Observations on the Life-History of Leucocytes.

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[PLATES 7—10.]

In presenting this preliminary communication it is necessary to explain that the term leucocyte is used in the widest sense, and is intended to include all the wandering nucleated cells and their immediate ancestors. After many endeavours to make the observations here recorded compatible with the current classification of these cells, it has been found imperative to use a term that will include them all, and, at any rate in most cases, to put aside for the moment the question as to which particular kinds of leucocyte are being dealt with.

The tissues used have been chiefly bone-marrow, lymphatic glands and spleen, but the leucocytes in various other tissues and the free leucocytes in the blood have also been examined. Most of the work has been done with material derived from the guinea-pig and the rat, but tissues from man, rabbit, mouse, crocodile, frog, *Triton* and *Axolotl* have also been used. In every case the tissues have been normal and, with one exception, derived from adult or nearly adult animals, the one exception being the testis of the early embryo of the guinea-pig.

In examining a section of bone-marrow one of the most striking, constant and frequent objects met with is the giant-cell or myeloplax. In the myeloplaxes there is, as a rule, an appearance of amitosis in the nucleus or nuclei (see fig. 1).

Occasionally, however, a myeloplax is found in which mitosis is taking place, and in such cases the mitotic figure is pluripolar but otherwise of the somatic type (see fig. 2). A further search shows that myeloplaxes with the chromatin of the nuclei in the ordinary somatic spireme form are not uncommon (see fig. 3). On the other hand, it is seen that the majority of the myeloplaxes divide amitotically. A portion or portions of the cell containing one or more nuclei are then separated off (see fig. 4).

Following the cells thus produced, one is forced to conclude that many of the smaller cells in the bone-marrow are derived from them. Among these are the cells commonly known as "polymorphic nuclear leucocytes" and many of the cells containing a single and more or less rounded nucleus (see fig. 5).

The polymorphic nuclear cells apparently continue to divide amitotically for a number of generations. It is impossible in the present brief communication to illustrate all these stages, but fig. 5 shows two of the most striking.

At present it is impossible to discriminate between the cells arising amitotically and those arising mitotically from the myeloplaxes.

Among the mononuclear cells of the bone-marrow and the germinal areas of lymphatic glands, mitotic division figures are very frequent. They are also present, but less numerous, among cells found in the blood and the spleen.

Many of these mitotic figures are of the ordinary somatic type, and nuclei showing the usual somatic spireme are not uncommon (see figs. 6, 7, and 8).

More rarely a division figure such as those shown in figs. 12, 13, 14, and 15 is met with. In many of these it is extremely difficult to make out even a single individual chromosome. In others, however, several can be made out quite distinctly. This is the case in figs. 12, 13, and 14. In fig. 15 the chromatin masses are much less clear, but even this is more distinct than is the case in a considerable proportion of such cells. Though it is not easy, on account of the small size of the cells and the difficulty of obtaining good fixation, to count the chromosomes in even a few among a great many cells, in those where anything approaching an estimation of the number is possible it is evident that the number is much smaller than that found in the somatic division figures occurring in the same animal. That reduction in the number of chromosomes takes place in leucocytes has already been pointed out in a previous communication.*

It would seem, on comparing these figures with fig. 16, which is the first maiotic (heterotype) division from the testis of a guinea-pig, that these two forms of division are similar to each other, but vastly different from the normal somatic division figures found in the same animal (see fig. 7).

The probability that these division figures are similar to that occurring in the first maiotic division in reproductive cells does not, however, depend only upon the forms of the chromosomes,† their number, or their arrangement upon the spindle. Prophases such as those shown in figs. 9 and 10 are not infrequent. In these the so-called spireme is seen to be distinctly split and to differ in other ways from the ordinary somatic spireme (see

* Farmer, Moore and Walker, "On the Cytology of Malignant Growths," 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 336.

† It may here be pointed out that several of the permanent forms of first maiotic (heterotype) chromosomes described by Moore and Arnold have been observed in the division figures dealt with here. (Moore and Arnold, "On the Existence of Permanent Forms among the Chromosomes of the First Maiotic Division in Certain Animals," 'Roy. Soc. Proc.,' 1906.)

fig. 6). In fig. 11 a corresponding stage in the prophase of the first maiotic division in the testis of a guinea-pig is given as a comparison.* Beyond this again one occasionally finds a cell in the diaster stage in which some at any rate of the chromosomes are longitudinally split (see fig. 17). This is typical of the first maiotic division.† In so far as the leucocytes are concerned, I have hitherto found this type of division only in the bone-marrow.

The typically somatic division figures and those that I have interpreted as heterotypes, however, even when taken together, are less numerous than another form of division of which figs. 18 to 23 are illustrations. This form of division is extremely common in bone-marrow and in lymphatic glands. Hitherto I have not found it in any other adult tissue or in any other tissue that I have examined, with one exception referred to later.

It is obvious, as will be seen from the figures, which are taken from cells occurring in the bone-marrow and lymphatic glands of the guinea-pig and rat, that the number of chromosomes is not more than half the normal somatic number, which is 32 in both cases. The shape of the chromosomes seems to vary slightly in different cells. Some are short, thick, and slightly curved rods (see figs. 18 and 22). Others are more or less oval, often very irregular. There seems to be every reason for regarding these divisions as similar to the second maiotic (homotype) division. Examples of this division from the testis of the guinea-pig are given in figs. 24, 25 and 26. The great frequency with which this division occurs suggests strongly that there are several generations of cells showing the reduced number of chromosomes. The fact that in many cells the chromosomes are rod-shaped while in others they are roughly oval, led me at first to believe that there might be a well-defined difference between the first and the succeeding generations after the first maiotic division. An examination of the testes of several animals, however, convinced me that the same difference might be demonstrated among the homotypes there (see figs. 24 and 25). How far this is to be regarded as a real difference or merely the result of a presentation of the cell to the eye or to faulty fixation in some cells is here beside the point, as we know that in these particular animals there is no further division after the second maiotic (homotype).‡

In comparing the foregoing observations with what occurs in the normal production of sexual elements in animals and plants, some remarkable

* Farmer and Moore, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants," 'Quart. Journ. of Mic. Science,' vol. 48, Part IV, February, 1905; Moore and Walker, "The Maiotic Process in Mammalia," 'Thompson-Yates Reports,' University of Liverpool, 1906.

† Farmer and Moore, Moore and Walker, *loc. cit.*

‡ Farmer and Moore, Moore and Walker, *loc. cit.*

similarities become evident. Amitosis occurs as one of the earliest known phenomena in the production of spermatozoa in some animals if not in all.* The first maiotic is, in spermatogenesis, immediately preceded by the ordinary somatic form of division. Then follows the second maiotic division, retaining half the somatic number of chromosomes as in the first maiotic. No further division has been recorded in the case of animals. Except as regards the latter point, what happens among the cells of the bone-marrow seems to be in many respects parallel to what happens in those of the testis, for the myeloplaxes very possibly, probably as I believe, correspond to the more or less syncytial condition of the cells sometimes observed in vertebrate testes, both arising amitotically.†

In plants, however, we find that though the series of complicated cell phenomena that occur in the production of sexual elements are practically identical to what occurs in animals, other cells than those destined to become mature sexual cells are involved in the maiotic phase. Without going into details that would here be out of place, it may be pointed out that in many cases in plants but comparatively few of the cells that pass through the first maiotic division, and thereafter show but half the somatic number of chromosomes, ever become converted into sexual elements, and also that the number of post-maiotic generations (those following the second maiotic or homotype division) is often very great, even if they can be considered in many cases as having any definite limit at all.

If the observations recorded above be correct, it would seem that the life-history of the leucocytes, in so far as I have been able to follow it, shows some remarkable points of resemblance to the life-history of those reduced cells in plants to which I have just referred. This comparison is carried even further by what has been observed with regard to the origin and history of the foot-cells of the testis.‡ All the observations recorded above were made upon adult tissues, but in the course of seeking for the origin of the foot-cells I examined the testes of very early embryos of the guinea-pig. Here, long before the formation of the tubules, the cells that are destined to become foot-cells are practically indistinguishable from certain of the stages observed in the leucocytes found in the bone-marrow and lymphatic glands of the adult animal. In these cells, besides the ordinary somatic division

* Meves, 'Anat. Anz.,' 1891, No. 22; 1894, 'Arch. m. Anat.'; Moore and Walker, *loc. cit.* Observations contained in a paper, not yet published, by Miss Embleton. They were carried out in the laboratories of the Cancer Research, University of Liverpool.

† Moore and Walker, *loc. cit.*

‡ Walker and Embleton, "On the Origin and Life-history of the Sertoli or Foot-cells of the Testis" (p. 50, *supra*).

figures, others are found exactly similar to those that I have described as second maiotic and post-maiotic in leucocytes (see figs. 27 and 28). In *Triton* testes it is possible to trace stages between the cells that have always been regarded as, and probably are, connective tissue cells and the cells that apparently perform the same function as the foot-cells of mammalian testes. In *Triton* also, during the earlier stages of the maiotic phase, the cells that are destined to become foot-cells are similar to certain leucocytes in the same animal. Accepting these observations as correct, and regarding the pockets of the amphibian testis as being directly comparable with the tubules of the mammalian testis, I am forced to the conclusion that either the leucocytes themselves or their immediate ancestors may give rise to connective tissue, the former probably being what really happens.

While, as has already been said, there are remarkable points of similarity between the life-histories of the leucocytes and those cells in plants which, though reduced, never become converted into sexual elements, it is also evident that there are some important points of difference. In plants, both the cells just referred to and those which are converted into definite sex cells have commenced the maiotic phase at the same time, and their immediate ancestry is common to both. In the case of leucocytes and certain connective tissues it is not at present demonstrable that anything of the kind happens. It may be that our present conception of what constitutes the whole of the maiotic phase, in animals at any rate, is too limited, and that the development of the mesoblast is in some way involved in its earliest stages. In this connection the fact that in some plants reduced cells may be differentiated into tissues that are somatic in characters and function is extremely suggestive, as are also the observations of Loeb upon the segmentations and the production of embryos in unfertilised eggs.

It does not seem out of place to mention here the bearing that these observations have upon what happens in cancer. As has been shown elsewhere,* one of the earliest phenomena observed in the development of cancer is the fusion of a leucocyte with a tissue cell and the subsequent division of the cell resulting from the fusion into two daughter cells, each possessing chromatic elements derived partly from the leucocyte and partly from the tissue cell. That in the subsequent generations of the cells produced from this fusion the characters of both ancestors should appear is exactly what would be expected. Among the cells of malignant growths all the forms of division here recorded as occurring among leucocytes and their immediate ancestors are to be found. As has also been stated before, some of the

* Farmer, Moore and Walker, "On the Behaviour of Leucocytes in Malignant Growths," 'Trans. of the Path. Soc. London,' vol. 56, Part III, 1905.

cells in malignant growths apparently go on dividing mitotically for a number of generations with the reduced number of chromosomes.*

Note.—January 8, 1906.—The following fixatives have been used and in every case the results described have been found with all the fixatives. Flemming's Fluid (strong formula), Hermann's Fluid, Acetic Acid and Absolute Alcohol, Corrosive Sublimate and Acetic Acid, and strong Formic Acid. As it is possible that these observations may interest some who are not conversant with cytological methods, it is perhaps permissible to point out that the greatest care must be taken with the processes of fixation, dehydration, imbedding, staining, etc. Extremely small pieces of tissue should be placed in the fixative within about a minute of the death of the animal or removal from the living body. The dehydration should be carried out in short stages, an increase of 10 per cent. of alcohol at a time being perhaps best. This does not of course apply to the tissues fixed in Acetic and Alcohol or strong Formic Acid (40 per cent.), from which the tissues are transferred immediately to absolute alcohol. At the same time it is necessary that the tissues should not be left in under 80 per cent. of alcohol for more than two or three hours after fixation. In imbedding, no higher temperature than 45° Centigrade should be used. These remarks apply particularly to mammalian tissues, but also to all animal tissues. Throughout the process of staining and mounting the greatest care must be taken that the sections do not become even partially dried upon the slides.

It is almost necessary to use a 10-inch tube microscope with a monochromatic light. I have used apochromatic objectives and eye-pieces specially constructed for the long tube by Zeiss. With a monochromatic light it is possible to obtain excellent definition with a 27 or even 40 compensated ocular and a 2 or 3 mm. apochromatic objective. Anything approaching this is impossible with the ordinary short tube.

In view of the enormous advantage gained by using a monochromatic light, the stains must be chosen with regard to the colour of the light used. The part of the spectrum between the blue and the green gives the shortest wave-lengths that can be conveniently used. As this gives a better definition than the parts of the spectrum with longer wave-lengths, red, yellow and orange stains give the best results.

DESCRIPTION OF PLATES.

Plate 7.

Fig. 1.—Myeloplax from bone-marrow of guinea-pig, showing nuclei apparently dividing amitotically.

Fig. 2.—Ditto, showing pluripolar spindle figure.

Fig. 3.—Ditto, showing two nuclei with somatic spiremes.

Plate 8.

Fig. 4.—Ditto, showing nuclei that have divided and the cytoplasm dividing (amitosis).

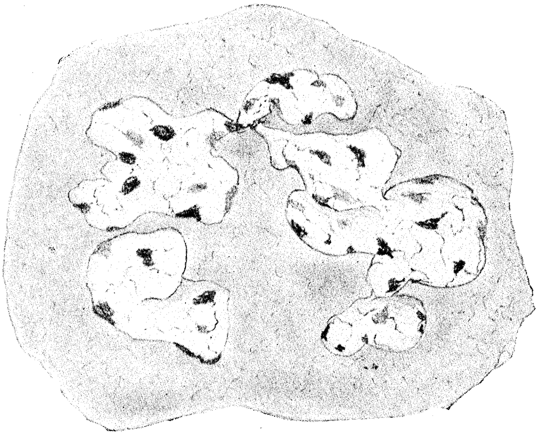
Fig. 5.—Adjacent cells in the bone-marrow of guinea-pig, showing stages of differentiation.

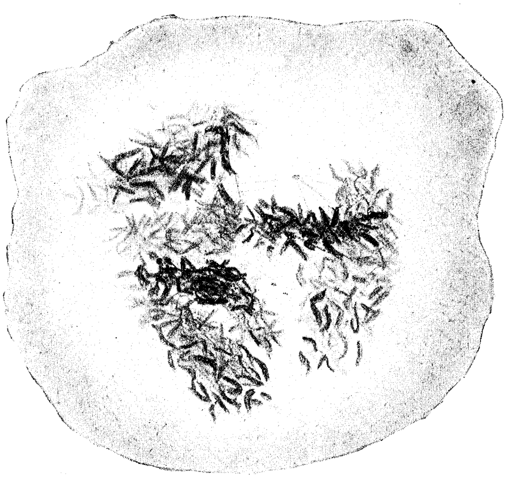
Fig. 6.—Mononuclear cell from bone-marrow of guinea-pig. The somatic spireme is formed.

Figs. 7 and 8.—Later stages in the somatic type of division in similar cells.

* Farmer, Moore and Walker, "On the Resemblances exhibited between the Cells of Malignant Growths in Man and those of Normal Reproductive Tissue," 'Roy. Soc. Proc.,' December, 1903.

FIG. 1.





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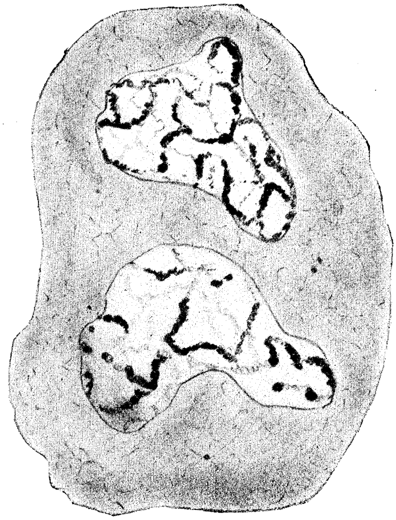

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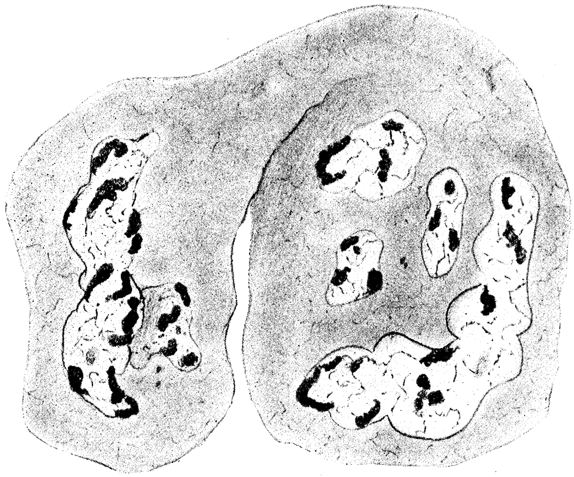

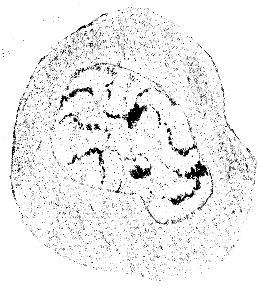

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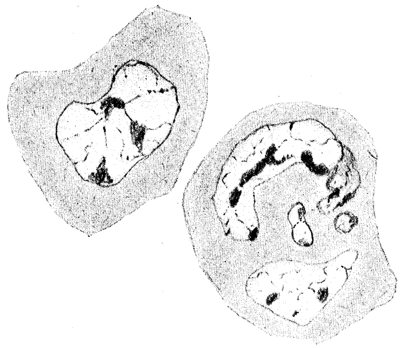

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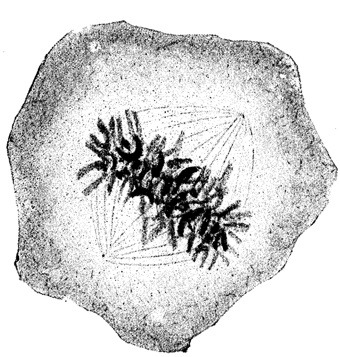

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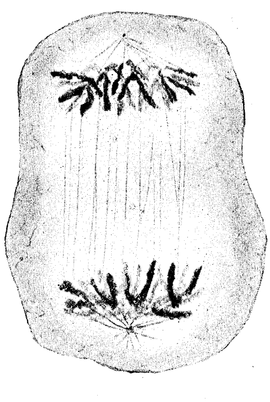

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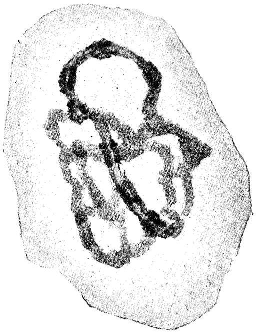

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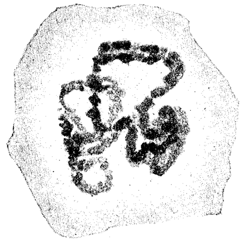

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FIG. 11.

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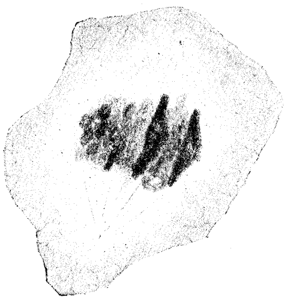

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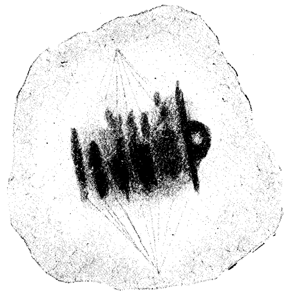
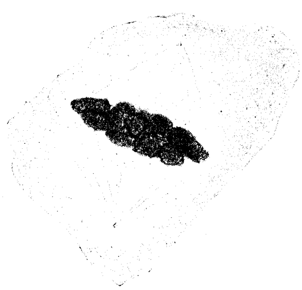


FIG. 14.

FIG. 15.

FIG. 16.

FIG. 17.



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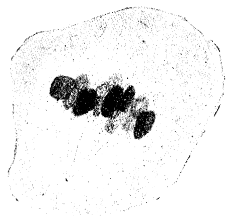

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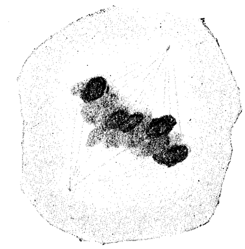

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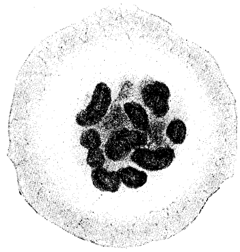

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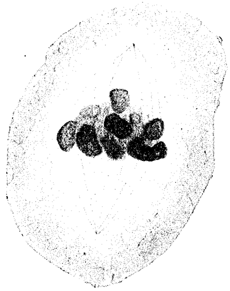

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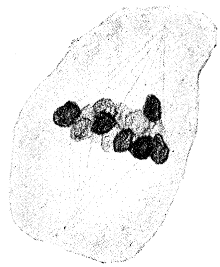

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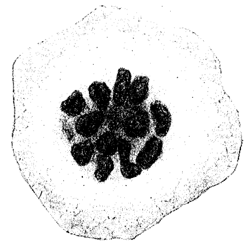

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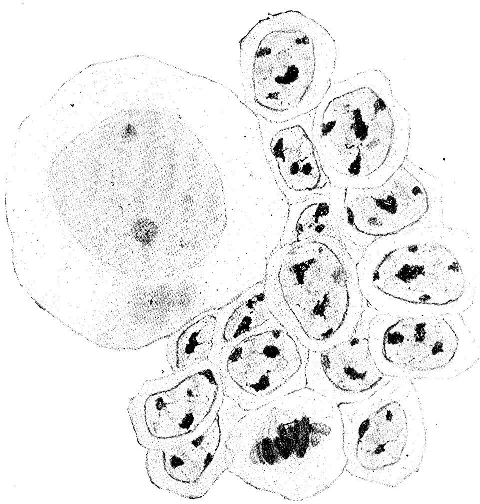

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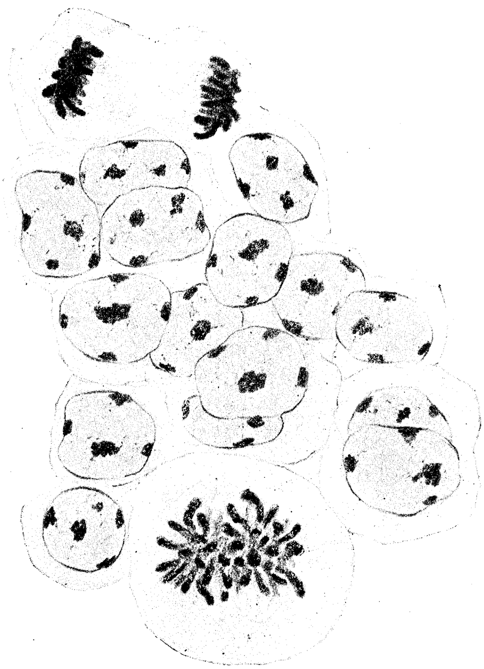


Fig. 28.

PLATE 9.

FIGS. 9 and 10.—Prophases of division in cells of bone-marrow of guinea-pig, showing splitting of the so-called spireme thread.

FIG. 11.—Similar stage in the first maiotic prophase of division in the testis of guinea-pig.

FIGS. 12 and 13.—First maiotic (heterotype) division figures from the bone-marrow of guinea-pig.

FIG. 14.—Ditto from bone-marrow of rat.

FIG. 15.—Division figure from bone-marrow of guinea-pig.

FIG. 16.—First maiotic division figure from testis of guinea-pig.

FIG. 17.—Diaster stage from cell in bone-marrow of guinea-pig. Some of the chromosomes are longitudinally split.

FIGS. 18, 19 and 20.—Second maiotic (homotype) division figures from bone-marrow of guinea-pig.

PLATE 10.

FIG. 21.—Ditto from germinal area of lymphatic gland of guinea-pig.

FIG. 22.—View of equatorial plane of a division figure similar to those shown in figs. 18 to 21.

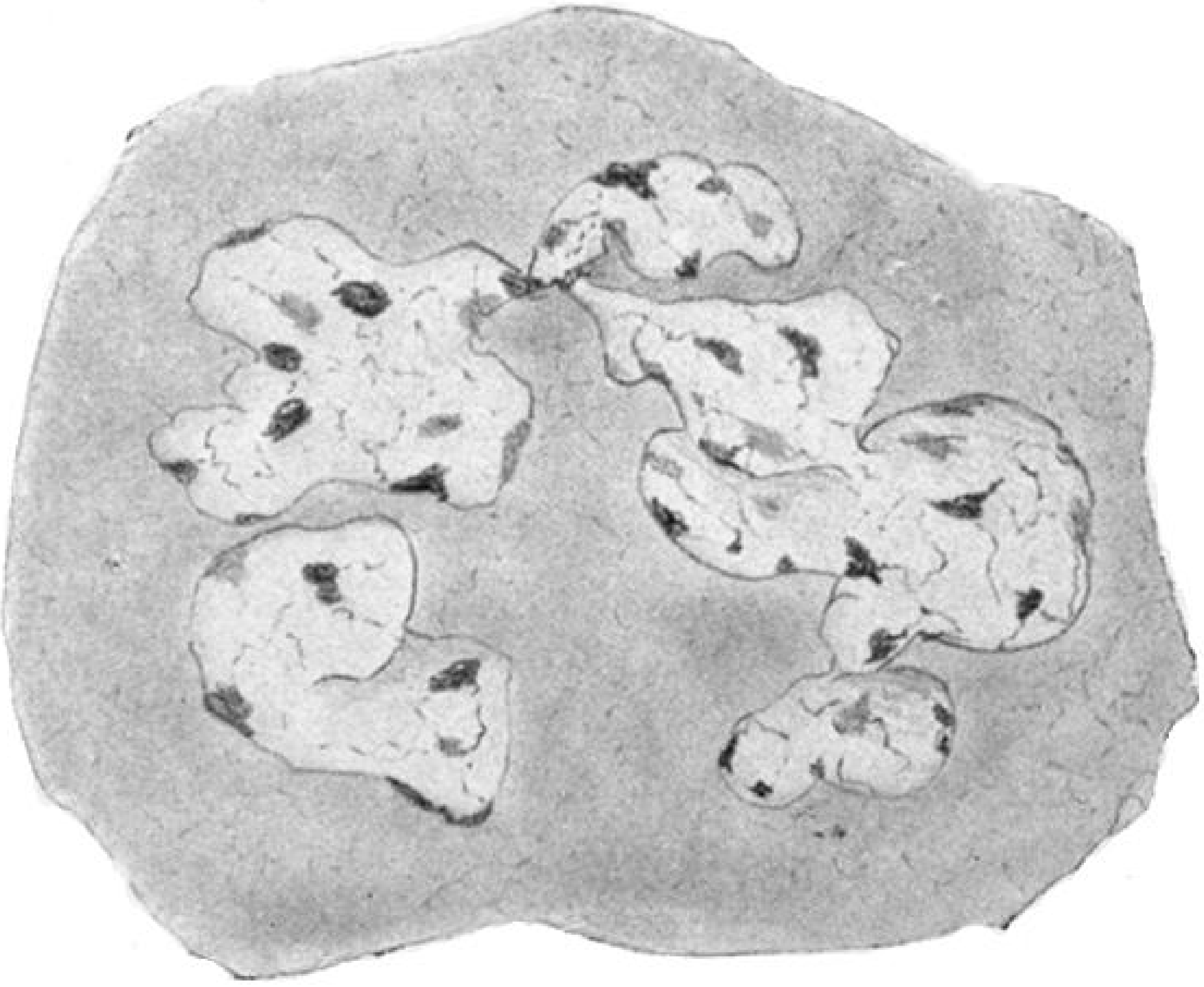
FIG. 23.—Diaster stage of a similar figure. Bone-marrow, guinea-pig. (Compare with fig. 8.)

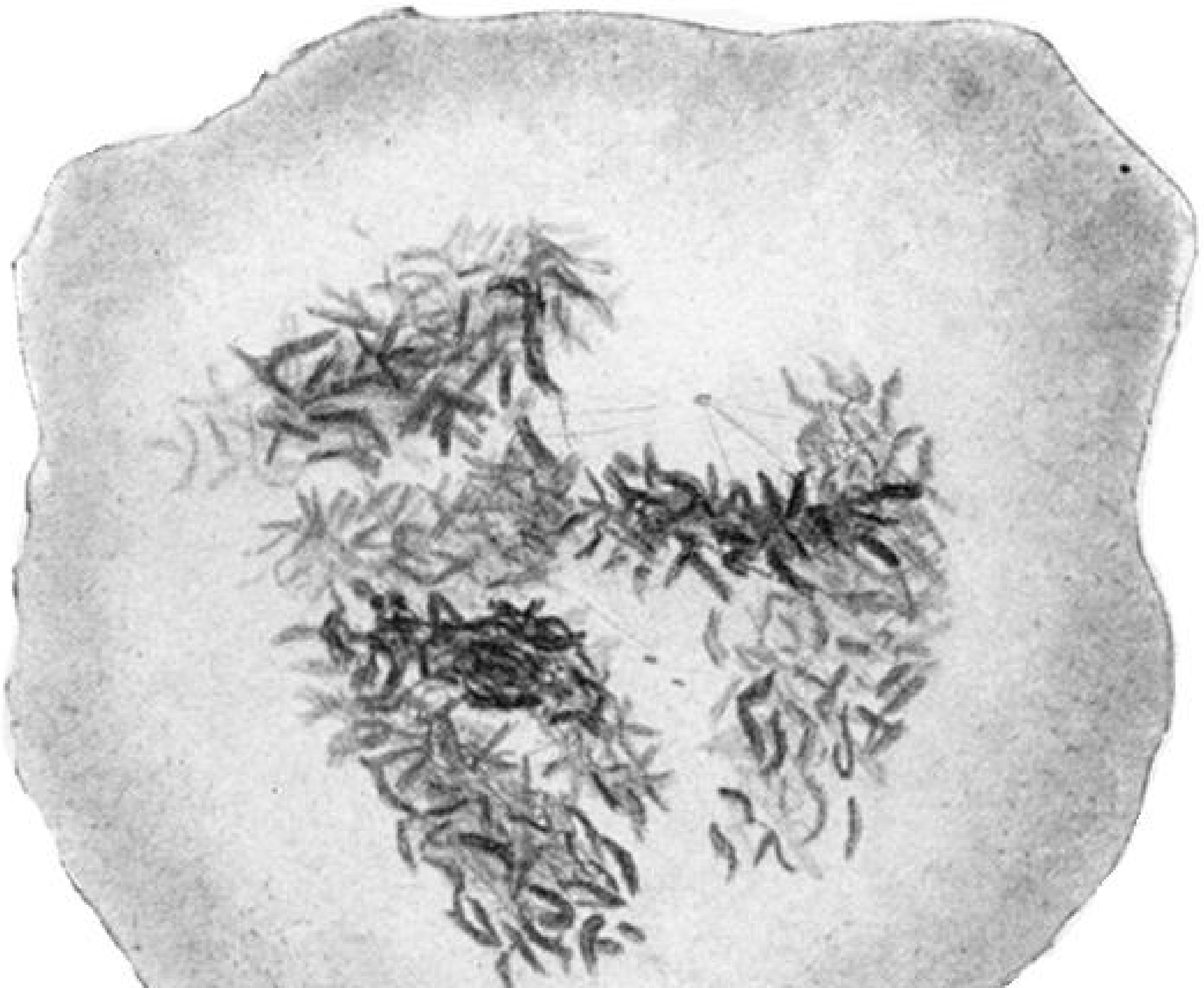
FIGS. 24, 25 and 26.—Second maiotic division figures from testis of guinea-pig.

FIG. 27.—Testis of embryo guinea-pig. A large male ovum and a homotype division figure among the cells which will form the foot-cells.

FIG. 28.—Ditto, showing somatic division figures.

Fig. 1.





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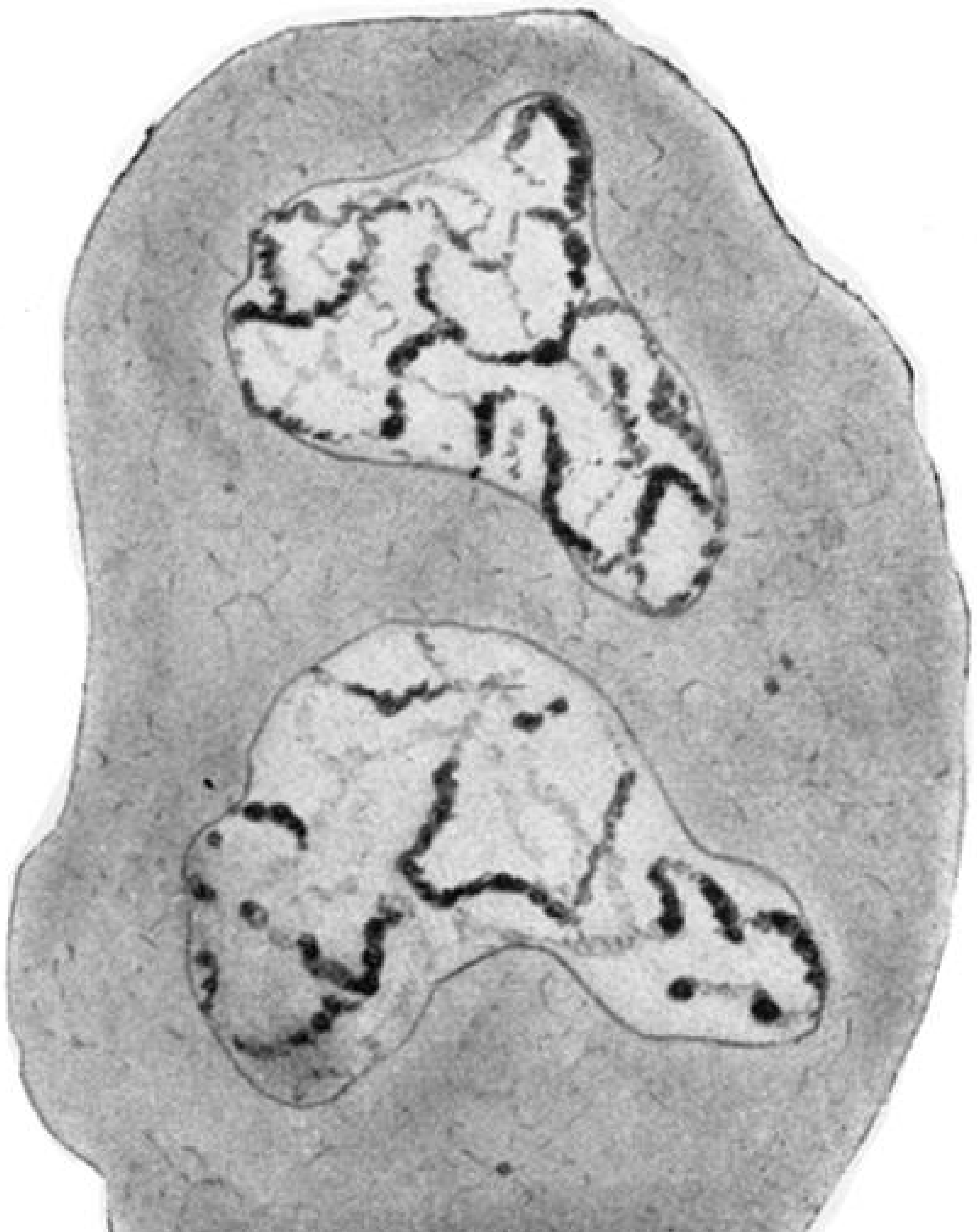

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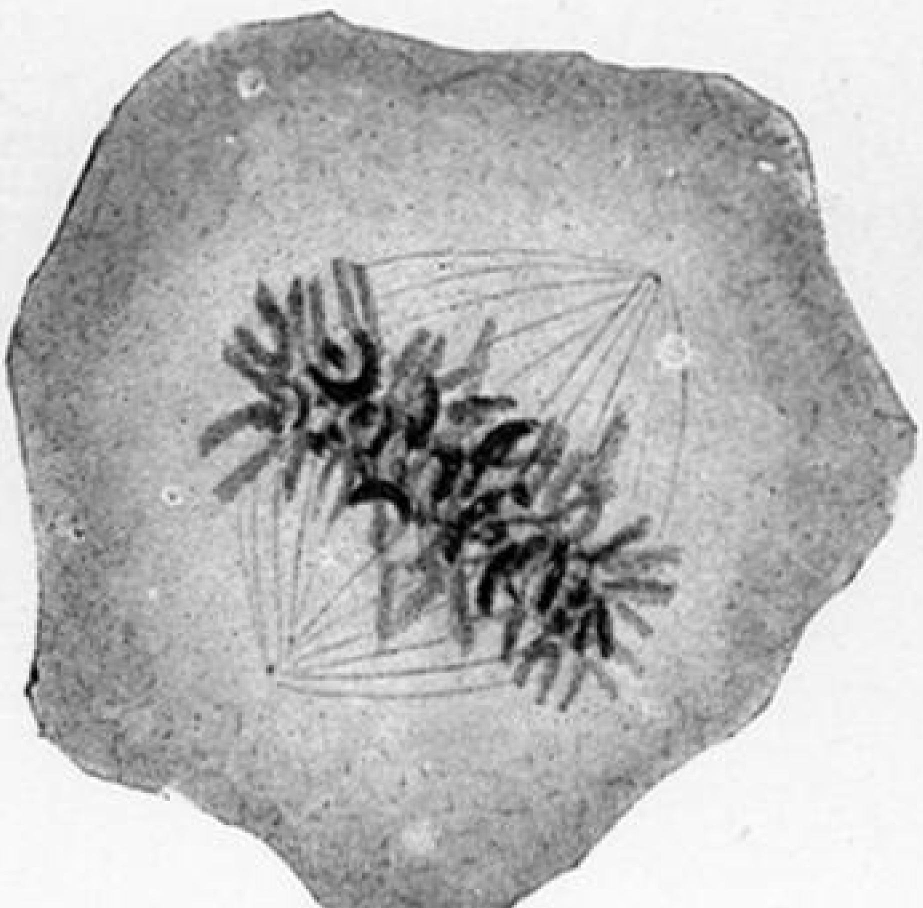

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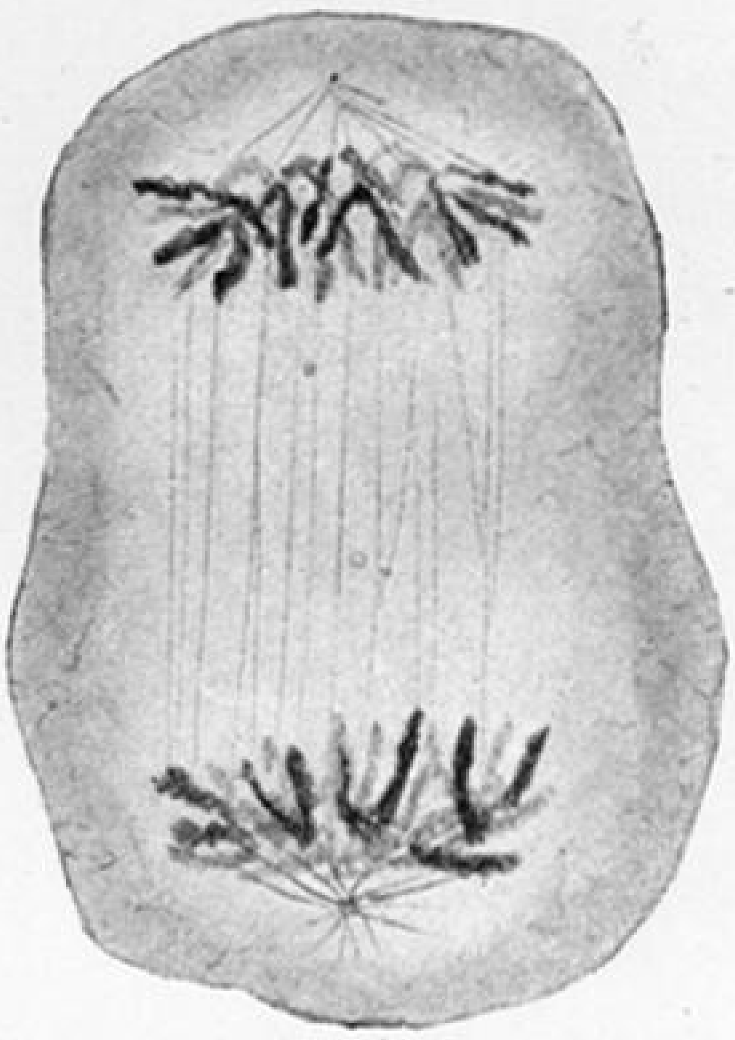

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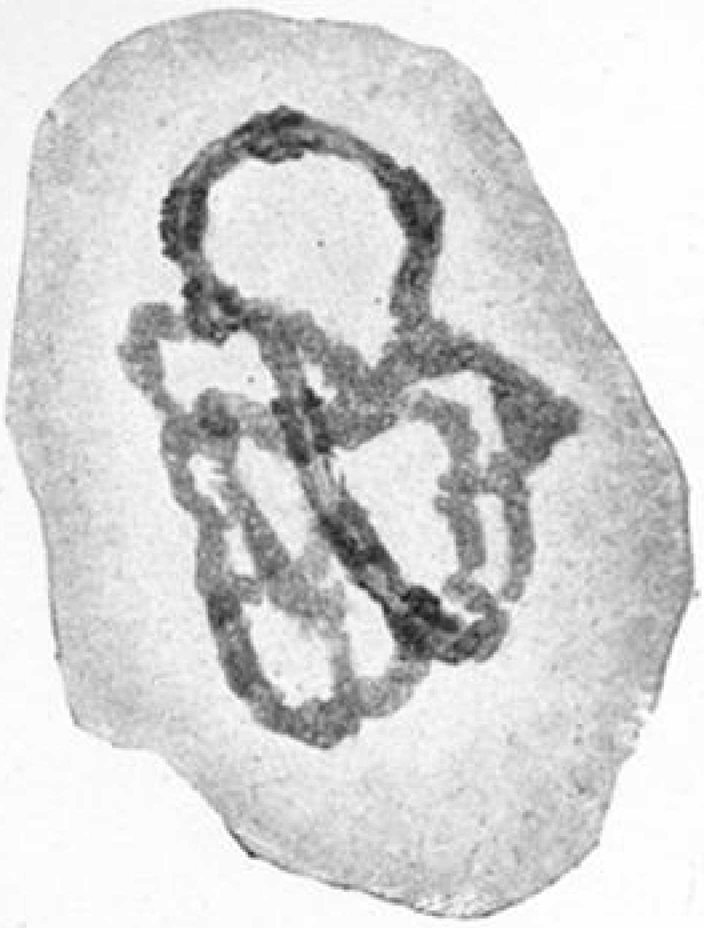

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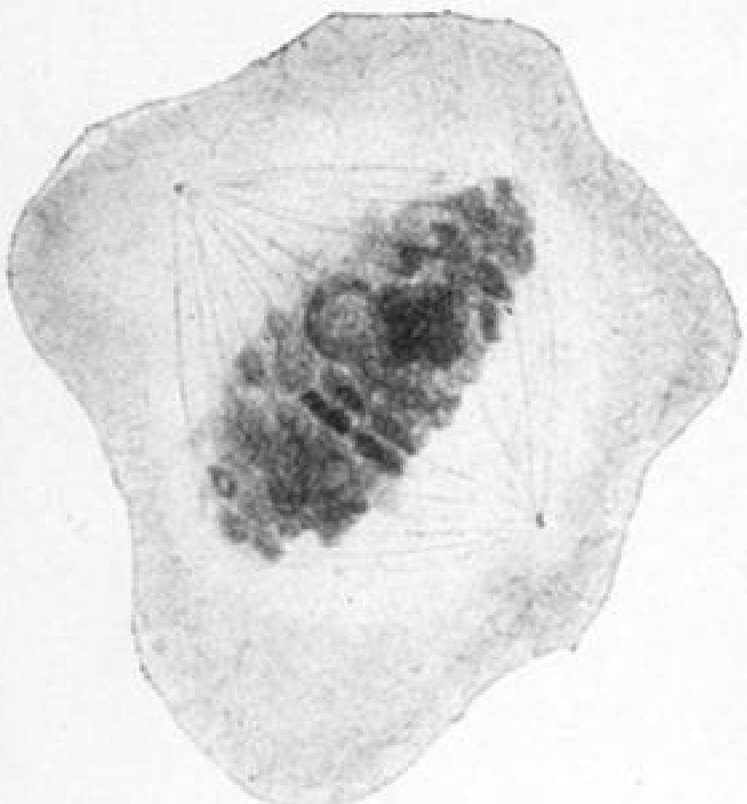

Fig. 15.

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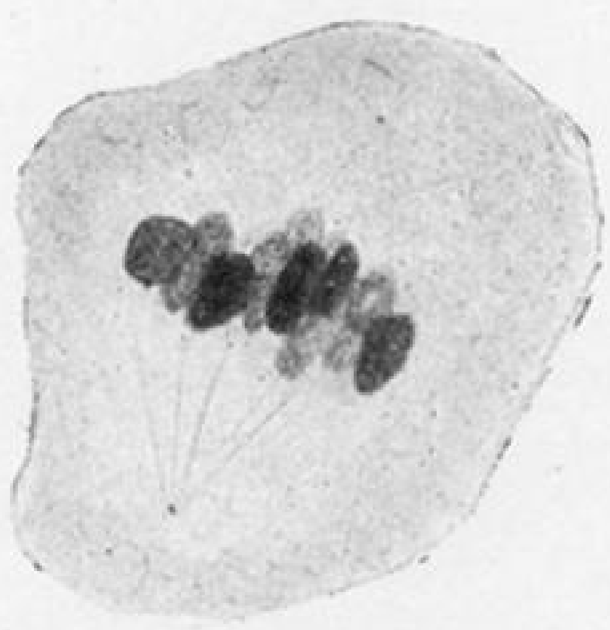

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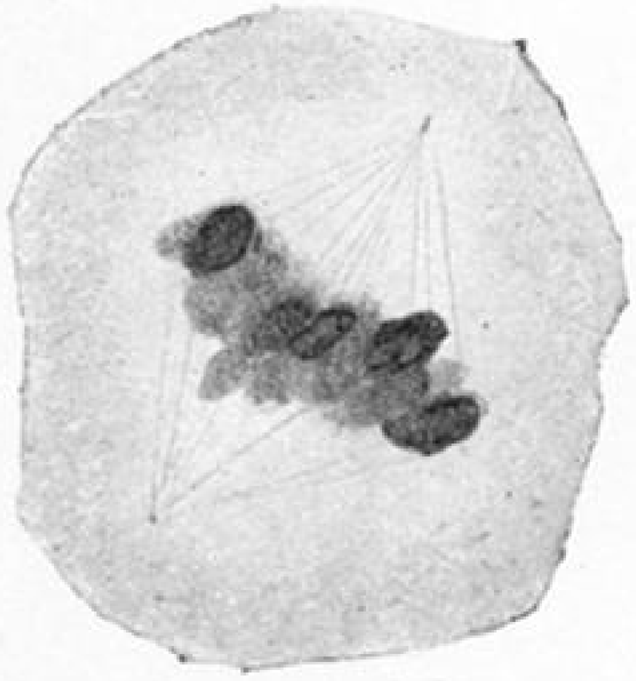

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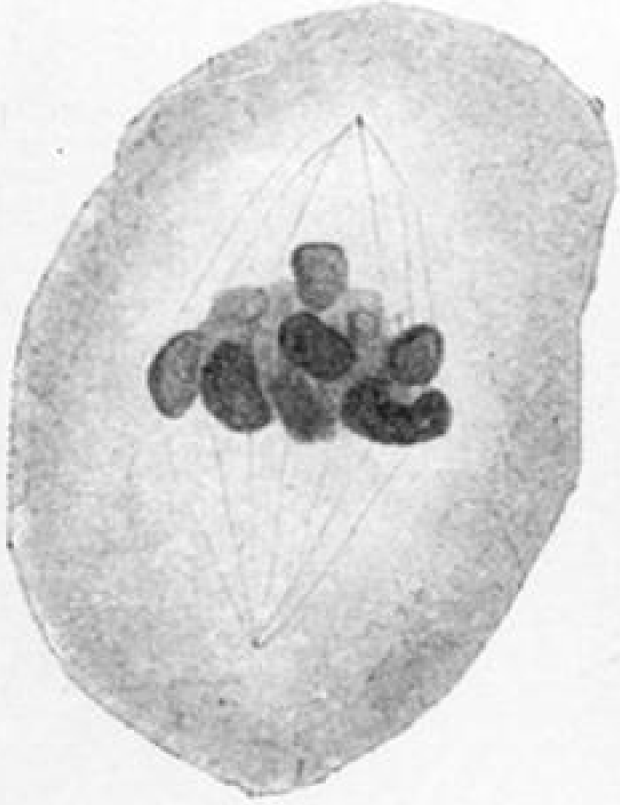

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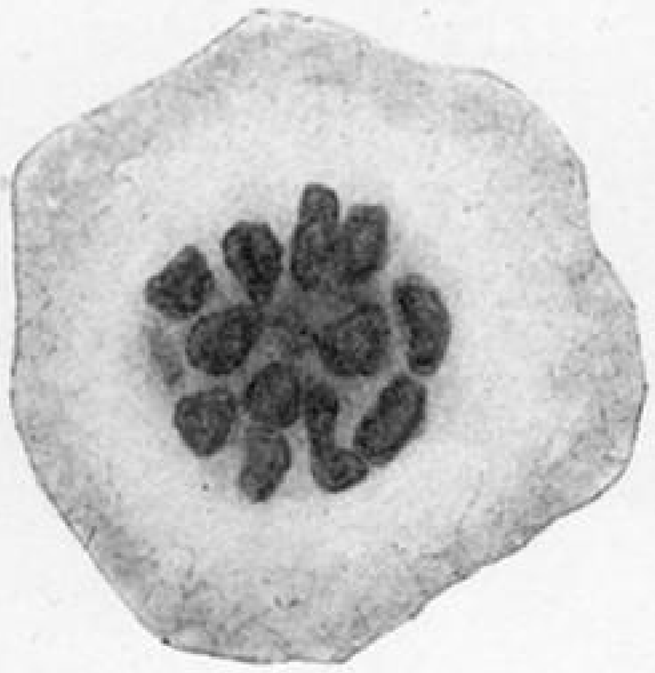

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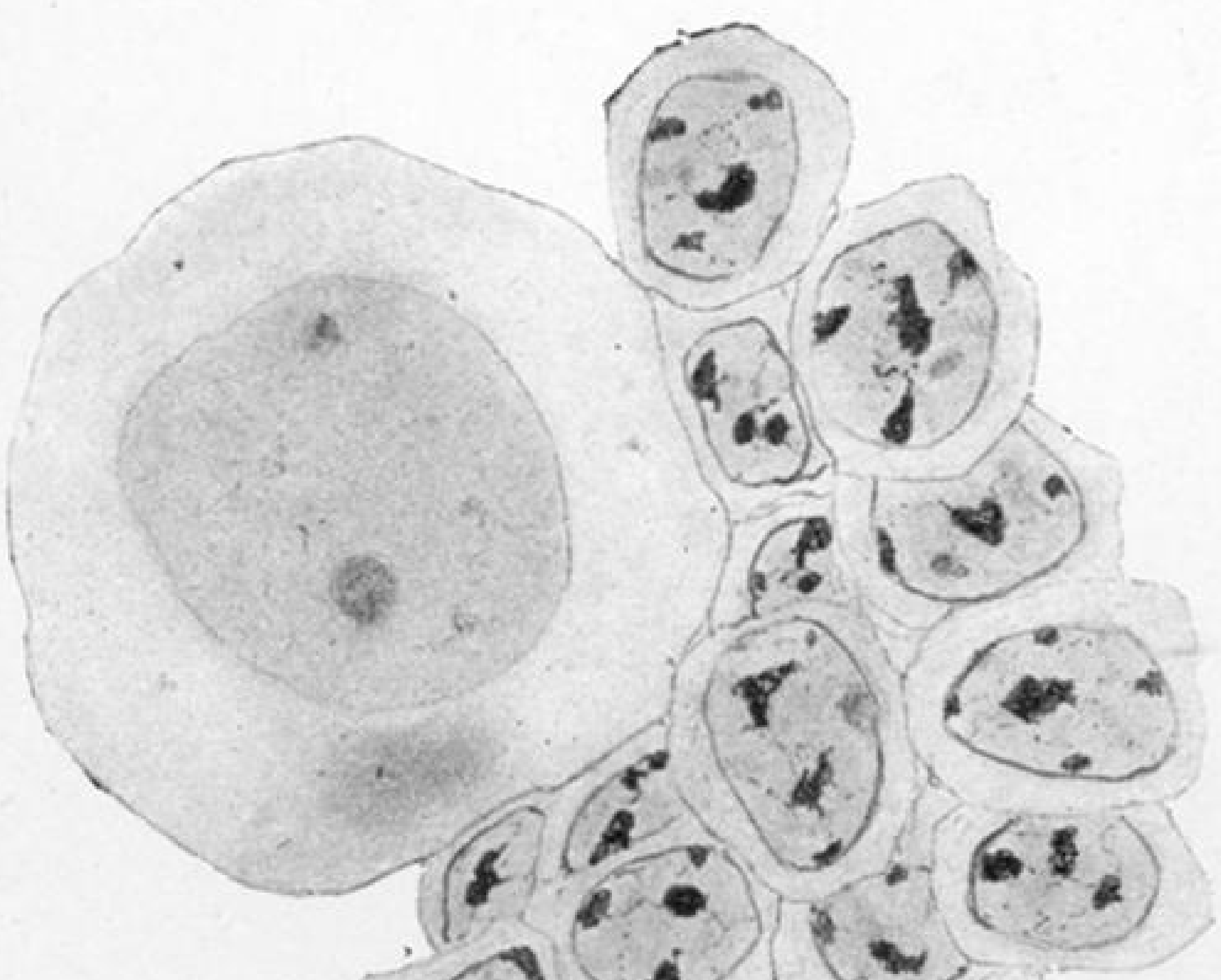

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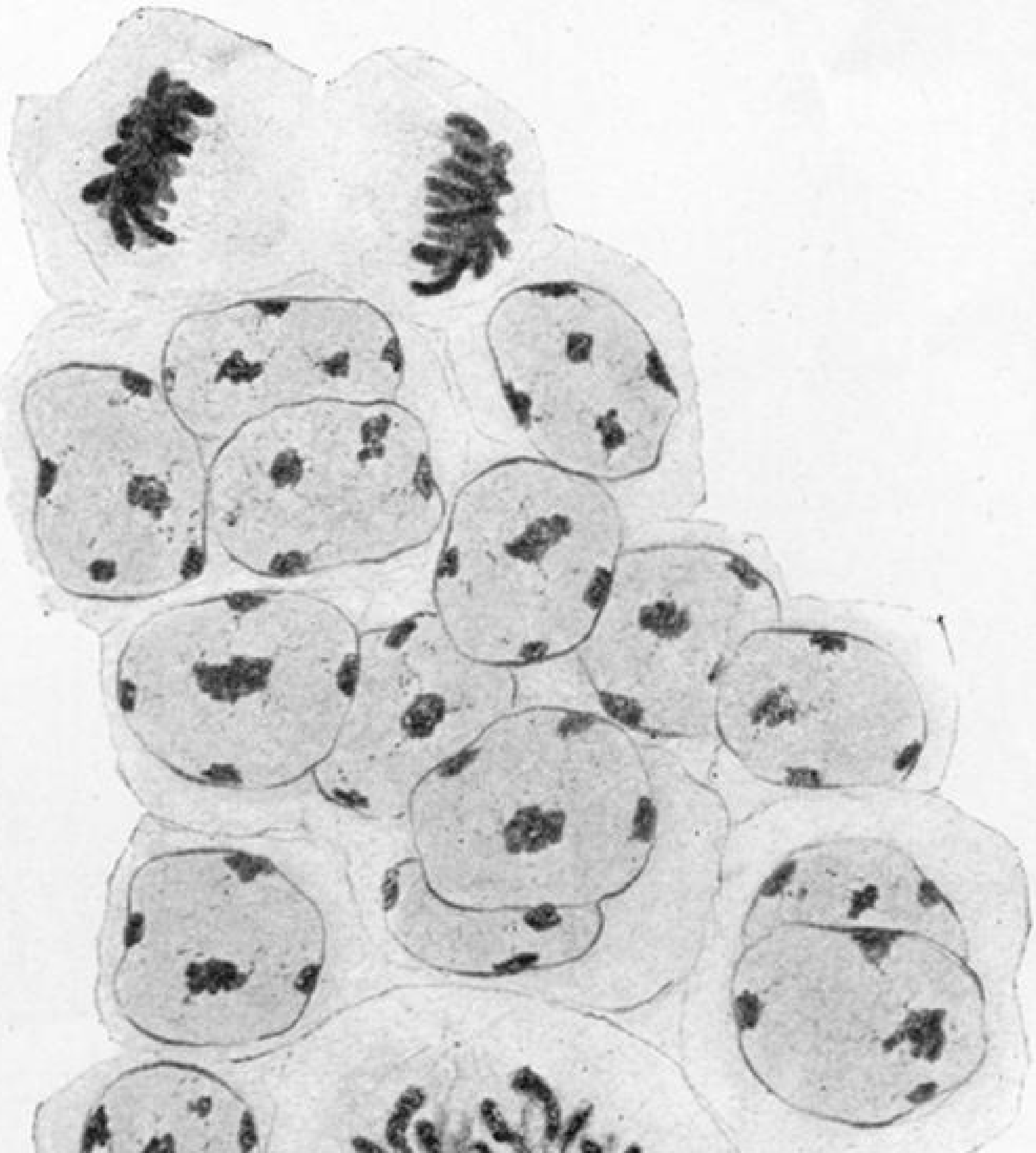

Fig. 28.