Gametogenesis of the Gall-Fly, Neuroterus lenticularis (Spathegaster baccarum).—Part I.

By Leonard Doncaster, M.A., Lecturer on Zoology, Birmingham University.

(Communicated by Prof. W. Bateson, F.R.S. Received September 21,—Read December 9, 1909.)

[Plates 1—3.]

In previous papers* I have described the maturation of the egg, with some observations on the spermatogenesis, of the Saw-fly, *Nematus ribesii*, in which the eggs may develop either parthenogenetically or after fertilisation. Some questions remained obscure or doubtful, and it seemed probable that an answer to them might be found in a study of one of the gall-flies (Cynipidæ). The gall-flies are remarkable in having in most species two generations in the year, of which one is bisexual, and the other consists wholly of females. The flies of the two generations are, further, so different from one another in structure that in most species they were originally described as belonging to different genera. The galls produced by the two generations are also very distinct.

The species chosen for this work was the very common *Neuroterus lenticularis*, of which the summer (bisexual) generation was originally described as *Spathegaster baccarum*. The galls from which the spring (agamic) generation emerge are lenticular growths found on the underside of oak leaves in October. From these galls the flies hatch early in April; they are exclusively females, and, if provided with buds of oak, readily lay their eggs deep down among the developing leaves and catkins. The eggs were dissected out, fixed in Petrunkewitsch's mixture (alcohol-acetic-sublimate) at various times after being laid, and cut into sections.

The galls of the summer form are spherical, sappy galls, found on the leaves and catkins in May and June. From them hatch males and females (the latter in nature largely preponderating in number), and after copulation the females deposit their eggs in the tissue of young leaves, always at the side of a small vein. The females of this generation differ considerably from the agamic females of the spring brood. The most conspicuous difference is in the ovipositor, which in the summer females is quite short, but in the agamic females is fully five times as long, and coiled up in the abdomen. The flies lay readily, and the eggs may be seen with a lens, so that small

* 'Q.J.M.S.,' vol. 49, 1906, p. 561, and vol. 51, 1907, p. 101.

pieces of the leaf containing them are easily cut out and fixed. Petrunkewitsch's solution was used, Flemming and others that were tried proving unsatisfactory.

For spermatogenesis and the development of the egg before deposition the larvæ and pupæ were removed from the galls at the end of May and early in June, opened and fixed immediately in Flemming (strong) and Petrunkewitsch. For this work Flemming's solution proved the better, but both fixatives gave good results. The chief stain used in all cases was Heidenhain's iron-hæmatoxylin, safranin being used as a control.

Since both males and females arise from the parthenogenetic eggs of the spring generation, it seemed desirable to determine whether both sexes arise from eggs laid by one female, and whether there are any differences in the maturation of the eggs corresponding to the difference in sex. With these objects in view, in collecting and preserving the eggs, in 1909 I kept those laid by individual females separate; and I also made some experiments in letting the flies lay on growing oak branches, and rearing the galls. In April I put single females in muslin bags or "sleeves" on the branches of an oak tree; removed the sleeves and marked the branches when the flies had died, and searched the leaves for galls at the beginning of June. In 1908 these experiments were wholly unsuccessful, perhaps because the tree chosen had not suitable buds for the flies to lay in, for in 1909 I found that they laid much more readily in buds from some trees than from others. The more advanced buds were preferred. In 1909 I sleeved out 40 flies on several different trees, including some quite young oaks from 1 to 4 feet in height. On June 1, I found galls on seven branches of two large oaks, but none on any of the small ones. The numbers of galls in the sleeved branches were respectively 6, 7, 7, 2, 4, 3, 3, and from these I reared respectively 5 ♀'s, 7 ♂'s, 6 ♂'s, 2 ♀'s, 4 ♀'s, 0, 0.

In the galls which did not hatch the larvæ either died or were parasitised.

I searched the trees carefully for galls on branches which had not been sleeved, and found none, so it may be concluded with confidence that these galls were produced by the flies sleeved out. Since in each case only one sex emerged from the galls produced by one fly, although the numbers are small I think it is justifiable to conclude that every fly of the agamic generation produces eggs of only one sex, *i.e.* gives origin to either males or females only, not to both. Cases of this kind are known in the Hemiptera (Aphididæ) and in Rotifers, but not hitherto, as far as I am aware, in the Hymenoptera.

The Somatic Mitoses.

In attempting to count the chromosomes in the mitoses in the egg, it was found difficult to determine the exact number, and with a view to checking this a number of counts were made of division-figures in the body tissues (developing wings, hypodermis) of male and female pupæ. Some of these mitotic figures are represented in Plate 1, figs. 1 and 2. In all cases the chromosomes are nearly equal in size, and the number is certainly approximately 20 in somatic mitoses. In mitoses in male pupæ or larvæ just before pupation, 50 counts gave an average of 19·2 chromosomes, 17 showing 20, and the remainder 19 or 18. In female pupæ of the summer generation, 40 counts gave an average of 19·4, 25 showing 20, and the rest 19 or 18. In the pupæ (female) of the spring generation, division-figures are scarce, as development proceeds much less quickly, but it is clear that the number is sensibly the same as in the summer brood (Plate 1, fig. 3).

The fact that in 50 counts of male mitoses only 17 showed 20 chromosomes, while 25 out of 40 had that number visible in females, might suggest the possibility that the male actually has one chromosome less than the female. In cases such as this, where the chromosomes are small and crowded, it is quite possible to count one elongated or bent chromosome as two, but some of the mitoses with 20 are so clear as to leave no doubt that this is the true number. The difference probably depends on the fact that the female pupa from which most of the counts were made had exceptionally fine mitoses, and was very well preserved, so as to give a smaller proportion of irregular figures than in the male pupæ used. It is possible that in the male one chromosome is smaller than the rest, and sometimes hidden, but I have not been able to convince myself of this.

Spermatogenesis.

The larvæ are found in the galls in the latter half of May; they develop very rapidly, for at the middle of the month they are exceedingly small, and by the end most of the galls contain pupæ. In very young pupæ, with no colour except in the eyes, the testes contain spermatocytes and the various maturation phases; a week later all the pupæ are grey or black, and the "meiotic phase" is over, only spermatids and nearly ripe spermatozoa being found. The full-grown larvæ also have spermatocytes in the testes, and some follicles show prophases of the maturation; for the spermatogonial stages, half-grown larvæ, about four or five days younger, are required. In very young larvæ I have not been able to distinguish the gonads.

The larvæ and pupæ were opened with needles and fixed entire in

Flemming's strong fluid, or sometimes in Petrunkewitsch's fluid; owing to their small size it was found that when the animals were well opened, fixation was quite successful after half an hour in strong Flemming, and if they remained too long the cells became over-fixed. The sections were stained in Heidenhain's iron-hæmatoxylin and sometimes in safranin as a control.

In larvæ, shortly before pupation, the testes contain only spermatocytes, and no division-figures are found. In younger larvæ it is not quite easy to distinguish the sexes, but the ovaries are larger, the cells are beginning to arrange themselves in strands to form the egg-tubes, and some cells are larger than the rest, with very large nuclei, and presumably are developing into eggs. The testes are smaller and all the cells appear alike. They have vesicular nuclei with a large nucleolus, and are much like the embryonic cells of the body-tissues. I have found only one larva* which shows the spermatogonial divisions, and these are remarkable from the large size of the chromosomes and spindle, and from the fact that there are clearly 10 chromosomes at each end in the anaphase (Plate 1, fig. 4, *a*, *b*, *c*). The chromosomes are elongated and rod-like, and when the spindle is cut across, the number 10 can be counted with great confidence at each end. In the same larva mitoses in the body-cells show clearly the diploid number (about 20); in this particular larva I have found none in which I can count the chromosomes with perfect accuracy, but in many mitoses at least 18 can be seen without any doubt, both in metaphase and anaphase. It appears, then, that while the germ-cells contain only 10 chromosomes at this stage, the body-cells contain 20. In female pupæ of the same age, as will be described below, 20 chromosomes appear both in oogonial and somatic divisions.

In larvæ, shortly before pupation, the testes are like those of young pupæ, but contain only primary spermatocytes; in some follicles these are in the

* Since the paper was sent for publication, I have found a second young larva showing 10 chromosomes in the spermatogonial mitoses both in metaphase and anaphase. I have also discovered that in the developing central nervous system of male larvæ (several different specimens) many, but not certainly all, of the mitotic figures show the haploid number of chromosomes (10). In female larvæ the mitoses of the nervous system contain the diploid number. I have not found any perfectly clear equatorial plate in the male nervous tissue showing the diploid number, but some figures suggest it, and it is possible that the supporting cells, like those of the hypodermis, have the full number, and the nerve-cells the reduced. It should also be mentioned that in both sexes there occur in places, in or just below the hypodermis, giant nuclei some 15 μ or 20 μ in diameter, and in one female larva I have found a division figure of one of these nuclei in anaphase with at least 50 chromosomes at each pole. A fuller description, with figures, will be given in the second part of this paper.—*November*, 1909.

resting condition, but in others chromatin is beginning to appear, and where it is aggregated into distinct masses the number of these approaches 20, but they are too irregular for an accurate count to be possible.

The testes in the young pupæ are divided into follicles, in the walls of which are very large nuclei with many chromatin granules. In each follicle the cells are nearly, but not quite, exactly at the same stage of development. This fact makes it very difficult to determine with certainty the exact course of events, for only here and there does one find a follicle in which slightly different stages are represented, and so get a clue as to the course of development. In pupæ which are not too far advanced, various stages occur in different follicles of the same testis from primary spermatocytes before the maturation divisions have begun, up to spermatids which are beginning their metamorphosis into spermatozoa. By comparing a number of testes of this age with one another, I have found a continuous series of stages, and think there is little doubt that in the account which follows they are placed in the correct order. The difficulty is increased by the fact that stages which appear closely similar occur at considerable intervals, and great care is needed in distinguishing one from the other.

The primary spermatocytes are rounded cells lying free in the cavity of the follicle; each has a large vesicular nucleus, containing a very faintly-staining reticulum, and often a nucleolus (Plate 1, fig. 5 *a*, *b*). At the approach of the "meiotic" divisions chromatin appears as small dots close to the nuclear membrane; these dots coalesce into irregular masses just inside the membrane, and these then form themselves into 10 chromosomes, which in some cases for a short time have an elongated band-like form (Plate 1, fig. 6). The cell is meanwhile becoming elongated and pear-shaped, the nucleus occupying the wider end. At the apex of the narrow end a minute black dot, the centrosome (centriole), may often be seen, and at a slightly later stage a second centrosome is seen at the broad end of the cell, close to the nucleus (fig. 7 *a*). I have not observed their origin. In the primary spermatocyte, when the chromatin is beginning to appear, a slightly larger black dot appears outside the nucleus, but as this may still be seen, in addition to the centrosomes, at the stage described, it cannot give origin to the centrosomes.

The chromosomes now become shorter, and place themselves in a radial arrangement, as in an equatorial plate; the nuclear membrane persists, but the nucleus is drawn out towards the narrow apex of the cell, so that it also becomes somewhat pear-shaped (Plate 1, fig. 7 *c*). At this stage, in some cells the narrow apex of the cell is elongated into a fine process, with the centrosome (centriole) at its tip (fig. 7 *c*), like that figured by Mark and

Copeland in the corresponding stage in the Bee.* Fine threads run down from this to the nucleus, but it is difficult to determine whether they penetrate inside the membrane or pass outside it, for at the narrow end the nuclear membrane becomes indistinct and confused with these fibres, while remaining clearly defined at the opposite wider pole.

The stage now described resembles the metaphase of a true division, such as occurs later, but is distinguished from it by the persistent nuclear membrane, and the position of the chromosomes at one end of the nucleus, near the broad end of the cell. In this case no nuclear division takes place, the nucleus becomes oval in shape, and the chromosomes generally contract to form a compact mass lying across its centre (figs. 7 *b*, 8). In some cells at least this chromatin mass seems to divide, half passing to each end of the oval nucleus, and as this returns to its resting condition it is common to find a cap of chromatic material at each pole. The chromatin may finally disperse, taking the form of granules, and thus giving rise to a condition not differing greatly in appearance from the primary spermatocyte in which the chromatin has begun to appear. Possibly the division of the chromatin inside the nucleus, which occasionally seems to occur, is the persistent remnant of a true nuclear division, or it may perhaps be compared with the "intranuclear karyokinesis" described by Kostaneckiț in parthenogenetic eggs of *Mactra*.

While the nucleus is returning to its resting condition, the apex of the pointed end of the cell, with the centrosome, becomes constricted off, apparently much in the same way as in the Bee. The amount of cytoplasm removed with the centrosome is very small, but it may sometimes be seen attached to the body of the cell by a narrow bridge containing spindle-fibres (fig. 7 *b*). In some cases the separation seems to be complete, and in follicles containing spermatocytes with a reconstituted nucleus small loose pieces of cytoplasm, sometimes with the centrosome visible at one end, are found scattered among the cells. In other cases these fragments seem to remain attached to the cell, but soon degenerate; in the prophase of the succeeding division they are perhaps represented by small knob-like excrescences sometimes found at the edge of the cell (figs. 8, 9, 10 *c*).

The cells with reconstituted nuclei may now be regarded as secondary spermatocytes, although no true nuclear division has occurred. In their resting condition, the duration of which it is not easy to determine, they do not differ greatly from the primary spermatocytes when the chromatin is beginning to appear; but they are generally more elongated,

* 'Proc. Amer. Acad. Arts and Sciences,' vol. 42, No. 5, fig. 8.

† 'Arch. Mikr. Anat.,' vol. 64, 1904, p. 1.

and very frequently the chromatin is closely packed round the nuclear membrane, especially at one pole, with fine strands radiating from this towards the more empty pole. At this stage they are much like the spermatids, in which the chromatin has a similar arrangement (*cf.* figs. 8 *c* and 17), but the nucleus is much larger, having the same size as that of the primary spermatocytes.

The chromatin next becomes grouped in the form of large elongate granules or small bands scattered under the nuclear membrane; their number is not easy to determine, but it approaches 20 (fig. 9). They then become combined, but whether by end-to-end pairing I cannot determine, into 10 very definite long bands having a more or less regular meridional arrangement under the membrane (fig. 10 *a*, *b*, *c*).

In one testis, in which a single follicle is much retarded, all the others containing well advanced spermatids, there are about 20 of these bands in each nucleus, and as they concentrate themselves to form chromosomes they appear to pair side by side to form 10 split chromosomes (Plate 1, figs. 18 *a*, *b*). It is possible that this lagging follicle is abnormal, for in all other cases where the bands can be counted their number is clearly about 10. The bands then become shorter, and arrange themselves across the nucleus in a fairly regular equatorial plate, in which 10 chromosomes radiate from a centre (figs. 11, 12).

The nucleus has now assumed the form of a wide spindle, stretching almost from end to end of the cell, but the membrane, though faint, appears still to persist. At each end of the nucleus a minute centrosome may be seen, and apparently within the nucleus is a system of spindle fibres extending from the centrosomes to the chromosomes, which lie across the centre. Commonly also at this stage a small deeply-stained dot may be seen outside the nuclear spindle, generally nearer one end. It is shown in figs. 13, 14, 15; where, as in figs. 13 *b*, 14 *a*, it appears within the spindle, it is in reality at a different level, and is always quite near the edge of the cell. The metaphase condition appears to last for some little time, for it is frequently found (fig. 13 *a*, *b*)

In the early anaphase the nuclear membrane has quite disappeared, and the chromosomes appear to split longitudinally, for in the equatorial plate they are arranged with their length across the spindle. The cell elongates somewhat, and the spindle extends completely from end to end. In early anaphase the chromosomes have the form of short rods converging towards the centrosomes (fig. 14 *a*, *b*); as they move apart they shorten, and as they aggregate round the centrosome they become still more concentrated. The cell meanwhile becomes constricted, a sheaf of spindle fibres extending from one daughter nucleus to the other, connecting the two halves (fig. 15 *a*, *b*). The two groups of chromosomes round themselves off into nuclei, having about

half the size of the spermatocyte nuclei; the cells divide completely, and become a pair of spermatids (figs. 16, 17). In late anaphases the small stained body described above outside the nucleus may usually be seen included in one of the daughter cells; it is found outside the nucleus or spindle during the whole process, so cannot be regarded as any kind of accessory chromosome (figs. 12, 13, 14, 15).

It will be seen that the spermatogenesis in *Neuroterus* presents the same remarkable features as have been described by Meves* and Mark and Copeland† in the Bee and Wasp. In each we find the first spermatocyte division suppressed, being represented by the extrusion of a centrosome with a small quantity of cytoplasm.

Neuroterus resembles the Wasp in forming two nearly similar spermatids from each spermatocyte, but differs from both Bee and Wasp in having a definite resting stage between the abortive and true divisions.

The presence of the small stained body, persisting from the primary spermatocyte and passing into half the spermatids, has not been recorded in either Bee or Wasp.

Development of the Egg in the Ovary.

In rather young larvæ of the summer generation the ovaries consist of masses of cells not yet divided into egg-tubes, but they are beginning to arrange themselves into strands from which the egg-tubes will develop. No clear distinction is yet visible between the cells which will become eggs and those which will form the accessory cells between the eggs. The nuclei are mostly large, reticular, with one or two conspicuous nucleoli; others are stil larger, and have a mass of chromatic material aggregated in the form of dots and fine threads at one side of the nucleus. Nuclei like this may also be found in the eggs in the pupal egg-tubes. Mitotic figures are frequent; they resemble those found in the body-tissues, and clearly have the diploid number of chromosomes (Plate 1, fig. 19 *a*, *b*).

In young pupæ of the summer generation, the stages in the development of the ovarian egg can be followed easily, for the upper ends of the egg-tubes contain very early stages, and at the lower end well developed eggs are found. In the upper ends of the tubes all the cells are alike, but lower down differentiation takes place, and some cells enlarge greatly, become filled with yolk, and begin to develop into eggs surrounded by follicle-cells. Alternating with the developing eggs are masses of cells with large nuclei, each usually containing a double nucleolus; these cells are all alike, and do not differ much

* 'Arch. Mikr. Anat.,' vol. 70, 1907, p. 414; vol. 71, 1908, p. 571.

† 'Proc. Amer. Acad. Arts and Sciences,' vol. 42, 1906, p. 103; vol. 43, 1907, p. 71.

from the primitive ova at the top of the egg-tube. The tube is thus moniliform, and the swellings contain alternately a developing egg and a mass of cells like primitive ova (fig. 20).

As the eggs develop they enlarge greatly by the deposition of yolk, and the nucleus appears as a relatively small vesicle, lying at one side. When ripe, the egg is prolonged at the front end into a hollow stalk with a small vesicle at the free end; the stalk is said to be held by the ovipositor as the egg is thrust into the hole bored in the leaf when the egg is being laid. The stalk thus partly blocks the opening, and in the spring eggs, which are generally somewhat compressed by the bud-scales, some of the yolk of the egg is frequently forced into the cavity of the stalk.

After one of the primitive ova has definitely begun to develop into an egg, as shown by the deposition of yolk, no division of the nucleus appears to take place until it is laid. In the follicle-cells which surround the eggs mitoses are not uncommon, and show the same number of chromosomes as in other somatic nuclei, viz., about 20.

In the females of the spring (agamic) generation, I have not observed the oogonia. In the pupæ the development does not differ materially from that of the summer generation. The egg-tubes are very similar, and the eggs when laid differ chiefly in being slightly larger. Since the pupæ develop very slowly, mitoses are more difficult to find, but the chromosome groups from an egg-follicle, represented in fig. 3, show that the somatic number is similar to that in the summer pupæ. Fig. 21 *a*, *b*, *c*, shows three figures of an egg-nucleus in which about 20 chromosomes appear to be visible, *a* and *b* being drawn from the same section at different levels, and *c* from the next section of the series.

Maturation and Fertilisation of the Egg.—Summer Generation.

The eggs laid by the flies of the summer generation in the early part of June are sunk in the tissue of the underside of young oak leaves, and the stalk of the egg commonly projects somewhat from the hole made by the ovipositor. These eggs are fertilised, and the large spermatazoon may almost always be found near the edge in sections of eggs preserved within about an hour and a half from the time of laying. The spermatozoon, after entering the egg, and while being converted into the male pronucleus, generally lies at the opposite side of the egg, or at least some distance removed, from the egg-nucleus, which during the same period is undergoing its maturation processes. During the first hour and a half after the egg is laid the nucleus is found near the edge; its position is somewhat variable, but it is commonly about midway between the ends of the egg (Plate 2, fig. 22).

In eggs preserved very shortly after being laid, the nucleus appears as a small darkly-stained body, either flattened against the edge of the egg or somewhat spindle shaped, with the longer axis of the spindle perpendicular to the edge. There is little doubt that the very narrow flattened nucleus is the earlier stage, but as both are found in eggs within half an hour of being laid, this is not perfectly certain. The nucleus now begins to enlarge, and instead of staining deeply and almost evenly throughout, chromatin bodies connected more or less conspicuously by a network appear within it (fig. 23). The succeeding stages are somewhat obscure, and are not unlike the maturation processes described by Henking* in the Gall-fly *Rhodites rosæ*. The nuclear membrane becomes faint and disappears, and the chromatin bodies segregate themselves to some extent into an inner and outer group, but whether by division of the individual chromosomes, or by a separation of chromosomes previously distinct, I cannot be sure (Plate 2, figs. 22, 24, 27, 30). The chromosomes of the inner group then separate themselves from those of the outer, and in so doing take the form of rods lying side by side or with their inner ends converging somewhat as in the anaphase of a typical mitosis (Plate 2, fig. 30). The outer part of the nucleus meanwhile has never become clearly separated into chromosomes, but appears as a group of chromatin bodies connected together by strands as in a reticular nucleus. Although Henking does not figure a stage exactly of this kind in *Rhodites*, yet he describes something similar. On pp. 149 and 150 he writes: "Die untere Tochterplatte lässt die Neunzahl unschwer erkennen, während in der äusseren Tochterplatte wiederum eine theilweise Verklebung eingetreten ist." In a nuclear division of this kind, in which there is never an equatorial plate, and in which the chromosomes of one half never become clearly separated, it is not easy to determine the chromosome number with certainty. But from a comparison of a large number of sections, especially when cut tangentially to the egg, it is fairly clear that the number of chromosomes in the inner group is about ten. In some sections not so many are visible, but that the number is really approximately ten there can be no doubt, *i.e.* the same number as in the spermatocyte divisions already described. In *Rhodites* Henking found nine.

The division just described does not lead to the production of two resting nuclei, but is succeeded immediately by further changes, which here also are obscure and hard to follow. Henking says that in *Rhodites* the chromosomes at each end of the first division-figure again divide, and thus give rise to four groups, of which the innermost forms a definite nucleus which sinks in as the female pronucleus, while the other three remain near

* 'Zeit. Wiss. Zoo.,' vol. 54, 1892, p. 147.

the egg margin as polar bodies. Such a process would not be very different from what occurs in the saw-flies and other Hymenoptera, but Henking confesses that he has never actually seen this second polar mitosis. "Die Theilung muss ausserordentlich rasch verlaufen; denn . . . . habe ich hier immer gefunden, dass die Theilung der Chromosomen bereits vollendet war." Like Henking, I have had difficulty in observing this second polar division, although stages with three groups of chromosomes and a developing pronucleus are abundant. It is clear that the outer and inner groups left from the first polar division do not divide simultaneously, and although I have many sections of these stages I have no clear figure of the division of the inner group, *i.e.* of the separation of the female pronucleus from the chromosomes of the second polar body. For some time I doubted whether any such division occurred, but the presence of three groups of polar chromosomes is difficult to explain without it, and I have obtained a few sections which suggest that such a division is taking place. It clearly occurs before the division of the outer chromosome group, for while the latter is still a confused mass near the edge of the egg, the inner group often appears very much drawn out (Plate 2, figs. 26, 30) as if undergoing division, and in other sections (fig. 31) the division is seen completed. This stage seems to follow immediately on the first division, so that the division of the inner group of chromosomes is part of the same process as the original separation into inner and outer groups. In Plate 2, fig. 29, a different phase is represented, in which the inner group looks as if it were forming a compact mitotic figure, but this appearance is not usual.

After the chromosomes which will give rise to the female pronucleus have sunk in to some extent, the outer group of chromosomes, lying near the edge of the egg, undergoes an irregular division (Plate 2, fig. 32), so giving rise to three groups altogether of polar chromosomes, but these are so confused and irregular that the number in each group is never ascertainable with certainty, and it appears as if some fusion often takes place between them (fig. 33).

There is one possibility which should be mentioned here, which is not entirely inconsistent with any of my sections. It is that the three groups of polar chromosomes are all derived by division of the outer group left by the first maturation division. I have no section which proves with certainty that the inner rod-like chromosomes described above are not converted direct into the female pronucleus, and that the three groups of polar chromosomes are not produced by a separation of the original outer group into two, followed by a second division of the outermost, and so

yielding three groups. If this is the case, the innermost of the three groups must be formed by a sorting out of certain chromosomes from the original outer group, followed by a division of the remainder. It has been seen that there is no evidence for more than one maturation division in the spermatogenesis, but the existence of only one such division in the egg does not seem consistent with the presence of the diploid number of chromosomes in the oogonia.

The further fate of the polar chromosomes seems to vary somewhat in different eggs. In some, at the time of the conjugation of the male and female pronuclei, they appear as a single large group surrounded by a field clear of yolk granules, and with the individual chromosomes long and thread-like. Very frequently there are two groups, as if the two inner had amalgamated, and occasionally one finds three or four chromatin masses, in these cases usually with the chromatin closely balled together. In rather later stages, during the segmentation (about five to eight hours), the polar chromosomes seem to shrivel into small irregular masses and then to disappear completely.

It may perhaps be suggested that the apparent abnormality of the whole of the maturation processes of the egg is due to defective methods of preservation, but I think it unlikely for the following reasons. In the first place, at a rather later stage the conjugation of the pronuclei and segmentation divisions are well preserved; and secondly, Henking found very similar abnormalities in *Rhodites* in eggs preserved by hot water and by Flemming. It seems unlikely that three so different methods of preservation should all give the same results if the phenomena were not genuine. The division-figures in the cells of the leaf-tissue are also well fixed.

While the maturation of the egg-nucleus is in progress the spermatozoon is being converted into the male pronucleus. In eggs preserved within half an hour after being laid it commonly appears as a long narrow rod, straight or slightly curved, and often extending through two or even three sections. It contracts to a small, oval, compact nucleus, which stains very deeply, and then gradually swells, while chromatin bodies become visible within it. At this stage the chromatin sometimes suggests a coiled thread, but the staining is too dense to see whether this is so with certainty (Plate 2, fig. 32). As it increases in size, the male pronucleus sinks into the egg to meet the female pronucleus, which has now reached a similar condition, and when they meet in the centre of the egg they are both very large and vesicular (Plate 3, fig. 34).

The chromatin masses become definite chromosomes, the nuclear membranes disappear, and the two groups of chromosomes mingle and begin to form

the first segmentation mitosis. Minute centrosomes are visible at the poles of the spindle, but I have not observed their origin. Sometimes at least the chromosomes in the equatorial plate of this first division are seen to be in two groups side by side, so that the complete mingling cannot take place till the first segmentation nuclei are reconstituted (fig. 35).

In the later segmentation mitoses the chromosomes are elongated, and appear to be about 18 to 20 in number; and in the nuclei just before mitosis a similar number of chromatin bands may be counted (figs. 36 to 38).

Maturation and Segmentation of the Egg of the Spring Generation.

My observations on the eggs of the spring generation are not yet complete, and cannot be finished until fresh material has been obtained. I give here a preliminary account of the results, some of which cannot be regarded as established with certainty, and leave the full description until the second part of this paper is published.

The eggs of the spring generation are parthenogenetic; they are laid in the developing buds of the oak in April, and as has been mentioned above, those laid by some females develop into males, those laid by others to females.

When the maturation divisions have been found they resemble the early stages of the maturation in the summer eggs, but I have never seen the division of the inner chromosome group, and am inclined to believe that it may sink in and become the egg-nucleus without further division, so that only one true maturation division takes place (Plate 3, figs. 39 to 43).

In other eggs I have been unable to find any trace of a maturation division, and in the later stages, during segmentation, no polar chromosomes are to be found in such eggs, although they are always to be found in the summer generation at the same stage. It seems probable, therefore, that there are two kinds of eggs, of which one undergoes a maturation division and the other does not, and this is confirmed by the study of the segmentation mitoses. In the eggs laid by the majority of spring females, 20 chromosomes are found in the segmentation mitoses (Plate 3, figs. 44 *a*, *b*; 45, 46 *a*, *b*, *c*), but in the eggs laid by one female the division-figures all contain 10 or about 10 chromosomes (figs. 47, 48 *a*, *b*, *c*), and in some of these eggs a double group of polar chromosomes is clearly recognisable at the edge of the egg in the place where the maturation mitoses have been found at an earlier stage. Most of my material was collected before I discovered that some individuals lay eggs which yield males, others which yield females, so that I have only one series of eggs laid by one female which show the reduced number in the segmentation divisions. But as far as they

go, the results indicate that one kind of parthenogenetic female lays eggs which undergo no maturation, or at least no reduction, while the other kind lays eggs which undergo a maturation division, and in which the segmentation mitoses show the reduced number (10) of chromosomes. It has already been shown that in the male of the summer generation the spermatogonial divisions have 10 chromosomes, although the body-cells have 20, so that it is probable that the eggs which have a maturation division give rise to males, and it is possible that the 10 chromosomes seen in their segmentation mitoses are bivalent. In the body-cells these would then split into their univalent components, giving the 20 chromosomes observed, but in the developing germ-cells the haploid number of bivalents would be retained until the single spermatocyte division. In the summer females, on the other hand, the oogonia show 20 chromosomes, and these are probably produced by females which lay eggs that undergo no reduction.

The complete proof of these facts must be left until a fresh supply of material can be obtained in the spring.

Summary and Discussion.

The chief results described in the foregoing pages are the following:—

1. The Gall-fly, *Neuroterus lenticularis*, has two generations in the year, the flies appearing in April and June respectively. The spring generation consists exclusively of females, which differ considerably from the females of the summer brood. Their parthenogenetic eggs are laid in oak buds, and all the eggs laid by any one female develop into individuals of the same sex in June, *i.e.* some of the spring females are male-producing, others are female-producing. The summer generation thus consists of males and females; their eggs are fertilised, and are laid in the tissue of young oak leaves, and give rise to galls very different from those produced in the spring. The flies from these galls hatch in April and thus complete the cycle.

2. Mitoses in the body-tissues of young pupæ show about 20 chromosomes, both in the spring parthenogenetic females, and the males and females of the summer brood.

3. In the spermatogonia of young male larvæ, mitoses show 10 chromosomes. In the primary spermatocytes of young pupæ 10 chromosomes appear. An imperfect mitotic figure is developed, but the nuclear membrane does not disappear; and after the metaphase is reached the nucleus returns to a "resting" condition. During this process the cell develops an elongation at one end, at the tip of which is one of the centrosomes (or centrioles); as the nucleus re-forms, the centrosome and a small piece of cytoplasm are separated off, as happens in the Bee and Wasp. The process is thus much

like that found in the Bee, except that the nucleus returns to a "resting" condition.

4. The spermatocytes now develop about 20 chromatin masses which form themselves into 10 band-like chromosomes. These shorten, form an equatorial plate across the cell, a typical spindle is produced; and the chromosomes divide so that 10 travel into each daughter nucleus. Two spermatids are produced, which are similar except that in some cases at least one of them receives a small extranuclear body of unknown nature, which is absent from the other. Both spermatids develop into spermatozoa.

5. In primitive ova in the young female larvæ of the summer generation, mitoses like those in the body-cells are found, with apparently 20 chromosomes. After the deposition of yolk has begun, no further nuclear divisions occur in the egg.

6. The maturation divisions of the summer eggs are difficult to follow, but apparently two divisions occur, giving rise to four groups of chromosomes, of which the three outer represent the three polar nuclei, while the innermost group sinks in to form the female pronucleus. This group probably consists of 10 chromosomes, but they are so crowded that the number commonly appears rather less in sections.

7. The male pronucleus and the female pronucleus meet and form the first segmentation spindle, in which, as in the later segmentation divisions, about 20 chromosomes appear. The polar chromosomes disintegrate and disappear.

8. The primitive ova of the spring generation have not been observed. In an egg from the egg-tube of a young pupa 20 chromosomes were seen in the nucleus.

9. The maturation of the spring egg has not yet been sufficiently studied, but it appears that some eggs undergo at least one maturation division, others probably none. In eggs in which maturation has occurred segmentation mitoses show 10 chromosomes; all the eggs laid by one individual female in which the chromosomes could be counted were of this type, and it is suggested that these develop into males. In the eggs laid by other females, however, 20 chromosomes appear in the segmentation divisions; in these, polar chromosomes appear to be absent, and it is probable that there has been no maturation division, and that these eggs would develop into females.

Most of the above facts have been shortly discussed in the sections where they are described, and only their more important general bearings remain to be considered. Of these, perhaps the chief is the relation of the facts observed in *Neuroterus* to what is already known in other Hymenoptera,

especially the Bee and other Aculeata. The life-history in the gall-flies is more complicated, involving an alternation of two different generations, but in some respects this simplifies our understanding of the facts. In the Bee and its relatives, fertilisation of the egg is facultative, and to all appearance, at least, the maturation of the egg is similar, whether fertilised or not. In the gall-flies the parthenogenetic eggs belong to a different generation, and since they give rise to both sexes, sex-determination in this generation, at least, does not depend on fertilisation. In the summer brood all eggs are fertilised and all give rise to females, so that here the gall-flies resemble the Bee. As in the Bee also, in the spermatogenesis there is only one maturation division of the nucleus, the number of chromosomes in the spermatogonial mitoses being the same as that entering the spermatids; in each case the first spermatocyte division is abortive, only a small portion of the cell being extruded. The Gall-fly differs from the Bee, but resembles the Wasp and Ant in producing two complete spermatids from each spermatocyte, while in the Bee one of the daughter nuclei degenerates without becoming a spermatozoon. It is tempting to speculate on the reason for this peculiarity in the Hive-bee, and a suggestion with regard to it will be made below after the parthenogenetic generation has been considered.

In the spring generation of *Neuroterus*, the eggs of which develop parthenogenetically, the evidence is unfortunately not yet complete. It has been seen that the eggs of some individuals give rise to females, those of others to males. The microscopical evidence, so far as it goes, suggests that in the eggs laid by some females there is possibly no maturation division, and in any case these eggs contain the diploid number of chromosomes; in eggs laid by other females a maturation division occurs, and the segmentation mitoses show the halved (haploid) number. It is suggested that the former type of egg develops into a female and the latter into a male, but since there is possibly only one maturation division in these, and since the body-cells of male pupæ contain the diploid number of chromosomes, it is possible that the chromosomes of the segmentation mitoses are bivalent. In the Bee also the body-cells of the male contain more chromosomes than the spermatogonia, in this case apparently four times as many (about 64 instead of 16).*

If it is the fact that parthenogenetic eggs of *Neuroterus* which have the diploid chromosome number in their segmentation mitoses develop into females, and those which undergo reduction become males, the sex-determination may be imagined as follows:—

* Meves, 'Arch. Mikr. Anat.,' vol. 70, 1907, p. 420.

The egg which is to undergo maturation may be regarded as containing both male and female determinants; the female determinant is removed in the polar mitosis and the egg remains male. The eggs which will undergo no reduction may be considered as containing only a female determinant, and this causes the egg to develop into a female.

If, as suggested, the ovarian eggs of some parthenogenetic females contain both male and female determinants, while those of others contain a female determinant but not a male, the difference between the two kinds of eggs must be caused by a difference of constitution in the females which lay them. Both kinds of female are produced from fertilised eggs, and the difference in constitution between them may be due to the existence of two kinds of spermatozoa in the males of the previous generation. The existence of spermatozoa of two kinds is indicated by a number of facts which will be further discussed below, but here it will suffice to refer to the evidence in the Hymenoptera and other parthenogenetic insects. In *Neuroterus* itself I have mentioned above that half the spermatids receive an extranuclear body which is not found in the other half, and it is conceivable that this is connected with the difference under discussion. But the fact that in the Bee half the spermatid nuclei degenerate, while in other related Hymenoptera all develop into spermatozoa, suggests that two kinds of spermatozoa may exist. And a similar condition has been described by Morgan* in *Phylloxera*, and confirmed in a fuller paper by von Baehr† in *Aphis*. They find that there is an odd number of chromosomes in the somatic nuclei of the male, and at the first division of the spermatocytes one chromosome goes over undivided to one end of the spindle, so that one daughter nucleus receives a "heterochromosome," while the other does not. In the second division the heterochromosome divides equally, but only those secondary spermatocytes which contain it develop further, the others degenerating. Thus, as in the Bee, half the daughter-cells of the primary spermatocytes atrophy, and since all fertilised eggs become females, Morgan supposes that the heterochromosome, which is contained in the functional spermatids, bears the female determinant. A different explanation of the facts will be offered below, but the facts themselves clearly indicate the existence of two kinds of spermatids, one of which, as in *Neuroterus*, contains a body lacking in the other. We may suppose, then, that in *Neuroterus*, one kind of spermatozoon, fertilising an egg of the summer generation, gives rise to female-producing (thelytokous) female, the other kind to a male-

* 'Proc. Soc. Exper. Biol. and Med.,' 1908, vol. 5, p. 56. (Full paper, 'Journ. Exp. Zoo.,' vol. 7, 1909, p. 239.)

† 'Zool. Anzeiger,' vol. 33, 1908, No. 15.

producing (arrhenotokous) female. In the Bee, as far as is known, all queens produce similar eggs, and this would be accounted for by the degeneration of one kind of spermatozoa.

The sex-determination in the Gall-fly would then take place as follows:—The spermatocytes of the male would bear a determinant for maleness, which may be represented by the symbol ♂. At the nuclear division this passes into one daughter-cell, its fellow being without it and thus containing no sex-determinant, a condition which may be represented by the symbol ⊙. The fertilisable eggs after maturation are supposed all to bear one determinant for femaleness, represented by the symbol ♀. The zygotes all develop into parthenogenetic females, but since half of them were fertilised by ♂-bearing spermatozoa, and half by spermatozoa with no sex-determinant (⊙), the former will have the constitution ♀ ♂, the latter ♀ ⊙. The females with constitution ♀ ♂ lay eggs which undergo maturation; the ♀ determinant is expelled, and the egg left with the ♂ determinant becomes a male. The females with constitution ♀ ⊙ lay eggs which undergo no reduction, but containing the ♀ determinant develop into females. The ♀ determinant is transmitted to the eggs of the summer generation, and since only one sex-determinant is present in the egg, it remains in the pronucleus when the polar mitoses take place, with the result that all eggs of the summer generation are ♀-bearing, as is assumed above.

It now remains to be seen whether this scheme is consistent with what is known of sex-determination, first in ordinary cases where all eggs are fertilised and secondly in the Bee and other aculeate Hymenoptera. The ordinary bisexual cases will be considered first.

As the result of my work on heredity and sex-determination in the moth, *Abraxas grossulariata*,* and of other similar cases, Bateson† suggested the hypothesis that the female is heterozygous in respect of sex, containing male and female determinants, the male homozygous containing only male. Eggs would thus be produced in equal numbers bearing maleness or femaleness, but all spermatozoa would bear the male determinant. This hypothesis would completely explain the cases which led to its formulation, but it is not completely consistent with the existence in many insects of two kinds of spermatozoa, one containing a heterochromosome and one without it. More recently, the evidence from some forms of sex-limited inheritance, such as colour-blindness‡ and congenital nystagmus,§ have made it clear that there

* 'Evol. Committee Roy. Soc., Report IV,' 1908, p. 53.

† 'Science,' 1908, N.S., vol. 27, p. 785.

‡ Bateson, "Mendel's Principles of Heredity," 'Camb. Univ. Press,' 1909, 1st impression, p. 230, inserted slip; 2nd impression, 1909, p. 195, note.

§ See Lloyd Owen, 'Ophthalmic Review,' vol. 1, 1882, p. 239.

are not only two kinds of eggs differing in their sex-determinants, but also two kinds of spermatozoa. In colour-blindness, for example, the affection is dominant in the male and recessive in the female; so that heterozygous males exhibit it, heterozygous females do not; but affected men do not transmit it to their sons. The sons of affected males are normal, but the daughters of affected males transmit the disease to some of their sons and, through some of the daughters, to their grandsons. It is clear, therefore, that among the spermatozoa of an affected male, those only bear the determiner for the disease which will give rise to female zygotes; but among the ova of a female heterozygous for the colour-blind condition, the colour-blind factor is borne indiscriminately by about half the ova, whether male-producing or female-producing.

These different facts all fall into line if we assume that the female contains both female and male sex-determinants, and produces equal numbers of ova bearing each; while the male contains no female determinant, but produces two kinds of spermatozoa, one bearing the male determinant, the other being without any determinant for either sex. Using the same symbols as before, viz., ♀ and ♂ for the female and male sex-determinants respectively, and the symbol ⊙ for absence of sex-determinant in a gamete, females would then have the constitution ♀ ♂, and would produce ♀ and ♂ eggs in equal numbers, males would be ♂ ⊙, and produce spermatozoa ♂ and ⊙ in equal numbers. In the insects with heterochromosomes, in the male the heterochromosome is regarded as bearing ♂, its absence being represented by ⊙; in the female the two heterochromosomes bear ♀ and ♂ respectively. If we regard the sex-determinants as equivalent to Mendelian allelomorphs, they must be considered as two pairs, each determinant being allelomorphic with its absence, *i.e.* ♀ with ⊙, and ♂ with ⊙, but ♀ and ♂ are spuriously allelomorphic with each other, so that when they coexist in the same zygote they cannot both enter the same gamete; just as in the case of *Abraxas*, the ♀ determinant and the *grossulariata* determinant exhibit spurious allelomorphism, and are never found in the same gamete. We shall then have females producing equal numbers of ♀ eggs and ♂ eggs, males producing equal numbers of ♂ and ⊙ spermatozoa, and it must further be assumed that ♀ eggs are fertilised by ♂ spermatozoa, giving females (♀ ♂), ♂ eggs by ⊙ spermatozoa, giving males (♂ ⊙).

This scheme is consistent with the facts in three categories of cases which have hitherto seemed irreconcilable, viz., *Abraxas* and those cases which resemble it; the insects with heterochromosomes, and the cases of sex-limited inheritance of which colour-blindness is the type. In *Abraxas* there is spurious allelomorphism between the ♀ determinant and the *grossulariata*

factor, so that ♀ eggs are without that character; ♂ eggs have it, but in the male, since the ♀ determinant is absent, there is no such spurious allelomorphism, and the *grossulariata* factor is indiscriminately distributed among the spermatozoa. In insects with heterochromosomes these may be regarded as bearing the ♂ and ♀ factors as suggested above. In colour-blindness, the factor for the disease can only be borne by a gamete which contains a sex-determinant, but the latter may be either ♂ or ♀; in the male, the factor is borne by the ♂-bearing spermatozoa, but not by those which have no sex-determinant (⊙); since the latter fertilise ♂-bearing ova and the former ♀-bearing, an affected male transmits the factor for the disease only to his daughters. But in the heterozygous female the colour-blind factor may be associated with either ♂ or ♀ eggs, and thus some of her children of each sex receive it.

The suggestion may here be made in passing that this hypothesis of sex-transmission may explain the cases not rarely met with, especially in the offspring of hybrids, in which all or nearly all the offspring are of one sex. If a ♀ egg were occasionally fertilised by a ⊙ spermatozoon, the resulting individual would be a female containing no male determinant, and either all its eggs would be ♀-bearing, or if any contained no sex-determinant they might be sterile or unfertilised. In crosses which give only male offspring (as in the case of the moths, *Tephrosia crepuscularia* and *T. bistortata* described by Tutt*), the ♀-bearing eggs may fail to attract the spermatozoa of the other species, and so only male offspring would result.

After this digression, we must now return to the case of the Bee, Wasp, and other parthenogenetic cases. In a previous paper† I have made the suggestion (which I find has also been made by Morgan) that the presence or absence of a spermatozoon in the egg might cause the maturation to take place differently. It is possible that when a spermatozoon is present the egg of the Bee undergoes normal "reduction" divisions, halving the chromosomes both quantitatively and qualitatively, and removing the ♂ determinant. But when the egg develops unfertilised the polar divisions may both be equational, leaving the haploid (reduced) number of bivalent chromosomes in the egg, and in this case expelling the ♀ determinant. This would be comparable with the supposed male eggs in the spring brood of *Neuroterus*, and in each case in the germ-cells of the male the reduced number would remain throughout, but in the body-cells the bivalent chromosomes would separate

* Tutt, 'Trans. Ent. Soc.,' 1898, Part I, p. 17. Quoted by Castle, "Heredity of Sex," 'Bull. Mus. Comp. Zoo. Harvard,' vol. 40, 1903, p. 206.

† "On the Maturation of the Egg in the Teuthredinidæ," 'Q.J.M.S.,' vol. 49, 1906, p. 586.

into their constituents, so giving the same number in male and female body-cells, as is observed. In the male Bee a single ♂ determinant would be present in the spermatocytes: this would pass into one spermatid, the other being left with none (⊙). The latter spermatids are the "male polar bodies" which degenerate. All the spermatozoa thus contain the ♂ factor, their presence in the egg causes the ♀ factor to remain in the polar divisions and the fertilised egg is thus ♀ ♂ and becomes a female.

This assumption that the presence of a spermatozoon in the egg causes a difference in the maturation process, and leads to the removal of the male instead of the female sex-determinant seems improbable at first sight, but there are considerations which reduce its improbability on further examination. If both eggs and spermatozoa are commonly of two kinds, the hypothesis of selective fertilisation cannot be avoided, and if there is some definite attraction between a ♂-bearing and ♀-bearing nucleus, the presence of a ♂-bearing spermatozoon (or male pronucleus) in the egg may attract the ♀ determinant to the inner end of the maturation spindle, although in an egg with no spermatozoon it would be expelled. Clearly some such attractive force exists, the nature of which is at present unknown, for otherwise in the saw-flies the male pronucleus might conjugate equally often with one of the polar nuclei, which to all appearance exactly resemble the egg-nucleus, instead of with the egg-nucleus itself. If it is assumed that, in cases where the egg matures before the entrance of the spermatozoon, the sex-determinant remaining in the egg attracts only one of the two kinds of spermatozoa, it does not seem a very improbable extension of the hypothesis that the presence of one kind of spermatozoon in the unmatured egg may cause the expulsion of the corresponding sex-determinant at the maturation division.

In Wasps and Ants* the spermatogenesis takes place much as in the Bee, except that two apparently similar spermatids are formed by the spermatocyte division, each of which becomes a spermatozoon. Their spermatogenesis thus closely resembles that of *Neuroterus*, and the most probable assumption seems to be that two kinds of spermatozoa are formed, ♂ and ⊙ respectively, but that only the ♂-bearing are functional. They would thus be exactly comparable with the Bee, except that the reduction of the useless spermatozoa is not carried so far. Other possibilities are of course not excluded, *e.g.*, that the ♂ determinant divides and passes into both spermatids, or that, as in *Neuroterus*, functional ♂ and ⊙ spermatozoa occur, and therefore that among the offspring of the female which founds the nest there may be two kinds of female individuals produced, one of which would contain no ♂ determinant. In ants and wasps the workers are not sharply separated from the queens

* Meves and Duesberg, 'Arch. Mikr. Anat.,' vol. 71, 1908, p. 572.

as they are in the Bee, and these purely female individuals might develop into workers which would not lay eggs. It is generally considered that when worker ants lay eggs, these always yield males (as in the Wasp and Bee), but Reichenbach* describes the case of a nest in which the workers produced females except at the season when males normally occur, and then males were produced. If some of the workers were of the ♀ ⊙ constitution, others ♀ ♂, this might thus be explained. The case clearly requires further investigation.

The hypothesis of sex-determination outlined above is doubtless highly speculative, but it has the advantage that it brings into line several sets of facts which have hitherto seemed irreconcilable, viz., the results obtained by breeding such cases as *Abraxas*, and also such cases as colour blindness, the cytological observations of Wilson and others on heterochromosomes, and the peculiar behaviour of the Bee and other Hymenoptera which have facultative parthenogenesis.

The fact that in *Neuroterus* the parthenogenetic generation is separated from the bisexual one, and that some parthenogenetic females give rise only to males, others only to females, supplies the clue, for it is clear that some difference must exist in the constitution of the spring-brood females to account for the difference in sex in the offspring of different individuals. That this factor is introduced by the male parent seems probable from the known fact that in so many cases spermatozoa of two kinds are produced.

In the Aphides a variety of conditions occurs in different cases, but here also the suggested explanation holds good. In all species fertilised eggs yield females, and these produce a varying number of parthogenetic generations. All these must be regarded as of constitution ♀ ♂. In some, the later parthenogenetic females give rise to both sexual females and males from the same individual;† in these it may be assumed that in the eggs which develop into sexual females the ♂ determinant is removed; from those which yield males the ♀ determinant. In other cases the parthenogenetic females give rise to "sexuparæ," some of which produce parthenogenetically only males, others sexual females. In these, the female-producing sexuparæ may have lost the ♂ determinant, while the male-producing sexuparæ contain both ♂ and ♀, but the ♀ determinant is removed with the polar body of the egg which yields a male. Morgan and von Baehr have shown in a species of *Phylloxera* and in *Aphis saliceti* that half the secondary spermatocytes degenerate, as do half the spermatids in the Bee, and in this case they have

* 'Biol. Centralblatt,' vol. 22, p. 461.

† N. M. Stevens, 'Carnegie Inst. Public., Washington,' No. 51, 1906; also von Baehr, *loc. cit.*

shown that those which degenerate lack the heterochromosome, *i.e.* those which we have represented by ⊙. The spermatozoa are thus all ♂-bearing, but since the sexual female lacks the ♂ element, all the eggs are ♀, and the resulting offspring (zygotes) are females with constitution ♀ ♂. Morgan assumes that the spermatozoon determines the sex, but this involves the belief that the male Aphid contains a ♀ determinant, and since we know that the parthenogenetic female contains the ♂ factor (since males are produced from it), this would involve the complex assumption of alternative sex-dominance. If my hypothesis is correct, it could be tested in Aphis by observing whether, in the single polar division by which a male egg is produced, one complete heterochromosome is extruded, thus leaving only one heterochromosome in the egg instead of the two which are characteristic of the parthenogenetic females.

In this discussion I have not attempted to deal with the case of the saw-flies (Tenthredinidæ), in which facultative parthenogenesis occurs. In former papers* I have discussed this question, but subsequent observations have led me to believe that the work requires extension and revision, and since this is still in progress, I prefer to leave any discussion until the results are clear.

A final word should be given to the recent hypothesis of Wilson† and Castle,‡ that the sex-determinants are not ♂- and ♀-bearing respectively, but that only one kind of determinant exists, and that the female contains one more such determinant than the male. Castle supposes that in some species the female contains two such determinants, the male one; in other species, the female one and the male none. The existence of two kinds of parthenogenetic females in *Neuroterus* cannot be explained on either assumption, but it is conceivable that both kinds of parthenogenetic female contain one sex-factor and the male none. The difference between the arrhenotokous and thelytokous female would then be that the eggs of the former contained a mechanism for expelling the sex-factor, while the thelytokous egg would retain it. The extranuclear body found in half the spermatids might be responsible for this difference. This, however, does not seem very probable. The hypothesis, also, cannot explain the almost invariable excess of affected males in cases of sex-limited inheritance, such as colour-blindness, which is accounted for if we assume that the factor for the disease is more often borne by ♂ than by ♀ ova. In general, the hypothesis does not seem to bring together such a wide range of phenomena as that suggested above.

* 'Q.J.M.S.,' vol. 49, 1906, p. 561 and vol. 51, 1907, p. 101.

† 'Science,' January, 1909, vol. 29, p. 53.

‡ 'Science,' March, 1909, vol. 29, p. 395. See also Morgan, 'Journ. Exp. Zoo., Sept., 1909, p. 332.

In conclusion, I wish to acknowledge the assistance received in the later part of my work from a grant from the "Endowment of Research Fund" of the Birmingham Natural History and Philosophical Society, and especially to record my indebtedness to Prof. Bateson, who has read the MS. of the paper and given valuable help in criticisms and suggestions.

EXPLANATION OF PLATES.

The figures, with the exception of Nos. 20 and 22, were drawn with Zeiss apochromatic objective, 3 mm. (apert. 1·40), and Zeiss ocular 12, giving a magnification of about 1600 diameters. When the magnification is less, it is mentioned in the description of the figure. The outlines of the larger figures were drawn with camera lucida, but the chromosome groups are drawn free-hand.

PLATE 1.

FIG. 1.—Chromosome group from anaphase of mitosis in developing wing of old larva, male.

FIG. 2.—Two similar groups from female pupa, summer generation.

FIG. 3.—Two similar groups (the two ends of one mitotic figure) from a pupa of the spring generation.

FIG. 4 *a*, *b*, *c*.—Three anaphases, two in face and one side-view, of spermatogonial mitoses, half-grown larva.

FIG. 5 *a*, *b*.—Two primary spermatocytes, after the growth period. Male pupa.

FIG. 6.—Prophase of first spermatocyte mitosis. Chromosomes appear as about 10 irregular bands.

FIG. 7 *a*.—Early stage of first (abortive) spermatocyte division. A centriole is seen at each end of the cell, and a small dot on the upper edge of the nucleus.

FIG. 7 *b*.—Later stage: the nucleus is re-forming, and the cytoplasmic bud with the centriole is being separated.

FIG. 7 *c*.—Rather earlier stage: pear-shaped nucleus and finger-like process with centriole at its tip.

FIG. 8 *a*, *b*, *c*.—Secondary spermatocytes, three stages of re-formation of the nucleus. In 8 *b* and *c* the cytoplasmic bud at one end, and the stained dot near the nucleus, are visible.

FIG. 9.—Early prophase of second mitosis. More than 10 chromatin bodies are visible, although part of the nucleus is not included in the section. At this stage the chromatin bodies are not always so definite as in this case.

FIG. 10 *a*, *b*, *c*.—Three prophases of second spermatocyte mitosis, showing 10 band-like chromosomes. In 10 *a* not all the chromosomes are represented; in 10 *c* the little knob on the lower side of the cell perhaps represents the "cytoplasmic bud."

FIGS. 11 and 12.—Equatorial plates of second mitosis; in 12 the stained dot is seen outside the circle of chromosomes.

FIG. 13 *a*, *b*.—Two metaphases in side view.

FIG. 14 *a*, *b*.—Anaphases in side view. In each the stained dot is seen near the lower end of the spindle.

FIG. 15 *a*, *b*.—Telophases: stained dot seen included in one daughter-cell.

FIG. 16.—Two telophases seen in pole-view.

FIG. 17.—Two early spermatids.

FIG. 18 *a*, *b*.—Two prophases from a probably abnormal follicle. In 18 *a* the double number of chromosomes is seen, in 18 *b* they appear to be pairing longitudinally. In 18 *b* not all the chromosomes are shown.

FIG. 19 *a*, *b*.—Anaphases from mitoses in ovary of half-grown female larva, summer generation. *a*, polar view; *b*, side view.

FIG. 20.—Part of egg tube from pupa of summer generation, showing alternation of developing egg on right, with undifferentiated primitive ova on left. (× about 650.)

PLATE 2.

FIG. 21 *a*, *b*, *c*.—Nucleus of developing egg of spring generation. *a* and *b* are optical sections of the nucleus at different levels, *c* part of the same nucleus in the next section of the series. In *b* the chromosomes shown in outline are ends of those seen in the same positions in *a*.

FIGS. 22 to 33 represent the maturation of the summer egg.

FIG. 22.—Outline of section of egg showing stalk and polar mitosis. (× about 650.)

FIG. 23.—Nucleus at edge preparing for maturation division.

FIG. 24 *a*, *b*.—Two successive sections of early stage of division.

FIG. 25 *a*, *b*.—Polar views of first maturation division. *a* represents the inner, *b* the outer chromosome group. Drawn from different levels in the same section. Fig. 27 represents nearly the same stage in side view.

FIG. 26 *a*, *b*, *c*.—Later stage of maturation. *a* and *b* are drawn from different levels in the same section, *c* from the next section of the series. *a* represents the innermost group, and some of the chromosomes in *b* are apparently continuous with those in *a*. *c* represents the outermost group.

FIG. 27.—Early stage of the first maturation division, side view.

FIG. 28 *a*, *b*, *c*, *d*.—*a* and *b* are the two groups of polar chromosomes drawn from the same section at different levels. *c* represents the spermatozoon becoming the male pronucleus in the next section; *d*, the insinking chromosomes forming the female pronucleus cut twice in the succeeding two sections.

FIG. 29.—Early stage of second maturation division, unusual condition.

FIG. 30 *a*, *b*.—Close of first and beginning of second maturation divisions; two successive sections of series. *a* shows outer group, *b* part of outer and elongated inner group.

FIG. 31.—End of second division of inner group. Chromosomes which form female pronucleus closely packed, below; inner polar chromosomes and outer group preparing to divide, above. Female pronucleus in next section to polar chromosomes. (× about 800.)

FIG. 32.—Close of second maturation division. Chromosomes of female pronucleus sinking in, outer group completing its division. *sp.* represents the spermatozoon becoming the male pronucleus, from the next section. (× 800.)

FIG. 33.—Female pronucleus (*pr. n.*) and three groups of polar chromosomes.

PLATE 3.

FIG. 34.—Meeting of male and female pronuclei.

FIG. 35.—Conjugation of pronuclei and first segmentation spindle. *a* and *b* mark the chromosome groups derived from the two pronuclei. In the section they lie at different levels, so that they are actually placed across the axis of the spindle.

FIG. 36 *a*, *b*.—Two polar views of telophases of segmentation mitoses, showing about 20 chromatin masses. Side view of same stage in fig. 38.

Fig. 1

Fig. 2

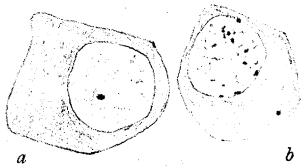
a b

Fig. 3

Fig. 4

a b c

Fig. 5



a b

Fig. 6

Fig. 7a

Fig. 7b

Fig. 7c

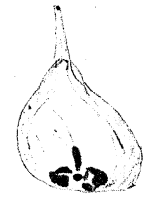
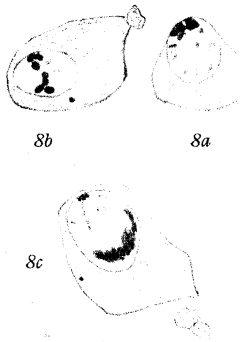


Fig. 8



8b 8a

8c

Fig. 9

Fig. 10

10a 10b 10c

Fig. 11

Fig. 12

Fig. 13

13a 13b

Fig. 14a

Fig. 14b

Fig. 15a

Fig. 15b

Fig. 16

Fig. 17

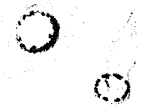
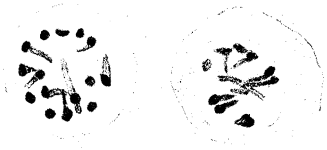


Fig. 18



18a 18b

Fig. 19

19a 19b

Fig. 20

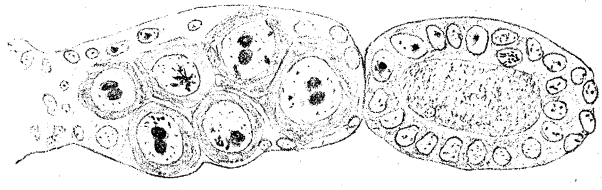


Fig. 22

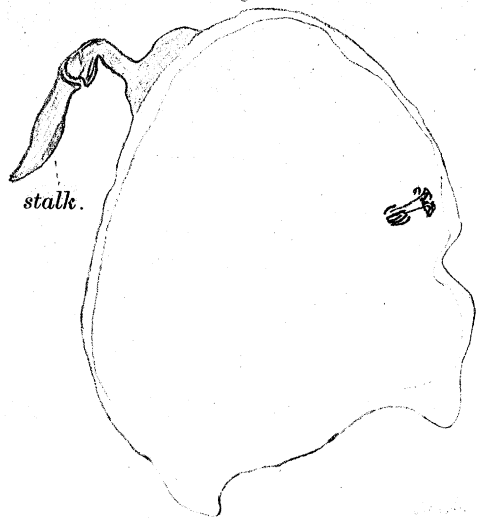


Fig. 21

Fig. 23

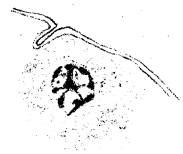


Fig. 25

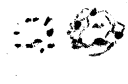


Fig. 24

Fig. 26

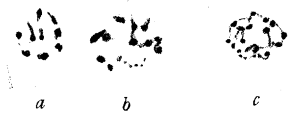


Fig. 27

Fig. 28

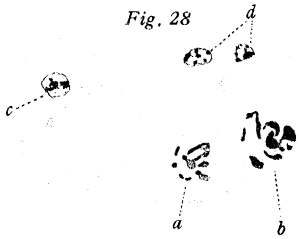


Fig. 29

Fig. 30

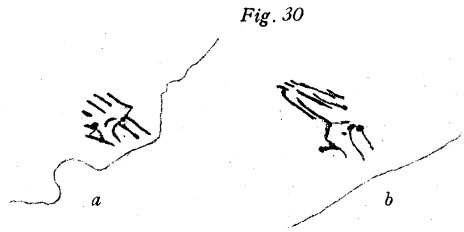


Fig. 31

Fig. 32

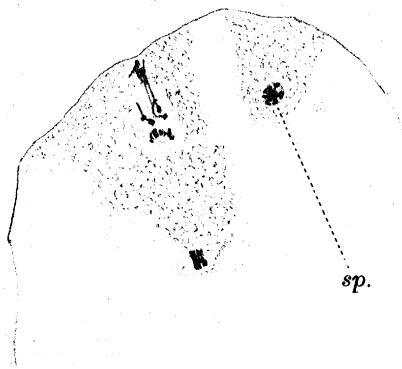
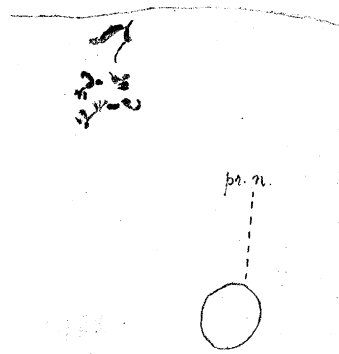


Fig. 33



London Stereoscopic Co. Imp.

Fig. 34

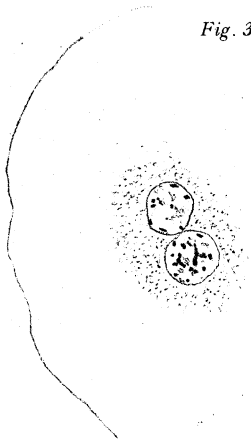


Fig. 35

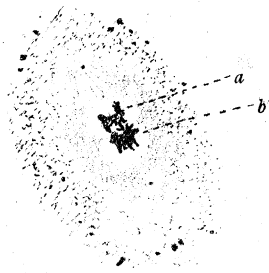


Fig. 36a

Fig. 36b

Fig. 37

Fig. 38

Fig. 39

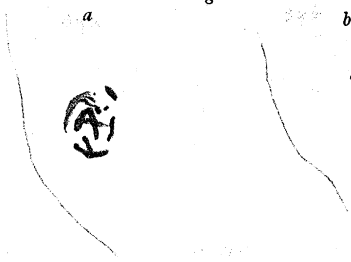


Fig. 40

Fig. 41

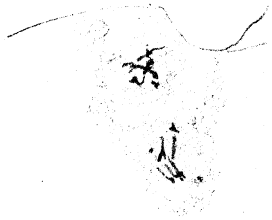


Fig. 42

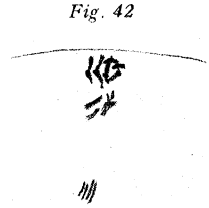


Fig. 43

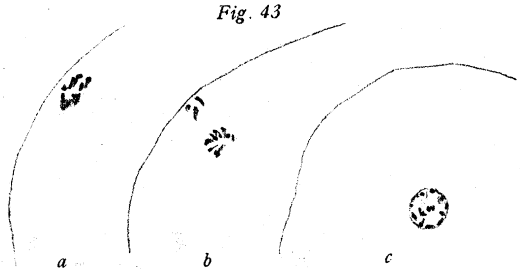


Fig. 44a

Fig. 44b

Fig. 45

Fig. 46

46a

46b

46c

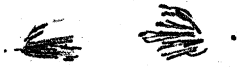


Fig. 47

Fig. 48a

Fig. 48b

Fig. 48c

FIGS. 37 and 38.—Two stages of segmentation mitoses.

FIGS. 39 to 48 represent eggs of the spring generation.

FIG. 39 *a*, *b*.—Two successive sections of chromosome group at edge of egg before maturation division.

FIG. 40.—Early stage of maturation division.

FIG. 41.—Late stage of maturation division.

FIG. 42.—Completion of maturation division. Chromosomes of egg-nucleus sinking in; polar chromosomes in two groups.

FIG. 43 *a*, *b*, *c*.—Polar chromosomes and egg-nucleus. *a* and *b*, successive sections of polar chromosomes; *c*, egg nucleus several sections removed. (× about 800.)

FIG. 44 *a*, *b*.—Two equatorial plates of segmentation mitoses, showing about 20 chromosomes.

FIG. 45.—Prophase of segmentation mitosis, showing numerous long coiled chromosomes.

FIG. 46 *a*, *b*, *c*.—Metaphase (*a*), early (*b*), and late anaphases (*c*) of normal segmentation mitoses with diploid number.

FIG. 47.—Equatorial plate in face of segmentation mitosis with haploid number.

FIG. 48 *a*, *b*, *c*.—Metaphase (*a*), anaphase (*b*) in side view, and anaphase in pole view (*c*) of segmentation mitoses with haploid number.

Preliminary Note upon the Cell Lamination of the Cerebral Cortex of Echidna, with an Enumeration of the Fibres in the Cranial Nerves.

By EDGAR SCHUSTER, D.Sc., Fellow of New College (Pathological Laboratory, Claybury Asylum, Essex, and Department of Comparative Anatomy, University Museum, Oxford).

(Communicated by Dr. F. W. Mott, F.R.S. Received September 30,—Read December 9, 1909.)

[PLATES 4 AND 5.]

Material.—The following notes are based on the study of the brain of an Echidna which died in the gardens of the Zoological Society in London.

Dr. F. W. Mott, F.R.S., kindly placed the brain in my hands with the suggestion that I should examine the cell lamination of the cortex and should estimate the numbers of fibres in the cranial nerves. For this and for his advice and help during the investigation I wish here to express my gratitude.

The right hemisphere was cut transversely into a series of sections 10 μ in thickness, from which sections were taken at intervals of about $\frac{1}{2}$ mm., stained with polychrome blue, and mounted. It may, perhaps, be mentioned

Fig. 1

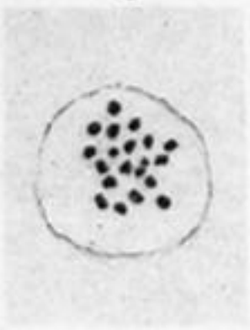
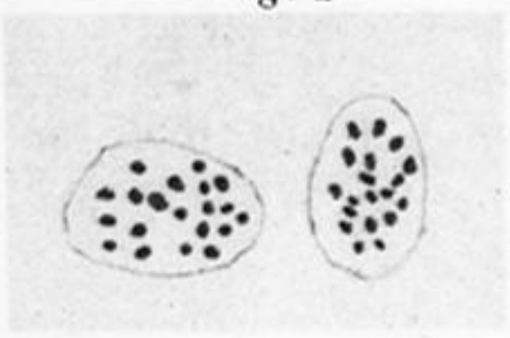


Fig. 2



a b

Fig. 3

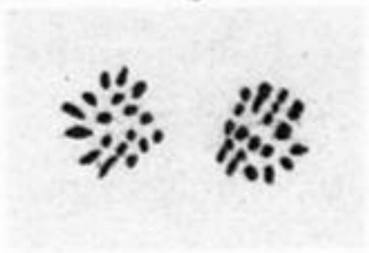
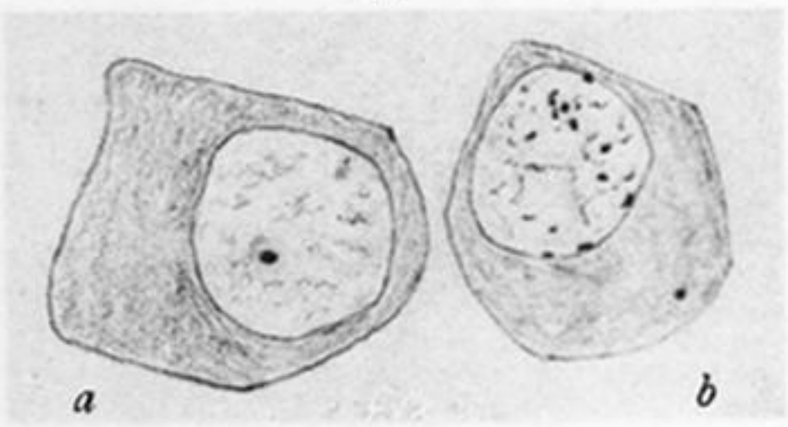


Fig. 4

a b c

Fig. 5



a b

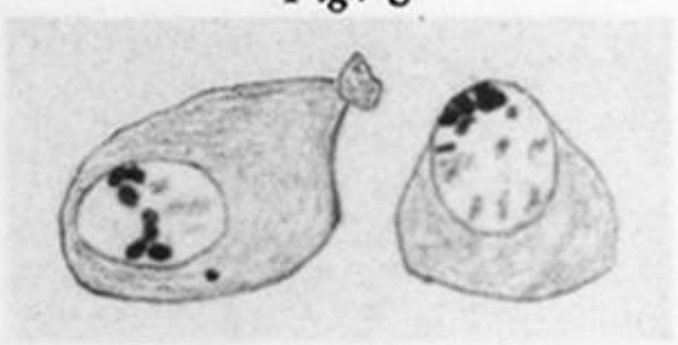
Fig. 6

Fig. 7a

Fig. 7b

Fig. 7c

Fig. 8

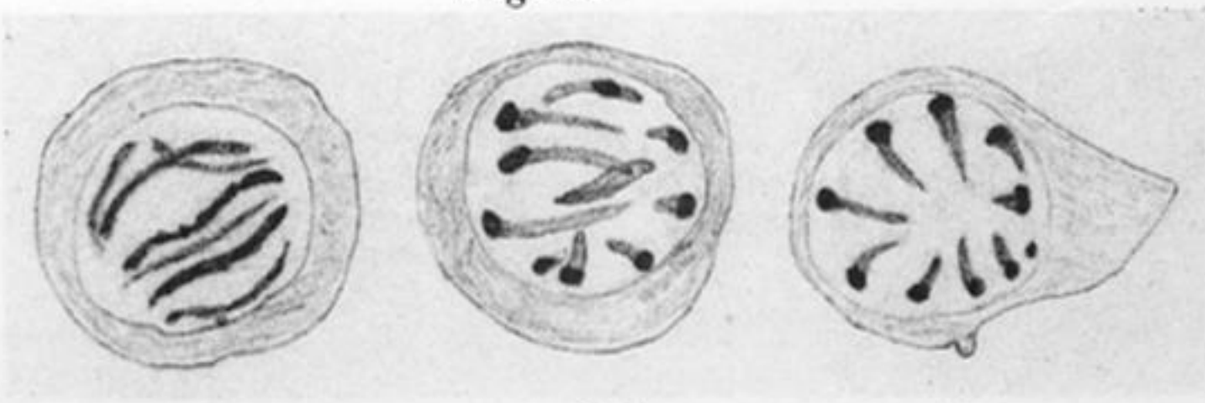


8b 8a

8c

Fig. 9

Fig. 10



10a 10b 10c

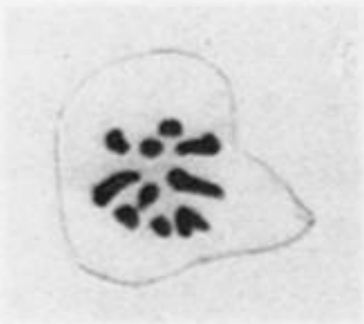
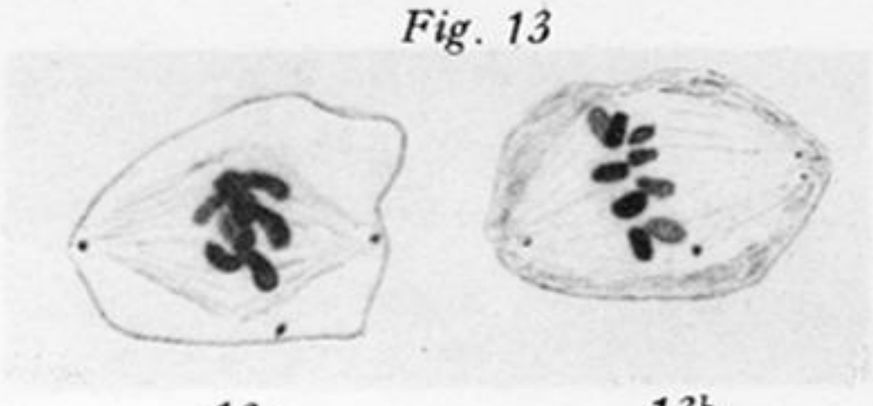


Fig. 11

Fig. 12

Fig. 13



13a 13b

Fig. 14a

Fig. 14b

Fig. 15a

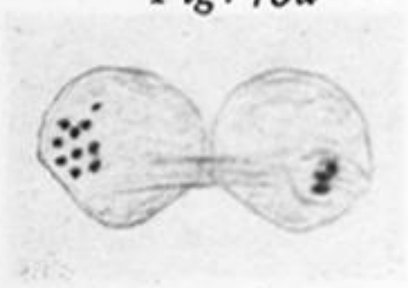


Fig. 15b

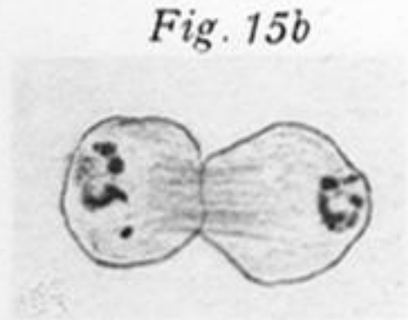


Fig. 16

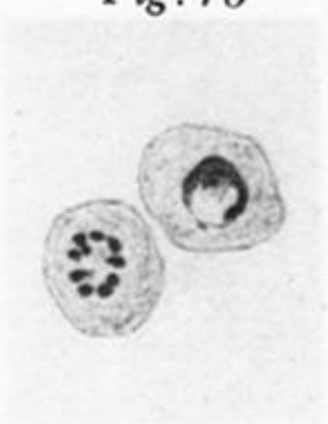
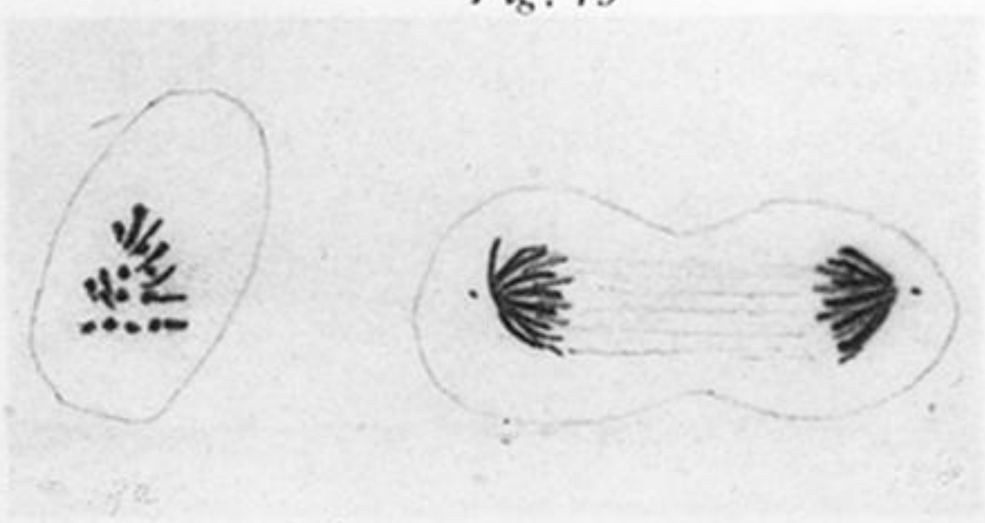


Fig. 17

Fig. 18

18a 18b

Fig. 19



19a 19b

Fig. 20

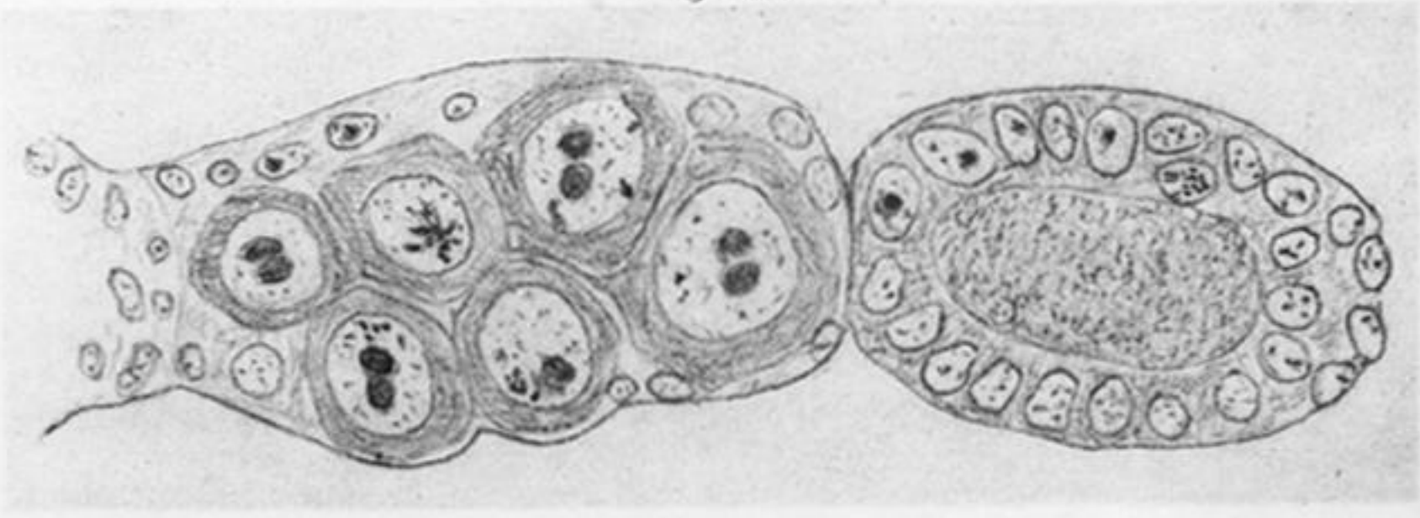


Fig. 21

Fig. 22

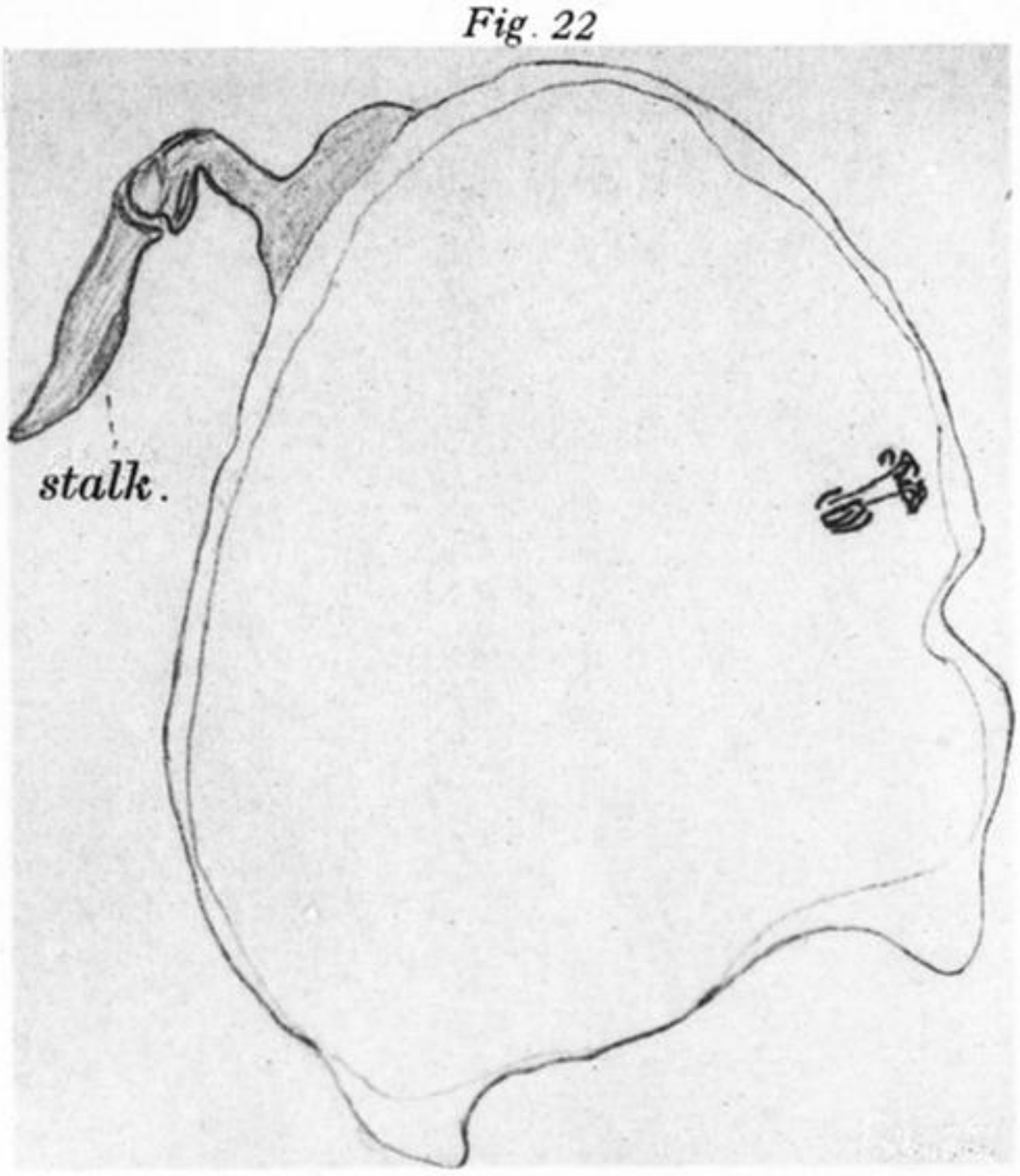


Fig. 23

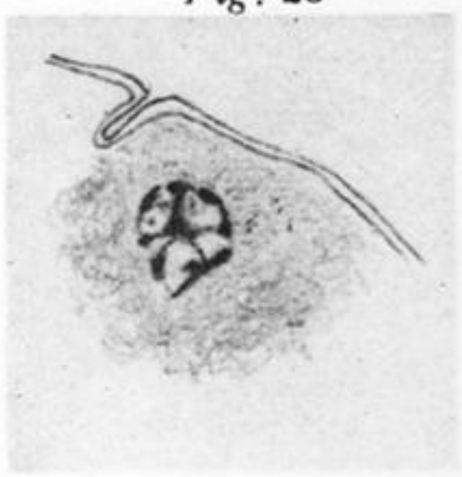
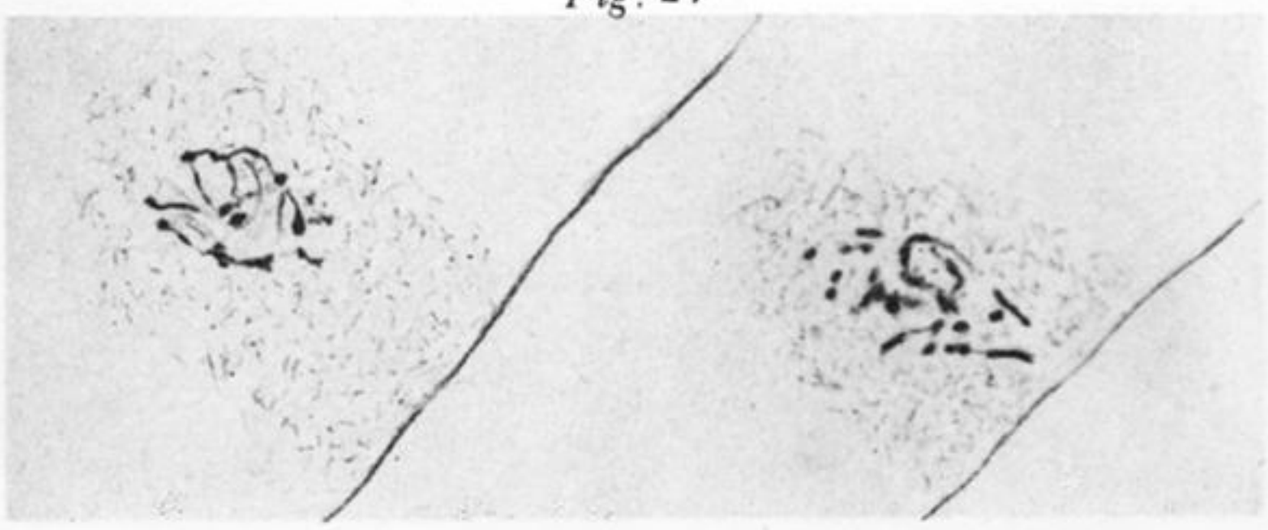


Fig. 25

a b

Fig. 24



24 a 24 b

Fig. 26

a b c

Fig. 27

Fig. 28

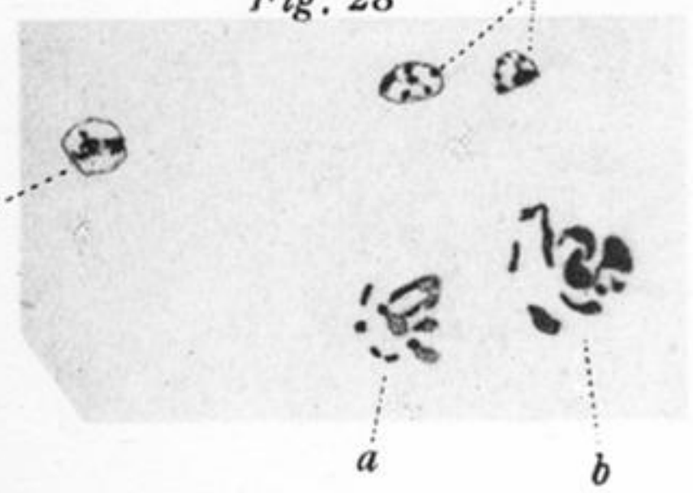


Fig. 29

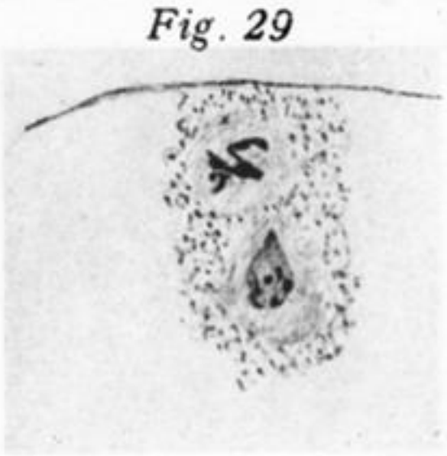


Fig. 30

Fig. 31

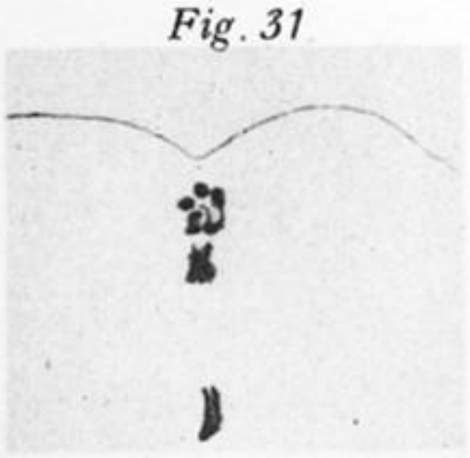


Fig. 32

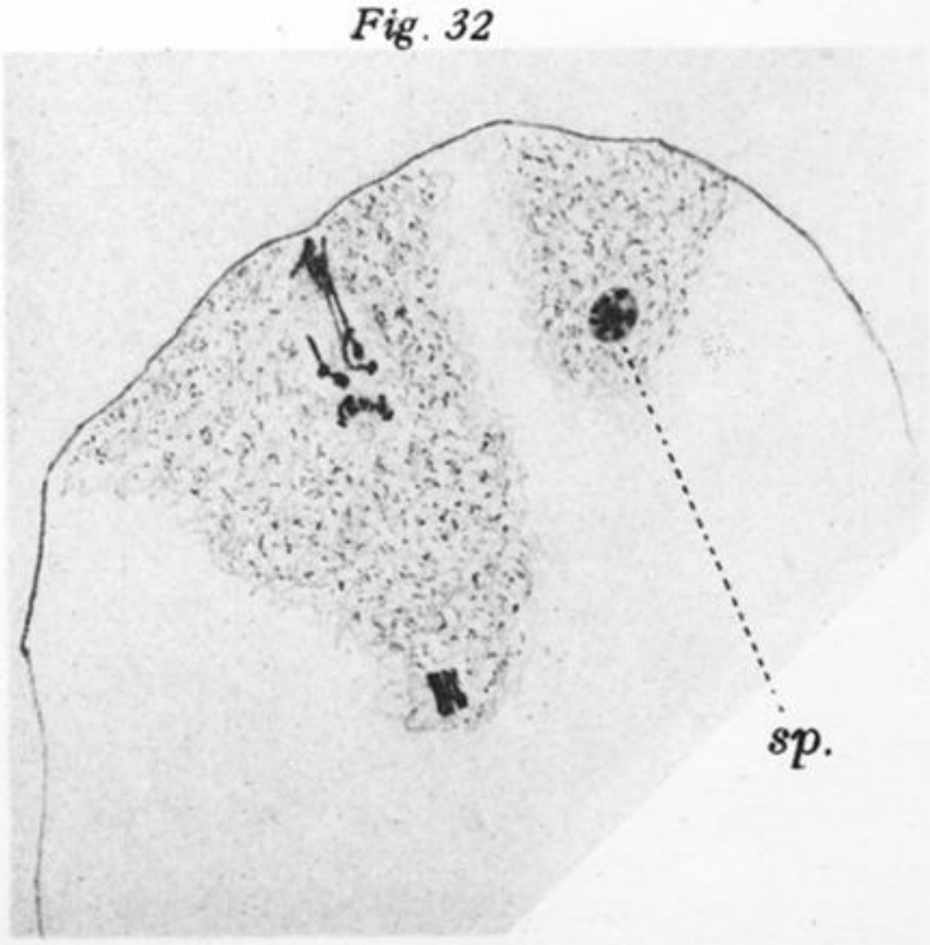


Fig. 33

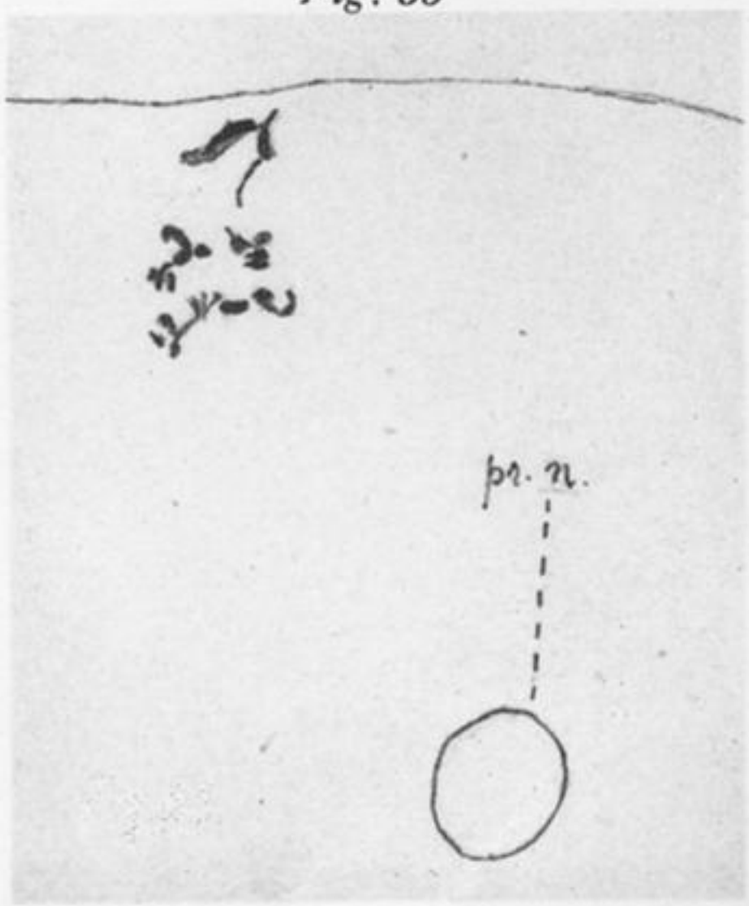


Fig. 34

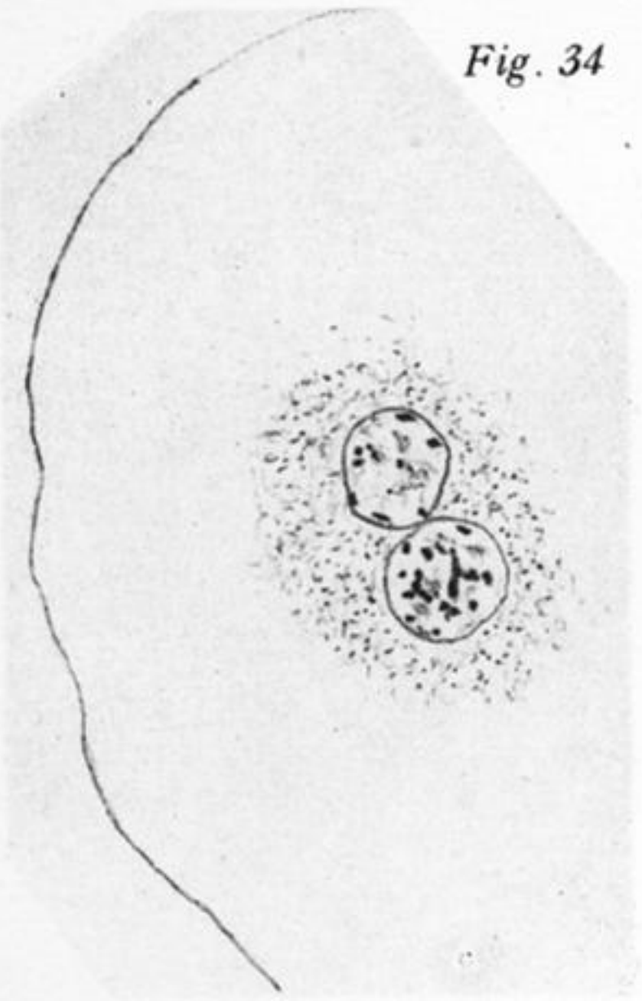


Fig. 35

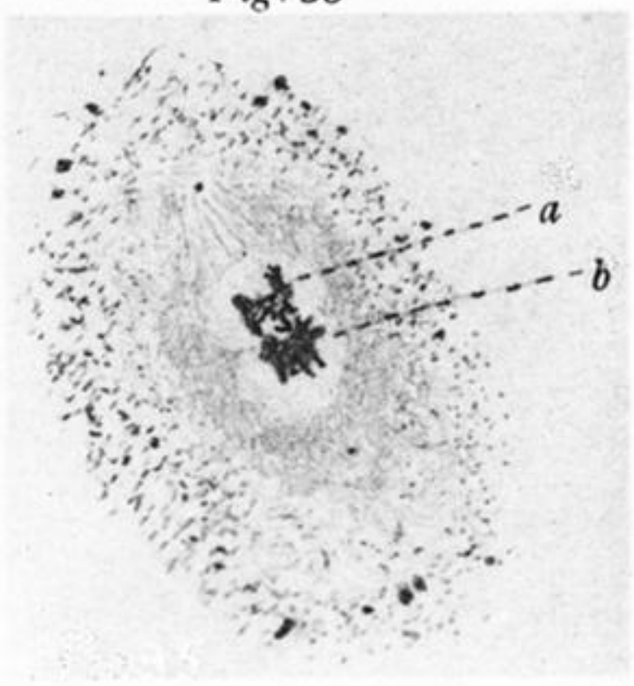


Fig. 36a

Fig. 36b

Fig. 37

Fig. 38

Fig. 39

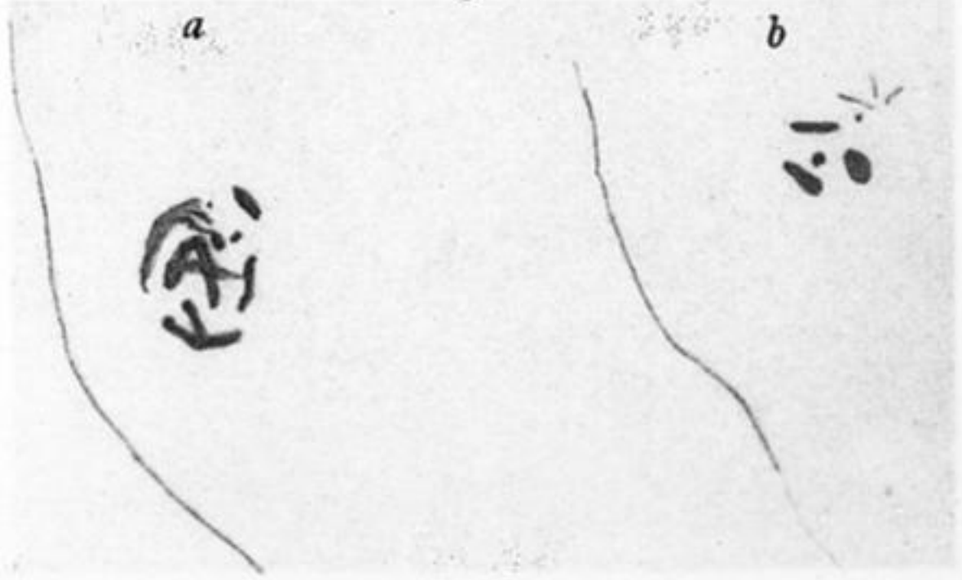


Fig. 40

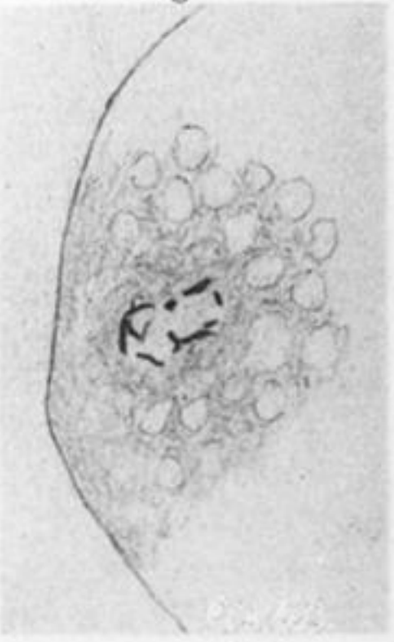


Fig. 41

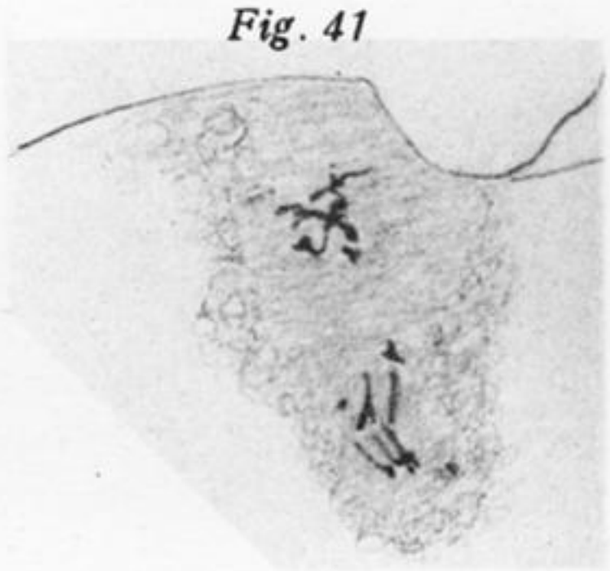


Fig. 42

Fig. 43

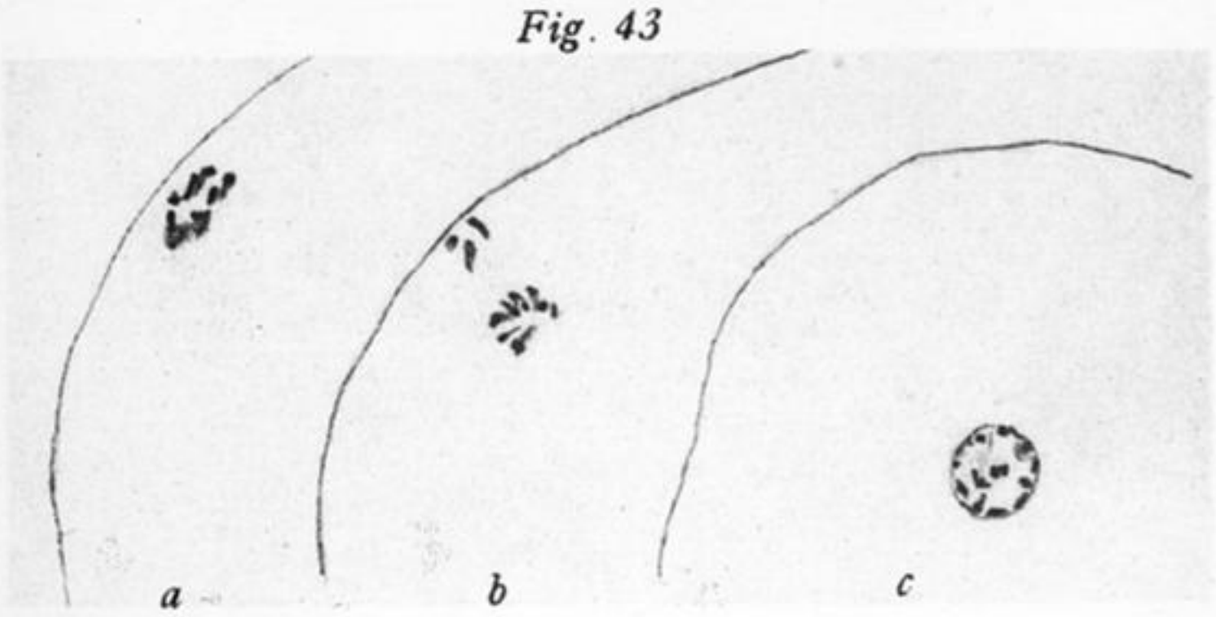


Fig. 44a

Fig. 44b

Fig. 45

Fig. 46

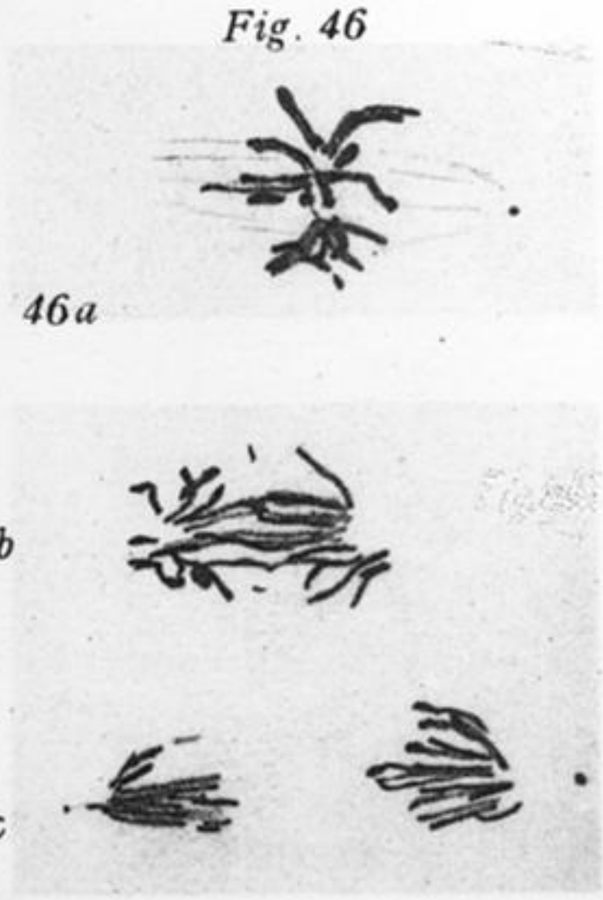


Fig. 47

Fig. 48a

Fig. 48b

Fig. 48c