Ca = 20·9. Calculated for $(C_4H_5O_5)_2Ca.H_2O$, C = 21·0. The free acid melts at about 100° C.

Examination for Carbohydrates.—Sugars seemed to be absent. A small quantity, however, of a mucilaginous carbohydrate similar to gastrolobin was separated.

Owing to the reported presence of quercitrin and saponin bodies, by Dunstan and Stockman respectively, special search was made for these bodies, but with negative results.

Observations on the Life-history of Leucocytes. Part II.—On the Origin of the Granules.

By C. E. Walker, Assistant-Director of the Cancer Research Laboratories, University of Liverpool.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received August 2, 1906,—Read February 7, 1907.)

[Plate 5.]

The granules that are so frequently found in leucocytes* generally seem to lie scattered quite irregularly in the cytoplasm of the cell in which they occur. It is possible, in the case of the leucocytes found in the spleen, lymphatic glands, and the blood of mammalia, that there never is any ordered arrangement of the granules. In the bone-marrow, however, where leucocytes containing granules are often extremely numerous, a section of properly preserved material will show that the granules in a large proportion of these cells are arranged in a more or less definite manner. The granules in these are, as a rule, oval in shape, and are seen to lie in sequence close to each other, so that a line drawn through their long axes would appear as a thread or wire coiled up irregularly in the cytoplasm of the cell (fig. 1). Given this line connecting them, the granules would exactly simulate beads threaded on a wire bent into irregular curves, and put into a small spheroidal space. There are many gradations in the regularity of this arrangement of the granules. It varies from the mere suggestion of some of them having been strung together to very definite order, and the joining of several end to end (fig. 2).

There are, again, other cells in which a large number of the granules are

* As in a previous communication ("Observations on the Life-history of Leucocytes," 'Roy. Soc. Proc.,' B, vol. 78, p. 53), the term "leucocyte" is used in the widest possible sense, and is intended to include all the wandering nucleated cells of the body.

joined together, forming in places a thick, deeply-staining thread, the axis of which is continuous with the axis of the strings of separate granules (fig. 3). In some such cells the separate granules and the thick thread appear to be about equal in amount and continuous with each other (fig. 4), while in others there are but very few granules, and the thread preponderates. From these it is possible to pass, by almost insensible gradations, to cells where there are no granules, but only a thick thread coiled up around the nucleus (fig. 6). In some of these cells it is practically impossible to distinguish the nucleus, so dense and darkly-staining is the coiled thread (fig. 5). So misleading are these figures that for several months I took them for, and drew them as, the spireme stages in the prophase of division in which the nuclear membrane had disappeared. It was only when I found nuclei in a resting condition existing in the cells which also contained this thread, that the present interpretation was arrived at. A careful examination of the cells in this stage, however, will frequently, though not always, demonstrate the presence of a resting nucleus. Where the nucleus cannot be seen, the reason is generally very obvious: the thread is so deeply stained with the basic stain, and is so closely coiled, that it entirely obscures the similarly stained nucleus. Moreover, while the thread stains very deeply, the nucleus, apparently, stains less readily than usual, thus adding to the probability of its being overlooked.

From this stage it is possible to pass again by practically insensible gradations to cells where the coiled-up thread occupies a space slightly larger than (fig. 7), equal to, or smaller than the nucleus, until we arrive at some where it seems to be about the same size in proportion to the nucleus as is the archoplasm in the case of the spermatid.

Though it has not been possible as yet to trace the origin of this thread farther, it is strongly suggested that it arises in the archoplasm, which is often seen to be connected with it (fig. 9).

During the whole of its existence the thread stains very deeply, and always with a basic in preference to an acid stain. It seems to show best with saffranin and basic fuchsin. When it has entirely broken up, the granules formed from it still stain in the same manner; but as they begin to lose their regular arrangement, so they seem to lose their affinity for the basic stain. It appears, however, that this happens but slowly in all such cells, and probably in many the granules never stain with the acid dyes. In bone-marrow large areas are often encountered where over 30 per cent. of the cells contain granules staining with a basic dye. In other parts of the body the cells containing such granules are not present in such large proportions, and we meet with more in which the granules take the acid dye.

The thread, which subsequently breaks up to form the granules, has only been met with in the cells in the bone-marrow, and has not been observed in leucocytes anywhere else in the body.

It would seem, then, that the granules arise from a threadwork forming part of the archoplasm, and which is probably derived from it originally. This thread grows and eventually breaks up to form the granules. During the whole of its existence it stains with a basic stain, reaching the maximum in staining capacity shortly before it breaks up. The staining capacity of the nucleus during this period seems to vary inversely with that of the thread. There seems to be no appreciable difference between the material forming the thread and the chromatin in an actively dividing cell. There is, however, no evidence of its having been derived from the nucleus.

The position, from a cytological point of view, is much obscured by the current methods of dealing with leucocytes. It is extremely difficult to compare the results obtained by the methods used by cytologists with what has been observed in specimens that have been dried, heated, fixed with alcohol, or otherwise treated in such a way as to materially alter the appearance and inter-relationship of the parts of the cell. The methods commonly used, however, have proved to be of the greatest value clinically, and with regard to the staining reaction of the granules, which have so largely entered into the current classification of leucocytes, there is nothing incompatible with the observations here recorded.

It has been seen that the staining reaction in some, at any rate, of the granular cells changes from basic to acid. It is highly probable that this change may be hastened or retarded by influences external to the cell while it is still in the body. This probability is increased by the fact that the proportion of acid to basic staining granules is increased by so crude an influence as additional heat and dryness after the cells have been removed from the body.

The presence, therefore, of varying proportions of cells containing the so-called acidophile and basiphile granules in different diseases is just what one would expect, and is no argument against a common origin of both from the thread here described as occurring in some of the cells in the bone-marrow.*

* The material used has been chiefly obtained from the guinea-pig and rat. The same fixatives were used as described in "Observations on the Life-history of Leucocytes" ('Roy. Soc. Proc.,' 1906). Attention is also drawn to the remarks upon preservation in the same paper, as smears or films of bone-marrow will not show the figures here described. No matter how carefully a smear or film be prepared, there must always be a grave risk that the cells will be partially dried, and the least approach to drying is fatal to the proper preservation of the cells.

I have to thank Professor Sherrington for his kind advice, which has been of the greatest value to me, particularly when beginning my researches with regard to leucocytes.

[*Addendum, July,* 1907.—I would take this opportunity of pointing out the relationship between certain structures which arise from true archoplasms. Among such structures are the archoplasmic vesicles found in the cells of the testis, which develop into the cephalic cap of the spermatozoon; the similar structures (Plimmer's Bodies) which appear in some of the cells in malignant growths; and the granules in leucocytes. Whether these phenomena are connected in some way with the Maiotic Phase is not clear, though something of the kind would seem to be suggested.

Since the above communication was presented, my attention has been redirected to a communication by Professor Mathews, of Chicago University, on "The Changes in Structure of the Pancreas Cell."* I give the following summary of the results obtained by Professor Mathews and myself.

Mathews.	*Walker.*
(1) Certain spiral twists or coils are found in the cytoplasm of the pancreas cells at certain periods. They probably arise from the nucleus.	An archoplasm, *i.e.*, an area in the cytoplasm showing a denser and more homogeneous structure than the rest, is found in certain leucocytes. This archoplasm does not arise from the nucleus.
(2) The threads, or some of them, subsequently collect in a mass, forming the "so-called nebenkerns of the pancreas."	A coiled up thread, arising in the archoplasm, may be observed in some leucocytes. The archoplasm in the leucocyte is something entirely different to the "nebenkern of the pancreas cell."
(3) These spiral twists or threads proceed to decompose.	The coiled archoplasmic thread proceeds to grow until it surrounds and obscures the nucleus. So far from any decomposition taking place, there is never any cessation in the growth of the thread until it has invaded the greater part of the cytoplasm.
(4) A stage apparently intervenes between the disappearance of the threads and the appearance of the granules in any given portion of the cytoplasm of the cell.	The thread breaks up directly into a number of short lengths, which subsequently acquire an oval, and, later, perhaps a spherical shape.
(5) It is probable that the "zymogen granules and the cytomitoplasm of the inner zone arise as the products of the decomposition of the threads."	The granules found in the leucocytes described arise by the direct breaking up into definite segments of the thread. In these cells there is no cytomitoplasm, and the thread from its first appearance never shows any sign of decomposition.

* 'Journal of Morphology,' vol. 15, 1899, Supplement.

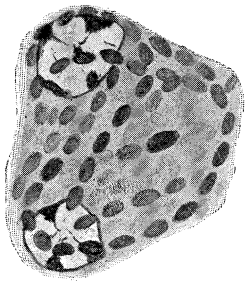

FIG. 1.

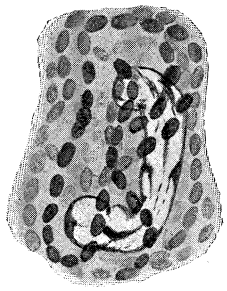

FIG. 2.

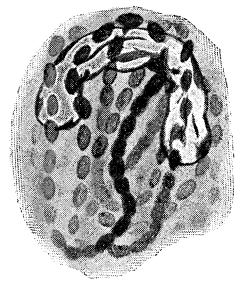

FIG. 3.

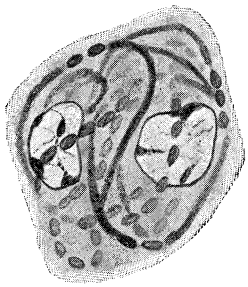

FIG. 4.

FIG. 5.

FIG. 6.

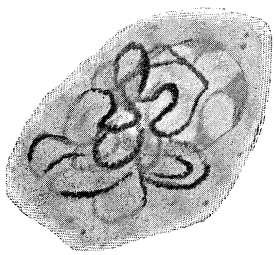

FIG. 7.

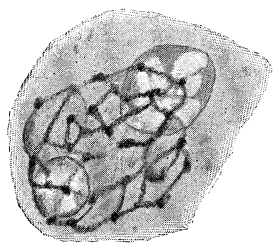

FIG. 8.

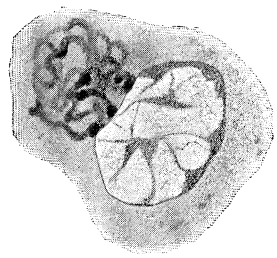

FIG. 9.

From the above it will be obvious that the second series cannot be regarded as any kind of confirmation of the first, for the sufficient reason that they possess nothing in common and deal with entirely different phenomena. For the same reason the two series do not contradict each other.]

DESCRIPTION OF PLATE.

(Drawn with a 2-mm. Zeiss apochromatic, 1·40 aperture, specially constructed for a 10-inch tube, and a 27 compens. ocular.)

All the cells illustrated are from the bone-marrow of the guinea-pig.

Fig. 1.—Cell showing the granules arranged more or less in strings.
„ 2.—The stringing of the granules is more marked, and some of them are joined end to end.
„ 3.—Still more of the granules are joined together, forming thick threads.
„ 4.—An earlier stage where granules and thread are about equal in quantity.
„ 5.—The thread before it has begun to break up. The nucleus is hidden.
„ 6.—The same stage with the nucleus showing.
„ 7.—An earlier stage. The nucleus here stains more faintly.
„ 8.—A slightly different form of thread, which is not uncommon.
„ 9.—A very early stage. The archoplasm is seen connected with the thread.

Observations on the Life-history of Leucocytes.—Part III.

By C. E. Walker, Assistant-Director, Cancer Research Laboratories, University of Liverpool.

(Communicated by Professor J. B. Farmer, F.R.S. Received May 15,—Read June 27, 1907.)

[Plate 6.]

The phenomena here recorded were first observed among the leucocytes of Axolotl, which had collected in a mass owing to the presence of a foreign body or to the infliction of a slight wound.*

Some among the mass of leucocytes thus obtained were seen to be sending out protrusions from their nuclei. Often where this occurred the action

* The methods used for obtaining leucocytes apart from the other cells of the body were adapted from those of other observers who have investigated inflammatory processes. Small celluloid tubes were introduced under the skin of the animal and removed after being left in the body for varying periods of time. The tubes and their contents were, after removal, dropped into a suitable fixative and sections made in the usual manner. Better and more uniform results were, however, obtained by fixing portions of the coagulated exudation from a small scratch upon the skin of the animal.

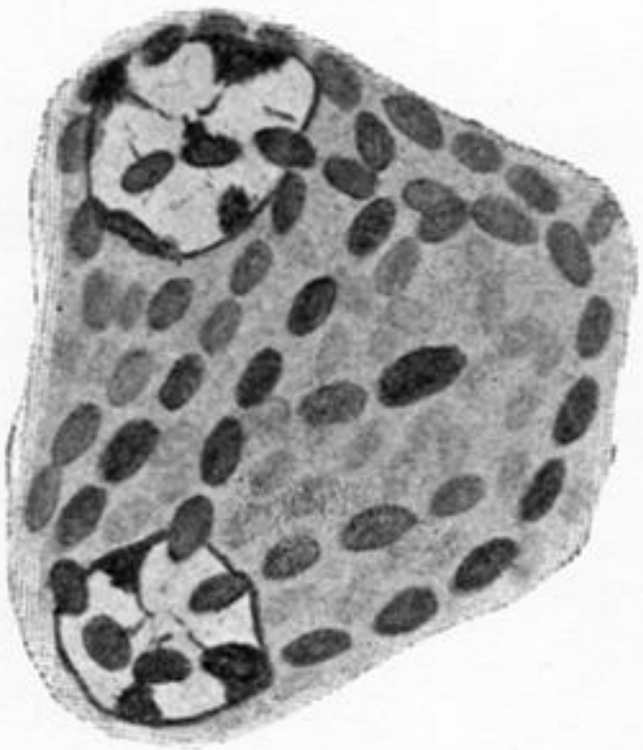

FIG. 1.

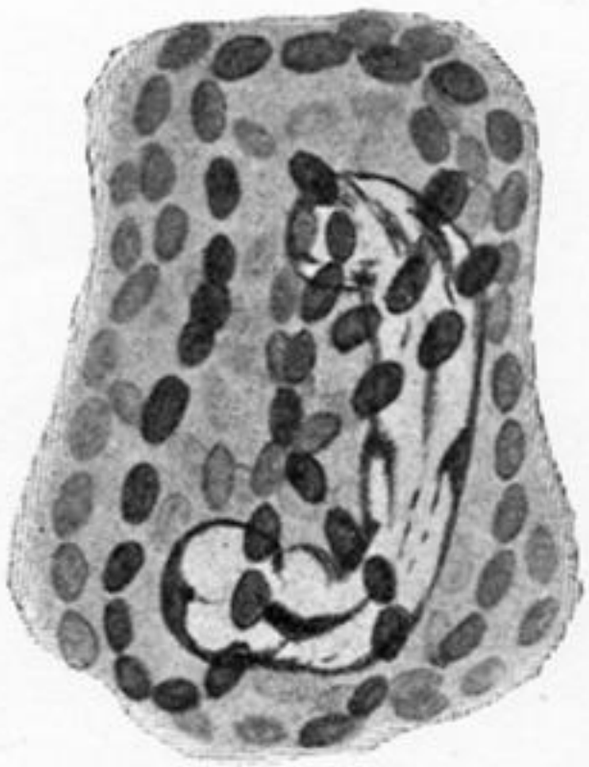

FIG. 2.

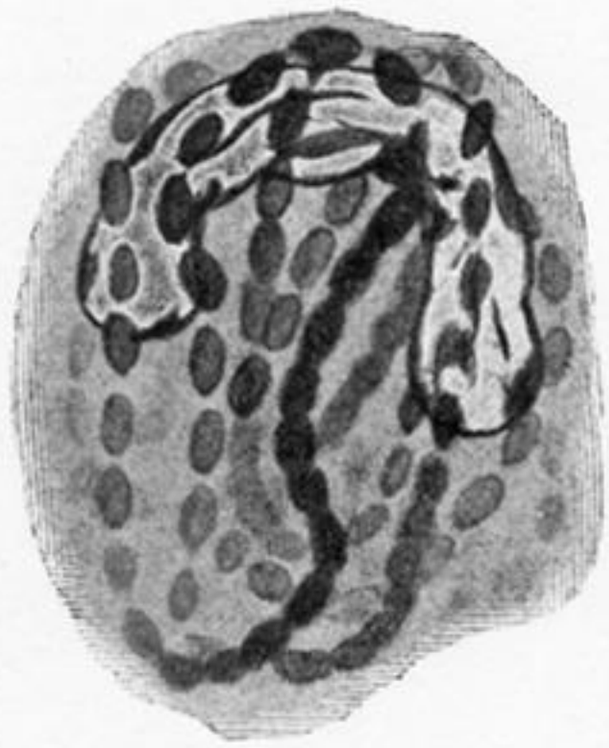

FIG. 3.

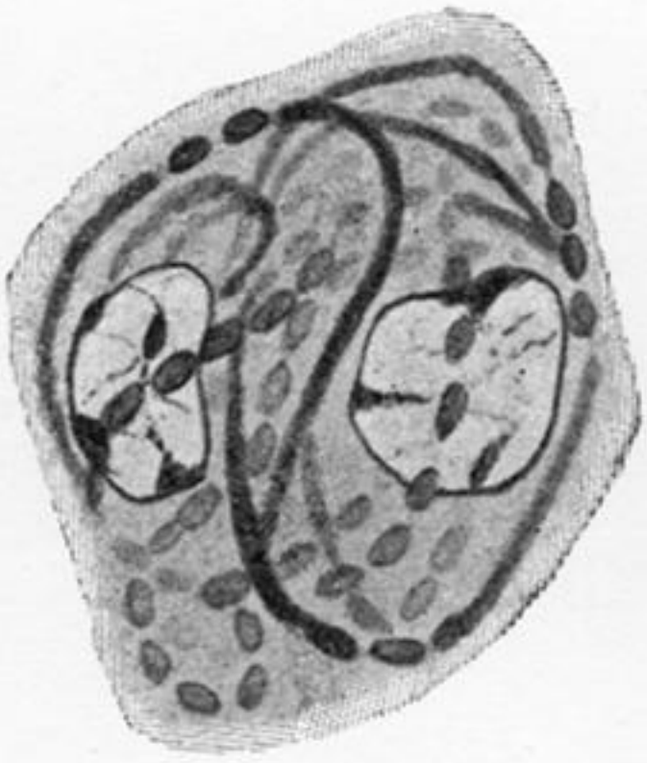

FIG. 4.

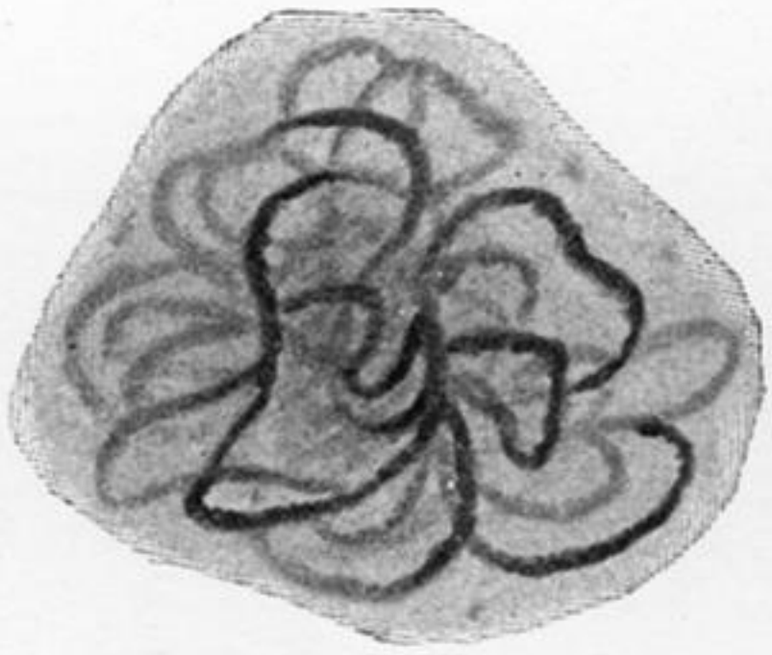

FIG. 5.

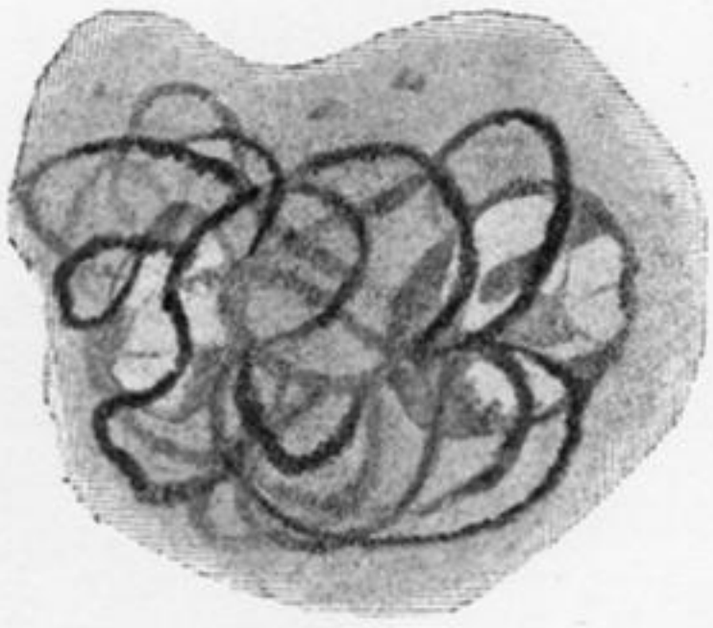

FIG. 6.

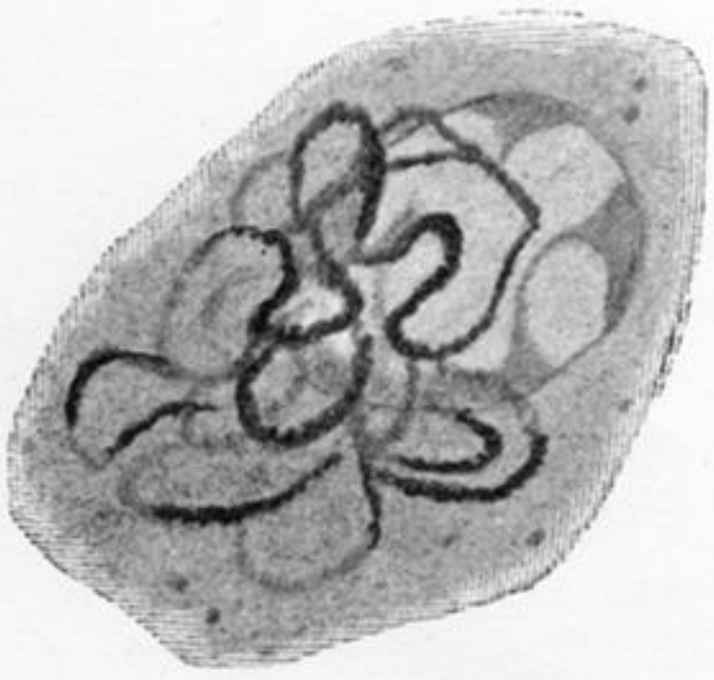

FIG. 7.

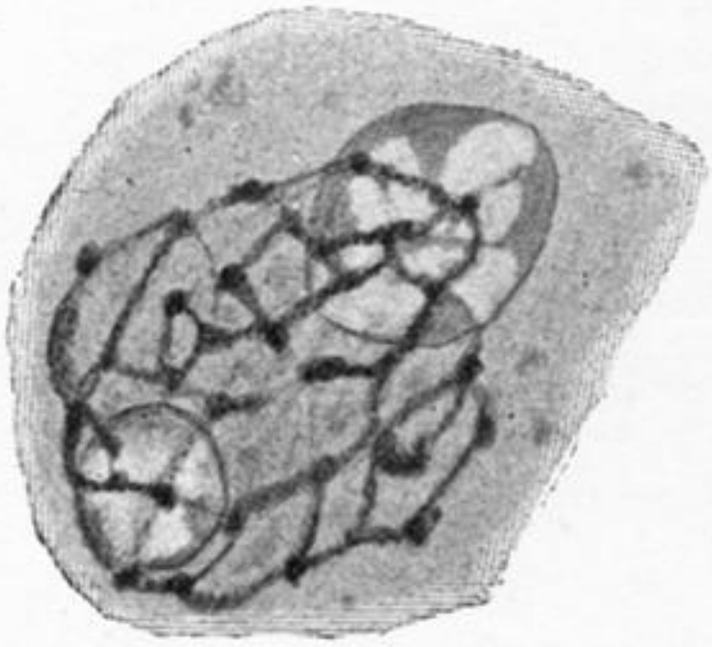

FIG. 8.

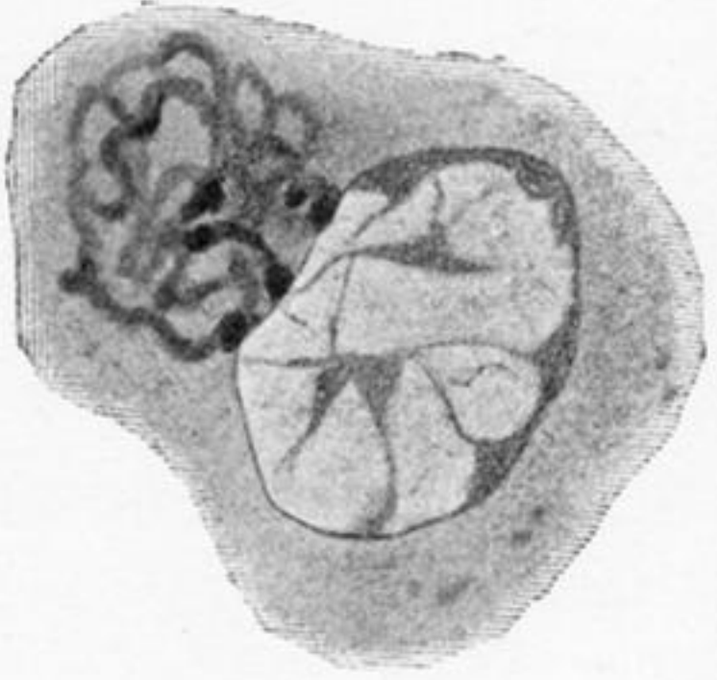

FIG. 9.