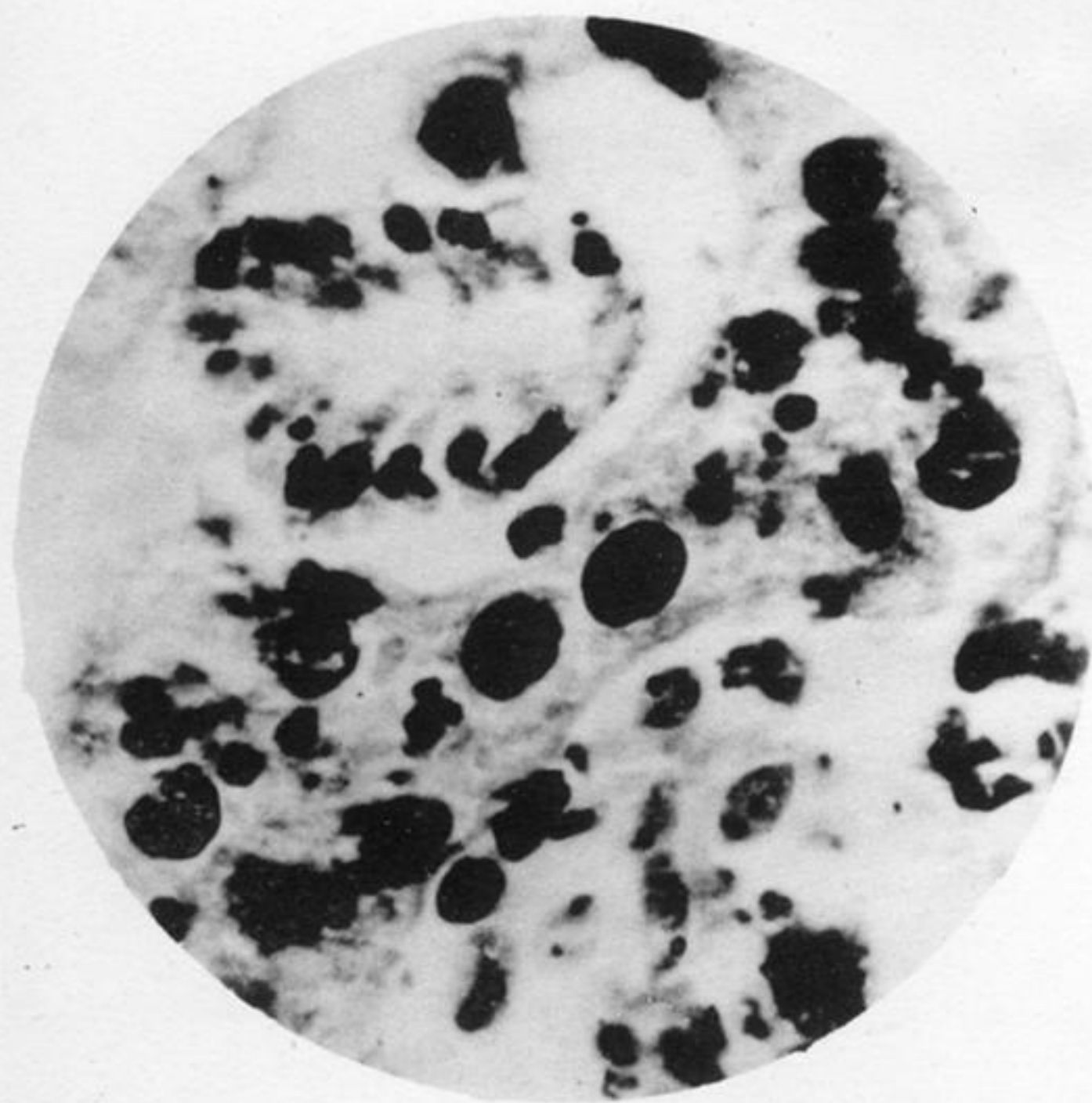


FIG. 9.

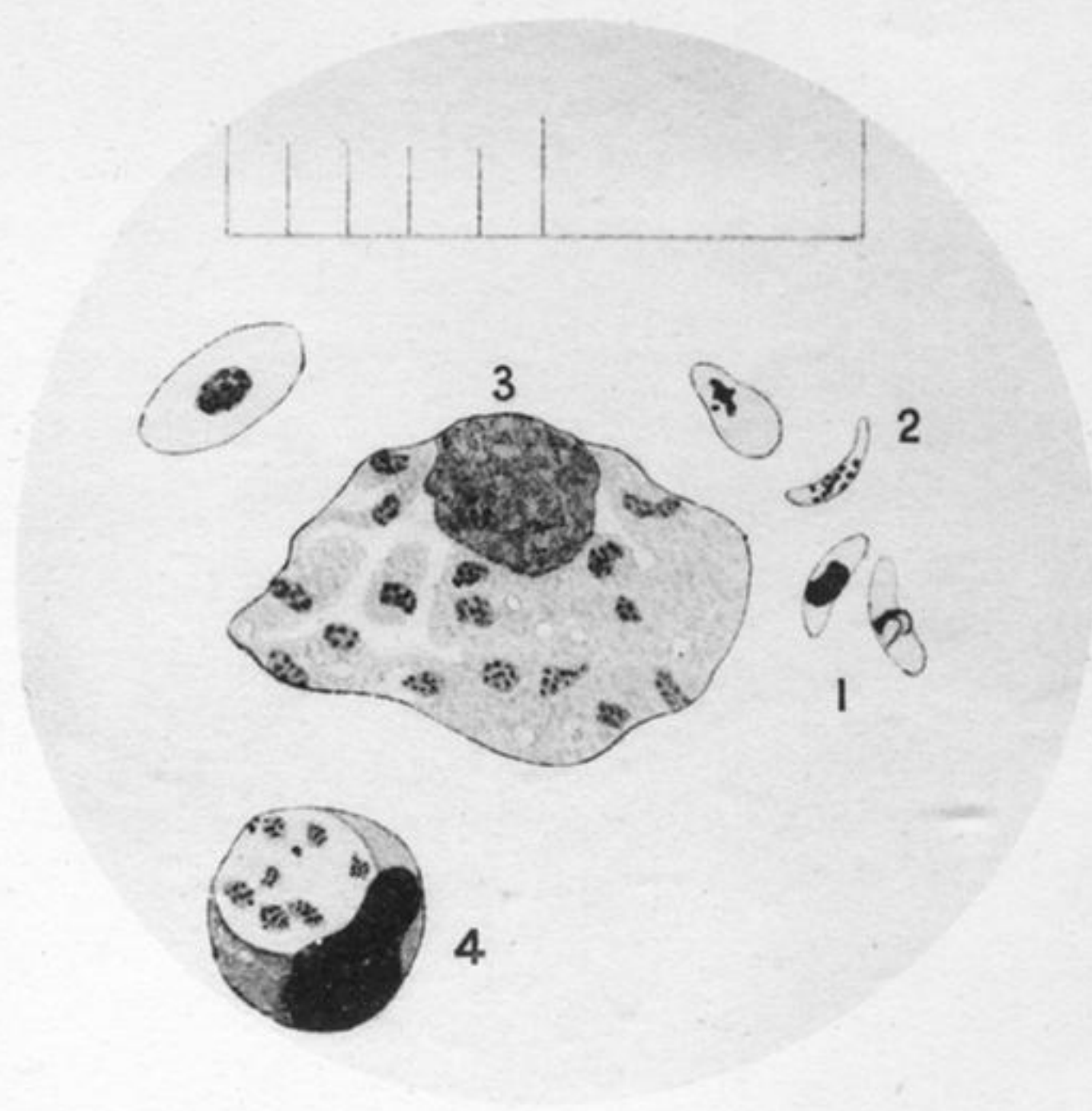


FIG. 10.