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.

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

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

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[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.

seemed to be mutual on the part of two contiguous leucocytes (fig. 1). These protrusions were quite different in character from the processes which are sent out by the nuclei in polymorphonuclear leucocytes and myeloplaxes, and from the appearances presented during the phenomenon of amitosis.

Shortly after the nuclear protrusions had reached the periphery of the cytoplasm, which was always reached at adjacent points of the contiguous cells, the ends of the protrusions joined.* After the joining of these two processes, a ring of chromatin masses appeared at either end, a short distance outside the points where the peripheries of the respective nuclei of the two cells would have been if no protrusions had been formed (fig. 2). Strands of chromatin extended from these masses just under the membrane of both nuclei, and also along the tube which joined them. Other strands seemed to pass along the tube between the two nuclei. After this apparatus had been fully formed, the chromatin in one of the two nuclei began to disappear, and the amount of chromatin in the other was frequently very markedly increased. This process continued until one nucleus was completely denuded of its chromatin, and the nuclear membrane collapsed (figs. 2 and 3). The chromatin ring at the end of the tube contiguous to the collapsing nucleus remained long after the other had disappeared, and sometimes the termination of the tube and the ring appeared in a shape something like an acorn on a stalk (fig. 3). While this process seemed to be quite constant in so far as the disappearance of the chromatin from one of the nuclei was concerned, the increase of the chromatin contained in the other nucleus was not always very apparent. Frequently, however, there was no doubt that a very large increase indeed had occurred.

The leucocytes of Axolotl upon which these observations were made were, of course, in a more or less abnormal condition, but in view of the relatively small size of mammalian cells when compared with those of Axolotl,† it appeared likely that what was very striking in the case of the large cell might easily be missed in the case of the small. Some slides of normal mammalian tissues (bone-marrow, spleen, lymphatic gland, etc.) were re-examined, and all the stages in the phenomenon described above, excepting

* It can but rarely happen that two contiguous cells will be in such a position in the section that the mutual sending out of nuclear processes will be presented to the observer in profile, as shown in fig. 1. Much more often either only one of the two cells will be found in the same section, or the two will be super-imposed either directly or at an angle. The phenomenon is therefore probably more common than would at first sight appear, as anything but a lateral presentation of the two cells would be difficult, often impossible, to interpret.

† Compare figs. 1—3 with figs. 4—9, which give a fairly accurate idea of the relative sizes of these cells.

the first, were found in the normal mammalian spleen (figs. 4—8). In addition, an apparently later stage was found, where the collapsed nuclear membrane had almost disappeared, leaving only a few shreds (fig. 9). Hitherto this phenomenon has not been observed in any other mammalian tissue than normal spleen. It is probable, had the series of observations upon what happened among the leucocytes of Axolotl been carried further, that the later stage observed in the mammalian spleen would have been found. One would also expect to find the same phenomenon taking place among the leucocytes gathered together in the mammalian body under certain abnormal conditions.

There are some points of difference between the appearance of the figures in Axolotl and those in mammalian spleen. The ring of chromatin masses in the latter class of cells does not appear to be constant, or even frequent, at that end of the tube nearest the nucleus which is destined to absorb the other. Again, the evidence that the process is at first mutual, is questionable in the case of mammalian leucocytes, as no undoubted cases (such as shown in fig. 1) have hitherto been discovered. It may be, therefore, that the process commences mutually between two cells, one eventually gaining the upper hand in the case of Axolotl, while in mammals the process may never be mutual at any stage; but one cell always plays an active part, the other remaining passive. No other material points of difference have yet been observed.

In both Axolotl and mammalian spleen, however, there is an immediate conclusion with regard to these observations that appears quite clear. The nuclear contents, at any rate the chromatin, and probably the linin, of the one cell are directly absorbed by the other. The probability that the linin as well as the chromatin is absorbed is very great. The chromatin masses forming the rings at either end of the tube, and the strands of chromatin extending from these masses, are so sharply defined that it would seem that something must be holding the chromatin together in a definite form. The equally sharply defined strands of chromatin that pass through the rings appear to be continuous with the chromatin and linin of the nucleus.

The process of absorption begins by the mutual throwing out of nuclear protrusions of a peculiar form by two contiguous leucocytes in the case of Axolotl, while in the mammalian spleen one leucocyte apparently sends out a protrusion from its nucleus, which attaches itself to the nucleus of another. In either case the obvious result is: (1) one cell with a nucleus containing a double complement of chromatin, and probably of linin, and (2) a mass of protoplasm without a nucleus, which represents what was originally the other cell.

The mass of protoplasm which is left without a nucleus may be dismissed in a few words. It closely resembles what has been described elsewhere as the red blood corpuscle after it has lost its nucleus, and before it has become differentiated to its concavo-convex shape.* Whether some red corpuscles arise in this way must remain doubtful at present. There is, however, nothing against this being the case in the observations referred to, as red corpuscles seem to arise in the spleen, as well as in the bone-marrow.

The case of the leucocyte with a nucleus containing a double complement of chromatin and linin, however, calls for a careful consideration.

In a previous communication upon the life-history of leucocytes, observations were recorded which seemed to show that some, at any rate, of the leucocytes in the bone-marrow passed through the Meiotic Phase, and, after the number of the chromosomes had thus been reduced to one-half the normal somatic number, many post-meiotic generations occurred both in the bone-marrow and in the lymphatic glands, in which the reduced number of chromosomes was retained.† A comparison was made between these phenomena occurring among leucocytes and what happens in the case of many plants. At the same time, it was suggested that, while the large number of post-meiotic generations, and the fact that but few of the cells of these generations were destined to conjugate with other reduced cells in the case of plants, offered a parallel with what apparently happened in the case of leucocytes, an important difference existed, in that there was no evidence that any among the post-meiotic generations of leucocytes ever reached a stage where they conjugated with other reduced cells.

The observations recorded in the present communication suggest that this comparison may now be carried much further, not only between certain phenomena exhibited by plants and leucocytes, but also between those exhibited by plants and unicellular organisms on the one hand and leucocytes on the other. This further comparison depends upon the interpretation placed upon the absorption of the nucleus of one leucocyte by another, and is only possible if this is regarded as a process of fertilisation.

There is a considerable amount of evidence that these leucocytes in which the fusion of nuclei takes place have passed through the Meiotic Phase. The members of this particular class of leucocytes are frequently found to be dividing mitotically, very commonly exhibiting the reduced number of chromosomes.

* C. E. Walker, "On the Origin and Differentiation of the Red Blood Corpuscles in Mammals," 'Trans. Path. Soc. London,' vol. 58, Part I, 1907.

† C. E. Walker, "Observations on the Life-history of Leucocytes," 'Roy. Soc. Proc.,' B, vol. 78, 1906.

This class of leucocytes, which is described in more detail elsewhere,* forms a large proportion of the cells in the spleen and the majority of those in the lymphatic glands.

It is necessary, before considering the probability of fertilisation being a correct interpretation, to dismiss the probability of the process being simply the destruction of one cell by another. This is a very common process, but seems always to take the form of engulphment of the whole or part of the cell destined to destruction into the cytoplasm of another cell. The usual sequence in the case of vertebrates is, that one leucocyte or other kind of cell engulphs another bodily into its cytoplasm. The engulphed cell is gradually disintegrated. The nucleus first shows signs of degeneration. At a later stage the whole cell somewhat resembles an archoplasmic vesicle or Plimmer's body, and eventually it disappears altogether, both nucleus and cytoplasm. The engulphed cell is never taken into the nucleus, but remains in the cytoplasm of the engulphing cell, and is there apparently digested. This phenomenon may be seen taking place in many different tissues, particularly where there is any disturbance from the normal condition. It is so totally unlike what has been here described as taking place among the leucocytes in Axolotl and mammalian spleen, that it is unnecessary to go into further details.† The process of engulphment and destruction of one cell by another is, in fact, morphologically a very simple and straightforward affair, and takes place without the development of any special apparatus on the part of either. On the other hand, the absorption of one leucocyte nucleus by another, as here described, seems to involve the development of a special and complicated apparatus, formed in such a manner that the chromatin and linin of one nucleus may be transferred directly to the other, without any process of digestion by the cytoplasm being possible.

It might possibly be suggested that certain prolongations of the nucleus and cytoplasm which are very common among the leucocytes found in the mammalian spleen might be confounded with the figures here described. A careful examination shows that they are of quite a different nature, and so dissimilar as to be hardly comparable.

The morphological evidence would therefore appear to be in favour of fertilisation, in that the nuclear contents of two cells are apparently fused without any disintegration of the contents of either nucleus taking place,

* C. E. Walker, "On the Origin and Differentiation of the Red Blood Corpuscles of Mammals," *loc. cit.* *Ibid.*, "Observations on the Life-history of Leucocytes," *loc. cit.*

† Figures illustrating the appearance of engulphed cells may be seen in the 'First Report on the Cytological Investigation of Cancer,' Moore and Walker, 1906, Cancer Research Laboratories, University of Liverpool.

and that the fused nuclear contents continue to exist in a common cytoplasm.

If we accept the interpretation that the apparent fusion of the nuclear contents of two leucocytes is to be regarded as the fertilisation of the one by the other, a remarkably complete, although complex series of phenomena is suggested; a series of phenomena which, however surprising it may appear at first, is found on careful examination to be analogous in many respects, even in detail, to several equally complicated series of phenomena that have been generally acknowledged to occur.

It is necessary here to describe briefly those phenomena in plants with which it is proposed to compare those occurring among leucocytes.

A certain group of cells belonging to the soma becomes differentiated and passes out of somatic co-ordination. Some of these, the tapetal cells, without passing through the Meiotic Phase, exhibit changes which render them different in function to the cells of the soma. They are destined to disintegration in a comparatively short time, quite independently of what happens in the soma or among the rest of the cells of this differentiated group. They serve a nutritive purpose. Other cells of the group pass through the Meiotic Phase. Of these, only a few of the individuals produced by many subsequent generations are destined to reach the stage where conjugation may take place with other cells in the same condition. The rest produce a comparatively unlimited number of post-meiotic generations, and carry out the function of nourishing and supporting those cells destined to conjugate.

Apparently a number of the cells belonging to the early generations of leucocytes do not pass through the Meiotic Phase, but though they are apparently out of co-ordination with the soma, they serve nutritive and other purposes, some among them developing granules.*

Other leucocytes, apparently belonging to later generations, pass through the Meiotic Phase. Of this group the greater number appear to be converted into red corpuscles, thus serving a nutritive purpose.† Others of this group, if the fusion of nuclear contents be regarded as fertilisation, conjugate with other reduced cells.

It may be that when, during the process of evolution, leucocytes or their immediate precursors were developed and passed out of co-ordination with the parent soma, these cells reverted to the characters of a remote protozoan

* C. E. Walker, "Observations on the Life-history of Leucocytes, Part II," *supra*.

† C. E. Walker, "On the Origin and Differentiation of the Red Blood Corpuscles in Mammals," *loc. cit.*

ancestor. This phylogenetic reversion of certain cells produced by the soma may have become constant, and new characters may have been developed from this fresh point of departure. This would account for the phenomena exhibited by the leucocytes in the vertebrates. It has been considered probable that spermatozoa are also examples of phylogenetic reversion to the characters of protozoan ancestors.

The possible conclusions to be derived from these observations may be briefly stated as follows: Certain cells, probably only existing in the bone-marrow in the case of the adult mammal, give rise to a group of cells which are, or will immediately pass, out of somatic co-ordination. Some of these are destined to comparatively rapid disintegration, probably including the nutrition of other cells among their functions. Others pass through the Meiotic Phase, and continue to divide, producing an apparently unlimited number of post-meiotic generations of cells possessing half the somatic number of chromosomes. The great majority of these are converted into red corpuscles, serving a nutritive purpose. The remainder are fertilised in the manner described in the observations here recorded, and, as is the case in many unicellular animals, after fertilisation divide a number of times, thus producing fresh generations of leucocytes, which may or may not subsequently pass through the Meiotic Phase again.

This would naturally lead to the further conclusion that the leucocytes passing out of co-ordination with the soma live as parasites upon the parent organism, and in themselves possess a complete life cycle.

These observations would appear to have some bearing upon what has been described as occurring in malignant growths. It has been stated that during the early stages of malignant growths, leucocytes, entering into the cytoplasm of some of the tissue cells, proceed to divide mitotically, synchronously with the nucleus of the cell that they have invaded, the chromosomes of the leucocyte and the tissue cell becoming mixed and being distributed between the daughter nuclei resulting from the mitosis.* This bastard form of fertilisation seems to suggest some properties in the leucocytes different to those possessed by any other cells in the body, excepting perhaps the sexual cells. This suggestion is strengthened by the observations here recorded.

A fusion between the cells of certain graftable tumours in mice has been described.† These tumours were said to be malignant, though some doubt has since been thrown upon this statement. A re-examination of the figures illustrating this alleged fusion of cancer cells of mice suggests very strongly that some of them may be fusions of leucocytes such

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* Farmer, Moore, and Walker, "On the Behaviour of Leucocytes in Malignant Growths," 'Trans. Path. Soc. London,' vol. 56, Part III, 1905. *Ibid.*, "On the Cytology of Malignant Growths," 'Roy. Soc. Proc.,' B, vol. 77, 1906. Moore and Walker, 'First Report on the Cytological Investigation of Cancer,' University of Liverpool, Cancer Research Laboratories, 1906.

† Bashford and Murray, 'Roy. Soc. Proc.,' 1904.

as are here described, and that the observers were mistaken in supposing that the cells in question were cancer cells. That a large congregation of leucocytes does occur in the case of Jensen's graftable tumour renders this extremely probable, for we have seen that among the leucocytes brought together by the introduction of a foreign body in the case of Axolotl, some of them conjugated. Moreover, nothing in the nature of a conjugation between cancer cells has been recorded by any other observers, although the alleged fusion has been diligently sought for during several years.

## DESCRIPTION OF PLATE.

FIG. 1.—Two leucocytes in Axolotl in which the nuclei are sending out protrusions.

FIG. 2.—The two nuclear protrusions have joined, rings of chromatin masses have appeared at either end of the tube thus formed, and the chromatin is disappearing from the lower of the two nuclei.

FIG. 3.—The nuclear membrane of the cell on the right is collapsing. The ring of chromatin masses has disappeared at the end of the tube nearest the nucleus that has absorbed the chromatin of the other.

FIG. 4.—Two leucocytes from the spleen of the guinea-pig, showing the nuclear protrusion from one attaching itself to the nucleus of the other.

FIG. 5.—A slightly later stage.

FIGS. 6, 7, and 8.—Stages showing the absorption of the contents of one nucleus by the other.

FIG. 9.—Showing the complete collapse of the nuclear membrane of one leucocyte, with only a few shreds of the membrane remaining.

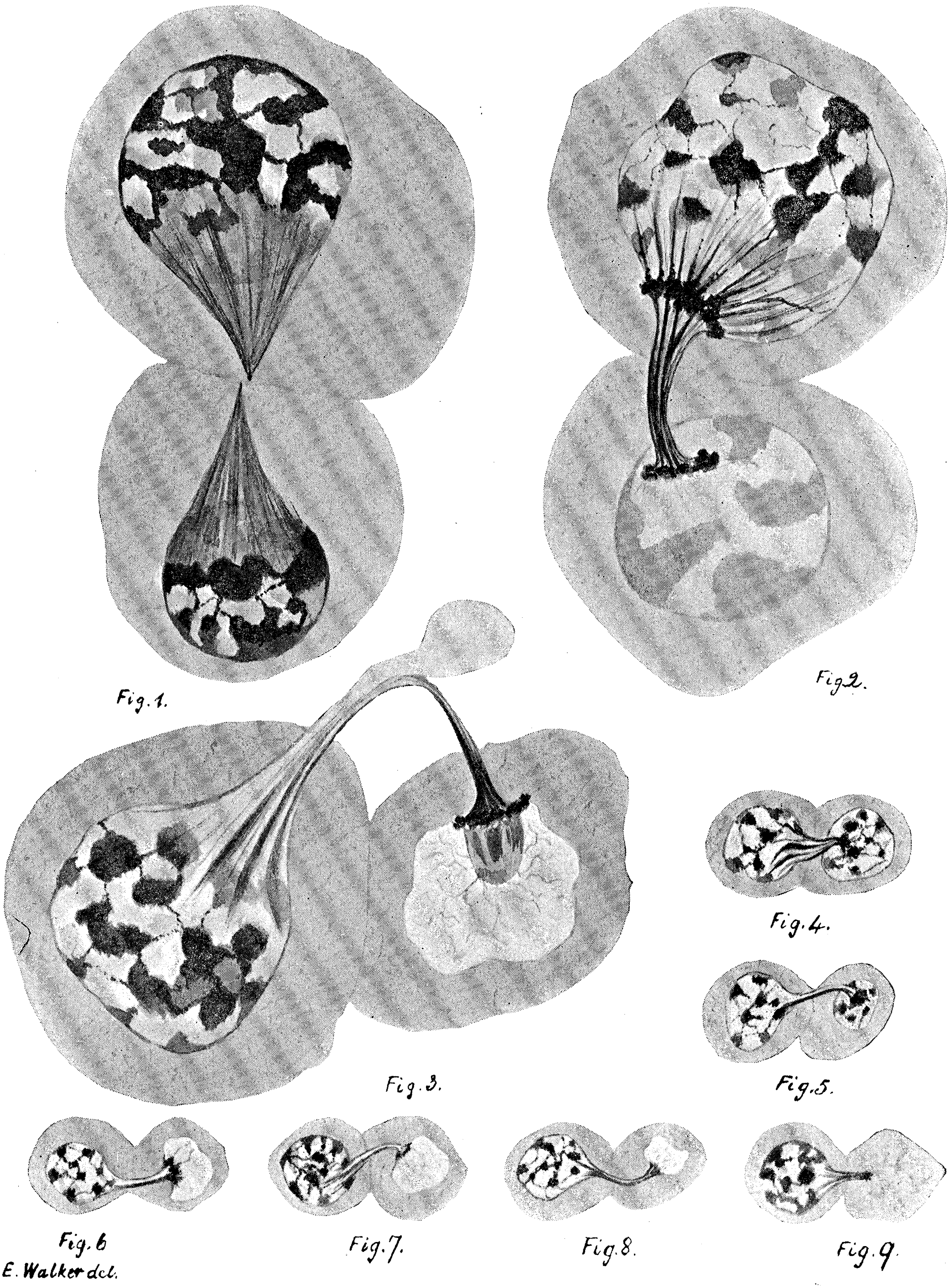
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
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Fig. 6
Fig. 7.
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Fig. 9.
C. E. Walker del.

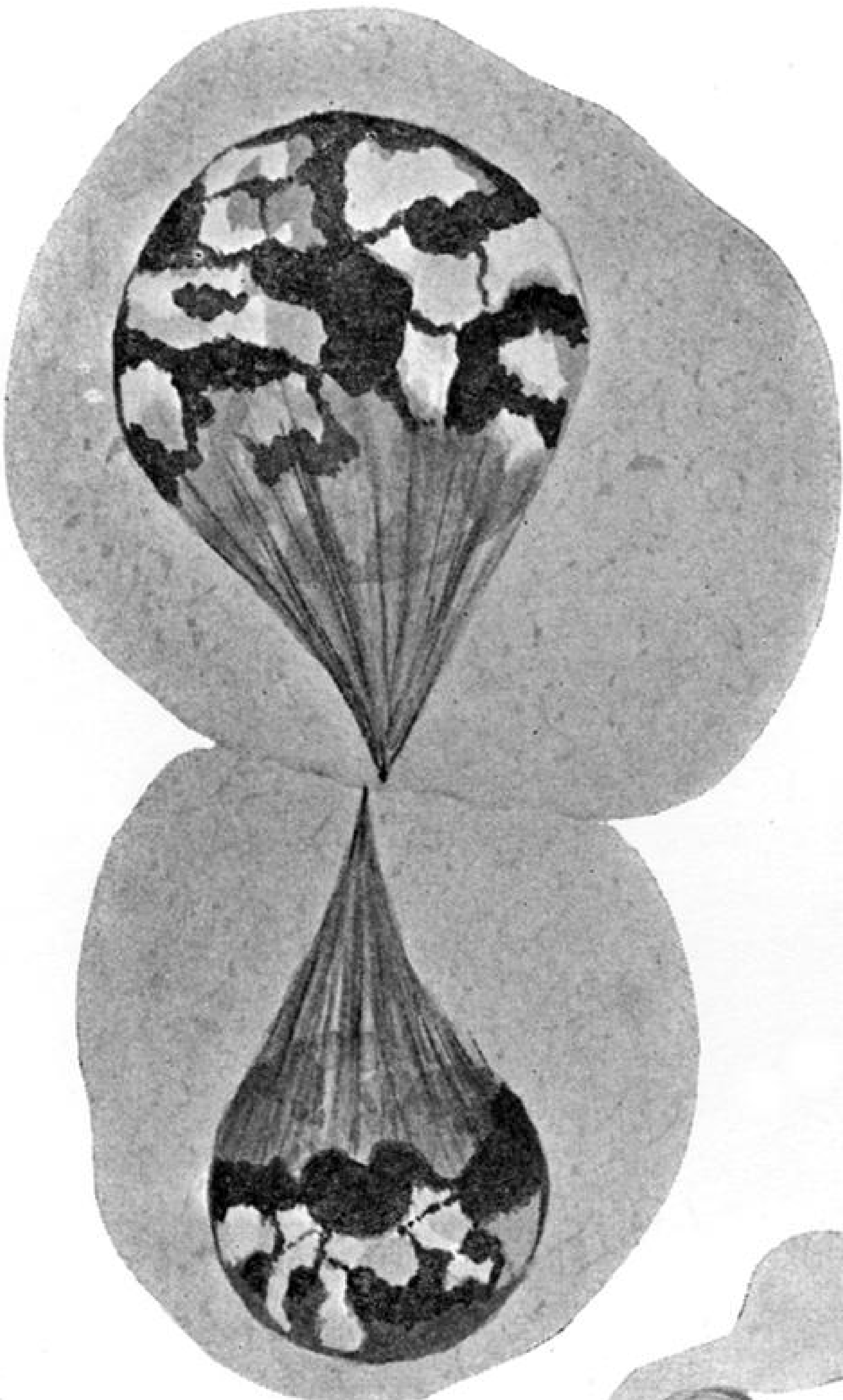

Fig. 1.

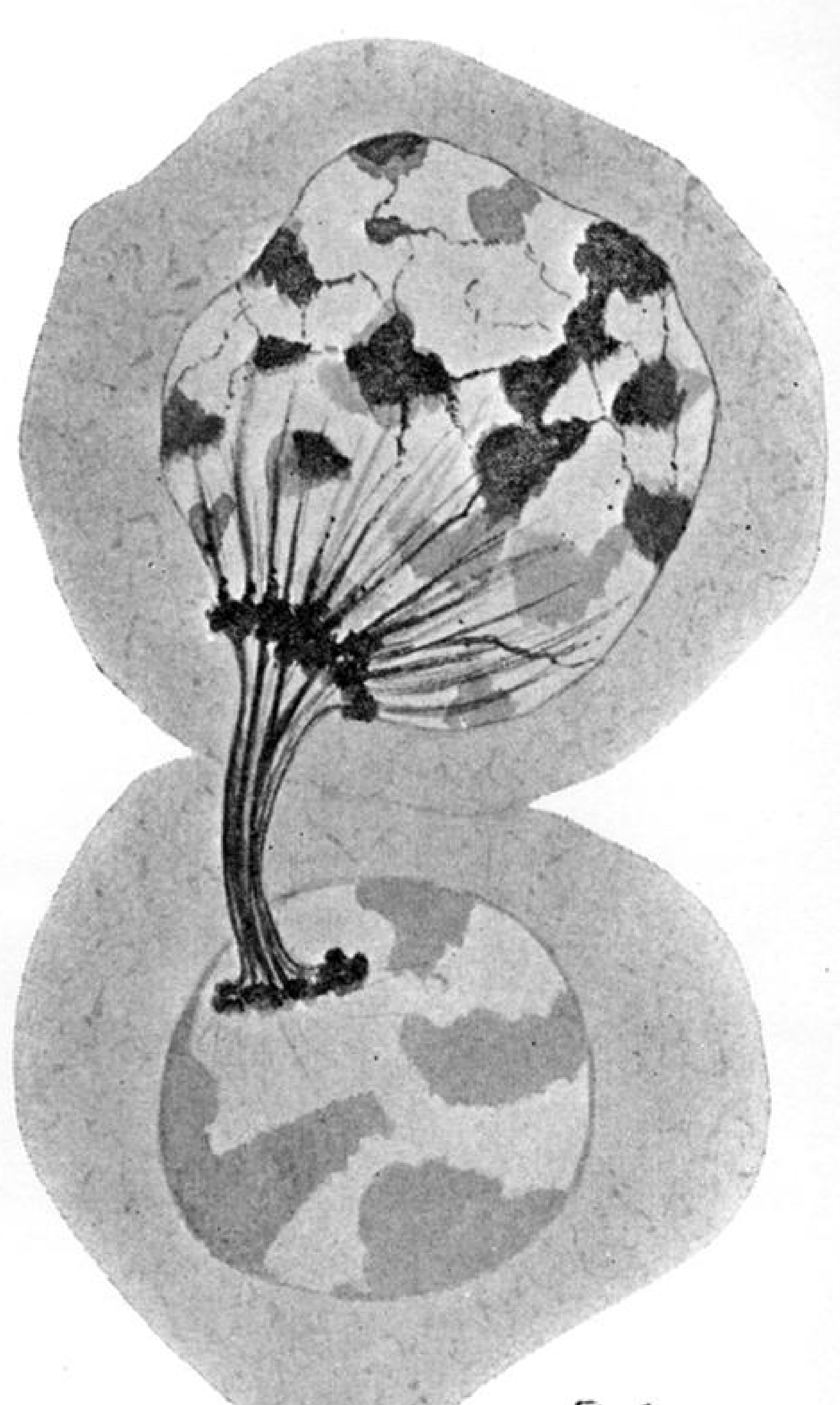

Fig. 2.

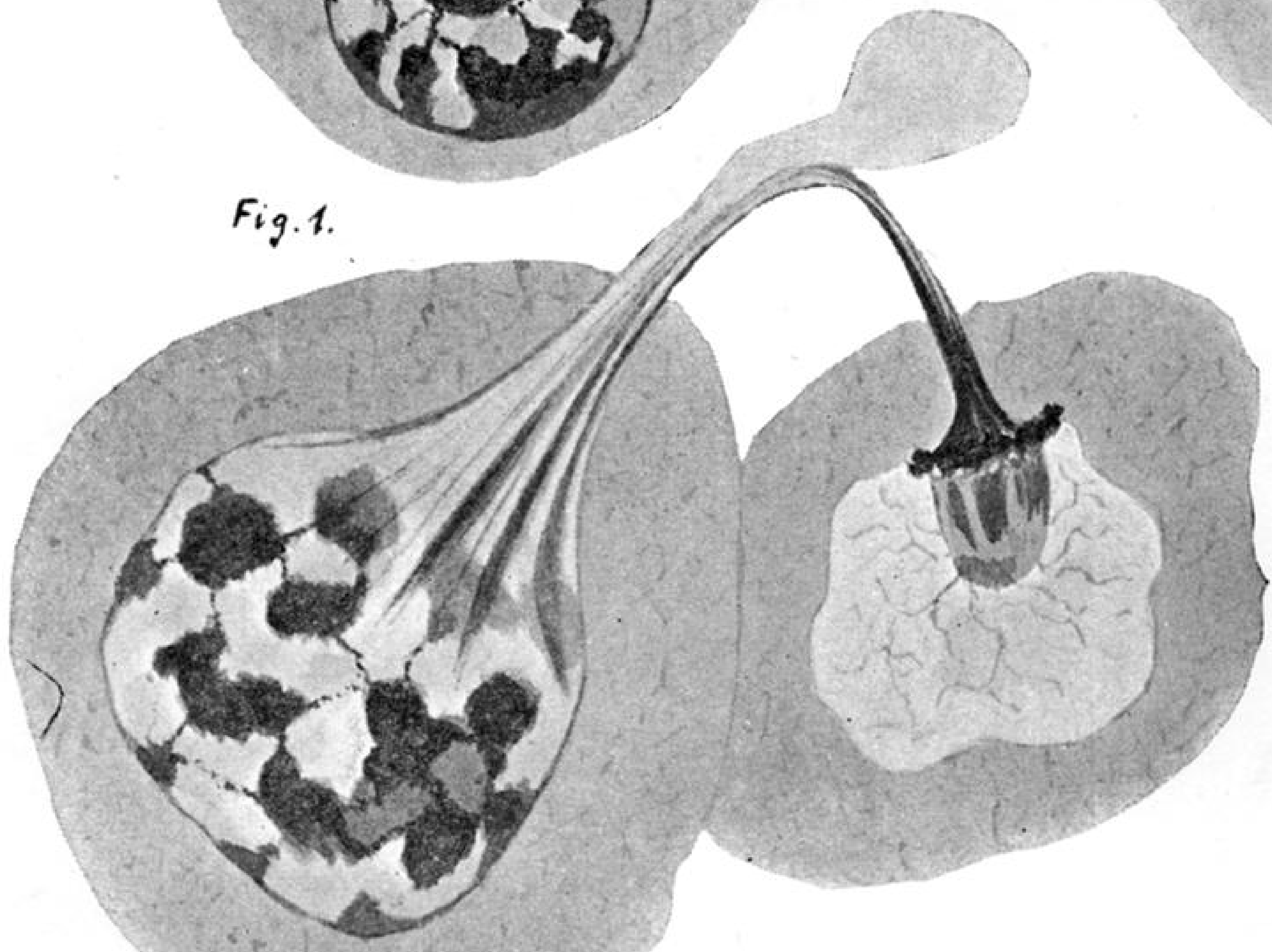

Fig. 3.

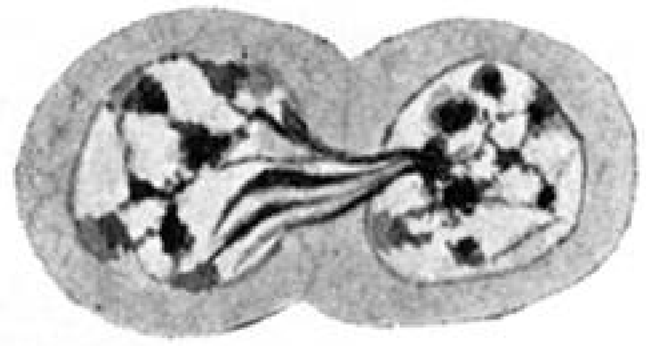

Fig. 4.

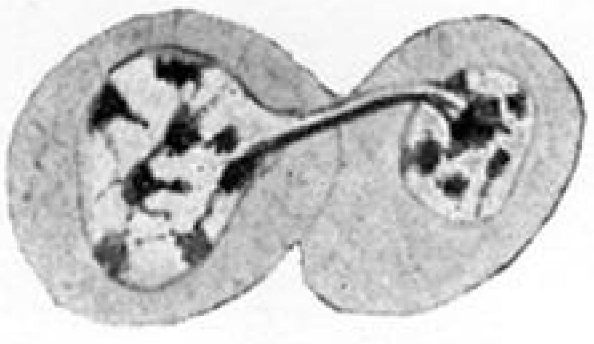

Fig. 5.

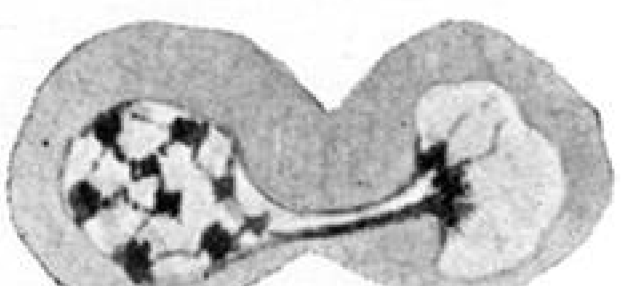

Fig. 6

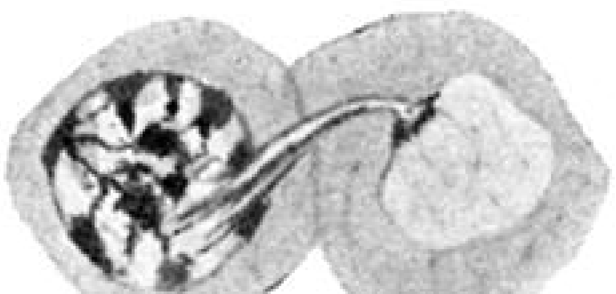

Fig. 7.

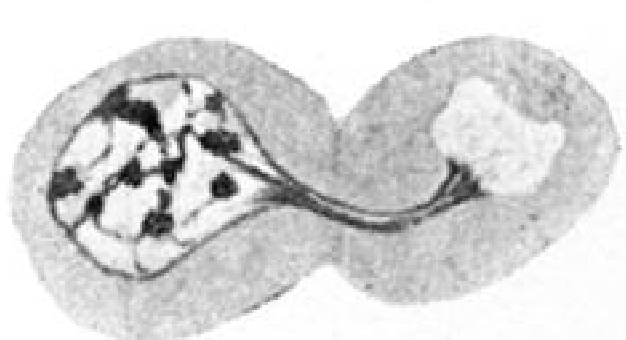

Fig. 8.

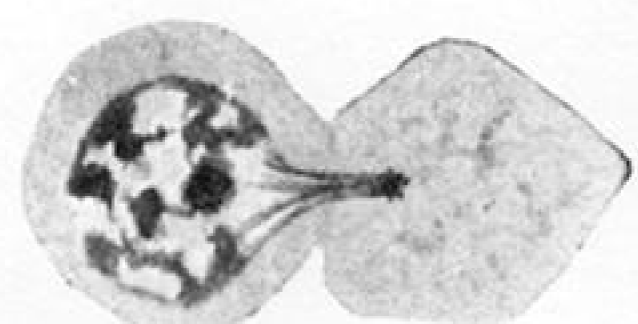

Fig. 9.