

Maturation of the Ovum in Echinus.
A cytological study with special reference to
the Reduction of the Chromosomes by
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Introduction.

The subject of maturation of the sexual cells is a thorny terrain. It can be attacked only by the highest powers of the microscope, and the facts can only be reached by a process of patient mental reconstruction of the various phases. Historically the subject has been overlaid by some brilliant but premature hypotheses, which, however much they may have stimulated research, have also tended to foster prepossessions. The necessary stimulus for research has been supplied by the fairly well founded hypothesis, that the chromatin of the nucleus is the hereditary substance, or at least the bearer from one generation to another of hereditary qualities. But apart from the interest connected with problems of heredity, and the meaning of fertilisation, the study of the intricate details of the process of maturation, goes to the bottom of all our knowledge of cellular morphology. The study of the maturation phenomena in *Echinus* was, in the first place, taken up merely with the motive of seeing some of the actual phases in the most readily obtainable material. But it was soon discovered that although the outward phases had been frequently studied, most of the finer details of the process, as seen in *Echinus*, were undescribed, and therefore it was considered worth while to make a study of the whole process. In view of the hopelessly diverging results for different forms obtained by different observers, an interest in the behaviour of the chromatin during maturation has declined of recent years, and the question of the centrosome has occupied more attention. Results which came out

led me to important theoretical conclusions, and tended to clear up the confusion at present prevailing, by providing a possible general explanation of apparently diverging facts. As the research was proceeding, Strasburger's latest work, "Reduktionstheilung, Spindelbildung, Centrosomen, und Cilienbildung in Pflanzenreich," (1900) came into my hands. In that work conclusions identical with my own, and foreshadowed in several previous botanical memoirs, are brought, by new comparative investigations to a focus, and are made a means of harmonising the apparently contradictory results in the case of plants. This obviously increased the importance of my own results, and inspired me to follow out, in spite of the large amount of labour involved, the whole series of phenomena, in order to obtain as complete a demonstration of the facts as possible.

Historical Resume.

I propose in the first place to sketch briefly the essential steps in the history of the subject, which have brought the problem of the maturation of the sexual cells to its present day form. The first step towards a real knowledge of the process was gained when about 1877 it was ascertained that the extrusion of the polar bodies was brought about by a mitotic division of the nucleus of the egg or germinal vesicle. The discovery of the polar bodies is attributed to Carus, who, in the twenties of last century, saw them in the egg of a snail. This observation was extended to various other forms, vertebrate and invertebrate, by different observers, and in 1841 J.P. Van Beneden recognised that the constancy of the appearance merited

a very special attention. The name 'Richtungsbläschen' was first used in 1848 by Fritz Müller on account of the fact that they were invariably found on the side of the ovum, where the first cleavage begins. Rathke, in the same year, suggested that Müller's directive bodies were thickened processes of the "Liquor vitelli" expelled from the egg. In 1847 Derbes, the first investigator of the sea urchin's egg, described the immature egg as consisting of three concentric zones, an inner, outer and middle. Later the middle disappears, the outer is the yolk, and the inner the "vesicule germinative". Then the egg is ready to receive effectively the action of the sperm. In other words the germinal spot is directly converted into the Nucleus of the mature egg. Opinion was long divided as to whether the germinal vesicle disappeared as a nuclear element with the maturation of the egg, or was retained in whole or part to become the progenitor of succeeding generations of nuclei, after being supplemented by the male element. As a consequence of the view that the germinal vesicle was entirely removed as a morphological element from the egg, may be mentioned Haeckel's hypothesis that there exists an anuclear stage after the disappearance of the germinal vesicle, which he regards as an ontogenetic reversion to the structureless Moner.

No advance was made until the seventies when the discoveries in regard to cell division drew the attention of observers to the early changes in the ovum. Radiations had been seen in the egg by many observers beginning with Derbes, and spindle figures were observed by several investigators, without their

assigning significance to them, before Bütschli described in worms and molluscs the formation of the spindle. He, however, like Van Beneden and other early observers, who believed that the germinal vesicle was altogether discharged from the ovum, considered that the whole spindle was ejected, and that a new nucleus was provided in fertilisation. Hertwig, in his first paper on the subject, (1875) sought to show, as Derbes had done before him, that the germinal vesicle was transformed into the segmentation nucleus, while the rest of the vesicle disappeared. But later, in 1877, grounding on Bütschli's earlier work, he and Giard, working independently and simultaneously, at length showed that the process was one of mitotic division. Bütschli, re-examining his material, confirmed the results, as did Fol (1877) in a description of the phenomena as seen in *Asterias Glacialis*. Further confirmation of the general results was given by various observers, Whitman, Strasburger, Mark, Nussbaum, Selenka and Flemming. Van Greeff, in 1876, described parthenogenesis in the echinoderm egg. Strasburger too described analagous phenomena in plants. Hertwig's early results, as re-stated by him in his paper of 1890, were as follows:-

- (1) The formation of the polar bodies is independent of, and in certain animals occurs long before, fertilisation, and is to be regarded as a ripening process.
- (2) The polar spindle is formed not from the whole germinal vesicle, but only from a small part of it, chiefly from the nuclear substance in the Germinal spot, while the greater part is broken up.
- (3) The polar bodies develop, not from the extrusion of the spindle, but by a twice

repeated cell division, in which both protoplasm and spindle take a place, as in ordinary cell division, but owing to the unequal size of the products of division, the process is rather one of cell budding than of cell division. (4) The Polar bodies are small cells composed of protoplasm and nucleus. Their number is three in some animals, owing to the division of the first polar body. (5) The egg is never anuclear, for after the extrusion of the second polar body, half the spindle remains in the egg. Thus there is no moner stage since the different nuclear generations directly spring from one another. The phenomena are of universal occurrence. Fol's account of the phases in the *Asterias Glacialis*, (1877) differs in some details from Hertwig's especially as to the origin of the spindle from Germinal Spot, and in this point Strasburger in *Phallusia* agreed with Fol, and Flemming's results (1882) confirmed the fact, that the germinal spot had no direct relations either to the spindle or the female pronucleus. Meantime the labours of Valette St George and others on spermatogenesis had proved that the spermatozoon was a modified cell, and investigations on fertilisation culminated in the discovery in 1875 by Hertwig that the act of fertilisation consisted in a fusion of the nuclei of the sexual cells.

The second step in the elucidation of the facts of maturation was taken in 1884, when Van Beneden established the morphological equivalence of the male and female chromatin in fertilisation, and discovered the fact of reduction. The nuclei of both ovum and spermatozoon possess in *Ascaris* only two instead

of four chromatin loops. They are only half nuclei or pronuclei, male and female. In fertilisation the two loops from each of the pronuclei pass into, but remain separate in, the segmentation nucleus. Van Beneden did not admit the Karyokinetic character of the polar divisions, and he pointed out for the first time the marked difference in the mitotic phases, especially in regard to the chromatin seen in those divisions and held that the process was only pseudo-karyokinetic, and as just mentioned regarded the sexual nuclei after maturation as only half nuclei. The fact of reduction was confirmed for various Nematodes by Carnoy, for *Ascaris megaloccephala* and *lumbricoides* by Boveri in 1887, and for various other forms, *Echinus* among them, by the same observer in the succeeding years. Flemming also, (1887) described the process of reduction in the spermatogenesis of *Salamandra maculata*. In that paper he shewed that the two segments resulting from the longitudinal division of the primary chromatic rods, in the prophases of the spermatocyte division, remain attached end to end, forming a ring which was resolved in the metaphase of the first maturation division. This modified form of karyokinesis he called heterotypical.

The view adopted by Van Beneden as to the manner in which reduction was effected - involved the degeneration or casting out of half the chromosomes during the growth of the germ cells - and this idea was shared by all the earlier observers.

Platner, (1886) in *Lepidoptera* and *Pulmonnata*, also

described the same phenomena, and led up (1889) to the third important step in the development of the maturation problem, when he compared the spermatocyte with the egg, and the two divisions resulting in the formation of the spermatids, with polar divisions of the egg. Mark, in 1881, had suggested that the polar bodies were to be regarded as abortive ova, and Balfour in his text-book, says:- "It has already been stated in the introduction that the male and female generative productions are homodynamous, but the conditions of the development of the Sexual products in the two sexes show that a single spermatozoon is not equivalent to an ovum, but rather that the whole of the spermatozoa derived from a spermospore, are together equivalent to one ovum."

It was reserved for Oscar Hertwig in his paper of 1890 to finally establish the identity of the divisions in a spermatogenesis and oogenesis. His main conclusions in matter of fact are (1) The sperm mother cell corresponds to the egg mother cell or unripe ovum. (2) During the long resting stage of the vesicular nucleus of both cells, the nuclear substance is prepared in a similar fashion for two cell divisions directly following one another. There is no casting out or degeneration of nuclear material. (3) During the two rapidly following divisions, no increase in chromatin ~~does~~ takes place since the resting stage between them is omitted, and the chromatin already prepared in the mother cell neither increases in mass nor divides longitudinally. Thus the final products of the twice repeated division contain only half the amount of chromatin

possessed by an ordinary cell dividing by simple division.

(4) The phenomena attending the two divisions are identical in both sexes, and therefore the products have the same morphological value. Thus (a) the two sperm daughter cells correspond to the egg and the first polar body. (b) The four sperm grand-daughter cells correspond to the ripe or mature egg and the three polar bodies. (5) the polar bodies are rudimentary egg cells.

The sexual cells are thus matured by two mitotic divisions rapidly following one another, after a long continued period of growth during which the nucleus of the cell has become very large and vesicular. The number of chromatin bodies emerging from the vesicle is one half the number characteristic of the Somatic cells, but there is no loss of chromatin substance. As the result of the double division there are formed in the one case four spermatids which become the spermatozoa, and in the other case 2 or 3 abortive ova or polar bodies and the ovum. In fertilisation by the union of the sperm nucleus with the germ nucleus, the number of chromosomes is restored.

Since 1890 the progress in research has been both intensive and extensive. A great many forms, both vertebrate and invertebrate, have been made to yield the minuter details of the phenomena of both spermatogenesis and oogenesis. The single celled organisms have been examined, and have shown analogous phenomena, and since Guignard discovered reduction in plants a great mass of facts has accumulated for the vegetable kingdom, which reveal singular resemblances and analogies to those described in

the animal kingdom. There is good reason now to believe that reduction in one form or another is of universal occurrence at certain stages in the generative cells of both animals and plants, and continual attempts are being made to point out the meaning of facts so profoundly significant, but there prevails still so great variety and confusion in the results obtained by different observers that as yet no general explanation has been reached. It is in respect of the evolutions of the chromatin rods in the prophases of the first maturation division that results are so contradictory, and it is just this point which is the crucial one for those who would explain reduction on a morphological basis. I do not propose to dilate in detail on the various attempts which have been made to explain this singular series of phenomena. I shall at this point merely refer to certain hypotheses in regard to the maturation divisions of the sexual cells, which have a direct relation to the actual facts to be described in the original part of my thesis.

First, the Hypothesis of the germ plasm.

Very briefly stated, Weissman's theory as far as it relates to reduction comes to this. The chromatin of the nucleus being the hereditary substance and identified with the idioplasm of Nägeli (Strasburger, Hertwig, Kölliker, Weissman) is a complex, representing a mass of different qualities which it is the function of mitosis to divide equally between the two daughter cells (Roux). The chromatin is made up of a large number of ultimate vital units (De Vries) which are grouped into various larger units of a higher order (Weissman). The chromosome or

idant is a complex of ids, these again of determinants, these again of ultimate units or biophors, each higher unit being vital and capable of self propagation. The union of the chromatin in fertilisation would lead to increasing complexity of the hereditary substance, but this is avoided by a periodic reduction. The maturation divisions effect this reduction, and Weissman prophesied in 1887 that it would be found that mitotic division was of two kinds; (1) the equal or equational-division in which the qualities were equally distributed and (2) a reducing or unequal division by means of which, the daughter cells would receive only half the number of qualities possessed by the mother cells; whereas in the one there would be an equal longitudinal cleavage of the chromatin thread, in the second the division would be transverse. The apparent confirmation of this speculation by Hertwig's 1890 paper, and later the complete realisation of it by Von Rath's results (1892) in the mole-cricket, and those of Ruchert and Haecker in the copepods, have given the theory a certain basis in fact, and the question of its validity in a modified form is still an open question. There are, however, a number of observers who, in the face of the apparently hopeless contradiction of results begin to doubt whether the question of longitudinal and transverse splitting of chromatin threads is of any significance at all. This is however rather to evade the issue, and it will be the object of my thesis to show that the results in *Echinus* may throw light on a certain series of results. Second the Hypothesis of the "Individuality of the Chromosomes".

This theory—first advocated by Rabl (1885) - was applied

by Boveri (1887-1890) to the question of reduction. Boveri held that the chromosomes possess an individuality from one nuclear generation to another, but there is no qualitative difference between them or any part of them. He formulated the law that for every living species the number of chromosomes is constant, and that this number always reappears in the nuclear division of homologous cells. The examination of a number of forms showed that not only in *Ascaris* but in all other forms examined the number of chromosomes was reduced to one half in the maturation stages. At what point did the reduction occur? In *Ascaris* he held that the original chromatin thread was twice longitudinally divided in the germinal vesicle, preparatory to two cell divisions, and that therefore the reduction occurred in the germinal vesicle, by some process unknown.

Third, the Hypothesis of the Plurivalency of Chromosomes.

Certain exceptions seem to run counter to Boveri's law; for instance, in *Ascaris megalocephala*, there are two types, namely, *Ascaris megalocephala univalens*, with two chromosomes in the germ nucleus, and *Ascaris megalocephala bivalens*, with only one. Brauer, in a Phyllopod *Artemia*, also found the number of chromosomes in the somatic nuclei of some individuals 168, while in others it was only 84. Haecker, in his "Zell Praxis" 1899 says that all these difficulties fall away when one accepts the fact that the chromatin substance of the nucleus has the tendency before each division to fall by segmentation into a number of segments characteristic and constant for each species. This segmentation is universally complete in the somatic tissues in

their adult condition, and in them therefore the normal number is always present. In the nuclei of the generative cells on the other hand, sometimes in the cleavage cells and even in older embryonic and larval elements, in general chiefly in large nuclei rich in chromatin, the last or even several segmentation stages may be left out, and thus may be formed bivalent or plurivalent chromosomes. The reduction of the chromatin elements in the germinal vesicle Ruchert and Haecker explain in this way. In accordance with Weissman's conception of the chromatin thread being composed of different and not identical "Idants", the thread can be represented by the formula $a - b - c - d$. As the result of longitudinal division either before, or after the thread is broken into segments, each idant is divided, and the double thread can now be represented $\frac{a - b - c - d}{a - b - c - d}$ or each chromosome $\frac{a}{a} \quad \frac{b}{b}$ etc. If now the last segmentation of the thread be omitted the formula changes to $\frac{ab}{ab} \quad \frac{cd}{cd}$ etc.

The reduction is only apparent, and the resulting chromatin group is formed by only one longitudinal division, not two, as Boveri finds it. Two chromosomes remain united end to end. There is no second longitudinal division at any time, and in the second nuclear division the two united chromosomes part company and there is a qualitative or a true reduction in Weissman's sense.

To sum up - It is quite certain that the chromosomes appear in reduced number during the maturation stages, and that this does not involve initial loss of substance. In explanation of the phenomena there are four theories:-

1. Reduction in bulk - Boveri

Reduction is effected in the germinal vesicle - cause unknown.

2. Reduction in mass - Hertwig

Reduction is effected between the two divisions - from omission of the resting stage the chromatin does not suffer the normal increase of growth.

3. Reduction in Quality - Weissmann

Reduction is effected in the 2nd division by a transverse in place of a longitudinal cleavage of the chromosomes, resulting in dissimilar distribution of hereditary qualities.

4. Reduction is apparent in the first, real in the second division - Haecker.

Reduction is effected in the 2nd mitosis by the separation of dissimilar chromosomes - which have not submitted to longitudinal cleavage.

I shall now proceed to describe the process of maturation as I have observed it in Echinus, reserving a fuller consideration of the above theories, until I have established my own results, and have referred to some more recent views as to the actual details of the process.

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Maturation in Echinus Esculentus (Sphaera Linn).

Previous observations on Maturation in Echinoderms.

The Echinoderm ovum has been the classical material for all observations on the living egg. The earliest observations, as I have noticed above, on the maturation of the Sea Urchin Egg were made by Derbes in 1847. Agassiz in 1864 described the polar bodies in both *Toxopneustes* and *Asteracanthion*. Between 1872 and 1882 Van Beneden examined the phenomena in *Asterias*, Hertwig in *Toxopneustus lividus* and *Asteracanthion*, Giard in *Psammechinus*, Fol in *Asterias Glacialis*, Greeff in *Asterias Rubens*, and Flemming in *Sphaerechinus brevispinosus*, *Echinus Miliaris*, and *Toxopneustes*. Since then the favourite material for the examination of the phenomena in the living egg has been *Asterias*. Notwithstanding this little is known as to the finer details of maturation. The early observations were made on the entire egg - either in the living state or fixed and cleared. The polar bodies in *Echinus* are normally thrown off within the ovary, and when the naked eggs are shed into the sea water, they remain entangled in the connective tissue of that organ. Sometimes it may happen that a partially immature ovary may be manipulated and some ova caught in the maturation stages. In the star fish on the other hand the eggs commence to show the phases when placed in sea water, and they can be watched. Again by shaking immature Sea Urchin Eggs the stages can be induced artificially. Boveri (1890) has figured a few stages after the formation of the first Polar spindle in *Echinus microtuberculatus*, but either the Chromosomes which are very minute in *Echinus*

sphaera are still more minute in *Echinus microtuberculatus* and cannot be further analysed, or he has not seen the figures which I have made out by my methods. Further the number of chromosomes is different. Matthews (1895) examined maturation ⁱⁿ of ~~a~~ *Asterias forbesii*. He was able to obtain only one ovary showing the stages up to the formation of the first polar spindle, but supplemented his observations by stages obtained by shaking the eggs. He describes the behaviour of the centrosomes, but gives no detail as to the Chromatin. Wilson in his atlas of "Fertilisation and Karyokinesis" shows a single photograph of a second polar spindle in *Toxopneustes*, and Boveri has drawn a single figure of the second polar spindle in his most recent work published in 1901. Haecker (1893) also gives an diagramatic drawing of the 1st polar spindle, but gives no description of maturation. In none of these figures is the finer constitution of the Chromatin elements represented. Cuenot and other observers have written on Oogenesis in Echinoderms, but their observations were confined strictly to the ovaries and the formation of its epithelium, and to certain points in the characters of the Nucleolus. Various observers have treated specially of Echinoderm Spermatogenesis (Jensen, Pictet and others) and many have studied the Morphology of the Spermatozoon, but of these Field (1895) brings the latest account. Owing to the excessive minuteness of the Chromosomes he seems to have confined himself to counting them in the different phases. Haecker 1893 published observations carried out on the living egg on the germinal vesicle and nuceolus of Echinoderms, but does not give any detail regarding maturation.

Personal observations.

Methods.

My material was obtained from animals freshly out of the water. Small pieces of close on 70 ovaries about 1 to $1\frac{1}{2}$ cm. in size were fixed, embedded in paraffin, and a few dozen sections cut from each. These were all carefully examined and when maturation was found to be proceeding some hundreds of sections were cut and gone over and the details built up from these. The fixative fluids used were Flemming's strong solution and Hermann's platinum chloride and osmic acid mixture; almost identical results were obtained by both and the small pieces of ovary-about a cubic centimetre or a little more-were well fixed throughout. At a later stage of the research by way of control, pieces of ovary were fixed in Boveri's picric and acetic acid mixture, and sublimate - acetic acid, as well as Lindsay Johnstone's fluid. The micro-acetic material was unsatisfactory, but the sublimate gave good results in some respects. The chromatin was however much better differentiated by the Osmic acid mixtures especially by Hermann's fluid, while in the sublimate material the centrosome gave quite a different picture as will be seen in the sequel.

Staining.

Osmic acid preparations being proverbially refractory to most staining reagents I have confined myself almost entirely to Heidenhain's iron haematoxylin method, but have used by way of control other stains. To facilitate staining I have always allowed my preparations to stand for sometime in old turpentine to

remove the Osmic acid. The best results were obtained by iron haematoxylin alone, the picture presenting the vivid black chromosomes on a blue-grey field. Heidenhain's preliminary stain with Bordeaux-red rather confuses the picture of the chromatin, and the only other contrast stain used was a very weak colouration by alcoholic solution of fuchsin S.

It is necessary here to refer to the recent criticism by Boveri (1901) of the iron haematoxylin stain. He shows, as every one knows who has used the method, that different degrees of washing out yield different results, and refers to the fact that structures may appear which owe their existence to a purely mechanical cause and not to any difference in chemical composition. Thus a part which is not readily accessible on account of its position to the differentiating fluid retains the stain while the parts in the neighbourhood are decolourized (Fig.2 Pl.I); further the fluid having a concentric effect in washing out, the superficial parts are decolourized while the central parts retain the black stain. Thus he explains the different accounts given of the structure of the centrosome, and points out that by strong extraction of the colour the chromosomes even may be apparently diminished in size owing to their peripheral parts being decolourised. This is weighty criticism in view of a number of the appearances, I shall have to describe, for he combats the generally accepted view, that the true appearances are obtained by strong washing out, and believes that in regard to the centrosomes the opposite is true. As to the chromosomes I may forestall criticism of my results by stating first,- that

I have obtained similar appearances both with the Osmic acid and the sublimate mixtures and by other stains besides the iron haematoxylin though the greatest vividness of differentiation has been ^{obtained} ~~occurred~~ by a combination of Hermann's or Flemming's fluid with iron haematoxylin, and second,- that the proof that I am dealing with realities and not illusions is to be found in the fact that the appearances described for the chromosomes represent a complete and unbroken series of the steps or stages of a process, that can be explained only by reference to the completed story.

I must state that the photographs which illustrate this Thesis so far as the mechanical processes are concerned are the work of John H. Teacher, M.B., Assistant to the Professor of Physiology in Glasgow University, but I am personally responsible for the placing and focussing of each one. The drawings are from my own hand. A very careful outline drawing was first made by aid of the Abbe drawing apparatus of Zeiss and the finer detail was then filled in freehand. The combination used was in every case Zeiss 2 mm. 1.40 numerical aperture, apochromatic objective, with either 8 or 12 compensating eye piece. The illuminating apparatus employed was a Zeiss 1 mm. numerical aperture, centreing achromatic condenser. The drawings necessarily implement the photographs, as these can only represent one plane in high magnifications. The drawing represents the whole thickness of the sections. The sections were cut in Paraffin and were of varying thickness. The object in most cases being to obtain the masses of chromatin entire, comparatively thick sections were

taken; 6 to 7 microns. Thinner sections down to 3 microns were employed to determine certain points regarding the achromatic structures.

In dealing with the subject I shall first describe the changes in the ovum leading up to the disappearance of the germinal vesicle, and after that treat in separate sections of the behaviour of the achromatic and of the chromatic structures.

My earliest preparations are from the growth period. Out of a large number of young Oocytes of the first order - I have only seen two or three in Mitotic division, and these only in the Spireme stage, so that I cannot speak as to the number of chromosomes in these divisions. The young ovum shows a delicate reticular protoplasmic structure (Fig. 1 Pl. I) The nucleus is already large and vesicular with a distinct nuclear membrane, and a deeply staining eccentric nucleolus. This being intensely black, contrasts strongly with the Granular and irregular Nuclear network, which refuses to take on the chromatin stain, and remains pink in preparations stained either with "fuchsin S" or Bordeaux red". There is frequently a second smaller deeply staining circular body in the nucleus, but it has no regularity in position and is not invariably present. I cannot in any of my preparations see the double nature of the threads described by Haecker. Close to the nucleus very frequently on the side of that body towards which the nucleolus lies, there is sometimes at this stage a body which presents much the appearance of the centrosome of a resting cell. It consists of either a single granule or pair of granules, sometimes a group of smaller granules,

enclosed in a circular area. While this may represent a centrosome it is impossible to distinguish it from similar bodies with central granules that may be found in other parts of the cell, which are undoubtedly cell inclusions, and therefore no structure can with certainty be identified as a centrosome.

Structure of the Protoplasm.

Wilson (1899) has shown that in the young ovum the protoplasm is granular, and that as the ovum grows in size an Alveolar structure is assumed. In the youngest ova of my fixed material, the protoplasm presents a granular appearance which is certainly not alveolar and can hardly be termed reticular. (Fig.1 Pl.I) In the fully grown egg the appearances vary according to the stain. In Fig.4 Pl.III the cytoplasm is represented as showing a reticulum which is composed of separate minute granules; the meshes of this reticulum bound alveolar spaces. These alveoli are on the whole rounded, and in this particular specimen, from which the iron haematoxylin was very thoroughly washed out and replaced by a slight counter stain by fuchsin, they were faintly red with a slightly darker periphery. In Fig.3 Pl.II on the other hand the appearances are different. The iron haematoxylin has not been so completely washed out, and the alveoli have retained the dark stain, showing up as rounded dark points separated by an unstained reticulum. Sometimes the centre of the alveolus is occupied by a black dot, as if the centre had not been decolourised. Thus my preparations fully bear out Wilson's latest conclusion (1899) regarding the structure of the Sea Urchin egg namely, that the condition of the cytoplasm conforms to

Bütschli's description. It has the same physical characters as an emulsion, that is, there is a fluid framework in which the microsomes are suspended, and the alveoli are filled with a fluid of different physical characters. When the alveoli are wholly destained all that is seen is the microsomic network, whereas when they are stained the alveoli stand out as the yolk granules embedded in the cytoplasm. Wilson shows however that the cytoplasm at certain periods may have a fibrillar structure, but to this point I shall return later.

The changes which the nucleus undergoes during the growth of the Oocyte, and storage of the deutoplasm, until it becomes the fully developed germinal vesicle are very complicated and uncertain. Many irregular figures suggest that the germinal vesicle may undergo changes of shape. They may well be artifacts. I shall only refer to certain facts regarding the chemical reaction of the nucleus which seem to be fully vouched for in my preparations. It has been shown by a number of observers that the staining reactions of the nucleus vary at different times. At one time the chromatin network will take the specific stains deeply, while at other times it remains unstained.

(Rückert 1892). My experience tends to support these statements though one must admit that very different effects are produced by different degrees of colouration with iron haematoxylin. The effect depends on the degree of extraction of the colour, but it is quite certain that at certain stages of the nucleus the network very readily parts with the black stain, and is left as an irregular granular reticulum of a blue-grey colour, or of a red

tint in preparations stained for contrast with rubin. The nucleolus on the other hand is exceedingly tenacious of the stain and appears as an intensely black spot. (Fig.3 Pl.II) Again at a stage I consider to be of later date the network shows a basis of delicate linin threads, with deeply stained chromatin particles arranged on the thread giving it a very irregular or feathery structure, while the nucleolus generally is less deeply stained and vacuolated (Fig.4 Pl.III). Finally when the nucleus is fully grown and maturation imminent, we find the contrast is exactly the opposite of that described for the young nucleus. The network is intensely black, consisting of particles of chromatin arranged in a very intricate and irregular fashion, while the nucleolus parts with the stain very readily and is left as an almost colourless apparently empty vesicle (Fig.7 Pl.V). Soon after the resolution of the nuclear membrane it disappears from view. Very similar changes are described in many other forms, for instance in the Turbellarians according to Francotte (1897) in *Polychoerus* according to Gardiner (1898); and according to Gathy (1900) in *Tubifex* (an Annelid) the nucleolus loses its capacity for staining with iron haematoxylin at the end of the growth period.

It is difficult to resist the conclusion that the chromatin substance is at first confined to the nucleolus, and later leaves it to form the chromatic basis of the nuclear network as a whole, and therefore also of the future chromosomes. The fate of the nucleolus in Echinoderm eggs has been variously interpreted. Derbes in 1847 thought it was directly converted

into the pronucleus of the mature egg and Hertwig (1877) took the same view. Fol (1877) and Flemming (1882) however proved that the chromatin itself became the future nucleus after it was provided with a new nuclear membrane. Recently Carnoy and Le Brun (1899) have maintained the view that in the amphibian egg where there is no chief nucleolus but a large number of smaller ones, certain of these become converted into the future chromosomes, thus reverting to the older view of Schultze (1887). It seems certainly true, as said above, that the chemical substance which is lodged in the nucleolus in the early ovum becomes later distributed into the germinal vesicle and so indirectly goes to form the chromosomes. This would be in keeping with the theory, that the substance of the nucleolus passes over to the chromosomes in division, and that after the division is over, it is again collected into the nucleolus of the daughter nucleus. Strasburger regards the body as a storehouse of reserve substances, which pass into the cell during division to form the "Kinoplasm" which goes to form the spindle, the Hautschicht, membranes, and cilia. We shall see later that the phenomena observed in the Sea Urchin egg may combine these two views. But in contradiction to both is Haecker's view. His observations on the living egg of the Sea Urchin reveal to him the nucleolus as a pulsating organ, in which periodically, through the whole growth period, small vacuoles appear; these run into a single central vacuole which increases and then diminishes in size. When the largest central vacuole appears the nucleolus removes itself to the periphery of the nucleus, and meantime the vacuole

comes into relation with the outer layers of the nucleolus, as if to bring its contents into relation with the nuclear sap, and further an indrawing of the wall of the germinal vesicle itself, suggested that there was a communication between the cytoplasm and nucleolus. From these and other observations Haecker regards the nucleolus as a secretory organ, collecting the by-products of Nuclear activity - not as a storehouse, or "Nuclein Laboratorium" (Fick 1899). So far as my observations go they tend to support the idea of the Nucleolus being a storehouse or laboratory of Nuclein.

Centrosome.

There has been a great deal of discussion as to this enigmatical structure in the Sea Urchin egg. Varying accounts have emanated from Boveri, Wilson, Fol, Bütschli, Reinke, Hill, Kostanecki, and Erlanger. Boveri says "Das Seeigel-ei ist von allen objecten die von mir bekannt sind, dasjenige, welches einer sicheren Darstellung der Centrosomen die grosstem Schwierigkeiten bereitet". This quotation is taken from his recent work "On the nature of centrosomes". He reconciles more or less the different accounts, and suggests a nomenclature which I shall adopt as being the latest and most authoritative.

The centrosome is composed of a special and peculiar substance the Centroplasma which according to the perfection of fixation, and the manner of staining presents different appearances. This accounts for the different forms under which the body has appeared. It stains best with iron haematoxylin and destains concentrically. When destaining has been carried far,

it shows as a discoidal area surrounded by a clear halo, and has a very fine alveolar structure. This is the form in which I have observed it in all my Osmic acid preparations, except that I do not see the halo, and when counterstained with rubin it has a red colour, which indefinitely fades away into the blueish grey astral rays and spindle fibres. This rounded body, as division proceeds, becomes enlarged, then lens-shaped, and ultimately flattens into a plate which lies along the side of the nucleus. In Polar view this is dumb-bell shaped, the enlarged ends are the daughter centrosomes which become surrounded with new radiations. In the maturation stages my preparations are not numerous enough to enable me to follow in detail, the behaviour of the centrosomes, and I have not been fortunate enough to see the division of the body in the first maturation spindle. The centrosome of Boveri corresponds to the centrosphere of Wilson. In another set of preparations less destained Boveri described the centrosome as a smaller body, showing in its centre a darkly staining particle the centriole, which corresponds to Wilson's centrosome. This as division proceeds, divides into two and goes through the usually described evolutions. In picro-acetic and sublino-acetic preparations, I have seen such a centriole but have been unable to trace its division. Again when destaining has been stopped early the whole centropiasm is black. This I have also seen in Picro-acetic and sublino-acetic material (Fig.2 Pl.I). The rays according to Boveri stop at the margin of his centrosome, and do not enter it so as to be inserted into the centriole. This is certainly the case, and in my cleavage

preparations fixed with Lindsay Johnstone's fluid, the central reticular body is sometimes seen to have completely dropped out, so that the astral rays are seen to end abruptly, leaving an absolutely round empty space occupied in the other eggs by the alveolar or reticular centroplasm. I do not presume to give an opinion on the much vexed question of the persistence of the centrosome as a special cell organ. The criticism that the upholders of that theory have never proved their case completely, seems to be weakened by Boveris' latest work, but one thing is clear that the centroplasm is a focus of protoplasmic activity, and is ultimately to be explained on physiological and not on mechanical grounds.

Changes in the germinal vesicle preparatory to division.

When the germinal vesicle has reached its full growth the nucleolus loses its staining capacity to chromatin stains, the nuclear network takes an intense stain, and the cytoplasm to its very outer edge is seen to have an alveolar structure. In many cases, presumably in stages close to the onset of maturation, the nuclear membrane is puckered. The germinal vesicle then moves towards the surface, and, as long ago described by Hertwig (1877) for *Asteracanthion*, at the spot nearest the surface a protoplasmic process projects into its interior. (Fig.5 - 9 Pls.IV,V,Va,VI). In Osmic acid preparations the nuclear membrane is seen to be indented and folded before the process. (Fig.7a & 7b, Pl.V).

This as it projects inwards spreads out in every direction from the neck, so that at the margins of the process are seen sections of peninsulae and islands. In sublimate material, the nuclear membrane is not so sharply differentiated and the inward folding of it is not so clearly seen. I have no doubt from my sections, such as figured in Fig. 7a, 7b. Pl.V. that this is a true invagination of the germinal vesicle by the cytoplasm. At the neck of the invagination the alveolar walls of the cytoplasm are drawn inwards towards the centre (Fig.5 Pl.IV) but in the process itself no distinct fibrillar structure is to be seen.(Fig.7b Pl.V) In the heart of this invagination is the Kinetic centre of the ovum. In Fig.9 Pl.VI there is seen a darker circular spot which is the only structure I have been able to detect as a possible centrosome. There is no centriole, but any detection of minute dark bodies is rendered uncertain by the fact, that darkly stained granules of the chromatin surround the invaginated mass. In the living egg the first change to be observed is that this process becomes less defined; in the sections it is seen that the whole mass is made up of looping fibres. In (Fig.11 Pl.VII) is represented a stage in which the chromatin reticulum has become finer, and at the neck of the process is an irregular mass which is destined to form the future chromosomes. All this time the germinal vesicle remains close to the periphery of the egg.

The next stage I can determine is the one represented in Fig.12 Pl.VII. The nuclear membrane has now entirely disappeared and in the irregular mass of looping fibres there are seen two asters. In each is a circular finely reticular area, the centro-

plasm - and from the periphery pass out in every direction very delicate interdigitating fibres. Between the two asters the fibres are drawn out to form an irregular spindle arrangement. Round this area the greater part of the nuclear reticulum which does not form chromosomes, but was related to the vegetative stage of the germinal vesicle is seen merging with the cytoplasm but still retaining its reticular character. Between the spindle and the surface, the chromosomal chromatin mass is seen in this case already segmented into a number of dyadal bodies.

This description corresponds exactly with Hertwig's original account, but differs from Fol's in that he describes no process projecting into the vesicle. It also differs from Matthew's description of what occurs in *Asterias forbesii*. He describes the two centrosomes probably passing out of the germinal vesicle, at the nearest point to the surface of the egg by the rupture of the nuclear membrane at that point. They then pass some distance from the nucleus, and are seen to have round them a faint halo of "Archoplasm". This later becomes distinct; radiations are developed; the whole Archoplasmic area divides, and the two parts being drawn asunder a spindle is spun out between them, which moves tangentially over the nucleus. As it grows the spindle fibres project into the vesicle; the nuclear membrane is dissolved, and the spindle then rotates to become the first polar amphiaster. In Haecker's text book (p.123) the process in the living egg is described in much the same fashion. A clear area is developed between the remains of the germinal vesicle and the surface, surrounded by a radiation which soon forms a double star which is the anlage of the amphiaster.

The invagination of the germinal vesicle in the egg of *Echinus* is obviously secondary. It must be related to the fact that the wall of the vesicle comes exceedingly close to the surface of the egg. The mounting of the vesicle to the surface is a fact which so far as I know, has not been explained. Haecker (1893) has suggested that it is due to the action of gravity, causing a movement in the elements of the egg, after the force connected with the exchange of material between nucleus and cytoplasm which keeps the vesicle in the centre of the egg, ceases with full growth. My preparations do not throw any light on the point.

Fig.13 Pl.VIII is a somewhat oblique section of the germinal vesicle at a later stage. It shows the two asters arranged tangentially to the surface of the egg, but between them, and extending towards the surface, is a finely reticular mass out of which the delicate wavy and interdigitating rays of the asters are evidently spun. Embedded in this reticulum are seen the chromatin segments. At a later stage (Fig.14 Pl.VIII) all these are drawn into the area between the asters which is seen now as a finely alveolar or reticular plate. Round this central plate is a complicated reticulum of fibres crossing and intercrossing but on the whole radiating from the central plate. In this reticular zone is also seen at a little distance from the plate, one centrosome surrounded by rays obviously part of the general reticulum. Fig.23 Pl.XV represents this plate very highly magnified - It shows distinctly a very fine reticular or alveolar structure - round the circumference of the plate is seen

the radial grouping of most of the fibres of the reticular zone - merging into the microsomal reticulum of the cytoplasm. At this stage one hardly ever sees a preparation in which both asters are cut in the same section. Griffin in *Thalassema* has described a similar disappearance of the spindle spun out between the centrosomes and the development of a central mass very like that which I have described between the asters. In most instances however the conditions are more like those described by Matthews in *Asterias forbesii*.

It is evident that when the nuclear membrane disappears and the rejected chromatin passes into the cytoplasm a profound effect is produced on the organisation of the egg. Whereas with the germinal vesicle still intact, the alveolar structure can be traced to the surface of the egg, we now find that round the transformed nucleus, and projecting into the centre of the egg, is a fibrillar mass which is sharply differentiated from the alveolar yolk. From this central area there also extends round the surface of the egg, a layer of differentiated protoplasm. 56 Fig. 60 Pl. XXXVIII shows this very well. It is a photograph of two ova lying side by side in the centre of a section and therefore exposed to exactly the same conditions of staining and fixing. The structure of the immature ovum with complete germinal vesicle, is seen to be what I have already described. In the other ovum in which the first polar spindle is formed, is to be seen the darker stained reticular matter extending from the neighbourhood of the spindle all round the periphery of the egg. This feature is very obvious owing to the fact that the section

has not been greatly decolourised, and the central area and peripheral layer are seen to be studded with very numerous microsomes. In other sections the boundary line between the alveolar yolk and the reticular zone, is sharply marked off by a collection of dark staining granules, which fade away into the alveolar walls. The central mass and surface layer have each a definite fate. The one is differentiated into the spindle and asters, while the surface layer is, I believe, associated with the formation of the membrane thrown off by the egg at the moment of fertilisation. The central mass of the yolk is unchanged in appearance, and the question is whether this reticular mass of protoplasm, is differentiated from the cytoplasm, or is derived from the rejected nuclear reticulum. I am inclined to think that it is in large measure formed from, or under the influence of the discarded nuclear material. This would be exactly in harmony with the results of Carnoy and Le Brun (1899) in Triton. Another evidence of the excitement produced in the egg at this stage is that accessory asters are formed which so far as I can see, have no relation to the formation of the definite asters of the spindle.

From experiments by R. Hertwig (1896) and Morgan (1896) it seems that under special artificial chemical stimulus, the cytoplasm may be excited to form asters and even in Hertwig's experiments amphiasters. Reinke (1894) also found that in the peritoneal cells of the larval Salamander three grades of asters are formed, primary, secondary, and tertiary. The last contribute to the secondary, and these again to the primary or definitive

asters. Carnoy described accessory asters during the formation of the second polar body in *Ascaris*, and Meade (1897) showed that a great number of such asters were formed before the formation of the first polar spindle in *Chaetopterus* (an annelid) which he thought contributed to the formation of the spindle asters. Watase (in *Macrobdella*) found as many as 13 asters in the cytoplasm with centres varying in size from the smallest microsome to the true centrosome. Griffin (1899) also describes the formation of accessory asters in *Thalassema*. These experiments and observations are held to afford strong evidence of the free formation of the centrosome, in which case both that body and its aster would be the expression rather than the cause of cell activities.

The secondary asters in *Echinus* at this stage I believe are produced in the cytoplasm under the influence of the nuclear material let loose on the disappearance of the nuclear membrane.

All this tallies better with Strasburger's views of the Kinoplasm than with any other theory. He thinks of protoplasm as of two kinds, Trophoplasm and Kinoplasm the first is vegetative in function and alveolar in structure, the second presides over the activities of the cell, forms centrosomes, mid-bodies, asters, and spindles, constitutes a peripheral layer from which membranes and cilia are derived, and is fibrillar in structure. This differentiation

of the protoplasm takes place when mitosis sets in. Further, he thinks, the nucleolus is a storehouse of reserve material, out of which, on need, the substance of the kinoplasm is drawn.

I have shown that the nucleolus at first contains all the chromatin substance, which later is found in the nuclear reticulum, part of which forms the Chromosomes, the remainder and larger portion being rejected - to form in turn a reticular zone out of which the asters and spindle are spun.

My conception of the meaning of the changes in the ovum does not, however, involve an acceptance of either Strasburger's Kinoplasmic, or of Boveri's Archoplasmic theory. It inclines rather to the view, that the same ground substance, under the influence of the chemical changes underlying vital activities, may take on different forms in response to varying physiological needs, and further, that whereas, during the vegetative period, the centre of these chemical activities lies in the Nucleolus, in the division period that centre is transferred to the centrosome, which is the expression of chemical activities resulting in the vital phenomena of division.

Fig.24.,Pl. XVI. represents a later stage. The spindle is not yet complete, but the two asters are situated radially and the reticular mass though still showing in some parts a radial distribution from the central plate is becoming more and more focus-sed on the centrosomes. The spindle in most forms is said to be fully formed before this radial position is assumed, and the whole spindle is said to rotate through 90 degrees. A very good example of this is seen in the egg of the Mouse as described by Sobotta (1895).

In my preparations there is no sign of such an arrangement. The spindle, as is the case also in *Thalassema*, (Griffin) is late in forming, and the asters move independently through the cytoplasm as I believe by successive new formation, the fibres arranging themselves round the centres of activity until the definitive position is reached.

The conditions described for the formation of the polar spindle are not unlike those accompanying the formation of the multipolar spindles described in the Pollen and the Spore-mother cells in many plants - by Farmer, Belajeff, Osterhout, Mottier, Nemeec and Byxbee, according to the description of these authors there is a filar zone round the nucleus out of which the multipolar figure is spun, the poles of which draw together to form the definitive bipolar spindle. I have seen one or two four-poled first maturation spindles, but I cannot make out that in the reticular zone there are more than two asters, which have any relation to the future spindles, and such four-poled spindles would thus merely

indicate the tendency to the formation of multiple centres of activity, or putting it in terms of the centrosome, to the formation of four centrosomes instead of two.

The whole process leading up to the formation of the first polar amphiaster is very complicated in *Echinus*, and extremely difficult to trace in the sections. It is as difficult to be sure of the phases in the living egg. The process does not seem to me to be so simple as it has been described for *Asterias*, indeed it is in many respects like what Mead has described for *Chaetopterus*. Dr. Teacher has recently studied the phases in the living egg, and has kindly let me see his results, which supplement my own. He has seen frequently a stage which I have described as follows, "near the surface of the ovum is a clear granular area having the appearance of ground glass, surrounded by a darker ring merging into the alveolar looking cytoplasm. This ring at its circumference is distinctly irregular and suggests delicate radiations from the central granular area." This is obviously the stage represented by the sections figured in plates VIII and XV, and corresponds to the transformed germinal vesicle. As this stage I could not make out distinct asters in the living egg, and in the sections the astral rays which lie within this area are of great delicacy. Dr. Teacher has seen in this phase many specimens with a number of asters in the cytoplasm around the transformed germinal vesicle, and has made out at the same time within the area itself astral formations which he believed to be the definitive asters of the spindle. While, therefore, I have in the foregoing description traced the centrosomes as

if they were persistent centres travelling through the cytoplasm I cannot exclude the possibility of their free formation as described by Mead.

Fig.25.Pl.XVIA represents the spindle now completed. In the photograph the remains of the reticular zone are still very distinct. The chromosomes are being drawn into the equatorial plate.

Fig.26.Plate XVII shows the now completed spindle in metaphase. It is relatively bulky, with blunt and rounded ends, and frequently when the section is through the side of it, the centrosome is not cut at either end. There is no central spindle. The chromosomes extend through the whole equatorial plate, and the peripheral rays of the asters are seen interdigitating opposite the equator. The fibres of the spindle itself are somewhat uneven, and in relation to the chromosomes there are darker bundles apparently of several fibres spun together. As metakinesis proceeds the waviness of the fibres becomes more distinct. The central centrosome becomes flattened, but I cannot determine the manner in which a division takes place. The outer centrosome diminishes in size. The central astral rays shorten, and ultimately disappear as the apex of the spindle is protruded. The lateral rays are obliterated progressively until the point of the spindle stands clear as in Figs. 34, 35, 36, Plates XXII and XXIII. The first appearance of the protrusion of the polar body is a tiny elevation into which the end of the spindle is directed. Later when the spindle has risen to the height of the equatorial plane there is seen

a depression on the surface of the egg where the constriction takes place, and in which afterwards the polar body lies. The rise of the spindle and its protrusion is very difficult to explain. It remains approximately of the same length throughout, and I do ^{not} see any special development of the central aster over the polar one. Wilson (1900) finds evidence in the protrusion of the spindle in favour of Dünér's (1895) theory that the divergence of the poles of the spindle in Mitosis is due to the progressive elongation of the central spindle. In Echinoderm ova, neither before nor after fertilization, is there a central spindle spun out between the centrosome but it is probable according to Wilson, that the difference is only a secondary one and that the spindle consists in part of continuous fibres and the waviness of the spindle fibres in the metakinesis would speak for the pushing hypothesis. In any event I cannot see how any hypothesis founded on mechanical principles, such as illustrated in Heidenhain's model, can explain the peculiar circumstances of the Polar Mitosis; and I see in them a demonstration of the impossibility of such an explanation.

Fig.38 Plate XXV shows the earliest phase of the second division which I have had the opportunity of observing. The asters are already separate, and a bunch of fibres from each is projected towards the chromosomes, which are immediately drawn into the equator of the spindle. Thus no resting stage intervenes between the two divisions. The whole figure is still surrounded by the remains of the reticular or kinoplasmic zone. The spindle when fully formed is slighter than the first polar spindle.

The central centrosome and aster progressively increase in size until the condition is found as in Fig.49 Pl.XXXII. The astral rays are thick and fairly straight and widely spreading. The behaviour of the outer centrosome and the manner of protrusion of the polar body is exactly as I have described for the first polar body. Figs. 46, 47, 48, 49.

Fig.50 Pl.XXXIII shows the condition of the nucleus long ago described by Hertwig after the extrusion of the second polar body. The first stage in the reconstitution of the nucleus is the formation of several small vesicles, which run together to form a single vesicle which is the mature nucleus. The description given of the process in the living egg is, that several small vesicles appear approximately in the middle of the radiations remaining in the egg. In the sections this is clearly seen not to be the case, but the vesicles surround the centrosome, and the astral rays are broken up into bundles passing out between them. Later these all disappear, and a single vesicle is left without any trace of centrosome or radiation in its neighbourhood.

This point may be of some theoretical interest in connection with the disappearance of the egg centrosome described in so many different forms. Boveri in his latest work already cited (1901) elaborates an hypothesis as to the phylogeny of the centrosome. Briefly it comes to this, the Protozoa possess a compound nucleus in which the chromatin element lies side by side with a body homologous to the centrosome. In such a nucleus the spindle is formed inside the Nuclear membrane.

In Metazoa the centrosome is individualized, and there is a chromatin nucleus, and a centrosomal nucleus which directs the cell division. The individualising of the centrosome however does not necessarily deprive the nucleus altogether of its generalized centrosome, and there are instances in which there may be a centro-nucleus combining both elements within itself. The mature nucleus of the Sea Urchin Egg is possibly a centro-nucleus, in which the centrosome lies latent, and in fertilization it is only the centrosome conveyed into the egg by the sperm nucleus, which initiates and directs the division of the segmentation nucleus. Under certain circumstances however as shown by R. Hertwig (1896) the unfertilised germ nucleus may by chemical means be stimulated to initiate the division, and Loeb has described parthenogenetic development under the influence of magnesium chloride solution. R. Hertwig holds that the centrosome is newly formed before division out of the nucleus, to be again taken into it after division is over. Boveri explains both cases by imagining a calling up of the latent centrosome into activity by artificial means. While in no way committing myself to the theory of the persistence of the centrosome, the appearances described suggest the possibility that when the vesicles unite to form the single large vesicular nucleus the centroplasma may be retained within it. If this were so, Boveris' explanation of the parthogenetic development of the Sea Urchin egg would not require so theoretical a foundation as that of the latency of the centrosome in a centro-nucleus. Fig.54 Pl.XXXVI shows an interesting abnormality of the second

polar body. It is here very distinctly a small cell, and precisely the same phenomena are seen in the reconstruction of the nucleus as in the egg.

I must now refer to a series of figures which accompany the constriction of the spindle in both maturation divisions. Associated with the disappearance of the spindle is formed the body called by Flemming the "Zwischenkorper." This plays a considerable role in spermatogenesis, but is figured also in a considerable number of the descriptions of polar body extrusion. I have seen it in various forms. In fig.49 Pl.XXXII are seen round the constricting spindle a series of points which afterwards, as seen in figures 50a Pl.XXXIII and 54 Pl.XXXVI, condense to form a ring round the remains of the spindle. It seems to persist for some time (Fig.38a Pl.XXV) and then disappears.

A final point still remains to be described. When the matured nucleus retires towards the centre of the egg all remains of the reticular or kinoplasmic zone have disappeared, and the nucleus lies surrounded by the alveolar yolk, while round the periphery of the egg the kinoplasmic girdle has narrowed down into a delicate layer of differentiated protoplasm. In sublimate material this is seen as a distinct layer, in which large microsomes are arranged regularly side by side. In the Osmic acid material the distinction is less sharp, but there is obviously a difference in the characters of the surface layer. I think there is little doubt that this layer has to do with the formation of the membrane thrown off when the selected sperma-

tozoon enters the egg, and as has been said I refer it to the Kinoplasmic zone - which is differentiated on the breaking down of the germinal vesicle. Fig.55 Pl.XXXVI, Fig.59 Pl.XXXVIII.

History of the Chromatin.

As has been described the greater part of the nuclear reticulum is rejected, and gives rise probably to the reticular zone round the transformed germinal vesicle. Close to the base of the neck of the invading cytoplasm is found an irregular mass of chromatin, just as Matthews describes for *Asterias*, which is presumably the chromatin destined to form the future chromosomes. (Fig.10 Pl.VI and Fig.11 Pl.VII) This condensation of Chromatin at one point corresponds to Moores' (1895) Synaptic phase, though only a part not the whole of the chromatin, as in spermatogenesis, is involved in the condensation. Emerging from this condensed mass are seen in fig.12 Pl.VII a series of separate elements as to the number of which I am not certain, but there are not more than at a later stage. The following stages Figs.13, 14, 15, 16, Pl.VIII and IX involve the collection of this mass of chromatin elements into the central plate, before described. Often one sees the chromatin collected to one side of this plate; sometimes the separate elements are widely scattered; in many instances as in Fig.19 Pl XI and Fig.22 Pl.XIV there are chain-like clusters, which strongly suggest that a thread is being broken up into segments, and in practically every ovum at this stage one sees compound masses which are breaking down into the separate elements which enter the equatorial plate of the spindle. I have between 50

and 60 sections of this stage and the relatively large number indicate that the prophase is protracted. From the very first these always present the same form. Fig.21 Pl.XIII shows a fragment of the thread composed of spheres, united by a less deeply staining substance. When separation is complete sixteen tetradal chromosomes of nearly uniform appearance are found. When seen from the side they have a dumb bell shape, when seen en face they are obviously the tetradal groups of authors. I have counted the chromosomes at the various stages again and again, and have always reached the number fifteen or sixteen. Fifteen is an improbable number, and I feel sure that the proper figure is sixteen. I have never succeeded in making the number eighteen which would be double the number (9) found by Boveri (1890) in *Echinus microtuberculatus*. R. Hertwig (1896) made the number of chromosomes emerging from the germ nucleus, in his experiments on the development of unfertilized Sea Urchin eggs, sixteen or eighteen which would agree with my results. Field (1893) in Echinoderm spermatogenesis counted 26 to 32 chromosomes in the spermatogonia, 16 to 18 in the spermatocytes and 8 or 9 in the spermatids. He confesses to great uncertainty in regard to these figures on account of the minuteness of the chromosomes and the last figure is quite out of harmony both with R. Hertwig's counts and my own.

Careful analysis of this tetrad body shows that it is composed of two short stout rods, placed side by side figs. 22a & 23a Pl.XIV & XV. The ends have the form of little spheres, and looking back to fig.21 Pl.XIII one may conclude, that they

correspond to the spheres seen in that thread united in pairs, but there is no transverse cleavage of the thread between the four spheres. A complete tetrad consisting of four independent round bodies as figured for *Ascaris*, or the Mole-cricket, there is certainly not. Further one cannot regard the two rods as separate and independent at this stage; they are bound together closely, and the figure is really a compound chromosome.

According to the above interpretation the tetrads thus arise by a single longitudinal split of the original thread. At no time are there any ring, or other irregular figures, as described in so many other cases. The possibility is not altogether excluded, that the groups might result from conjugation of the dyadal bodies in pairs, as described by Wilcox (1895) in the grasshopper, and Calkins (1895) in the earth-worm. In almost all other cases however the tetrad groups arise by the shortening up of longitudinally split rods, and the interpretation I have adopted certainly accords best with the facts, and with what is known to occur in the ordinary type of Mitotic division. In two instances only out of a large number of prophase stages have I seen a figure other than those described. In one section just before the spindle is formed (fig.24a Pl.XVI) there is a double comma form, which appears in all other sections at a later stage.

As the compound chromosomes are gathered into the equatorial plate they lie irregularly, and in the metakinesis they do not seem to be resolved simultaneously, for in all my sections of this stage, about sixty in number, figures in

different phases are seen, and as the chromosomes lie throughout the whole equatorial plate, and not only round the periphery of the spindle, various irregular bodies are seen which are portions only of whole chromosomes. The relatively large number of sections obtained in this stage indicates that it is of long duration.

The varied figures (Fig.26a Pl.XVII and figs.31 & 32 Pl.XX) are capable of only one satisfactory explanation, keeping in view that the end result is always the same. The little rods come to be placed radially on the spindle. Their central ends move apart to form a T shaped figure. The cross piece of the T representing the separating limbs, opening out on the spindle, the stem of the T the outer directed, and still united portions of the chromosomes. As separation proceeds the stem of the T is pulled down until the figure is like two commas placed end to end. It is obvious that this evolution will open out the chromosome along the plane of the original longitudinal split from within outwards, as is seen in a series of drawings Fig.32 Pl. XX of the chromosomes in profile view, but when observed en face (same figure) it is equally clear that a second longitudinal split has simultaneously been effected along a new plane, from without inwards giving the double V shaped figures I have drawn and represented in figs.31 & 32 Pl.XX, and the different photographs in plates XVII, XVIII, XIX. If we describe the appearance in terms of the minute terminal spheres of each rod, we see that the spheres come to lie in a row exactly as Wheeler (1897) describes in *Myzostoma glabrum*.

The equatorial bodies then divide (fig. 32) but the terminal spheres of each rod remain undivided, and are drawn away from the equatorial spheres, so that the whole chromosome is lengthened out very greatly, and the apical spheres are carried far away from the equatorial, delicate less-deeply-staining threads uniting them together. The equatorial spheres after remaining long in contact in the equator, then part and give rise to V shaped figures with a single apical, and two equatorial spheres, one at the end of each limb. These figures then shorten up by the contraction of the elongated thread, and in the final anaphase condense into short stumpy masses. (Fig.33 Pl.XXI, Fig. 36 Pl.XXIII, Fig.37 Pl.XXIV) These when analysed show that the apical sphere has also divided, and we have produced small tetradal bodies exactly like those in the prophases of the division, but of smaller size. In reality just like the earlier bodies, they are short somewhat curved rods with dilated extremities placed side by side. Those at the outer pole pass into the first polar body; and those remaining in the egg persist, enlarge somewhat, and pass otherwise unchanged into the second polar spindle. Sometimes during the metakinesis the second longitudinal split is not so evident, and then long drawn out threads are seen, the double nature of which is difficult to make out. Ultimately however the two halves separate in the anaphase exactly as in other cases. Exactly similar figures have been described by quite a number of observers in other forms, for instance and especially distinctly by Klinckowström (1897) Francotte (1897), Van der Stricht (1898), Griffin (1899),

Gathy (1900), but, as I shall describe in the sequel, their interpretation has been different and leads to very different theoretical conclusions. The transition between the first polar and the second polar spindle is very rapid, so that the number of sections found in this stage are relatively few.

The little compound chromosomes are drawn into the equatorial plate of the second spindle Fig.38 Pl.XXV and there different appearances are seen according to the plane of the section. In fig.41 Pl.XXVII we have apparently little tetrads which are really the lobed ends of the small slightly curved chromosomes. In fig.42 Pl.XXVIII again the rods are seen lying back to back. These rods I have every reason to believe from the various figures I have drawn (Fig.43 Pl.XXVIII & Figs.44 & 45 Pl.XXIX) open out just as in the first spindle, only there is no second longitudinal split, and therefore the division is homotypical. A single preparation rather suggests that the rods may sometimes be simply separated along the plane of cleavage. It may well be that both methods are adopted, according to whether the body lies radially or tangentially to the spindle. The result is the same; the separation is effected in the plane of cleavage established in the anaphase of the first division.

Similar figures in the second division have been described by the authors above mentioned and in other instances also the short slightly curved rods have somewhat the appearance of tetradal groups. When the daughter chromosomes have separated they pass to the poles at the spindle. Those at the external pole pass out with the second polar body, and remain as

short stout distinctly bilobed bodies in many instances, after the second polar body is cut off. (Fig.51A Pl.XXXIV) Those remaining in the ovum however at once begin to lengthen, and in the Telophase are seen (Fig.42 Pl.XXXII) as long bent rods. These are gathered into the series of vesicles already described. Within each of these vesicles are seen elongated curved rods, and round the walls, there are tiny particles of chromatin forming an incomplete membrane. (Fig.50a Pl.XXXIII) Later when the vesicles are fused the nucleus is seen to be bounded nearly all round by semi-circle loops of chromatin and in the centre the reticulum is becoming restored. (Fig.51a Pl.XXXIV) At a later stage (fig.53 Pl.XXXV) the reticulum takes on the form of irregular feathery strands, beset with chromatin granules of varying size, accumulated here and there to form irregular net-knots or chromatin nucleoli. All trace of the separate chromosomes is absolutely lost in this network.

The phenomena attending fertilization and cleavage are so well known that I do not intend to enter on that subject, but I wish to refer to the behaviour of the chromatin threads in the metaphase of the cleavage division. The primary rods segment into about thirty two chromosomes. I have counted them in cross sections of the spindle a good many times, and generally reach that figure, which would make my count of the chromosomes in the maturation stages fall in exactly with the general law.

Each chromosome when divided forms first a V shaped figure (Fig.56 Pl.XXXVII). This mounts on the spindle so that

a loop is formed with its apex directed outwards (Fig.54); and the ends of this loop are drawn out to the poles of the spindle; the threads lengthening as they go. Finally the daughter chromosomes separate by the breaking apart of the thread at the point which corresponded to the apex of the loop. (Fig.58 . Pl.XXXVIII) This is exactly the manner in which I have described the short stout chromosomes of both maturation divisions as opening out on the spindle. The difference between the two types consists only in the stoutness of the chromatin rods in the polar mitoses, the occurrence of a second longitudinal split in the first division, and consequently the absence of the usual longitudinal cleavage in the second division.

Summary of Results.

The chromatin thread, which is derived only from a portion of the mass of chromatin in the germinal vesicle, is found issuing from the synaptic phase split longitudinally and segmented into sixteen bodies - half the number of the chromatin rods in the nuclei of the cleavage divisions. These bodies consist of two short rods placed side by side, and each rod is composed of two spheres united by a less deeply stained portion of the thread. The two rods are intimately associated so as to form a tetrad-like mass, and the whole figure is to be considered a compound chromosome.

After a relatively long prophase, each of these is resolved in the first polar metaphase, in such a manner that while the body is opened up along the original cleavage plane, another longitudinal cleft is effected, which is completed in

the anaphase, and the final result is another compound chromosome, exactly like the original from which it sprang except in size. Each of the sixteen double rods, which remain in the ovum after the extrusion of the first polar body, is resolved in the second polar spindle into its two elements without further cleavage taking place.

In the telophase of the second division the elements which remain in the ovum after the extrusion of the second polar body, elongate into rods which become bent on themselves, while those in the second polar body remain condensed as small bilobed rods.

The maturation phases differ from the ordinary cleavage mitoses in respect of (a) the thickening and condensation of the chromatin rods, (b) the second longitudinal splitting which occurs in the first metakinesis, and (c) the absence of longitudinal cleavage in the second metakinesis. The second mitosis thus merely distributes the grand daughter chromosomes formed by the second longitudinal splitting in the first mitosis.

There is thus no reducing division. The only reduction which occurs is effected in the germinal vesicle in association with a kind of condensation called the "synapsis", in which the chromatin destined to form the chromosomes of the polar divisions is diminished in bulk.

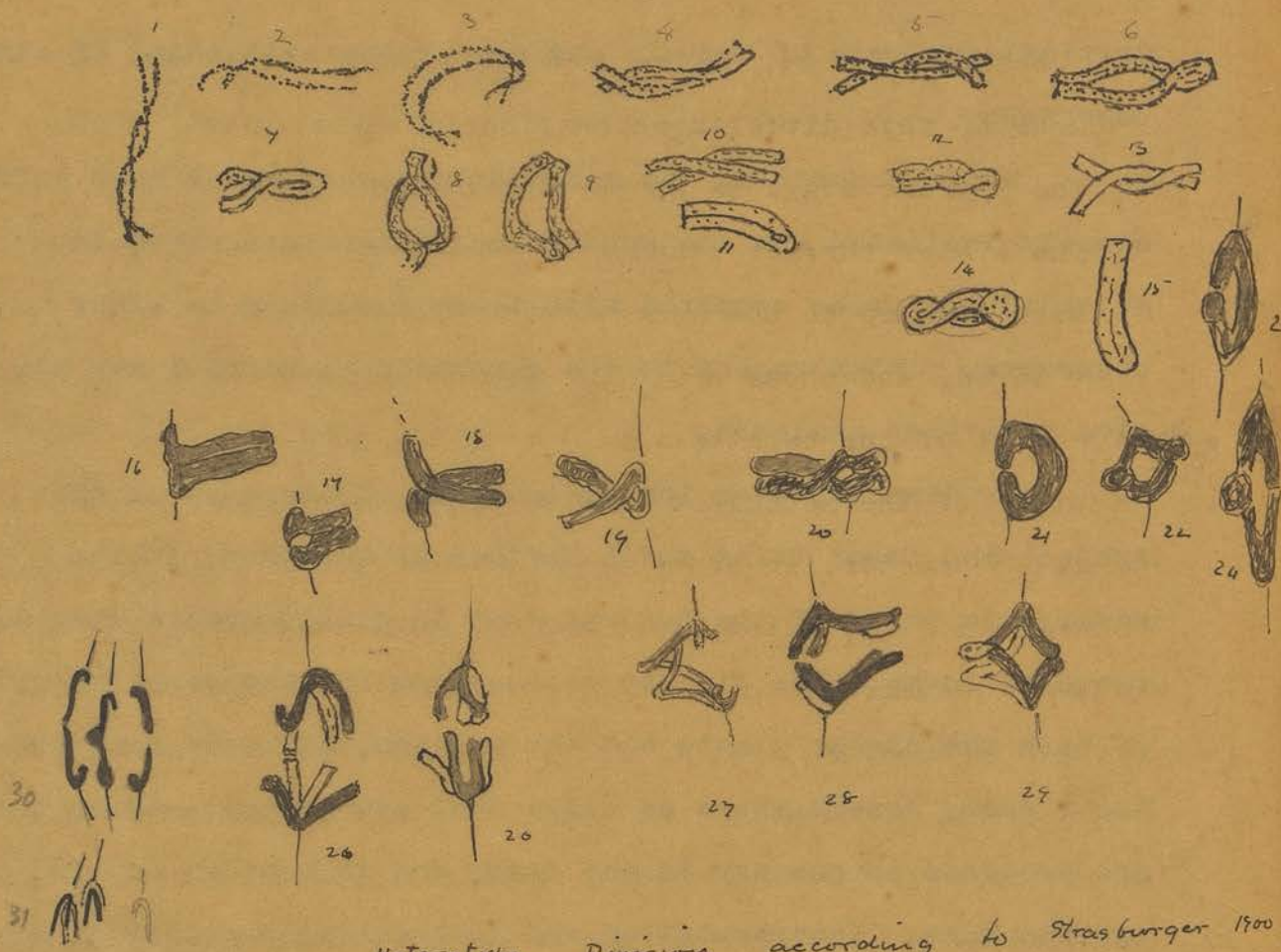
Critical analysis of Results and comparison with those of other observers.

In describing the achromatic structures I have sufficiently indicated how the appearances I have described in my material are to be compared with those described by other observers. With regard to the chromatin elements I may now give a further analysis.

Glancing over the whole field of research on the subject the first thing which strikes an observer, is the remarkable unity of the process even in detail over a very large range of forms. The figures represented for the great majority of both the higher plants and the metazoa, and even some protozoa, show resemblances so close that one cannot imagine they are produced in one way in one form, and in another way in another form. Interpretation and theoretical conclusions may differ, the process is identical throughout.

It has been insisted that the solution of the problem of reduction lies in the determination of the origin of the tetrads, but as these in typical form occur in a relatively small number of cases, it seems that the solution rather lies in a closer analysis of the heterotypical division, such as has lately been done for plants by Strasburger.

Heterotypical division was first described by Flemming in 1887 as a form of mitosis occurring in the spermatocytes of the salamander, and in all cases in which tetrads are not formed a heterotypical division in some sort, ushers in the first maturation division with its reduced number of chromosomes, and



Heterotypic Division according to Strasburger 1900

Strasburger *Über Reduktionsteilung, Spindelbildung, etc.* - Fischer *Jena* 1900

- 1 to 15 Various shapes taken in prophases by double rods
- 16 to 23 Various positions of insertion on spindle
- 24 - 29 Result in every case a double figure.

Heterotypic Nuclear Division according to Farmer & Moore *Anat. Anzeiger* Vol. XI 1896



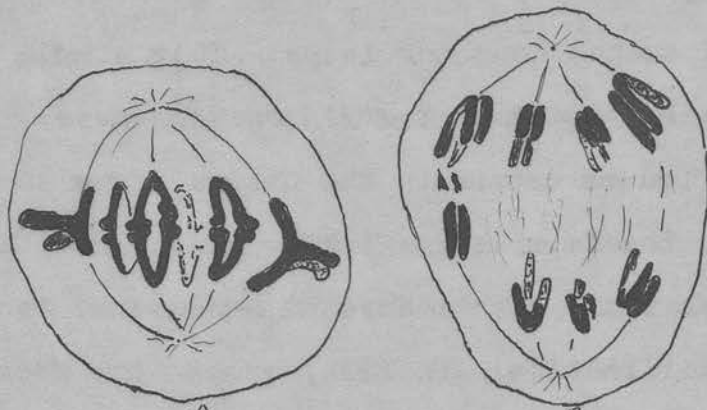
this is true of plants as well as animals. The distinctive features of this division as originally stated are:-

(1) The Spireme stage is not so tight as in other kinds of cells
 (2) The sister threads round which the segments split are fused by their ends up to the metakinesis. (3) The monaster stage is short lived, and shows a radial arrangement only indistinctly on account of the twisted position of the threads. (4) The end stage of the metakinesis is very prolonged, and has a very special character, in consequence of the fusion of the ends of the threads. (5) A temporary and not understood second longitudinal cleavage of the threads appears in the anaphase. The outstanding feature of the heterotype was considered at first as being the incomplete separation of the two halves of the longitudinally split rods resulting in the formation of ring chromosomes but the figures may assume very various forms according as the loop is bent, or drawn out so as to obliterate the hollow of it. Again the rings or their derivatives may be attached to the spindle in different fashions, so that in their resolution, different irregular figures emerge. This is shown in the series of diagrams opposite, copied from the paper of Farmer and Moore, who first clearly pointed out the essential resemblance of the heterotype in plants and animals. The feature described by Flemming, namely, the second longitudinal cleavage found in the anaphase has until recently had very little significance attributed to it.

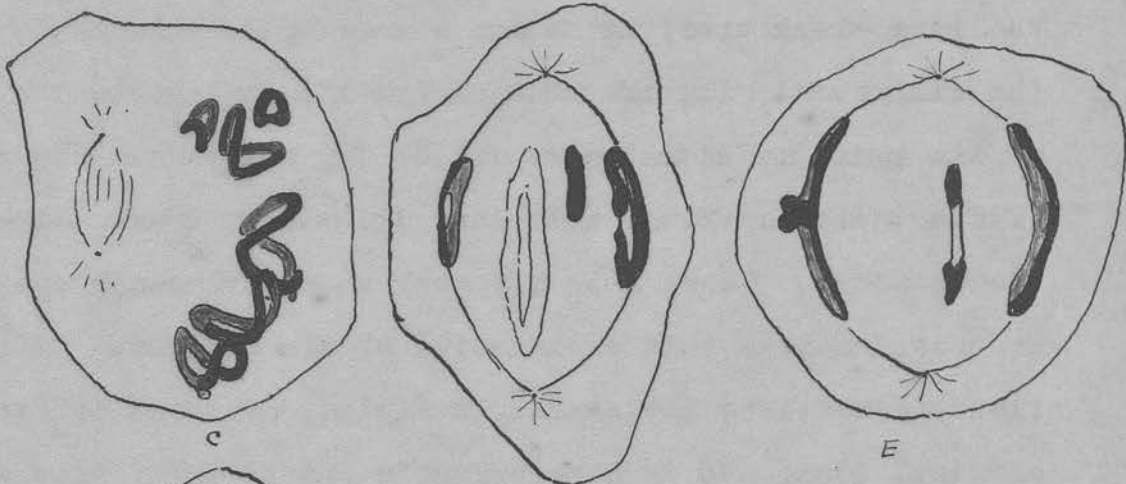
The simplest idea of heterotypical division is that the two halves of the ring shaped chromosome, are drawn out



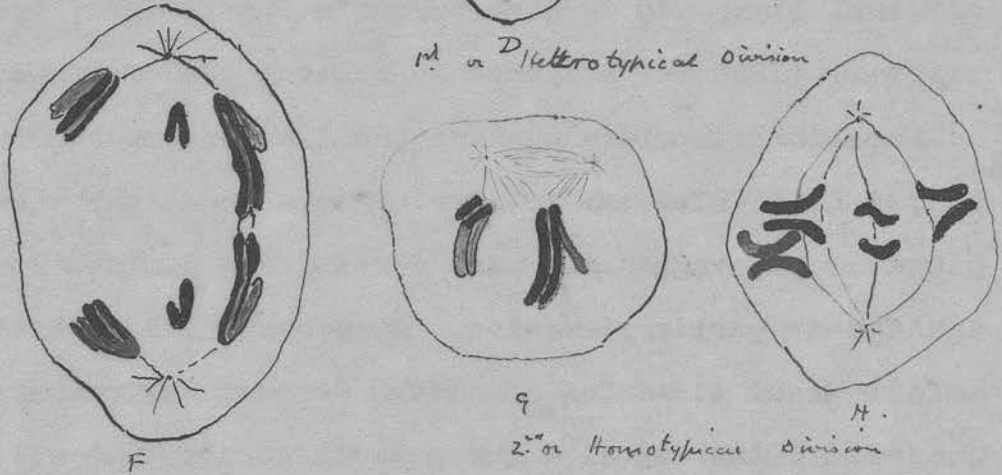
into U or V shaped daughter loops. This simple explanation will not explain many of the figures observed. Farmer, (1895) describing the phenomena in the Lilies, gave an interpretation involving a double cleavage taking place simultaneously in different planes, as the compound chromosome is resolved into its daughter elements. In 1896, along with Moore, he gave another explanation of the phases, which only involved one split. The idea elaborated, which was shared by Miss Sargant, was that the elliptical ring was bent on itself, applied to the spindle at its apex, and then drawn out to the poles from the point of bending. The original ends were ultimately broken across at the equator. Moore, in his work on elasmobranch spermatogenesis, adopted this explanation of his figures. Gregoire, (1899) describing the stages in Lilies, reverted to Farmer's original idea, and in Strasburger's latest work, from a careful examination of the prophase in a large number of plant forms, he absolutely decides against the idea of the bending up of the ring. In Echinus, where no ring is seen at any time, the explanation is easier and more direct, and decides conclusively against such an explanation. Strasburger, in addition to examining a large series of cases, reviews the results of other observers, and comes to the general result that all the processes can be referred to one type, namely, (1) As the result of the primary longitudinal cleavage of the chromosome thread, two rods, wavy or curved, are formed. These straighten and ultimately shorten down into stout rods. In shortening, various adhesions and twistings may take place, so as to form rings or



A
McGregor Amphiuma Jour. Morph. vol XV (1899)



1st or 2nd Heterotypical Division



9
2nd or Homotypical Division

twisted threads. (2) According to the position assumed by these various figures on the spindle, the character of the resulting metaphase figures depends. (a) If the chromosome is placed radially in the form of two rods side by side, they are drawn apart in the plane of the first cleavage, and at the same time, a second slit is effected from the free end inwards. The result is the formation of V shaped daughter chromosomes, which in the anaphase break apart at the apex to complete the second longitudinal cleft. (b) If placed tangentially the result depends on the point of attachment of the "Zugfasern", but invariably as the limbs are drawn apart, a second longitudinal cleavage reveals itself, and two daughter V's are formed. The first type (a) is exactly what I have described in *Echinus*; the second form (b) is exactly that described in amphibians (1899).

Flemming in (1887) and Meves (1896) in *Salamander*, McGregor (1899) in *Amphiuma*, Kingsbury (1899) in *Desmognathus* give us identical conclusions. All describe and figure a second longitudinal cleavage of the chromosomes in the dyaster stage, and this cleavage is preparatory to the second division. The nucleus is partly reconstructed between the divisions, and the longitudinal cleft is lost sight of to reappear in the second division either by re-establishment of the old or by a new longitudinal splitting. Kingsbury was able to trace the longitudinal cleft directly into the second division, owing to the fact that the nucleus is not so far reconstructed. More recently (July, 1900) Janssens described the phases in *Triton*, and took the further step of interpreting the process in exactly

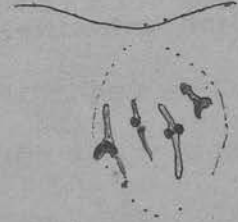


1. Polar Spindle



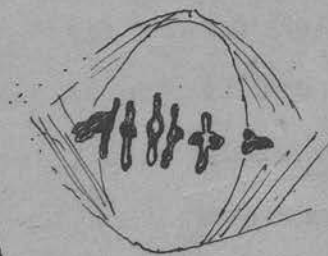
2. Polar Spindle

Fick über die Reifung und Befruchtung des Axolotteles Zeit. Wiss. Zool. Bd. 61



Born Triton taeniatus

Arch. f. Mikr. Anat. Bd. 43 1894



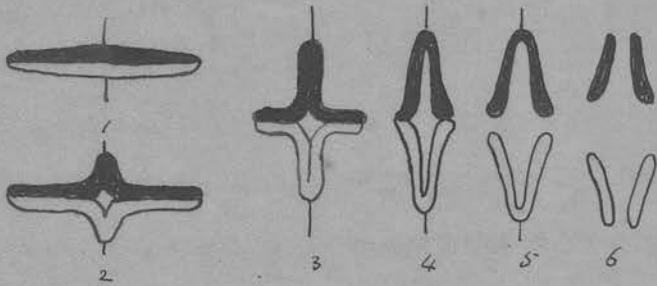
Carnoy & Le Brun

Amphibians

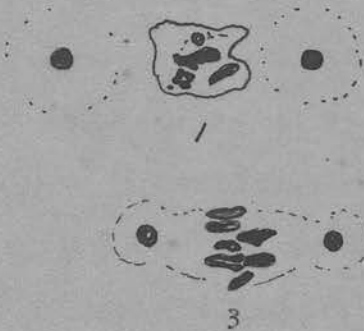
La Cellule XVI 1899

the same terms as Gregoire in lilies. Flemming, (1887) in his first paper described tetrads, but regarded them as abnormal. Vom Rath (1893) redescribed these bodies as normal appearances, but his results were not maintained by Meves (1896), who failed to find the least evidence of tetrads in amphibian spermatogenesis, though he in a short paper described tetradal figures as an abnormality in the early oocytes.

Amphibian oogenesis has been attacked by Fick (1893) and Born (1894) and Carnoy and Le Brun (1899). Practically identical figures are given by all three, but the later authors give much more complete details, and offer a new interpretation. They describe the chromosomes as condensing after some intermediate phases into short rods. These are complex structures formed by the fusion of a considerable number of separate elements. These short rods, or rather blocks, place themselves in the equatorial plane of the spindle in a circle round its periphery, and orient themselves so as to be placed with their thicker and larger ends on the spindle, the other end being directed outwards. Once installed in this position, the chromosomes go through varied movements, during which they submit to a double longitudinal splitting. The one is effected in the equatorial plane, the other in the axis of the spindle and perpendicular to the first. The equatorial division shows itself first and begins in the large part attached to the spindle, rising insensibly into the stalk. The second occurs later, and begins at the summit of the stalk, descending by degrees till a kind of tetrad is formed. Further complicated changes are



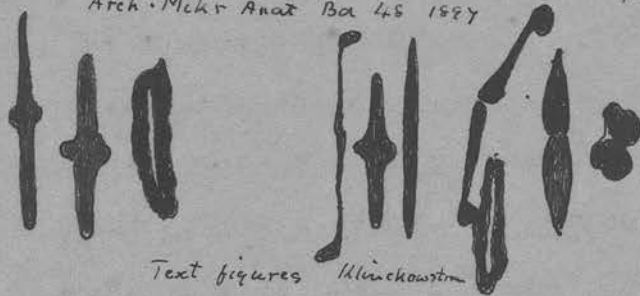
Diagram



Polar view

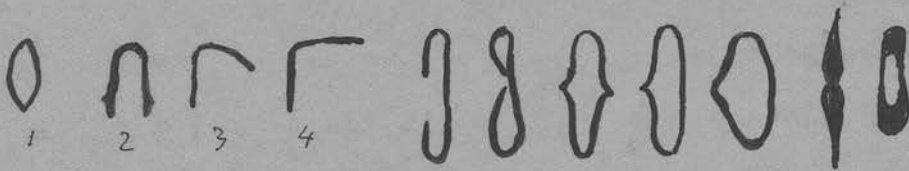
1 Polar spindle

Klinckowstrom Prosthecaraeus Vittahus
Arch. Micro Anat. Bd. 45 1887



Text figures Klinckowstrom

2 Polar spindle, so called crossforms.



Equatorial plate
2 Polar spindle

Van der Straet Thysanozoon
Arch. de Biol. Thysan.

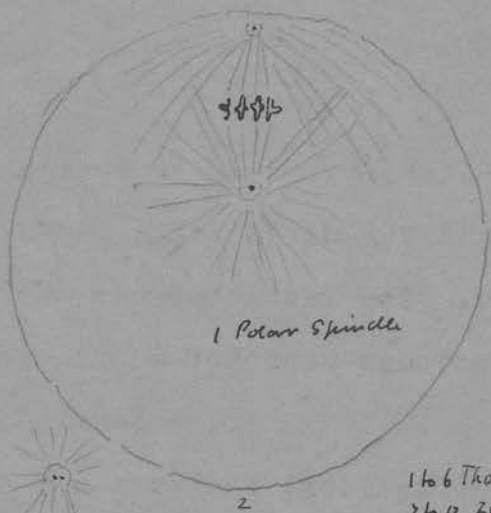
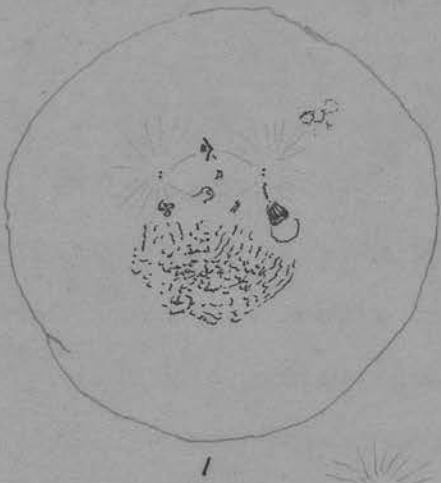
1891

described, which result in double V's originating in the wings of the "equatorial crown". These are separated and carried to the poles. A partial reconstruction takes place in the anaphase but V's again appear in the telophase, and the double V's remaining in the ovum are separated from one another in the second polar spindle. The whole description shows a very complicated process, and exactly what I have found in Echinus in a much simpler form, because as there are no V's or twisted threads to complicate the picture, I may say that only the initial stages described by Carnoy and Le Brun are found in Echinus, and the drawings opposite, traced from their paper, show how exactly similar the appearances are.

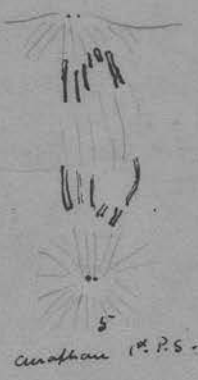
This is the only positive evidence of the occurrence of a simultaneous double split of the compound chromosome of the heterotypical division in animals. It will be seen that my interpretation, agrees exactly with that of Carnoy and Le Brun and Janssens; and further, that the same idea has enabled Strasburger to reduce the heterotype in the higher plants to one common plan.

Now Echinus falls exactly into line in every essential respect with another considerable series of cases recently described.

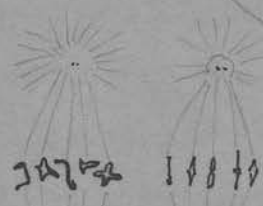
- | | | | |
|-----|---------------------|-----------------|------|
| (1) | Prostheceraeus - | Klinckowstrom | 1897 |
| (2) | Various Polyclads - | Francotte | 1897 |
| (3) | Thysanozoon | Van der Stricht | 1898 |
| (4) | Thalassema, | Griffin. | 1899 |
| (5) | Zirphaea, | Griffin. | 1899 |
| (6) | Tubifex & Clepsine, | Gathy. | 1900 |



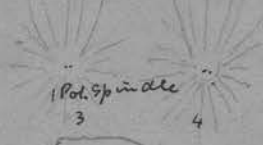
1 to 6 Thalassomai
7 to 12 Zierphaen



Centrophase (P.S.)



1 Pol. Spindle



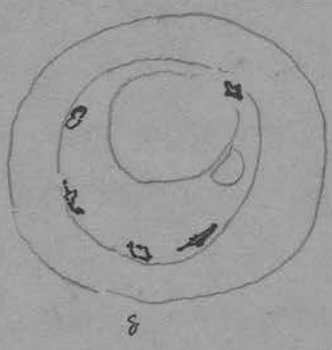
6



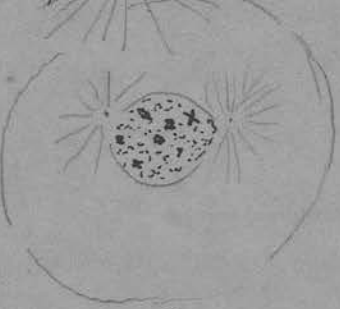
2 Pol. spindle



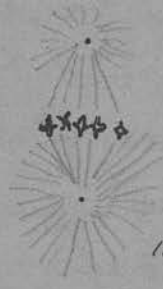
12



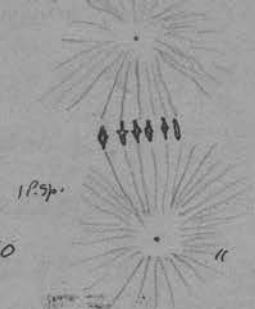
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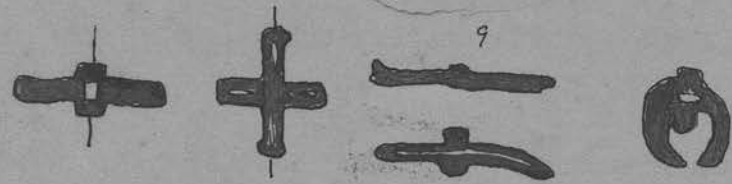
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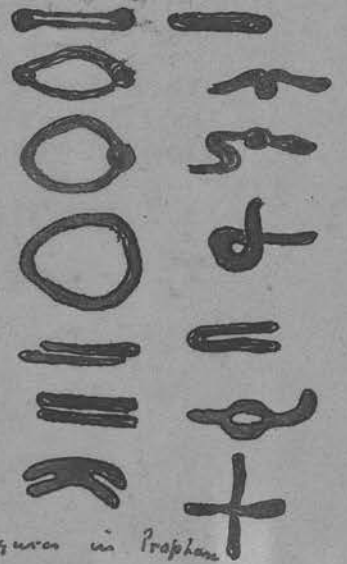
10



11



Griffen
A B C
Figures in Metaphase



Figures in Prophase

Diagrammatic Test figures

In every one of these the figures belong to the same type, except that Van der Stricht and Griffin describe rings in the prophases. The last observer has not given details, because the chromosomes were too minute for analysis.

The first four authors all explain their results according to the Diagram given opposite. The double rods resulting from the compression of the ring, are placed with the longitudinal cleft in the plane of the equator of the spindle and are drawn apart by their middle points to form U shaped or V shaped figures, and the breaking apart of the U's or V's at the apex in the anaphase, is held to be a transverse division of the original long chromosomes. Griffin alone says that the possibility of a second longitudinal cleavage is not absolutely excluded. Figures (b) and (a) indicate such a cleavage, but as the T shaped figure is rare, he held to the other explanation. It is obvious that it is extremely unlikely that such exactly similar appearances should arise in different ways. I believe that the demonstration I have given of the nature of the process, as it is seen in Echinus, might if applied to them reconcile all these instances, with what is known to occur in amphibia and the higher plants. A certain part of the contradiction in results would thus be removed, and instead of these cases being held to prove a reducing division in Weissmann's sense, they would, as does Echinus, disprove it.

The figures given by Linville (1899) for certain pulmonate gastropods are very similar to those described in

this group, but the origin of the figures is not completely worked out. He decides for a longitudinal division in the 1st division, and the elements are doubled in the anaphase while these again are distributed in the second division.

On the other hand in *Helix pomatia* Bolles Lee (1897) describes appearances which lead him to conclusions different from most other observers. He finds transverse divisions in both mitoses, but no reduction in the number of chromosomes. He holds that there is both quantitative and qualitative reduction. His figures have a strong family resemblance to those in *Echinus*, but the chromosomes are very lumpy and solid, and do not show the compound character of their prototypes which I have described.

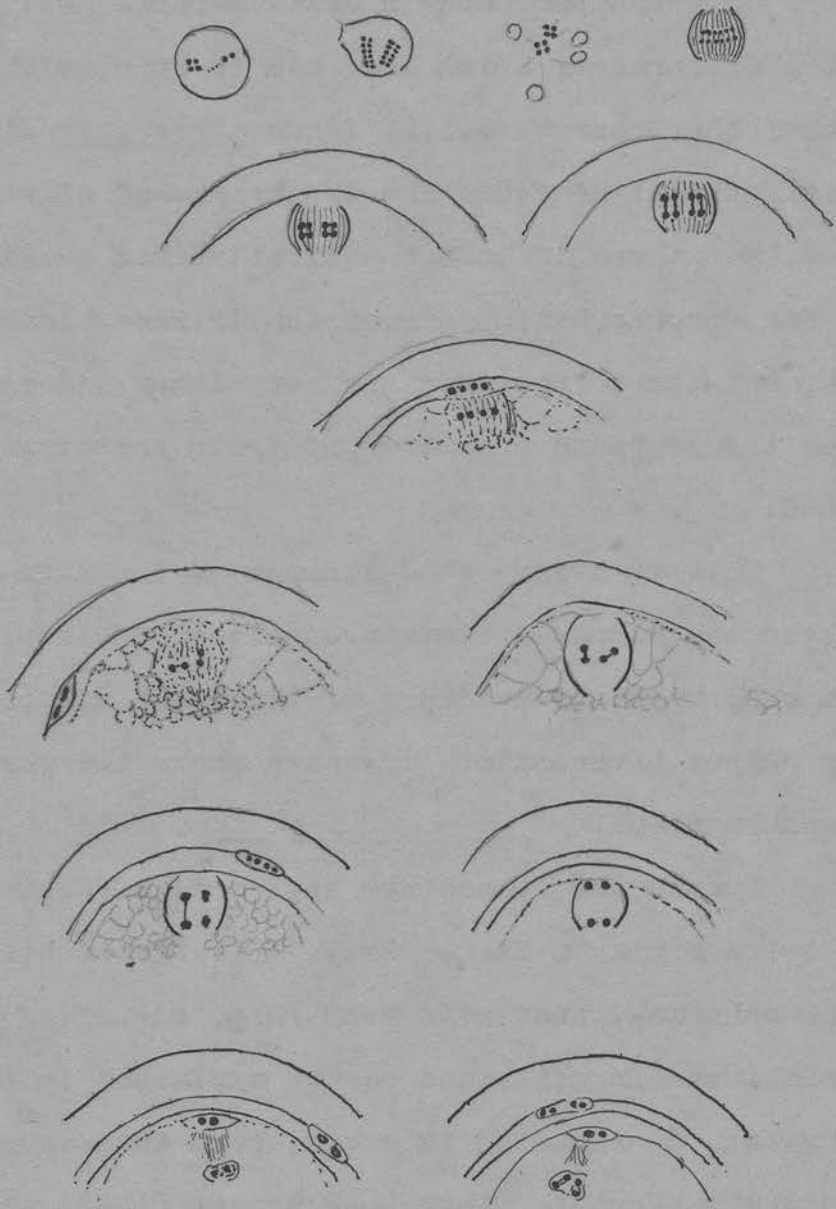
This is a very good example of the extraordinary variety in the manner of interpretations of closely similar appearances, which is evidence of the great difficulty of reaching any degree of certainty in cases where the chromosomes are small and numerous.

I come now to another series of cases in which the so called tetrads play a large part. A figure consisting of four separate spherical bodies is very rare, occurring only in *Ascaris* and the *Insecta*, and can be explained in two different ways. First, in *Ascaris*, it seems, from the researches of Boveri (1897), Hertwig (1890) and Brauer (1893), that the primary chromatin rods split twice longitudinally, preparatory to two rapidly following divisions which succeed one another without a pause. Two groups of four rods are formed, which con-

ASCARIS

MEGALOCEPHALA

Bouvier Zell. Studien H. I 1887.



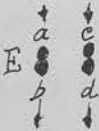
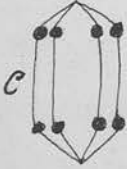
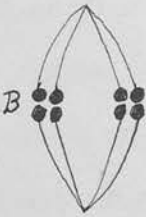
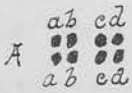
dense into two tetrads (see figure opposite). In the first maturation spindle, two of these are linked together as dyads, and pass to the poles of the spindle. The dyads retained in the ovum are resolved into monads in the second maturation division. Whilst there is a mass reduction there is thus no reducing division—no dissimilar distribution of the "Ids" of the original spireme thread.

(Second) Henking (1891) described in *Pyrrhocoris* tetrad groups which arose in another way, by a single longitudinal and transverse cleavage of the spireme thread, and interpreted the first division as a reducing, the second as an equation division. Vom Rath (1892) followed this account by a description of the process in *Gryllotalpa*, the mole-cricket, in which he figured the halves of the split rods remaining united to form rings. The chromatin material was then condensed on to four parts of the rings, which broke up to form typical tetrads. These were distributed as dyads in the first polar, and monads in the second polar spindle. Vom Rath held that each of these bodies represented a single chromosome, and both divisions were "reducing". There is thus not an "equation division" but a dissimilar distribution of the "Ids" of the spireme thread. In neither of these cases is the first maturation mitosis of the heterotypical form. Vom Rath's results were partly corroborated, partly modified, by Wilcox (1896) for the spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*. The difference between the two interpretations is that Wilcox found the tetrad formed by conjugation of dyads, and reduction consisted there-

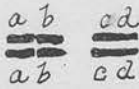
fore, not in the unequal distribution of sister "Ids", lying next each other in the spireme thread, but of any "Ids" indifferently from any part of the spireme thread. Paulmier (1899) in *Anasa* described the formation of the tetrads more in the fashion described for the copepods by Ruckert and Haecker, except that there is no spireme stage, and his first maturation division is unequal, owing to the manner in which the tetrad groups are placed on the spindle, separation taking place in the original transverse plane. The second division is an equal division, the separation being effected along the original longitudinal plane of the tetrad. It is to be noticed that his tetrads are not composed of four separate elements, but are compound bodies, the elements of which are condensed into a homogeneous mass. Ruckert (1894) and Haecker (1892-1893) examined a considerable series of copepods. They found the early stages to differ in the various forms, but the end result was always the same, namely, a condensation of the elements into tetrad groups. The early stages differed according as the split of the primary rod was complete or incomplete at one or both ends, the result being the formation of double rods, in the first case, angles in the second, rings in the third, as will be seen by reference to the diagram borrowed from Haecker.

Among the copepods the case of *Cyclops brevicornis* (Haecker 1895) requires special mention. The splitting was here complete, and double rods were formed, which divided transversely, and then united again by their ends, so that the original tetrad figure was replaced by a pair of rods lying side

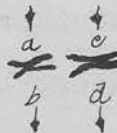
Animal Type.
Grylotalpa



Cyclops Type

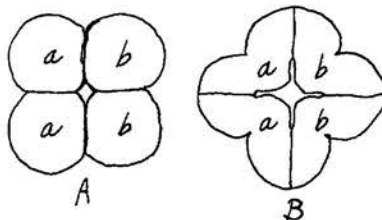


Plant Type.
Thysanozoon.



From Haeker "Praxis und Theorie der Zellen- und Befruchtung" etc.

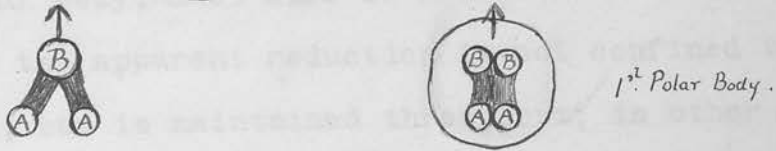
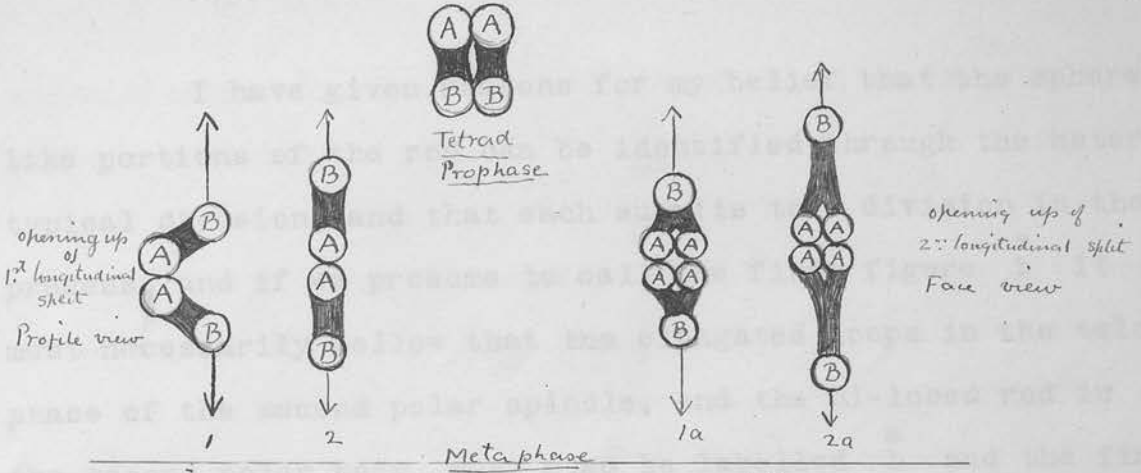
by side. In the first maturation spindle each half of the double rod was drawn apart into a V shaped loop, which was resolved in the second maturation division without further cleavage. In the Metakinesis of the 2nd Polar spindle the chromosomes take the form of "Pseudo-double rods or Cross figures". Rückert and Haecker, in explanation of their results, adopted the "apparent reduction" hypothesis already explained, that is, the reduction is only apparent in the first division, and is realised in the second, by a suppression of a second longitudinal splitting. It is to be particularly noticed that in Cyclops Haecker makes the chromosomes of the 2nd division bivalent. The diagram opposite shows how Haecker reconciles the various types. The third row of figures represents the series of appearances supposed to be seen in Thysanozoon, and stands as a type for all the cases already referred to. It is interesting to note that except in insects, this type, which Haecker calls the plant type, has claimed all the more recently described cases. In this type, as before explained, the typical tetrad formation is absent, and Haecker homologises the rings described in the pro-phases with the tetrad groups, by making them equivalent to the 4 elements of these bodies. Griffin has attempted to establish the same analogy by imagining his cross figures as derived from a crushed ring, the four limbs of which represent the four bodies in the tetrad.



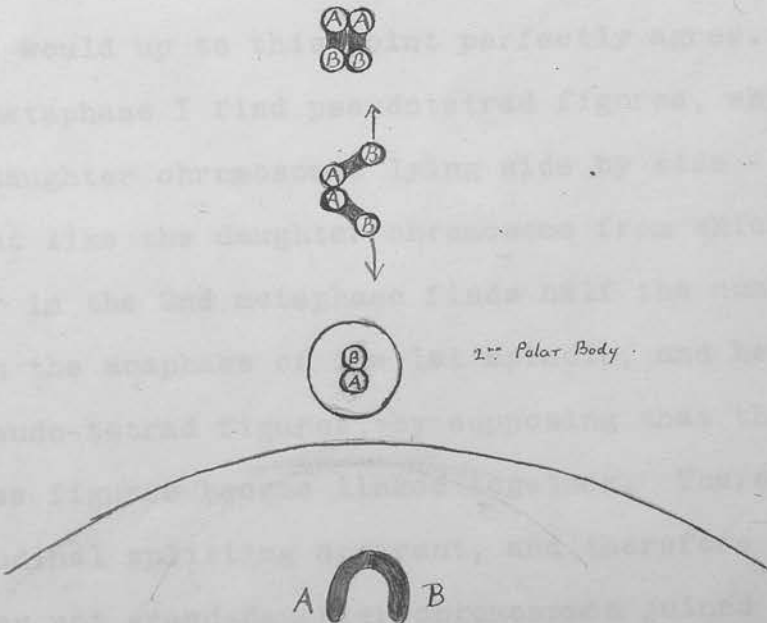
Echinus will not fall into any of Haecker's types. The special value of the observations in Echinus seems to me to be that the heterotypical division is present without previous ring formation on the one hand, and on the other, distinct tetrads are formed which certainly submit to a second longitudinal division.

I shall now endeavour to explain my results in terms of the tetrad, but I must first of all refer to Boveri's and to Wilson's figures of the second polar spindle in Echinus microtuberculatus and Toxopneustes. They both show obvious dyads in the equatorial plate, exactly as seen in Ascaris and Gryllootalpa, and this was exactly the interpretation I was at first inclined to give to the appearances, until I discovered the compound nature of the bodies, which at once transferred Echinus from the group represented by the insects, to that represented by the more recently described case of the turbellarians, that is, to Haecker's plant type, though, as I have said above, it differs from that type in certain important particulars, and agrees very closely with the Cyclops type.

Each half of the compound chromosome or tetrad is a short rod, showing at its ends small spherical bodies. If these spheres are to be interpreted as separate elements of the tetrad, as there is absolutely no trace of a second longitudinal division, I cannot represent the figure as in ascaris $\frac{a}{a} | \frac{a}{a}$. If one adopts Haecker's idea of a suppression of the last transverse segmentation of the spireme thread, the figure could with perfect propriety be represented $\frac{a/a}{b}$ always supposing each sphere to be an equivalent of a single chromosome. Following up this formula through the first and second divisions, it would work out as follows;



2nd Maturation Division



I have given reasons for my belief that the sphere-like portions of the rod can be identified through the heterotypical division, and that each submits to a division in the process, and if we presume to call the first figure $\begin{smallmatrix} a \\ b \end{smallmatrix}$ it must necessarily follow that the elongated loops in the telophase of the second polar spindle, and the bi-lobed rod in the second polar body, must also be labelled $\begin{smallmatrix} a \\ b \end{smallmatrix}$ and the final result is that the apparent reduction is not confined to the first division, but is maintained throughout, in other words that the chromosomes are coupled in pairs, and go through their evolutions as linked chromosomes. Now returning to the case of *Cyclops brevicornis*. Haecker regards each half of the double rod of the 1st metaphase as the result of a fusion of two elements end to end - so that each is bivalent - though they go through their evolutions as if they were univalent rods. Thus if each half of my tetrad figure is bivalent, our results would up to this point perfectly agree. In the second polar metaphase I find pseudotetrad figures, which are the grand-daughter chromosomes lying side by side - each being bivalent like the daughter chromosome from which it sprung. Haecker in the 2nd metaphase finds half the number of elements seen in the anaphase of the 1st spindle, and he accounts for his pseudo-tetrad figures, by supposing that the previous anaphase figures become linked together. There is no second longitudinal splitting apparent, and therefore the elements are daughter not grand-daughter chromosomes joined together. Each of the daughter chromosomes is bivalent - so that when united,

a complicated redistribution of the elements is brought about -
 according to the formula

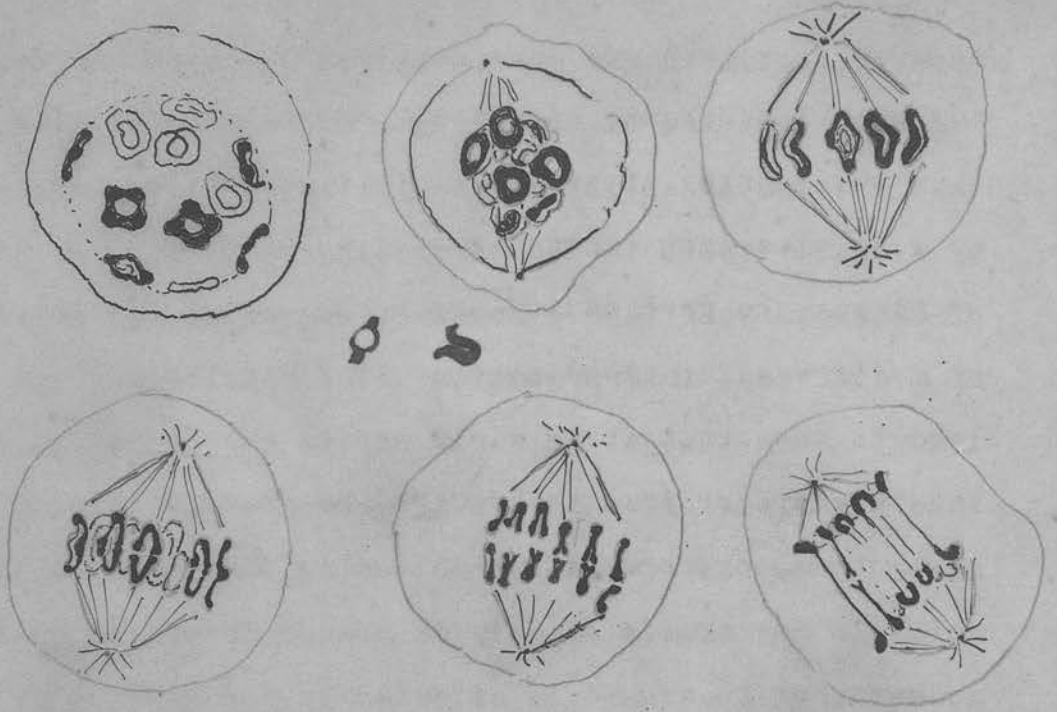


and separation being effected in the plane of the last transverse segmentation of the spireme thread - there is a true reducing division - Haecker suggests several possibilities in explanation of the figures, that just given being his choice. It does not seem very convincing to me, and the figures lack the inevitable sequence which is so apparent in Echinus.

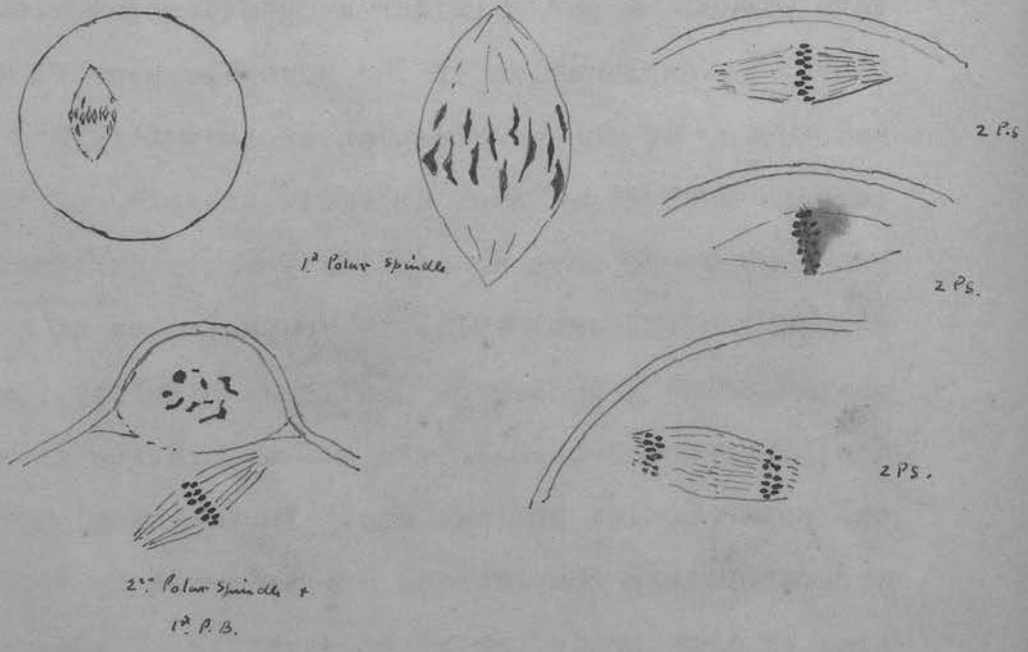
Returning to my own results we can only on theoretical grounds assume that each sphere represents a separate chromosome; but the idea certainly provides a plausible explanation, though of course such an interpretation deprives the process of any significance such as Weissmann and others have attributed to it. If we look upon the tetrad as a single chromosome longitudinally divided, then we cannot get beyond the statement that during maturation the chromatin substance, after being subjected to a synaptic phase, condenses into masses, which are half the number of the segments characteristic of the cleavage nuclei, and that these masses adopt a special form in the prophases of the first division, preparatory to the occurrence of a double longitudinal splitting.

Wilson, in his book on the "Cell in Inheritance and development", second edition (1900) P.256, suggests a possible way in which the results in Ascaris and the Copepods could be harmonised. Brauer, in his study of the spermatogenesis of

Ascaris, produced the most complete evidence for double longitudinal splitting of the chromatin thread, but also showed that the double splitting was initiated at an earlier stage, by a double split in the chromatin granules. The recent work of Sabaschnikoff (1897) however, suggested the possibility of a different interpretation. The results of this observer tend to show that at an early period the thread is broken up into granules. From this stage the granules emerge in quadruple form, to arrange themselves in the doubly split spireme, which he represents exactly as Brauer does. There is the possibility that this is effected by conjugation of separate granules or pairs of granules. If this were maintained, then as Weissman's theory involves really in essence the reduction of the "Ids" in the "Idants", the elements of the tetrads would not be equivalent, and a true or qualitative reduction would take place. A very similar suggestion was made by Bolles Lee (1897) in explanation of the possible significance of the scattering of the chromosomes of the divisions of the spermatogonia in Helix. Such an early grouping of the chromatin granules would have no significance in Echinus, for, if a double longitudinal split is demonstrated at a later stage, whatever the grouping of qualities might be, there is an equal distribution of them in the two maturation divisions between the polar bodies and the egg. In the same way I am not concerned whether the tetrads are produced by longitudinal splitting of the thread, or by conjugation of separate elements, for in either case the ultimate distribution is equal and similar.



Moore Elasmobranch Spermatozoen Quart. Jour. Microsc. Sc. 38 1896



Sobotta . Mat. des eggs of Mouse Arch. Mikr. Anat. Bot. 45 1895

"Another consideration" writes Wilson, "suggested to me by Professor T.H.Morgan, opens still another possibility, which seems well worthy of test by further research. As already stated (p.88) the long chromosomes of *Ascaris* are plurivalent, since in all but the germ cells each breaks up into a much larger number of smaller chromosomes. If therefore the latter corresponds to the chromosomes of other forms, in which tetrads occur (e.g. *Cyclops* or *Artemia*) the so called tetrad of *Ascaris* is a compound body, and the true process of reduction must be sought in the origin of the smaller elements of which it is composed, which are directly comparable with Sabaschnikoff's 'granules'".

Perhaps in my analysis of the compound tetrad figures in *Echinus* I may have shown the road to the solution of the contradictions, not by tracing the origin of the figure, but by resolving it into its finer elements during its later phases.

It would be tedious and unprofitable to attempt a complete recapitulation of all the cases described. In many forms, owing to the difficulty of obtaining an absolutely complete series of stages, the evidence is incomplete, while in others the minuteness of the chromosomes is a barrier to finer analysis. I have already referred to most of the well authenticated cases, and in the tracings which accompany these pages, a number of the figures from other types are given. From these it will clearly be seen that in the vast majority of cases, the heterotypical chromosomal forms are practically identical.

I must however refer in a brief but somewhat more

connected form to the facts as represented by the botanists regarding the heterotypical division. In the earlier days of investigation into the mitoses occurring in the pollen-mother cells of higher plants, Strasburger (1888) and Guignard (1891) described a longitudinal splitting at the beginning of each division, and in regard to reduction of the chromosomes, they did not find that the pollen mitoses differed from the process in vegetative cells.

Belajeff (1894) was the first to point out that the V shaped figures of the heterotype were not due to the rods or rings being curved progressively in their ascent to the poles of the spindle.

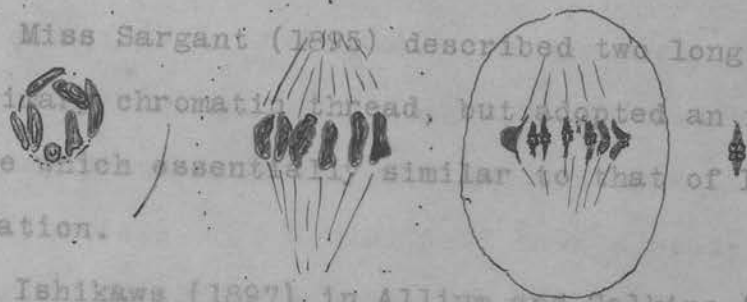
Farmer, (1895) as I have already said, first suggested the idea of a double longitudinal cleavage simultaneously progressing, but in his paper in conjunction with Moore, he elaborated the idea that the double rod of the prophase was produced by bending of the ring on itself and the fusion of the two halves. In the metaphase the rods were separated along the plane of fusion, so that only a single longitudinal cleavage was involved, and the separating elements were the original daughter chromosomes. He held that there was a longitudinal splitting of the chromosomes in the second division.

Strasburger (1895) gave an explanation involving two longitudinal cleavages, the second split completing itself in the anaphase preparatory to the second division.

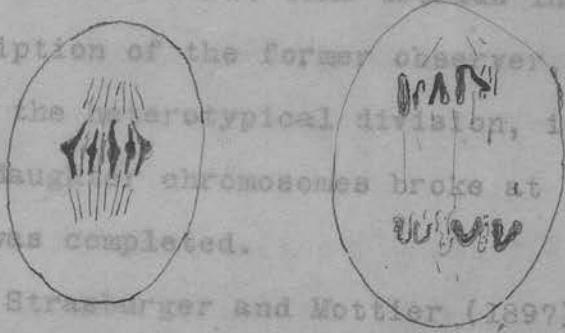
Dixon (1895) gave a somewhat different explanation, involving a longitudinal split taking place for the first time

in the metaphase.

Miss Sargent (1895) described two longitudinal splits in the primary chromatin thread, but adopted an idea of the heterotype which essentially similar to that of Farmer's second interpretation.

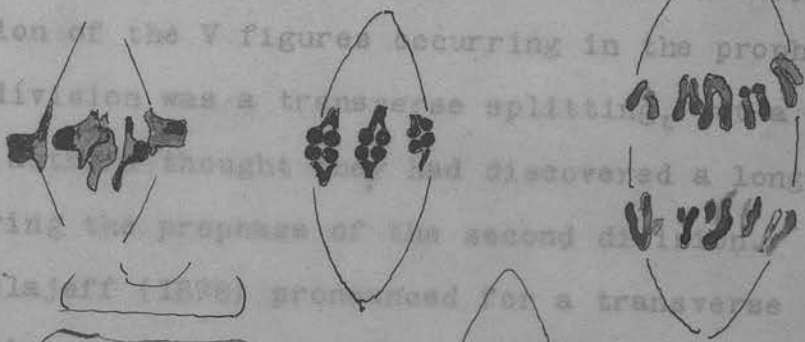


Ishikawa (1897) in Allium and Calkins (1897) in Pteris, described for the first time tetrads in plants. According to the description of the former observer, these tetrads were resolved in the heterotypical division, in such a fashion that when the daughter chromosomes broke at their apex, a transverse cleavage was completed.



Strasburger and Mottler (1897) under the influence of the Farmer of *Hermetium* in *Lilium Antheren* Flora Bd 180 1898

solution along the same plane admitted the possibility, that the separation of the V figures occurring in the prophase of the second division was a transverse splitting. A few months later these two thought and discovered a longitudinal division during the prophase of the second division.



Belajeff (1898) proposed of a transverse division in Weisemann's sense, but Guignard, (1898) for *Najas major*, returned to the division proposed by Strasburger in 1896 for the Lilies.



Partial return to rest

2 Mal. Sp.

Gregoire (1898) made a re-examination of the phenomena in the Liliaceae, and concluded for a double cleavage, the daughter V's in the first division being separated without further cleavage in the second.

The difficult point in the heterotype in higher plants-

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Strasburger and Mottier (1897) under the influence of the idea of the bending of the ring, and its subsequent resolution along the same plane admitted the possibility, that the separation of the V figures occurring in the prophases of the second division was a transverse splitting, but a few months later these authors thought they had discovered a longitudinal division during the prophase of the second division.

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The difficult point in the heterotype in higher plants -

a single longitudinal split being admitted in the prophases - is to account for the V shaped forms and their varieties.

Strasburger, after changing his ground several times, returns in his final pronouncement (1900) to his ideas of 1895 with some modifications. He finds, as I have already said, the V figures arise in two ways, according to the position assumed by the prophase figures on the spindle, but in every case a second longitudinal cleavage takes place.

An examination of his plates shows how remarkably closely the figures I have drawn for *Echinus* resemble, even in their details, those he has figured for the plant forms, and the general statement of his conclusion, is as remarkably in conformity with my own. He says (p.81) "The special peculiarity of the first nuclear division of the spore and pollen mother cells, which follows the numerical reduction of the chromosomes, consists in this, that the daughter chromosomes, which arise by a longitudinal splitting of the mother chromosomes, are inclined to a premature separation, and that they directly suffer a second longitudinal cleavage.

"The second nuclear division which follows on the reduction of the chromosomes has only the mission of distributing to the grand-daughter cells the grand-daughter chromosomes already produced in the first division.

The two divisions differ from ordinary mitotic division only in the double longitudinal splitting in the first mitosis, and the condition thus created for the second division". Again (p.99) "the pith of the heterotypical division lies in the two

longitudinal clefts, not in the form of the chromosomes."

"The cause of the two cleavages of the chromosomes so rapidly following one another, which again conditions the rapid sequence of the two nuclear divisions, must lie in the process of reduction which precedes the maturation division."

I have now established my own results, and shown how they stand in comparison with those of other observers. In conclusion I shall consider what light they seem to throw on the theoretical aspect of the question. The earlier theories as to the significance of the polar cells, such as Minot's and Van Beneden's sex-theory, and Balfour's hypothesis that the ovum has acquired the function of extruding the polar bodies to prevent the occurrence of parthenogenesis,- need not be more than mentioned, for they are inconsistent with the later facts discovered in connection with reduction of Chromosomes, and with such facts as (1) that the polar bodies have the morphological value of eggs, and (2) that they are equivalent to functional spermatozoa, and (3) that in spermatogenesis there is no loss of substance, though the Chromosomes are reduced in number.

The fact of numerical reduction of the Chromosomes in a generation of cells, preceding the union of the nuclei of the sexual cells, is, I believe, established. And it is very generally recognized that this is "a provision to hold constant the number of chromosomes characteristic of the species; for, if it did not occur, the number would be doubled in each succeeding generation through union of the germ cells" (Wilson p.243) How this provision is effected is, however, undecided. In other

words it is not yet known whether this reduction in number involves only a reduction in bulk, or a reduction also in the mass of the chromatin and further whether the reduction in mass is merely a quantitative reduction, involving a similar distribution of chromatin elements in the maturation mitoses, or involves at the same time a qualitative reduction, or dissimilar distribution of chromatin elements, representing specific qualities in the hereditary substance. The theories of Weissmann and Oscar Hertwig both relate reduction directly to fertilization and hence to heredity. Both regard the chromatin as the hereditary substance, and both are agreed that reduction, however produced serves to prevent the summation of the hereditary substance in successive generations. I have shown that there is reason to believe, that no unequal division of the chromatin elements takes place during maturation, and therefore there is no dissimilar distribution of hereditary qualities. Hence, as I think, Weissmann's theory of reducing or qualitative, divisions must be definitely given up.

On the other hand Hertwig's conception of a mass reduction, caused by the omission of the growth period in the resting stage between the two divisions, might be true for Echinus. But it does not explain the omission itself of the resting stage, nor the peculiar character of the chromatin phases from the synapsis onwards.

These earlier phases would thus have only a physiological significance, and would condition the reduction; whereas, according to the view I adopt, with Strasburger, all the features of

the maturation mitoses, are conditioned by the primary reduction already occurring in the synaptic phase.

Again, many cases are known, in which a resting stage does intervene, and, in the Cryptogams the whole sexual generation possesses the reduced number of chromosomes, while, in Cyclops, Haecker found the reduced number in the progenitors of the sexual cells already differentiated in the blastula stage.

Thus we fall back upon the idea of reduction in bulk, which underlies Boveri's conception. His original statement left undetermined the cause of this reduction in bulk. We may look upon the process as purely physiological, but I have shown in the foregoing pages, that the theory of plurivalency of chromosomes provides for it, a simple and tenable explanation.

It is certainly a fact that, after the resting stage, the same number of chromosomes emerge from the nucleus as entered it from the previous mitosis, even without going so far as to admit an individuality of the chromosomes from one cell generation to another.

The evidence for such individuality is to be found in Van Beneden's original discovery of the independence of the male and female chromatin threads in the fertilization of *Ascaris*, which, confirmed later by Boveri, has since been extended by Herla and Zoja, up to the twelve-cell stage.

Further evidence is contained in Boveri's observations on abnormalities in the fertilization, also of *Ascaris*.

Again, Rückert and Haecker have demonstrated the same for Cyclops. Rückert discovered a double character of the

nucleus even up to the formation of the germinal layers.

In the Sea Urchin, however, as in most other forms the union of the sexual nuclei takes place in the reticular stage, and all trace of separate chromosomes is lost, though, for a considerable time, the sperm chromatin retains its independence. Yet even here there are indications of the truth of the law in its general statement. Boveri (1893-95), and Morgan (1895) were able to rear larvae from fertilized enucleated fragments of sea urchin eggs, and the nuclei always contained half the typical number of chromosomes.

Richard Hertwig (1896) also observed 16 to 18 chromosomes emerging from the nucleus of unfertilized sea urchin eggs. I have myself counted 16 chromosomes entering, so that some correspondence, between them, is suggested.

The instance of the parthenogenetic development of *Artemia*, described by Brauer, (1893) has a still more direct bearing on the point at issue. There are two types of development in this form. In one, first polar body carries 84 dyadial chromosomes with it, 84 similar bodies being left within the egg. An attempt at a second division then occurs, but is not completed, and a reticular cleavage nucleus is formed, from the 84 double chromosomes.

The cleavage nuclei all contain 84 chromosomes; and that number is also characteristic of the nuclei in the later stages of the individuals developing in this way. In the other type a second polar spindle is formed in the usual fashion, and the 84 double chromosomes give rise to two groups of 84 single

chromosomes. Both these groups remain in the egg, and become reconstructed into two vesicular nuclei. These arrange themselves side by side, and become resolved, in the first cleavage spindle into two groups of 84 chromosomes, 168 in all. Each of these divides longitudinally, and the daughter chromosomes are distributed as in ordinary mitosis. Thus 168 chromosomes enter the daughter nuclei, and again in their descendants, the same number persistently re-appears. I have suggested that in *Echinus* the reduction may be considered as only apparent throughout, each element being supposed bi-valent. The process involves a reduction in bulk only, brought about by an unusual condensation and tendency to adhesion of the chromatin elements, as evidenced by the phenomena of the Synaptic phase, the formation of ring chromosomes, and the condensed tetradal figures. It may be that this condition of the chromatin has only physiological significance, indicating a re-combination, or re-distribution of the Chromatin particles preparatory to fertilization; but in the light of the morphological principle that the same number of chromosomes always emerge from the resting nucleus as entered it, I think it justifiable to add to this purely physiological conception, one that is morphological in its bearing. The end of maturation is to maintain constancy in the number of chromosomes, and this end is attained by the physiological alteration expressing itself in the form of the chromosomes. In other terms, the chromatin thread segments into half the number of rods characteristic of the nuclei of the somatic cells, but each of these rods represents two elements,

providing for a change of form without loss of substance, and that the features of the maturation mitoses are secondarily due to this phenomenon.

This is similar to Hertwig's conception of mass reduction, except that I do not see the necessity for supposing an actual diminution in quantity of the hereditary substance, as if it were so much ponderable material.

It is on this point perhaps that the idea of apparent Reduction may be attacked from the side of theory. If the Chromatin has a fixed morphological constitution, and is identified with the hereditary substance; if the hereditary substance is handed on in an altered mass from one generation to another, and there is an equal contribution of that substance from each of the germ nuclei in fertilization, then in terms of matter a reduction in mass is necessary in thought. Further, if there is a direct continuity of the germ cells then the ovum must contain an equal quantity or mass of the hereditary substance as the fertilized ovum from which it sprang, for the chromatin is equally divided and equally distributed in mitosis.

But here the facts at once break down the identification of the chromatin as the hereditary substance *sensu stricto*, for in the ovum the greater part of the chromatin is rejected as chromosomal material. Further the chromatin is revealed as an active physiological agent, it is increased in mass for a definite physiological end, and part of it disappears as such when that end is attained. It seems to follow that reduction

in mass is not required in thought for the chromatin, but only for the hypothetical hereditary substance. It does not follow however that the chromatin is not the chief agent in hereditary transmission. I believe it is so but in virtue of its physiological not its morphological constitution.

I must lastly show how this relates to another conception of the significance of reduction. It seems that reduction is not always closely related to fertilization in time. Haecker, for instance, found in Cyclops that the whole generation of sexual cells differentiated already in the blastula stage, possess the reduced number, and among plants the nuclei of the whole sexual generation in Cryptogams have half the number possessed by the nuclei of the asexual generation.

Various attempts have been made to explain the process on phylogenetic grounds. Whitman (1875 p. 262) interpreted the formation of the polar bodies as a relic of the primitive mode of asexual reproduction. Giard (quoted from Hertwig, 1890) regarded the ovum as behaving like a Protozoon, and repeating the protozoan stage in the evolution of the Metazoa.

Neither of these conceptions takes account of the process of reduction as now understood. Strasburger (1894) enumerated the theory that the generation of cells, with the reduced number of chromosomes represents a separate generation, now a mere remnant included within the body. This generation from analogy with plants, is the sexual generation, and the reduced number of chromosomes is the ancestral number, belonging to the ancestral type, before sexual differentiation took place.

The somatic number of nuclei arose with the union of the chromosomes of the germ cells.

Beard (1895) followed this up on his own line in his theory of the "Antithetic Alteration of Generations".

I shall not attempt in this essay any detailed criticism of these theories as applied to the Metazoa, for I should be led away from my main purpose into a general discussion of the larger problem of fertilization with part only of which I have professed to deal.

I shall refer only to one point, though it is the main one of Strasburger's hypothesis which bears directly on my results, and the suggested explanation of them.

According to his conception, the reduced number of chromosomes is the primary or ancestral number, whereas all I have written makes it obvious that if there is any foundation at all for my suggestion, the larger or somatic number is the primary - the reduced the secondary or acquired number.

This is the view adopted by Hertwig and it is certainly supported by the fact that apparently in some Protozoa - reduction takes place before, in others after conjugation.

Again the inconsistency in the number of chromosomes in closely allied species, speaks against there being phylogenetic meaning in their mere number - for instance in *Echinus microtuberculatus* according to Boveri there are 9 in the Maturation nuclei and 18 in the nuclei of the somatic cells - while I find - 16 in the Maturation nuclei, and 32 in the cleavage stages.

Are we to imagine that natural selection has brought about this difference for some unknown physiological end? To do so would be to attach an importance to the mere number of Chromosomes which our present knowledge does not justify.

On the other hand, the number of chromosomes being established it is of importance that it should not be indefinitely multiplied, in successive generations - indeed such a possibility is unthinkable. Therefore it does not seem unreasonable to suppose that - granted the principle that the same number of chromosomes emerge from as entered into the resting nucleus - such a form of reduction as I have shown possibly occurs in Echinus, may have ^{been} secondarily acquired through natural selection, to serve the physiological end of maintaining constancy in the number of chromosomes.

Thus though the suggestion of 'apparent reduction' may be at variance with Strasburger's phylogenetic hypothesis, it is perhaps not thereby deprived of phylogenetic significance.

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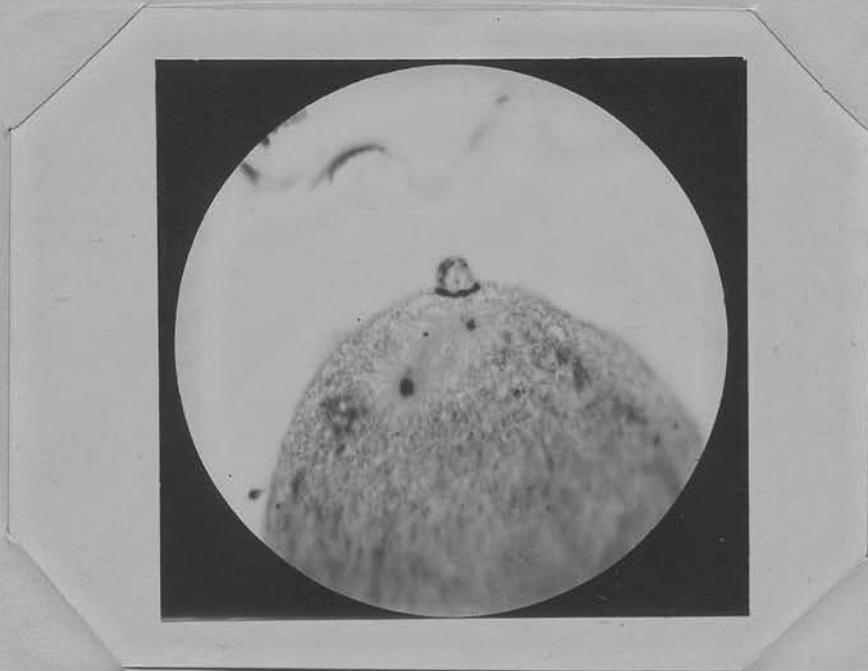
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Fig. I

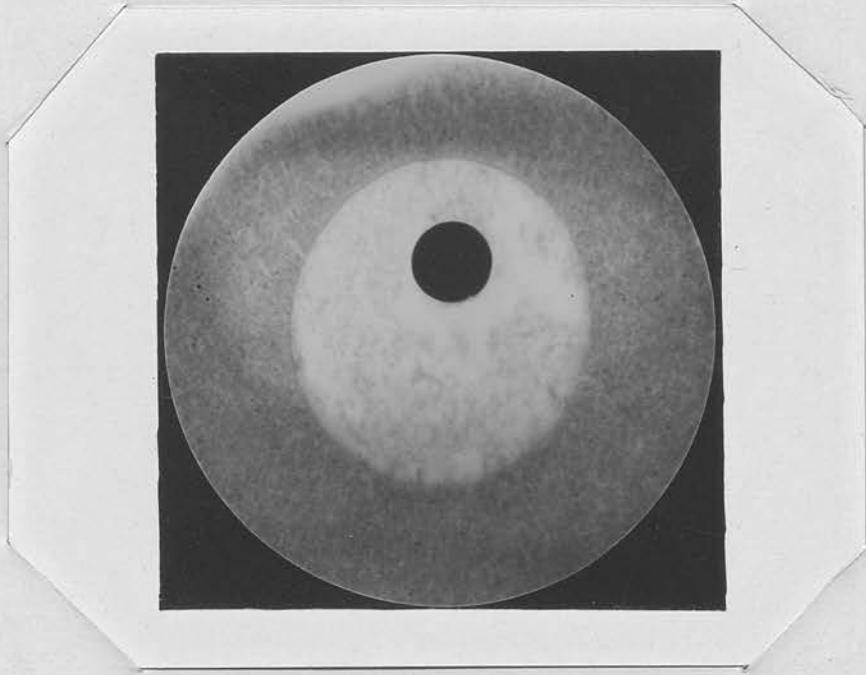
Young ovum - showing granular Protoplasm
vesicular nucleus - Nucleolus only taking chromatin
Stain x 1200 D

Fig. II



2nd Polar Spindle & 1st Polar Body - showing Centrosomes
deeply stained from insufficient washing out of the
Iron Haematoxylin stain - Shows also the colour retained
between the ovum & Polar body - Sublimis-acetic preparation

Fig. III A

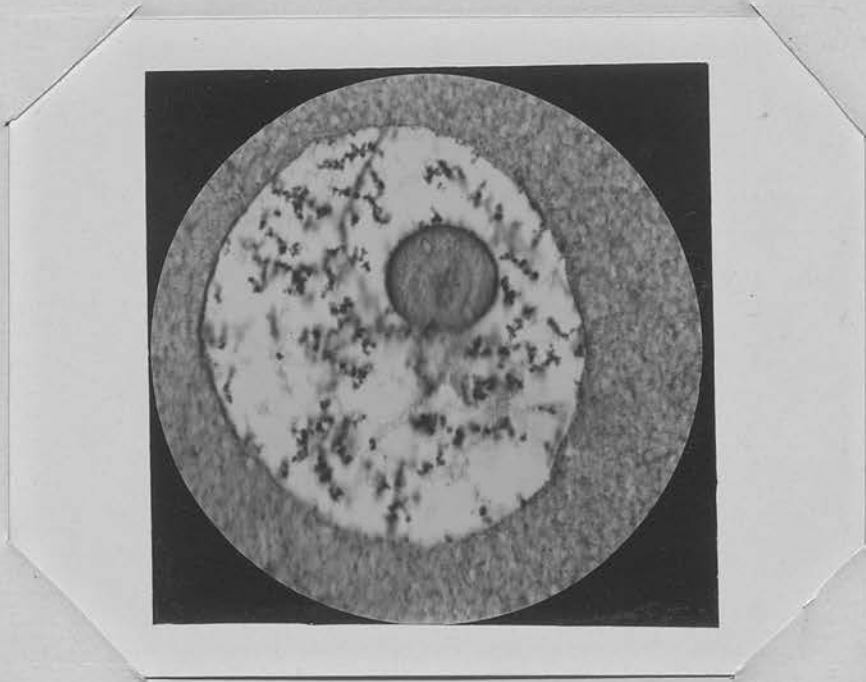


X. 780 D.

Fig. III B

X. 1000 D.
Germinal vesicle showing intensely stained
Nucleolus - and Nuclear Reticulum entirely

Fig IV A



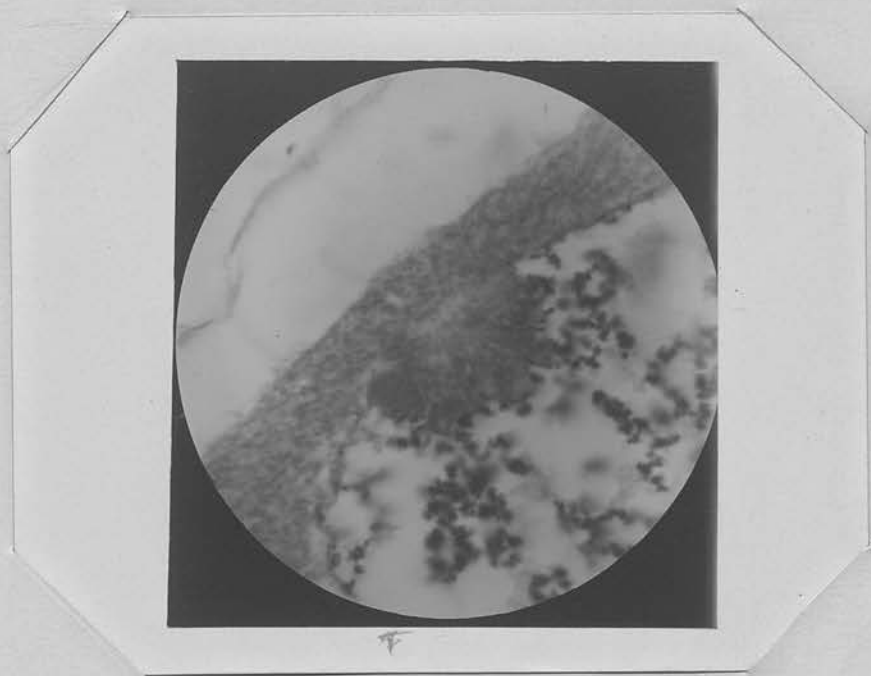
X 480

Fig IV B

X 950 D

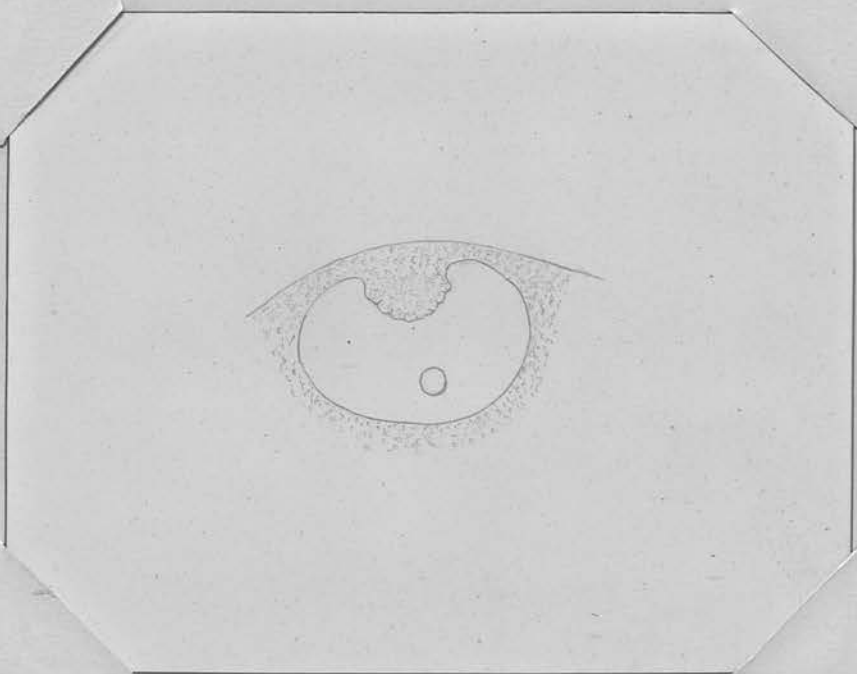
Germinal Vesicle at end of growth period.
Nucleolus has lost its capacity of taking the
chromatin stain in such measure - Nuclear
Reticulum now beset with deeply staining

Fig V



x 1240, 3

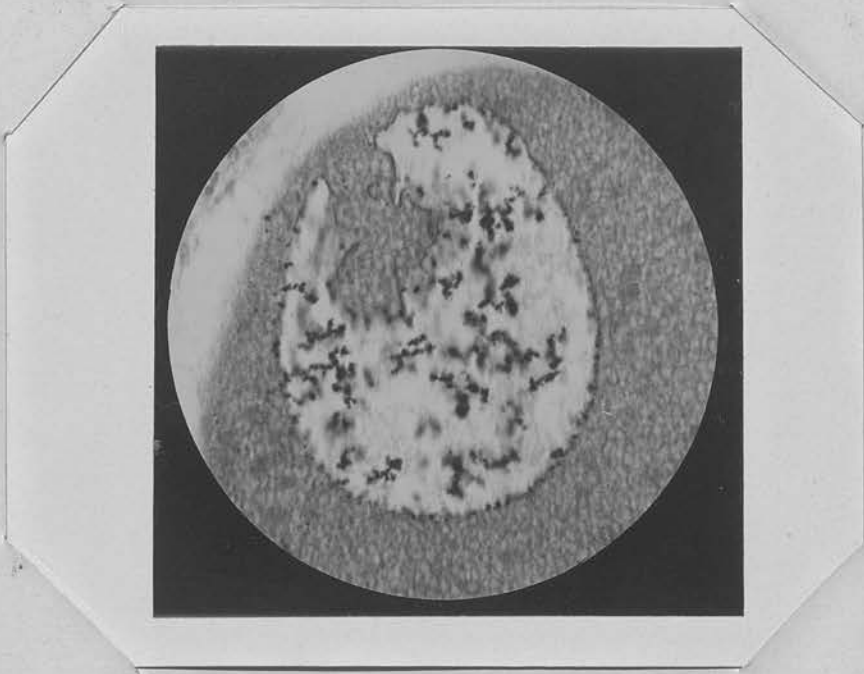
Fig VI



x 350 -

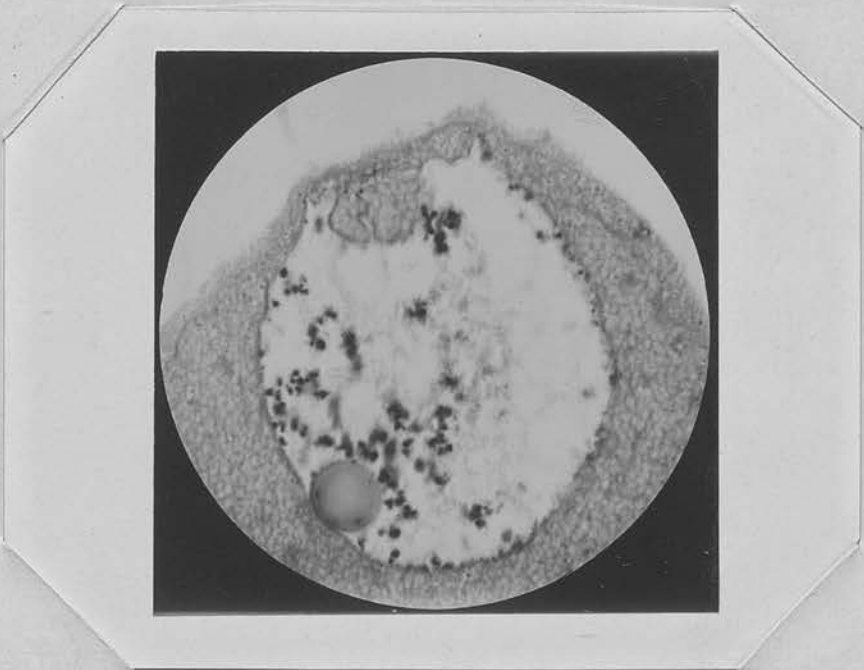
Drawn from the living egg.
 Invagination of Germinal Vesicle -

Fig VII A



X 780

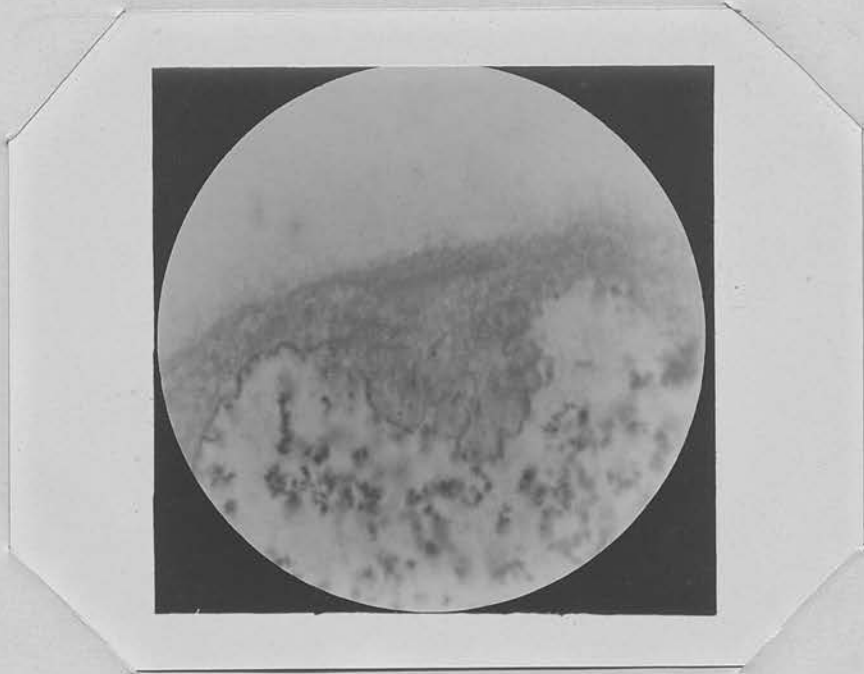
Fig VII B



X 750

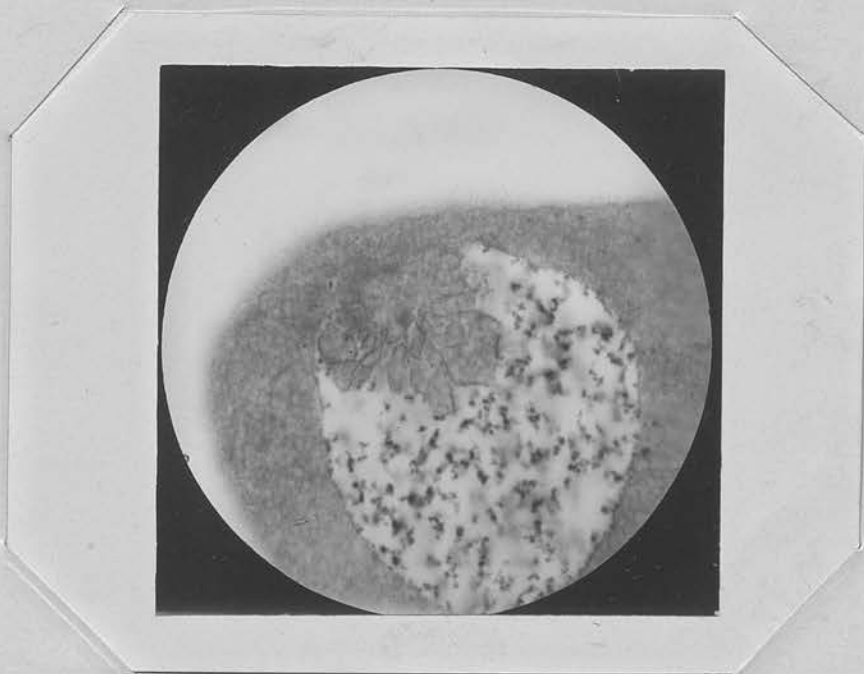
Invagination of Germinal Vesicle.

Fig VII c



X 1240 μ

Fig VIII



X 725 μ

Invasion of germinal vesicle

Fig. IX

X 425

In magnification of the terminal vesicle in the center of the mass is seen a dark granule round which there is a general radial arrangement - which may be a centrosome

Fig. X

X 1200

Two adjoining sections of the same worm showing the chromatin which is destined to form the chromosomes collecting in an irregular thread like mass, at the neck of the invagination. In A the nucleolus is seen.

Fig. XI

x 960 D

Transitory of Germinal vesicle

Fig. XII

x 950 D

Transitory of Germinal vesicle later

Nuclear membrane has disappeared.

Chromatin destined for chromosomes

concentrated into an irregular mass.

Fig. XIII

X 1200 $\bar{\sigma}$

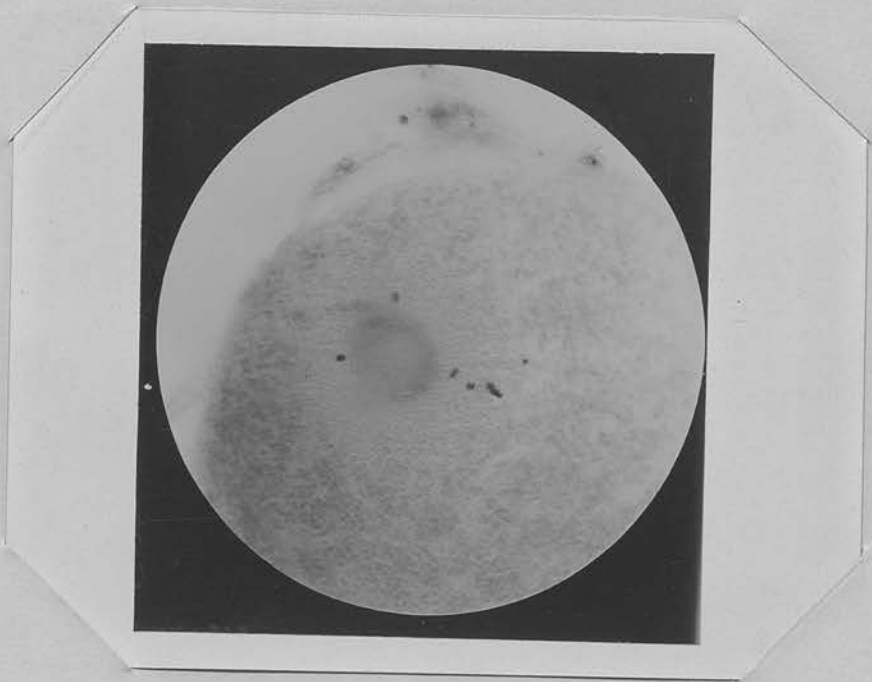
Two asters & reticular area - tetrads
not drawn into area between asters.

Fig. XIV

X 1200 $\bar{\sigma}$.

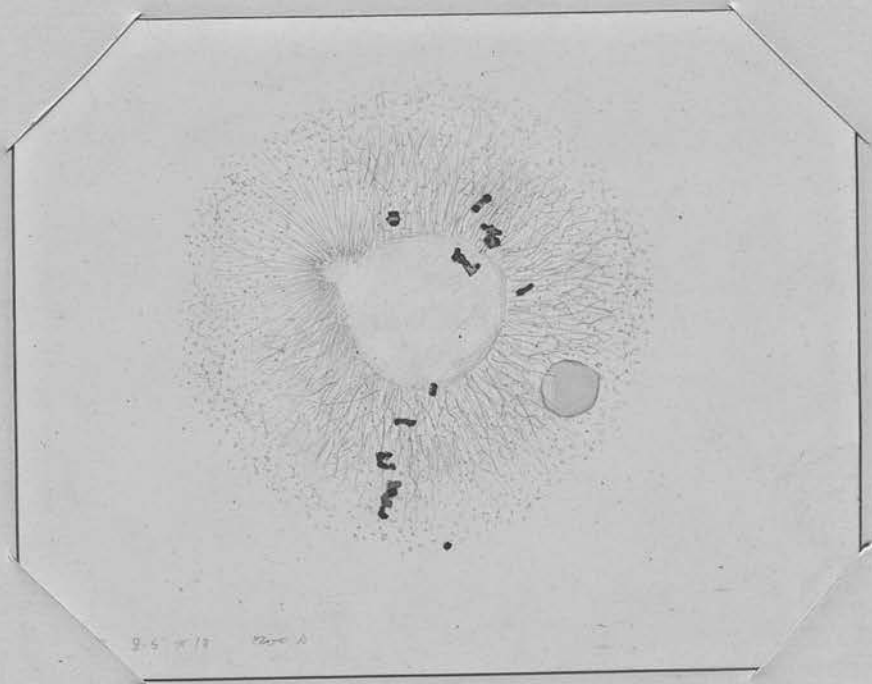
Tetrads in central plate surrounded by
reticular zone, one aster in section.

Fig. 15



x 225

Fig 15a



x 1200 D

Central plate - with reticular zone
one aster - the nucleolar was in the
adjoining section to that photographed
Irregular masses of chromatin not yet
drawn into central plate.

Fig 16

X 725 D.

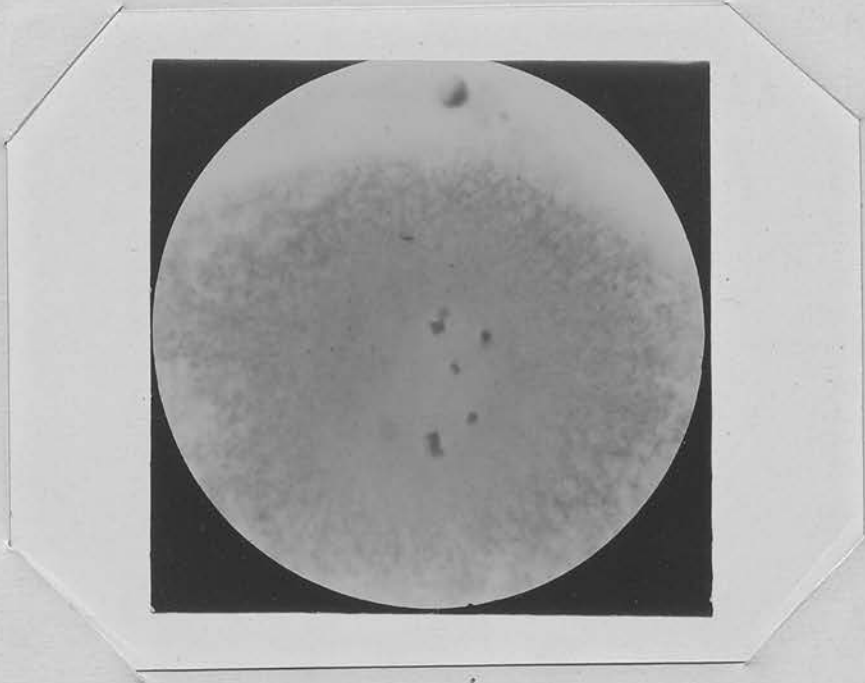
Fig 17



X 725 D.

Tetrads in central plate surrounded by reticular zone. Fig. 17 is seen the remains of the Germinal Spot.

Fig 18



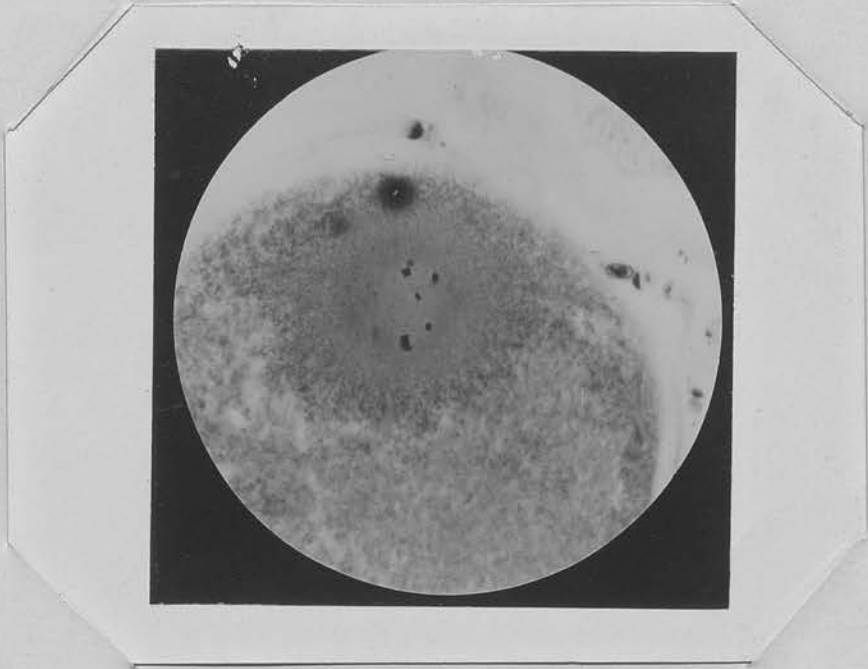
X 1200 D

Fig 19

X 1200 D

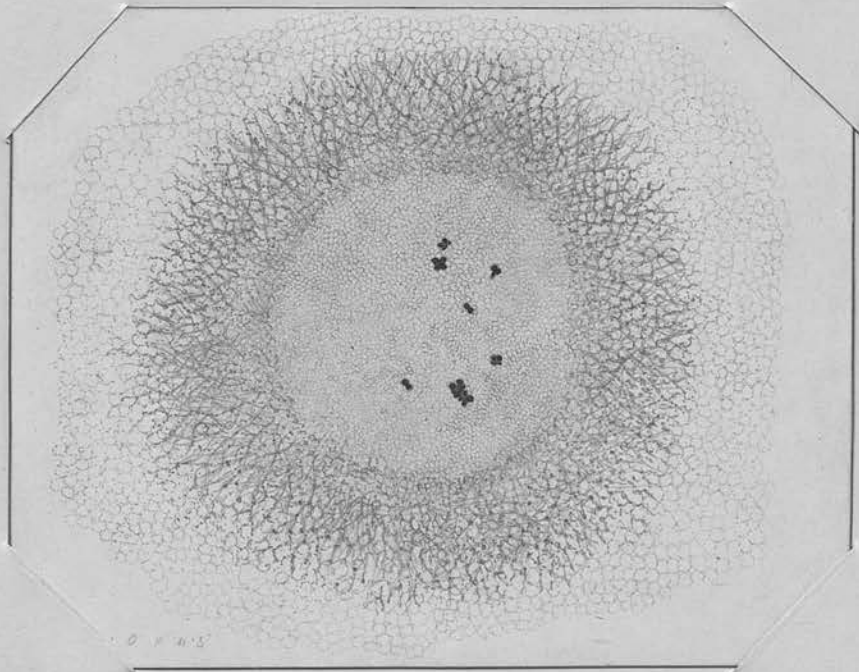
Tetrads .

Fig 20



X 725 D

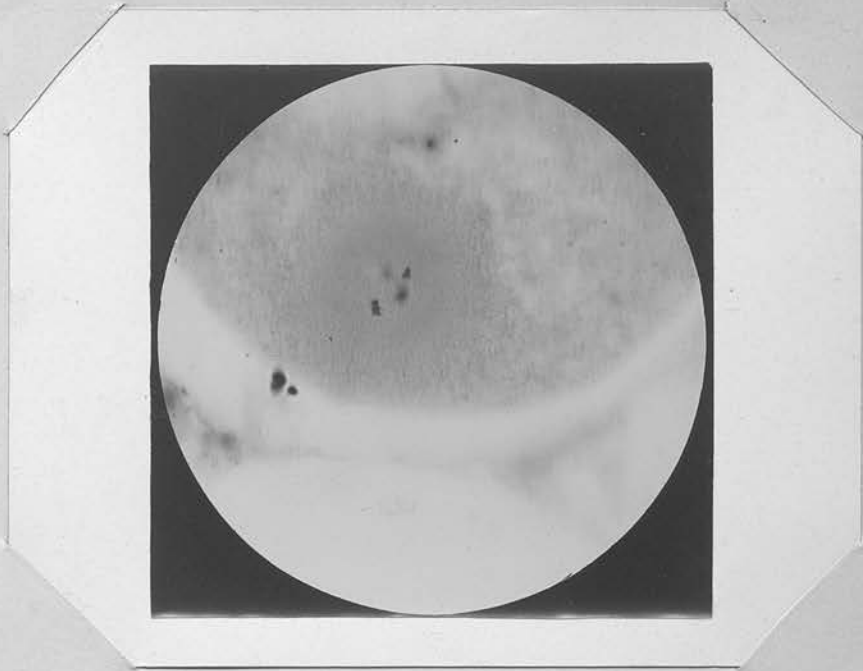
Fig 20A



X 1200 D

Tetrads

Fig 21 1



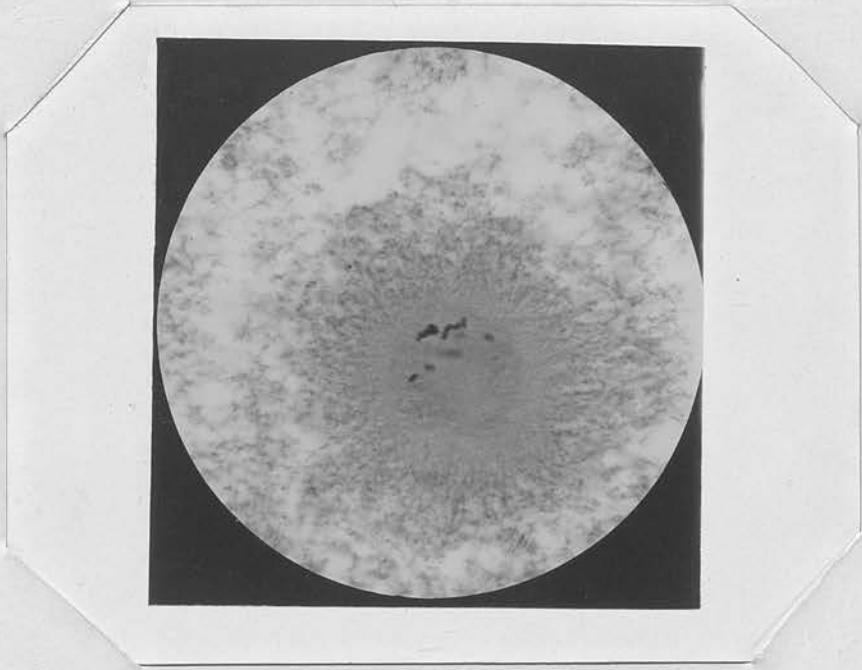
X 725
~~1560~~ D

Fig 21 A

X 1560 D

Portion of thread longitudinally split
showing that is composed of
rounded spheres joined by a less deeply
staining portion.

Fig 22



X 725 0

Fig 22 A

X 1200

Prothare figures tetrads when seen
en face - dyads when seen in profile

Fig 23



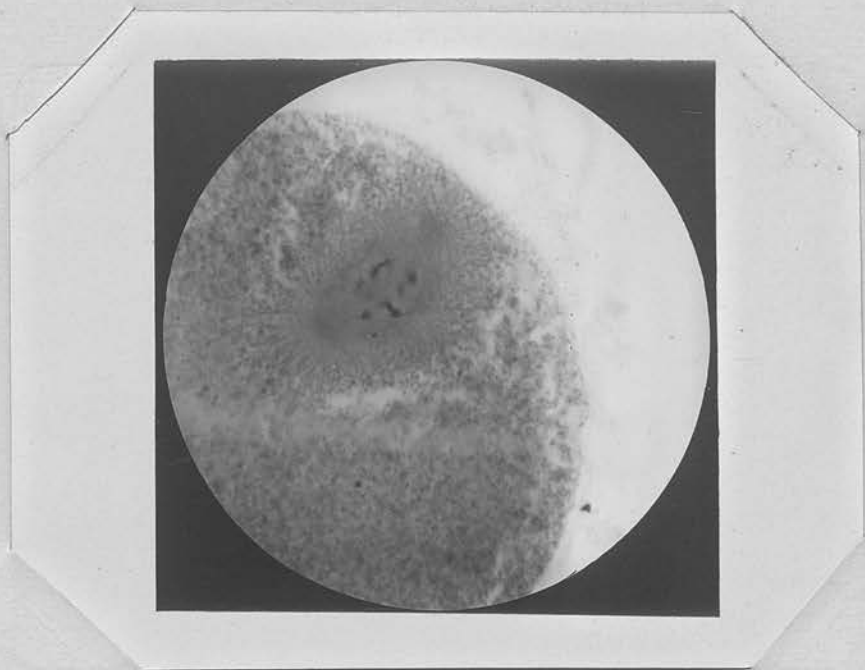
X 1240 D

Fig 23A

X 1500 D

Tetrads in central plate - surrounded
by the reticular zone.

Fig 24



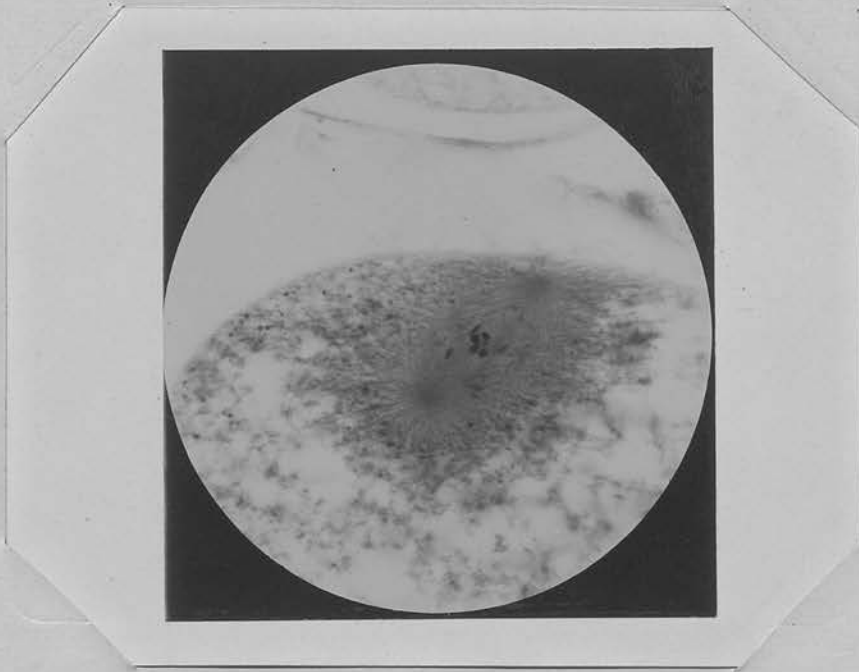
x 720

Fig 24a

x 1200 0 .

The centrosomes are seen in a radial position - the spindle is not formed, the chromosomes are being collected into the equatorial plate.

Fig 25



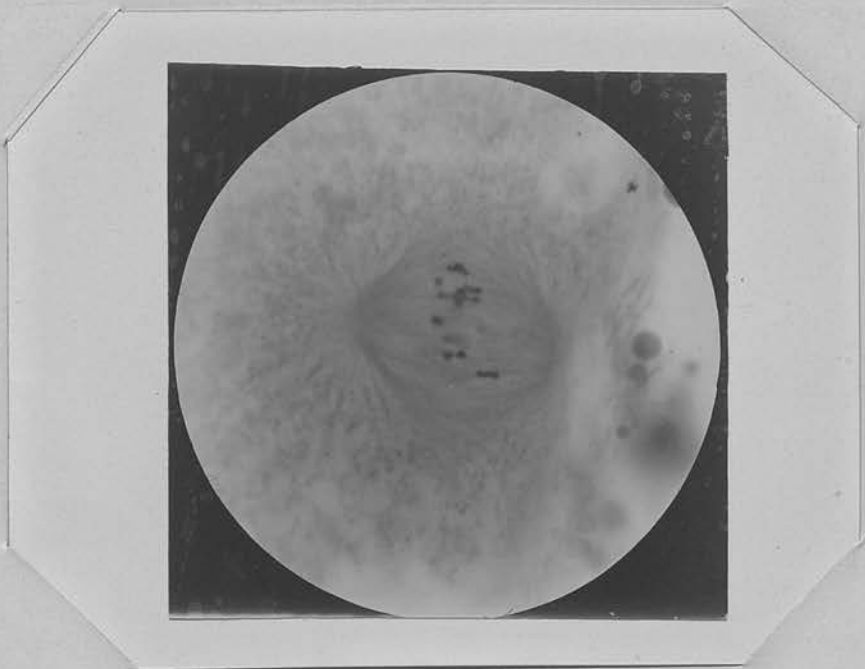
x 725 δ

Fig 25a

x 1700 δ

Early stage of 1st Polar spindle

Fig. 26



X 1240 D

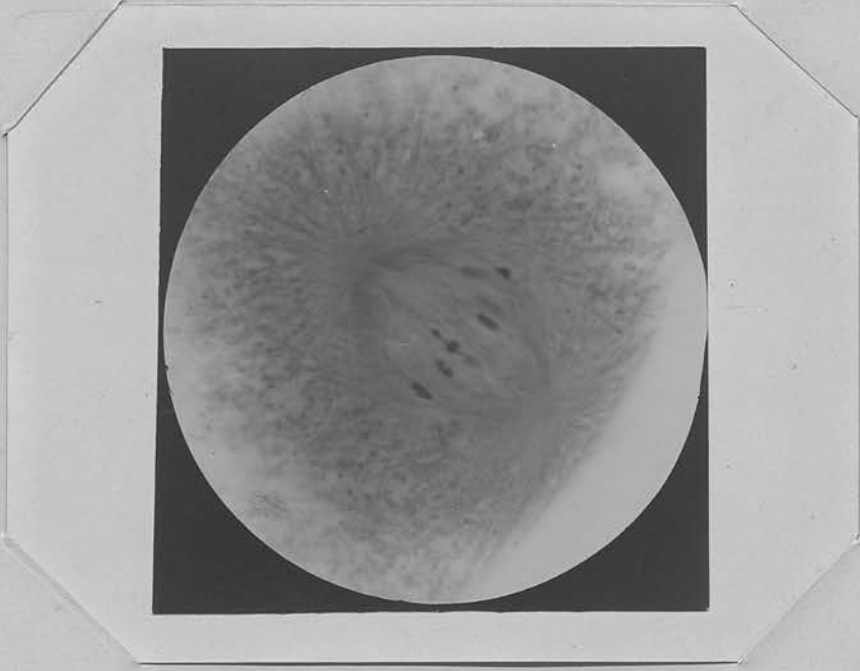
Photograph & engraving of
transverse section of 1st Polar protuberance

The structure of double corona - figures are
very distinct - and the double and
occasional double V figure is very clearly
defined.

Fig. 26a

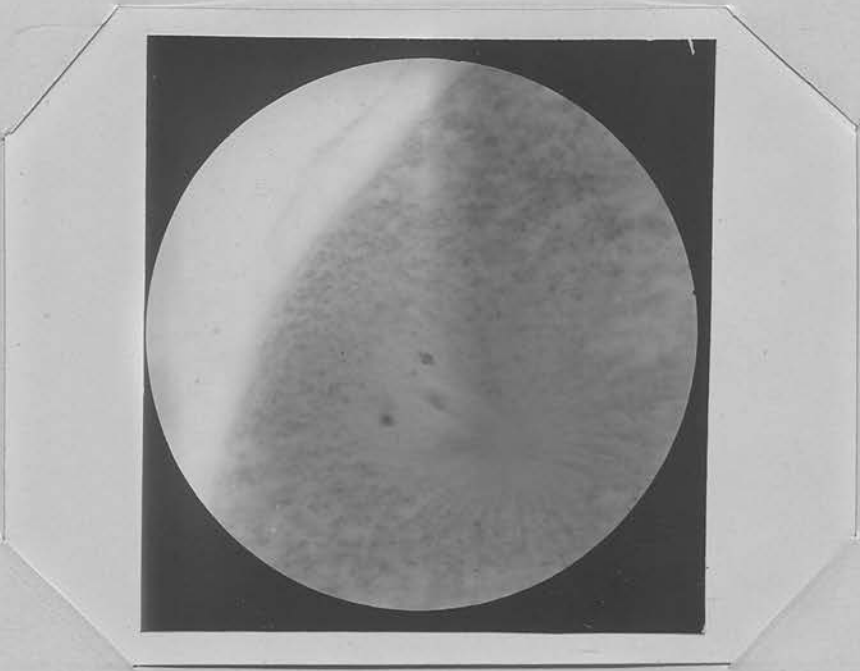
X 1200 D

Fig. 27



X 1240 \times

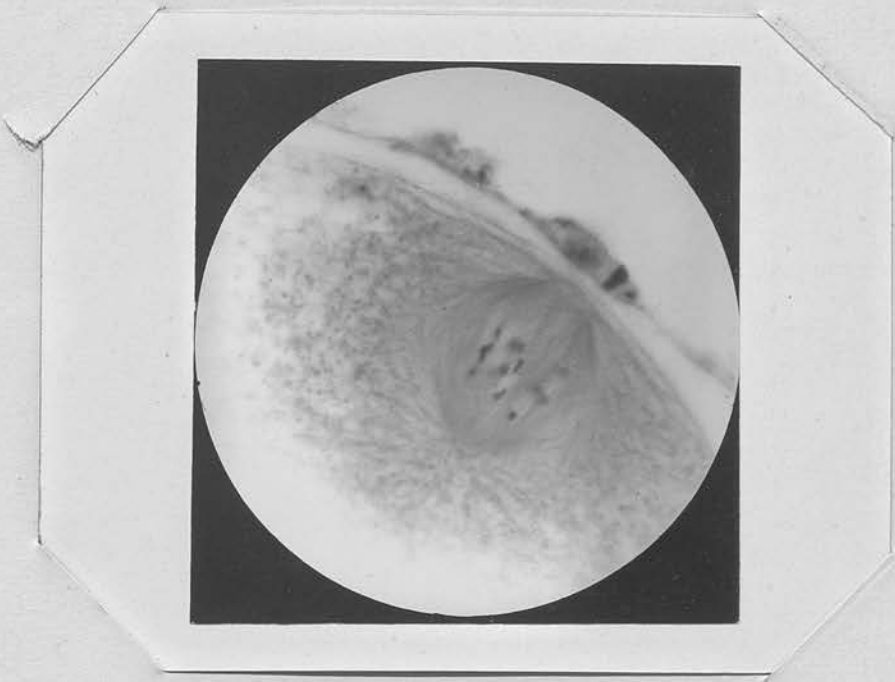
Fig. 28



X 1200 \times

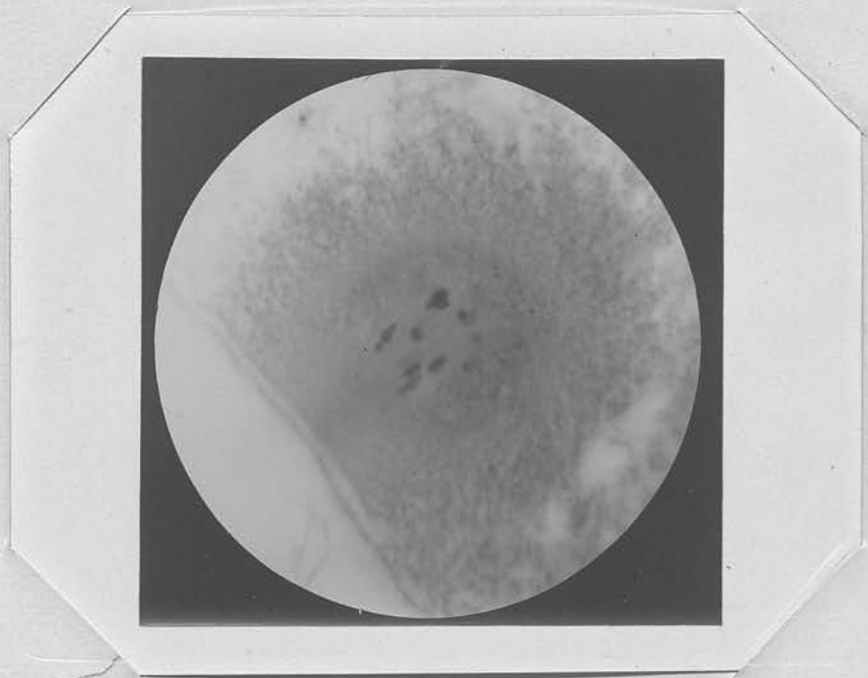
Photographs of 1st Polar Sporophore
to show the different regions

Fig 29



X 1260 D
1st Polar Mithras

Fig 30



X 1260 D

The 2^d polar Mithras - showing
the various regions observed.

Fig 31

X 1500 D

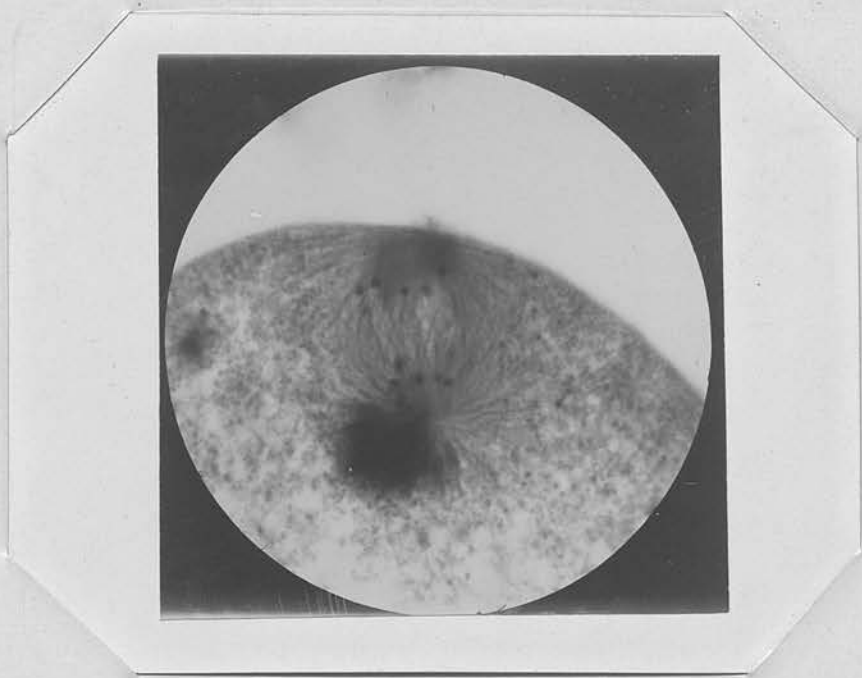
Diagrammatic representation of the 1st Polar spindle
in Metaphase. All the figures are accurately drawn,
but made to appear on the same plane.

Fig 32

X 1500 D

In this figures all the figures observed
have been collected from a large number
of sections - and arranged in series -
The Profile views show the opening up of the primary
longitudinal split - The face views show the secondary split.

Fig. 33



x. 1240 D

Fig. 33A

x. 1200 D
 Anaphase of the 1st Polar Spindle
 Photograph and drawing of the same ovum.

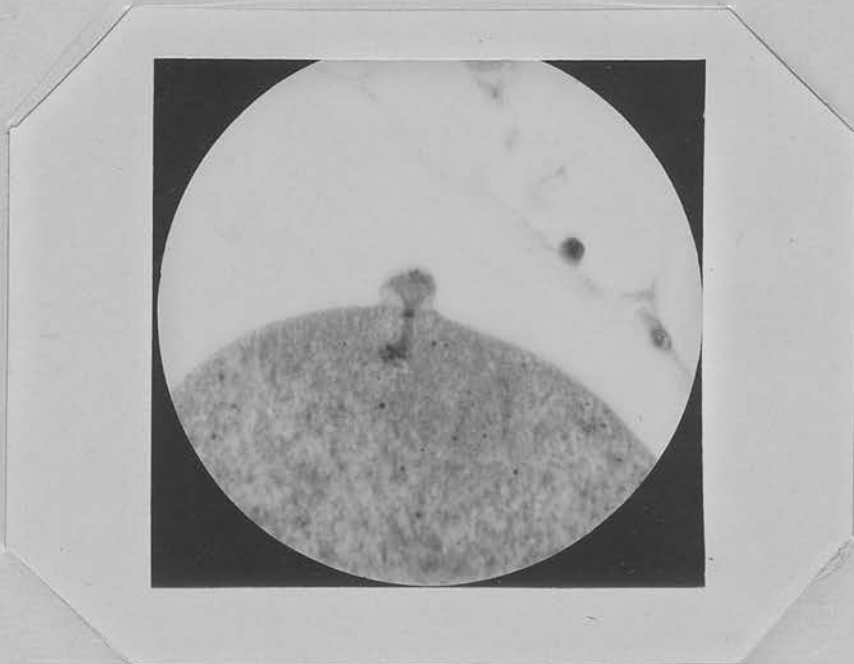
The compound character of the chromosomes is
 distinct in the photograph - especially in the one
 sharply focussed viz. the second from the ^{left} end
 of the outer row.

Fig 34



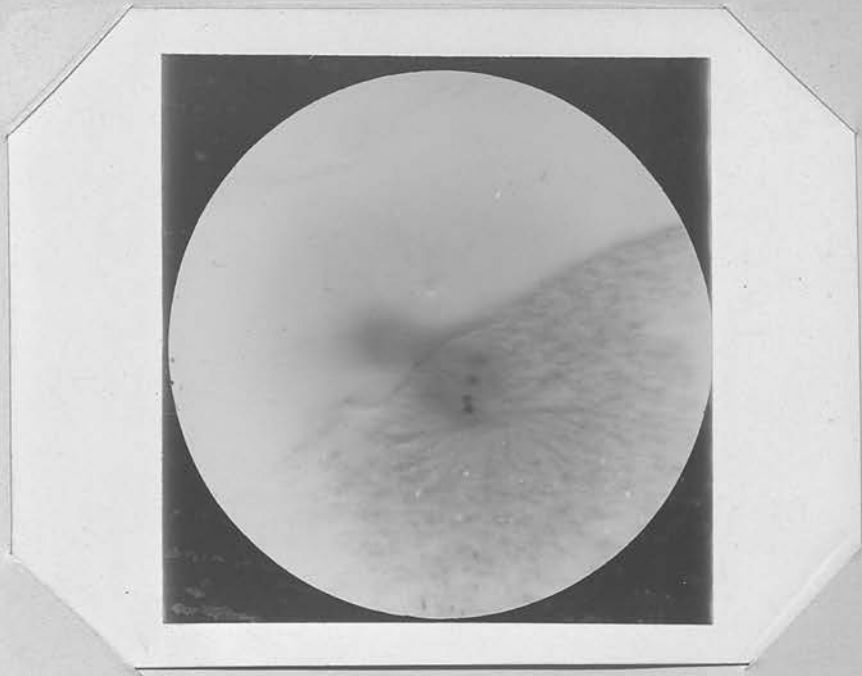
X 725 Δ
Extension of the 1st Polar body.

Fig 35



X 725
Extension of 1st Polar body showing
ring zona shell = Zwischenkörper

Fig 36



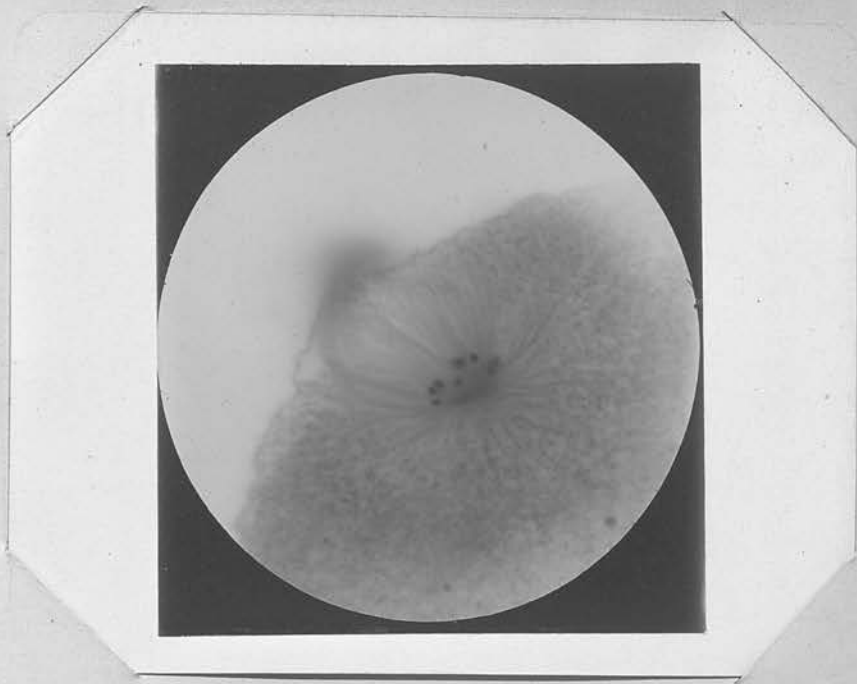
X 1240

Fig 36a

X 1500 D

Photograph and drawing of same worm photographed in Fig 34
The umbilic nature of the chlorosomes can be quite distinctly apprehended
in the photograph - The eye equ readily resolves them as drawn

Fig 37



X 1240 D

Photograph of central aster in 1st polar
telophase

Fig 37a

Drawing of ^{X 1150 D} an aster in same stage as above
Central aster after extrusion of the
1st polar body. showing the small double rod-
chromosomes - These are not ^{been} fully resolved by the
photographic plate, but the compound character of
the minute masses can be made out.

Fig. 38

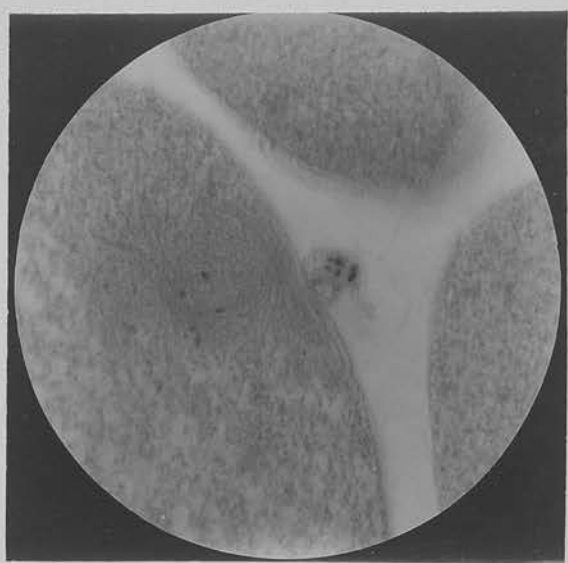


Fig. 38a

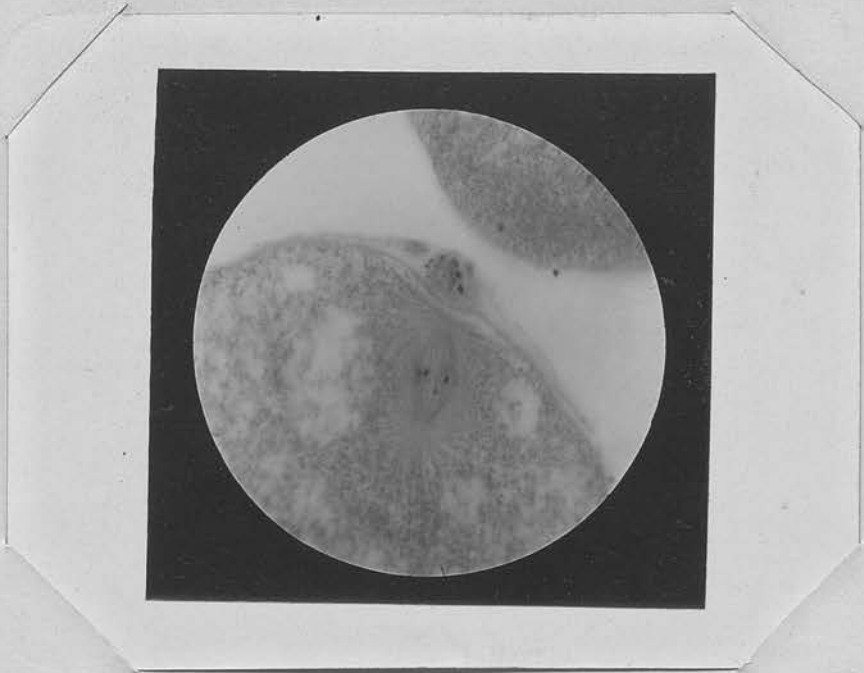
X 425 D

Early stage of 2nd or Polar Spindle

X 1200 D

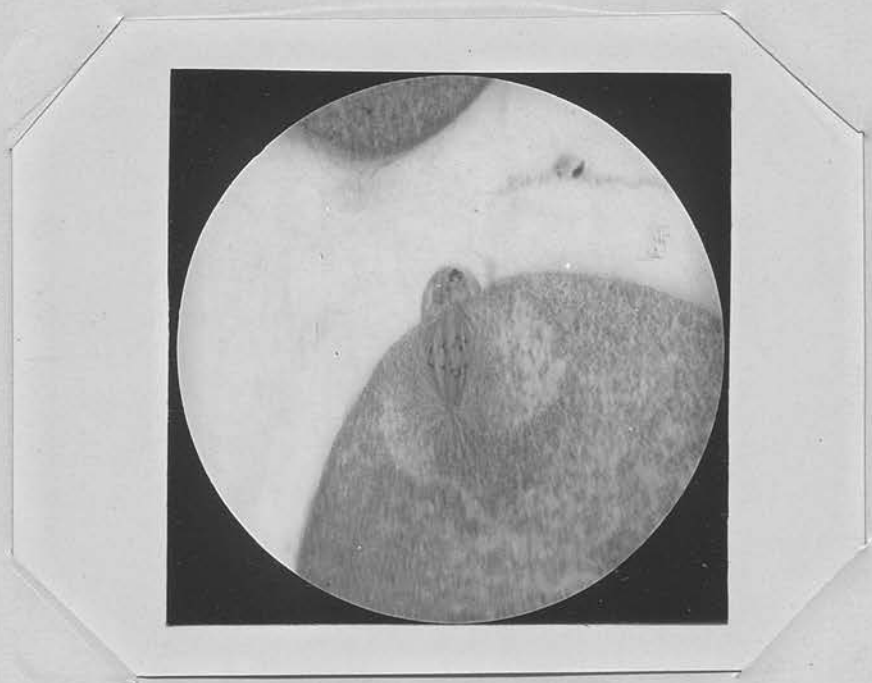
1st Polar body shows the double-rod chromosomes - Remaining of the spindle
 The acetylsaccharine ring structure body seen between same poles body in the Translucent region
 The Spindle is not yet formed. The spindle rays
 are longer where spindle forms the chromosomes, & are
 seen distinctly in the discharging. Chromosomes have coarsely
 the form seen in 1st polar chromosome. There is no selection of plate
 of the medium between the two divisions.

Fig 39



X 425

Fig 40

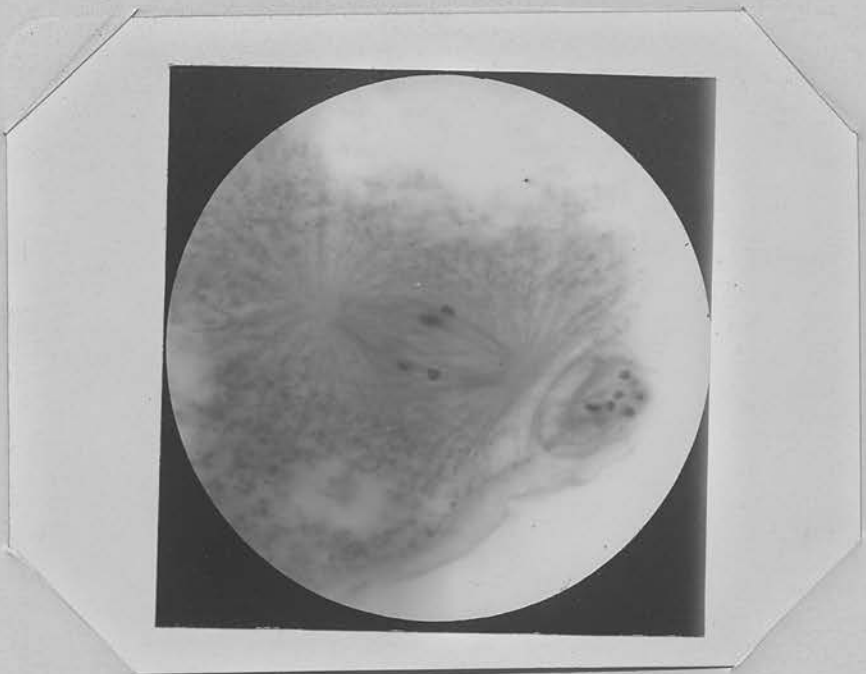


X 425

Photographs at relatively low magnification
 of metaphase and anaphase of 2nd polar spindle
 to show general features.

Fig 41

Fig 41 a



X 1240 B

X 1200 D

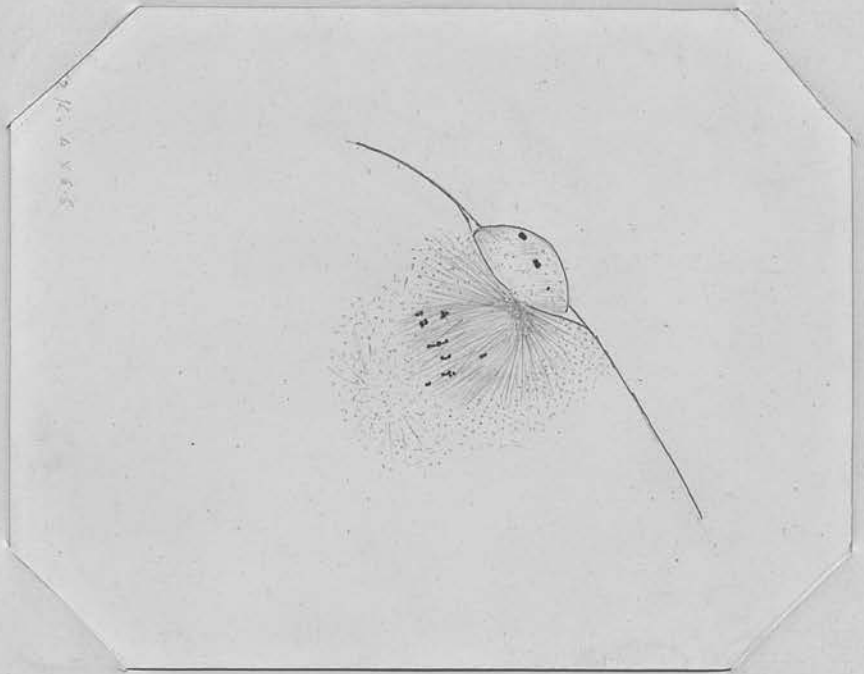
2nd Polar spindle and 1st Polar body.

1st Polar body shows the double rod or tetrad chromosome

2nd polar spindle in metaphase shows the double rod chromosome which have a characteristic appearance - each consisting of two thick curved rods

located side by side - and in this section the rods in the center look horizontal

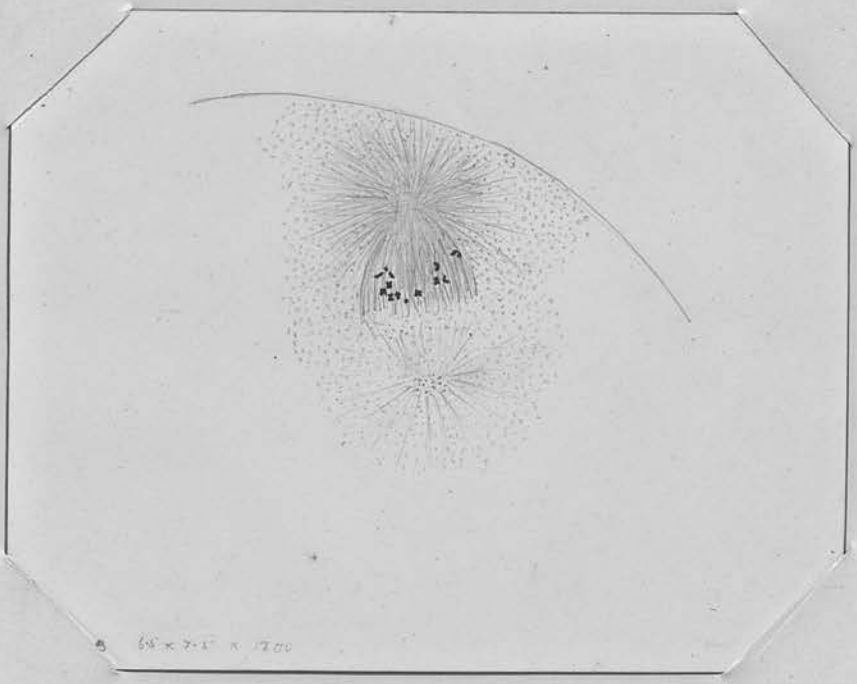
Fig 44



x 1200

2 K. 4 x 8.5

Fig 45



x 1200

201 x 1.2 x 39

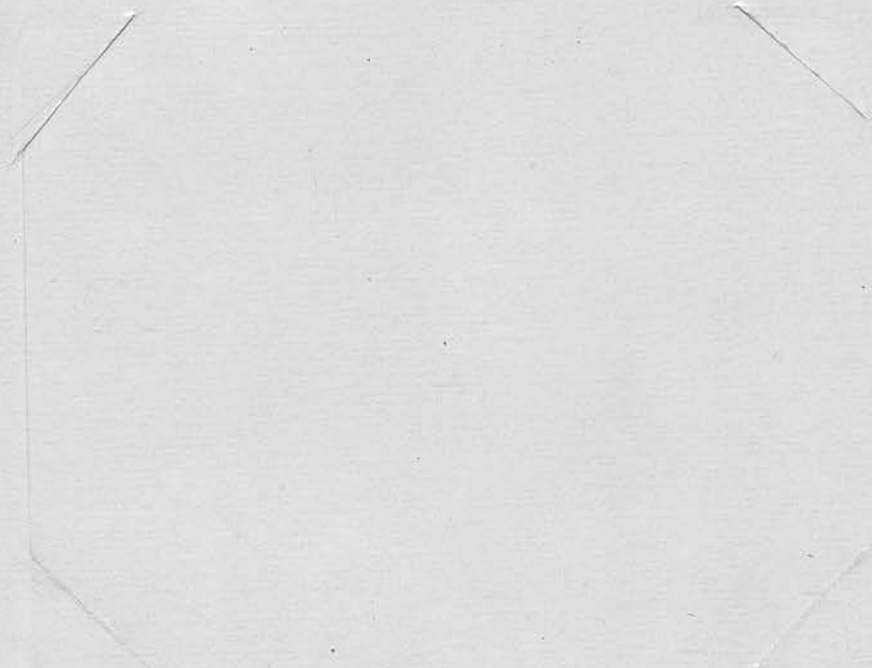
Two somewhat oblique sections of
 the 2nd Polar spiracle showing
 the acute rod form of the
 chrysosomes - and manner in
 which they separate.

Fig 46



X 1240 D

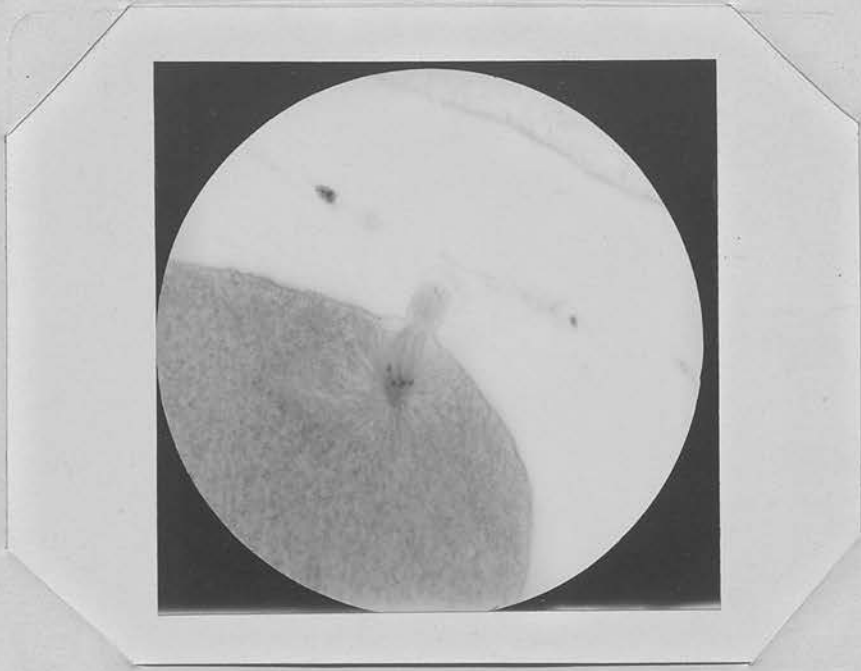
Fig 46a



X 1200 D

Early anaphase 2nd polar spindle.
 In polar body shows the double rod chromosomes
 outer centromere & rays disappearing -
 chromosome in spindle in the form of short
 slightly curved rods.

Fig 47



x 475 D

Fig 47d

x 1200 D

Extension 2nd Polar body. Constriction of spindle
commencing - Chromosomes in form of thick curved
rods.

Fig 48

X 1200

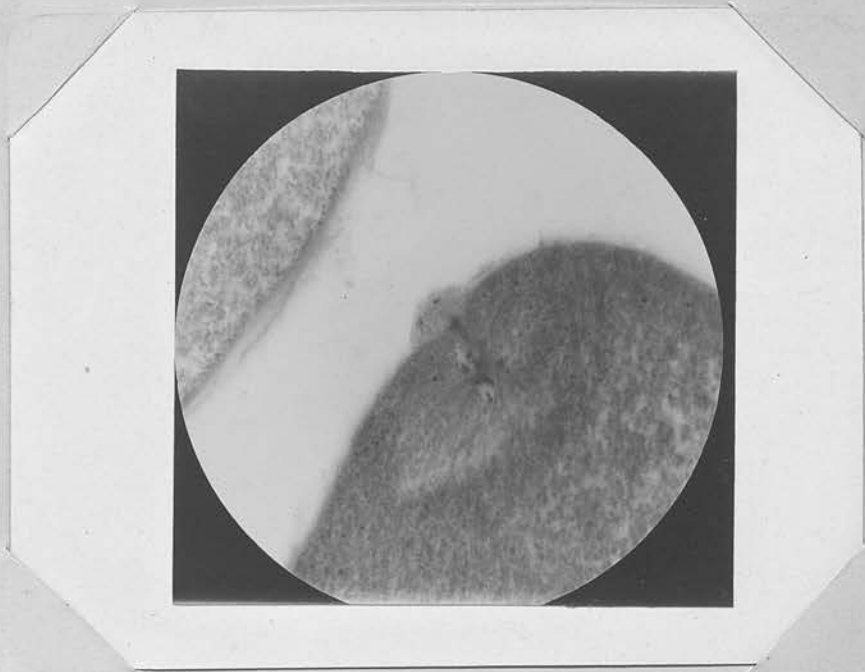
Late anaphase 2nd Polar spindle. outer centrosome
and aster has disappeared - Projection from surface
of egg receiving apex of spindle - Chromosomes in the
form of short curved rods

Fig 49

X 1200

Telophase 2nd Polar spindle. 1st Polar body is seen in the
section with its double rod chromosomes. 2nd polar body is not cut.
Spindle is ~~seen~~ constricted - & around it is a row of minute black
granules = the "Zwischenkörper". Central centrosome & aster enlarged.
The curved chromosomes have become considerably drawn out into
loops.

Fig 50



x 725

Fig 50a

x 1200

pt. stage in reconstruction of egg nucleus - There are a number of separate vesicles with loops of chromatin + scattered granules - The aster is broken up - The centrosome lies surrounded by the vesicles. Remains of the spindle seen with ring-like "wickerwork" -

Fig 51

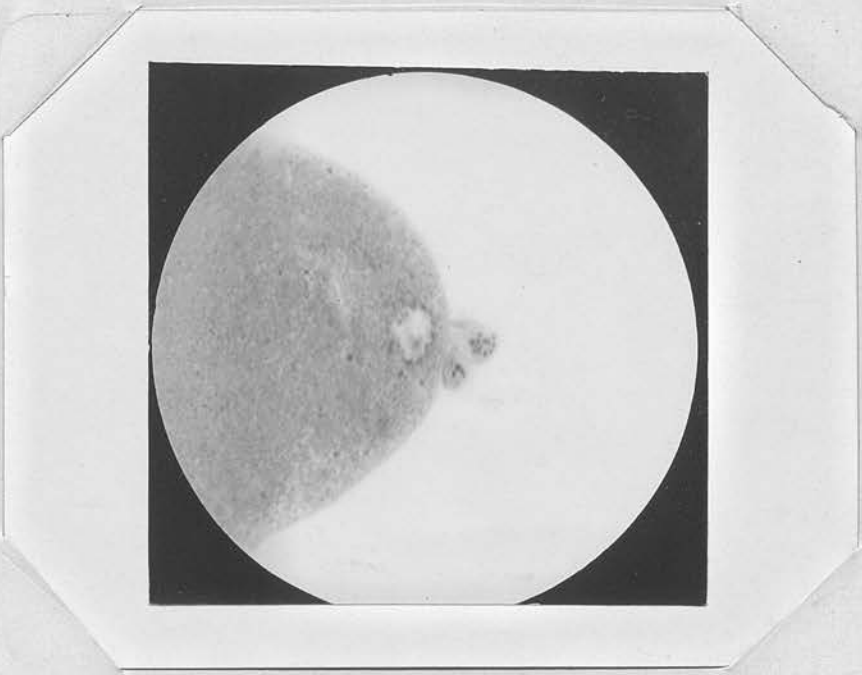


Fig 51a

X 725-

X 1200

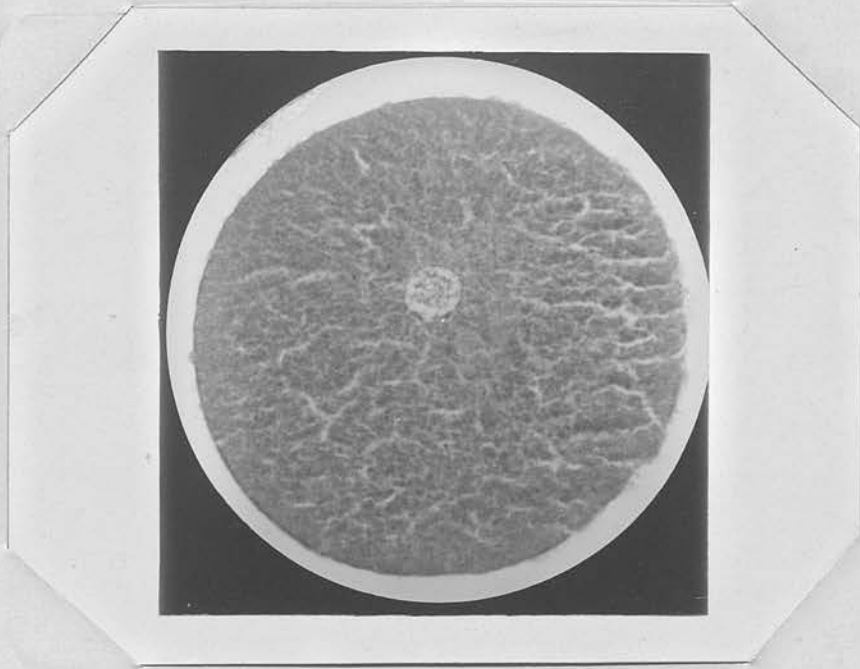
Two Polar Bodies and single vitelline egg.

Nucleus - In 1st Polar Body some of the chromatin grains show the double row form - In the 2nd.

They are all single but some are distinctly double in both there are abscise threads the remains of the spindle - In Egg nucleus there are loops of chromatin

and scattered granules some the wall. In the center

Fig 52

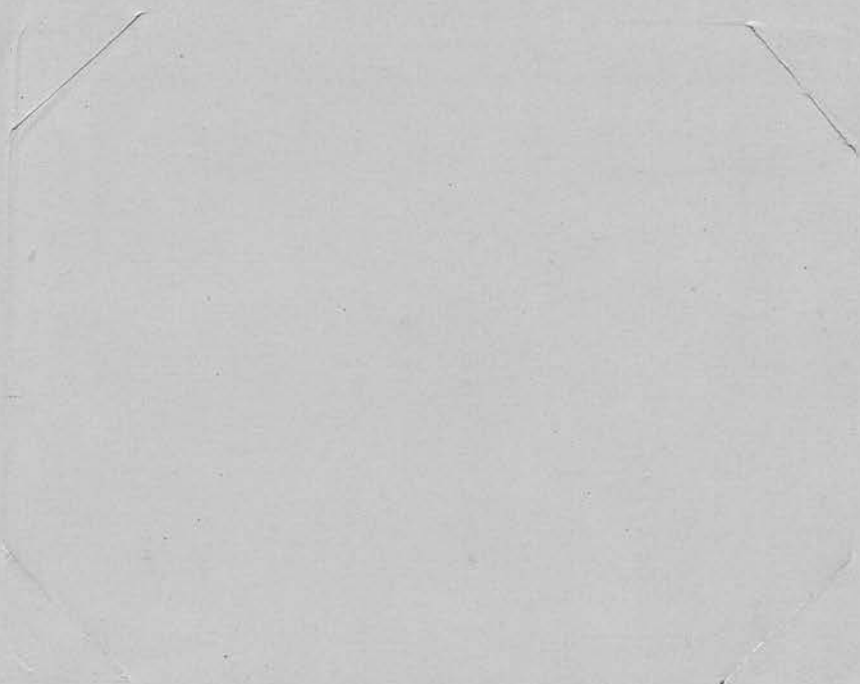


x
Matured ovum. The nucleus has returned
to the centre of the egg - and its nuclear
reticulum is restored.

Fig 53

x 1200
Nucleus of matured ovum. All trace
of radiation is gone - and the nuclear
reticulum is restored.

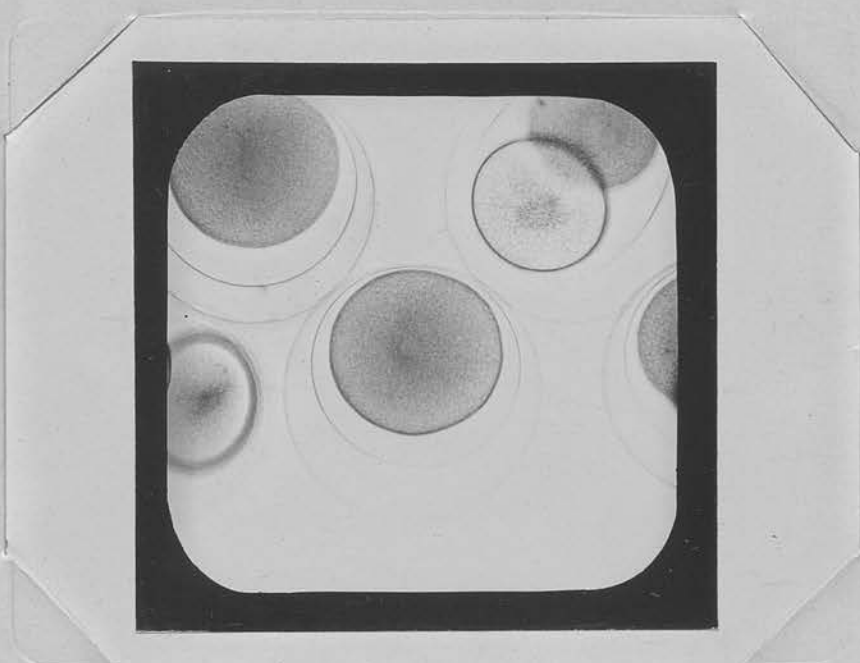
Fig. 54



x 1200 D

Abnormality of 2nd Polar body. Its nucleus is being reconstructed in a manner identical with that of the ovum. Remains of spindle & mid-body - well seen.

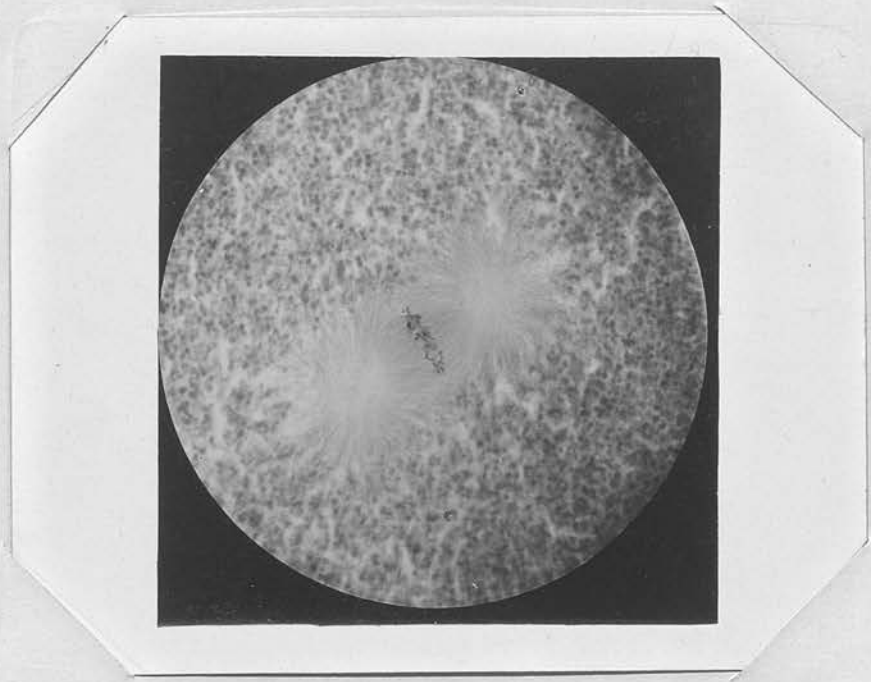
Fig. 55



x 150 D

Photograph of fertilized ovum - showing the membranes thrown off by the egg when the selected spermatozoon enters it -

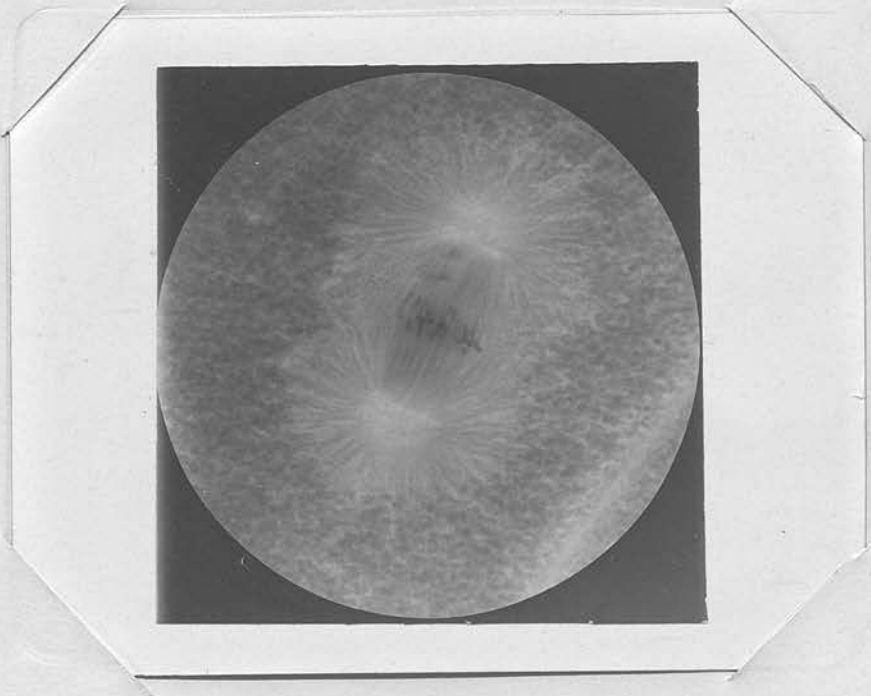
Fig 56



x 640

Early Metaphase of 1st Cleavage Nucleus

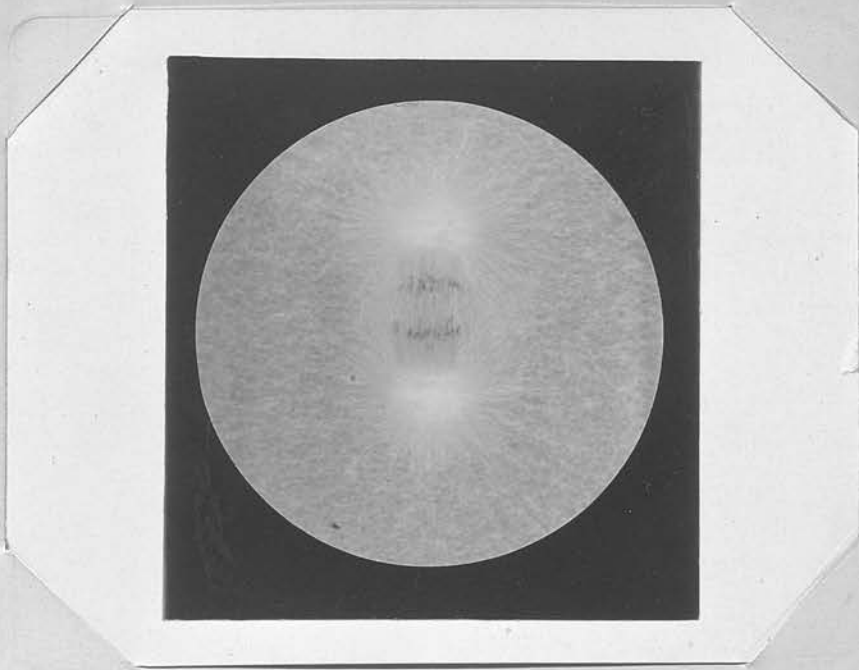
Fig 57



x 725

Metaphase 1st cleavage Nucleus showing the manner in which ^{the ends of} the looped chromosomes are drawn out along the spindle - the daughter chromosomes being formed by the breaking of the loops at its apex.

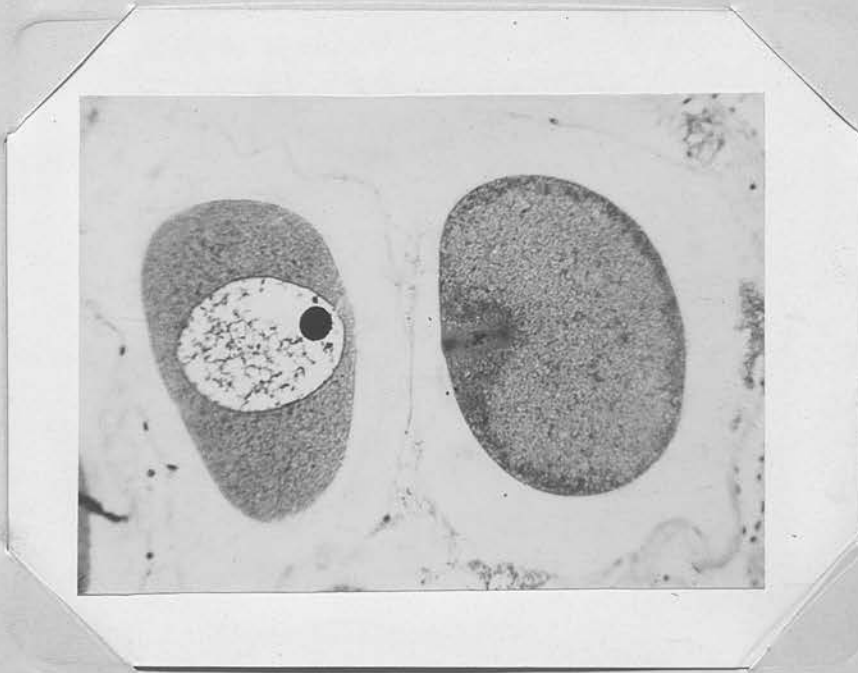
Fig 58



x 640

Early anaphase 1st cleavage nucleus

Fig 59



x 320 Δ.

- A. Omm with geminal vesicle entire shows alveolar structure of its cytoplasm - & no differentiated surface layer.
- B. Omm with 1st polar spindle formed - showing kinoplasmic zone & surface layer of differentiated protoplasm.