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THE SPERMATOGENESIS OF ICHTHYOPHIS GLUTINOSUS (LINN.)

Part II.

THE MEIOTIC DIVISIONS.

By

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With 47 figures in the text.

(Eingegangen am 14. Juni 1937.)

Primary Spermatocytes.

The repeated divisions of the spermatogonia result in the production of a large number of cells which, at a particular generation cease to divide further and enter on a phase of growth and internal differentiation whose culmination is the two divisions of the meiotic process. The peculiarity of these divisions and their general significance are now found in all treatises on Cytology. A review of the literature on Amphibian spermatogenesis reveals a large amount of work done in the Urodela and Anura while the interesting group Apoda has not been examined at all. So the facts which a member of this group reveal may be of interest.

It has already been remarked (SESHACHAR, 1936) that spermatogonia occur either singly (primary spermatogonia) or in groups (secondary spermatogonia) along the wall of the locule. The initiation of the processes of growth and differentiation takes place when the cells are arranged in two rows along the wall of the locule (fig. 3) and most of the earlier prophasic changes take place when the cells are in this position. The cells are conical with the apices turned towards each other while their bases are turned away. The leptotene and very often the amphitene as well as the pachytene stages are passed through while in this position and it is only when the compact pachytene chromosomes are showing signs of becoming resolved into their component threads, i.e., when the diplotene stage begins, that the cells leave their peripheral position and abandon their compact arrangement migrating deeper into the locule. It is also to be noticed that in later stages the cell mass encloses a space within it, unoccupied by the fatty matrix that fills the rest of the space in the locule. This is probably to be explained by the fact that the original conical cells of the leptotene stage become converted into those of the rounded or hexagonal shape, thus developing intercellular spaces, which by a simultaneous rearrangement of the cells themselves, become fused to form a single large space. The two divisions of the meiotic

phase as well as spermateleosis take place while in this position, with the cells embedded in the intralocular matrix. Fig. 1 shows the arrangement of cells in different stages of spermatogenesis in the locule.

A section of a testis lobe at any time of the year except during winter reveals all stages of spermatogenesis and we shall begin our account of

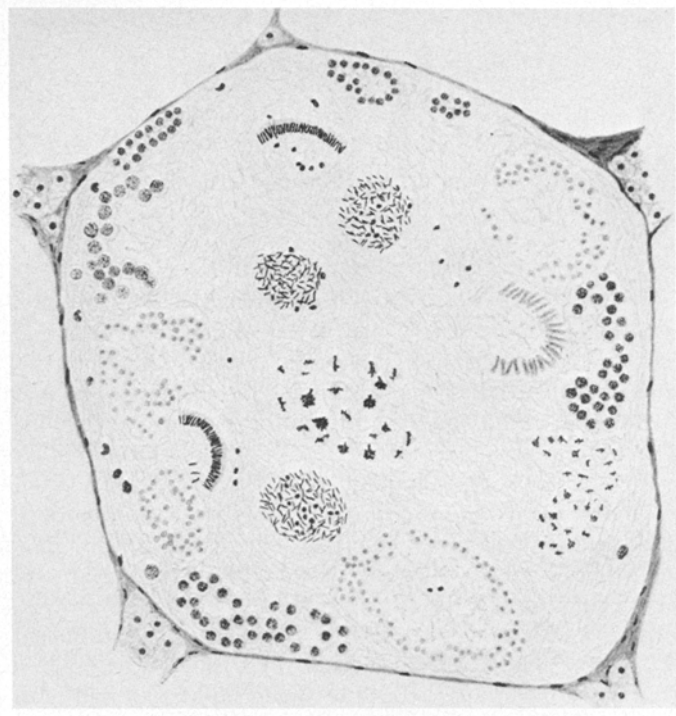

Fig. 1. A locule of the testis of *Ichthyophis glutinosus* showing cells in different stages of spermatogenesis. The cells are scattered in the form of groups in the fatty matrix of the locule. In the centre is a group of cells in which the nuclei are in metaphase I. There are three sperm masses about to be shed. Nuclei in the pachytene and diffuse stages, metaphase plates of the second division as well as spermatids in various stages of development are also seen. × 150.

the meiotic divisions where we left off in the first part of this study, i.e., the spermatocytes of the first order or primary spermatocytes.

Many authors (Meves, 1897; MacGregor, 1899; Janssens, 1901, 1905; King, 1907; Champy, 1913 and Saez, Rojas and de Robertis, 1936) describe a stage of rest which is of a variable duration and which is intercalated between the last generation of secondary spermatogonia and the primary spermatocytes. In this stage, the nucleus shows a number of chromatin blocks, angular in outline, arranged in the form of a coarse network. Some authors (Witschi, 1924; Saez and others, 1936) find a diminishing in the staining capacity on the part of the nucleus.

This stage of rest is of some importance and can be recognised easily in a mass of cells like the spermatogonia which pass through their

divisions more or less rapidly and where no regular stage of rest is seen for any considerable length of time. The resting stage, therefore, where the nucleus, having definitely abandoned further divisions under a stimulus yet unknown, enters upon a period of growth and differentiation, can be said to initiate the meiotic processes. The number of divisions that the spermatogonia pass through before they become spermatocytes is limited to 8 (SESHACHAR, 1936) though it varies in other Amphibia.

In *Ichthyophis*, a stage of rest occurs in the nuclei after the spermatogonial divisions but the staining capacity on the part of the nucleus does not diminish. The nucleus is filled with coarse and angular blocks of chromatin (fig. 2) which are connected with neighbouring blocks by thin linin threads. The cells are still conical and the nucleus which is rounded and never polymorphic lies at the base while the sphere is situated at the apex.

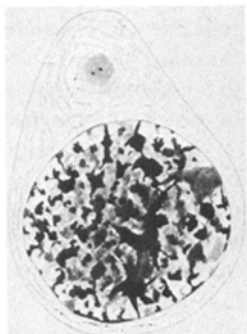

Fig. 2. A primary spermatocyte at the beginning of growth; the nucleus is in a stage of rest. The chromatin is in the form of large angular blocks. × 2400.

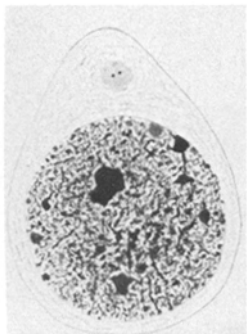

Fig. 3. Primary spermatocyte showing the nucleus with fine chromatin grains. Filament formation is not yet seen. × 2400.

Prophase. The prophase of the first meiotic division is probably the longest that occurs in any cell and JANSSENS (1901) is not far wrong when he says that it extends to weeks or even months. While it is possible to estimate the time taken by these prophasic changes in certain amphibians where there is a regular and restricted breeding season and a seriation of stages in their testis, it is a matter of impossibility to do it in *Ichthyophis* where breeding extends almost throughout the year and the ripening of the germ cells is an almost continuous process. But from the fact that in a locule with groups of cells in various stages of spermatogenesis most of them are either in the prophase of the first meiotic division or in spermateleosis, one would be not far wrong in concluding that these stages extend over a great length of time.

The initiation of the processes of prophase consists in a disintegration of the large coarse blocks of chromatin into very small ones so that the nucleus takes on the more or less homogeneous appearance of a fine network (MEVES, 1897; KING, 1907; TERNI, 1911; STIEVE, 1920). The granules are very small and thin linin threads pass between them and connect them. This stage where as yet no formation of threads and no polarisation of any kind is seen is characteristic of *Ichthyophis* (fig. 3

and 4) and is probably of longer duration than in certain other Amphibia like *Desmognathus* (KINGSBURY, 1902), *Triton* (JANSSENS, 1901) and *Alytes* (JANSSENS and WILLEMS, 1909) where the formation of the filaments and the polar orientation quickly follow the resting stage.

Spindle bridges connecting the spheres of adjacent spermatocytes (presumably the descendents of a single spermatogonium) described in a number of Amphibia by MEVES (1897), RAWITZ (1895), MACGREGOR (1899), EISEN (1900), TERNI (1911) and CHAMPY (1924) are conspicuously absent in *Ichthyophis*. One would expect to see them in this animal where the products of division of a cell lie huddled together but careful examination has always revealed the spheres of spermatocytes quite independent from one another and unconnected in any manner with those of neighbouring cells.

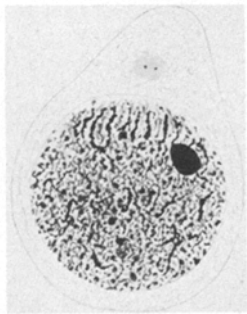

Fig. 4. Primary spermatocyte showing the beginnings of filament formation. It will be seen that the filaments begin to be formed at the proximal pole and become directed towards the sphere even as they are forming. × 2400.

Leptotene. The next stage shows the granules being built up into threads. This building up of the leptotene threads is very interesting in the primary spermatocytes of *Ichthyophis*. In most Amphibia the threads make their appearance first and are later orientated towards the pole at which the sphere is located, so that the early leptotene is characterised by an unpolarised tangle of threads. The time which elapses between the two processes is very little but many authors (JANSSENS, 1901 and 1905; KING, 1907; JANSSENS and WILLEMS, 1909; STIEVE, 1920; WITSCHI, 1924 and SAEZ and others, 1936) have figured a directionless skein of leptotene threads directed towards the pole. *Ichthyophis* differs from these animals in this respect. The construction of the leptotene filaments takes place in such a manner that the polar orientation is seen even from the start. The granules are seen organised into threads first at or near the proximal pole while deeper inside the nucleus and at the opposite pole, the small granules are discrete and are not formed into filaments. Fig. 4 shows this feature of the early leptotene nucleus very clearly. It will be seen that this figure in similar to fig. 10 of *Geotriton* by TERNI (1911) and fig. 13 of *Amblystoma* by CARRICK (1934) where also probably the two processes of filament formation and polar orientation go hand in hand as in *Ichthyophis*.

Two or more nucleoli are usually seen in the nucleus. Their position in the nucleus is not definite and though many times a distinct space may be made out around the nucleolus, nucleoli without this space and apparently connected with the filaments are not uncommon. As filament formation proceeds from the proximal to the distal pole, the nucleus takes on the characteristic appearance noticed in many Amphibia where

the polar orientation is more or less clear. This has been called the leptotene bouquet by JANSSENS (1905) and by the time filament formation is complete the leptotene bouquet has attained its perfect orientation. The filaments can be studied very clearly at the proximal pole where they do not interlock but lie parallel with their ends pointing towards the nuclear membrane nearest the sphere and each of them is seen to consist of irregular granules strung together in a linear series (fig. 5). No duality of either the filament or the granules can be seen at this stage.

SATO (1932) describes in *Diemyctylus pyrrhogaster* a flattening of the nuclear membrane at the proximal pole. I know of no other author who has described such

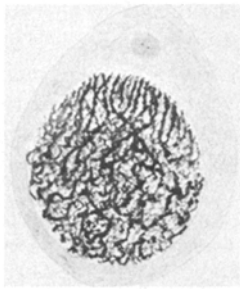

Fig. 5.

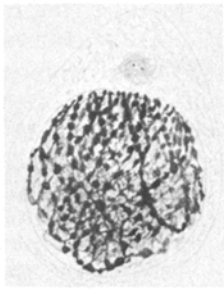

Fig. 6.

Fig. 7.

Fig. 5. Primary spermatocyte showing the nucleus in early leptotene. At the proximal pole, the filaments are clearer than at the distal where the nucleus still exhibits the appearance of a tangle of threads. × 2400.

Fig. 6. A typical leptotene bouquet. The filaments show a beaded appearance and all of them point towards the pole. × 2400.

Fig. 7. An amphitene nucleus. The parallel union of the filaments at the pole is seen. Distally the filaments are free. × 2400.

a phenomenon in any other amphibian nor have I found it in *Ichthyophis*. This same author (1934) has figured the prophase stages of the primary spermatocytes in *Triturus ensicauda* and does not show polar orientation of the chromosomes at any stage. Probably it does not occur in this animal. If so, *Triturus* is one of the most unique among amphibians, for I do not know of any other amphibian example where the polarisation of the filaments either at the leptotene or at the pachytene stage has not been described.

In *Ichthyophis* polarisation of the threads begins very early in the history of the spermatocytes and is in evidence for a long time. The nuclear membrane at the proximal pole retains a spherical contour as in the rest of its surface and is not flattened as in *Diemyctylus pyrrhogaster* (figs. 4, 5, 6, 7 and 8).

The nucleus with the fully formed leptotene filaments has a characteristic appearance and is a deeply staining tangle of numerous thin threads. It is impossible to count the number of threads even in thin sections passing through the proximal pole of the nucleus partly on account of their very large number (presumably 84 parallel threads occur at the pole, two for each chromosome) and partly on account of their dense

crowding. It is also impossible to trace the threads from the pole into the body of the nucleus all through their ramifications in this region back again towards the pole. In the distal aspect of the nucleus the individuality of the threads is not clear on account of their repeated crossing.

Amphitene. Now a very characteristic transformation takes place in the nucleus. A tendency to a thickening of the filaments at the proximal pole is evident and gradually this thickening becomes more and more clear.

The thickening of the threads results from a side-by-side union of the thin threads at the pole as well as from their contraction and condensation. Two threads come together, the fusion beginning at the ends nearest the polar membrane of the nucleus and proceeding inwards. This process of conjugation of two of the leptotene threads to form a thicker thread is called Synapsis and is the process that results in the formation of the thick bivalent chromosomes of the nucleus of the later stages.

Many authors (KING, 1907; LEVY, 1915; WITSCHI, 1924; STOHLER, 1928) have described in relation with synapsis, a more or less pronounced contraction of the chromosomes and a restriction of the filaments to one pole of the nucleus. This stage which has been called Synizesis, is according to these authors, of normal occurrence in spermatogenesis. Contrary is the opinion of MEVES (1897), KINGSBURY (1902), JANSSENS (1901, 1905, 1909) and SAEZ and others (1936) that wherever these stages occur they are not in the nature of normal nuclear phenomena but are caused by imperfect fixation. The present position of our knowledge regarding the status of synizetic condensation of nuclear matter is still quite indecisive and while evidence has accumulated on the one hand for the belief that synizesis, at least in plants, is a normal feature of meiotic phenomena, it is becoming increasingly clear that the nucleus during synapsis being of a highly sensitive nature, is most liable to break down under the violent action of many of the fixatives used, giving us pictures similar to the synizetic contractions observed by those authors who regard them as normal occurrences.

In *Ichthyophis* I have never found such a synizetic contraction in normal spermatocytes. Even Bouin's fluid and its modifications have not produced the artefacts that SAEZ and others (1936) have observed in *Bufo arenarum*. It is highly probable that the nuclei of the primary spermatocytes of *Ichthyophis* are more resistent to the action of the fixatives than those of many other Amphibia and it is not unlikely that when the history of the nature of the synizetic phenomena comes to be written, it will ultimately rest on the question of the resistance that the nuclei of the spermatocytes offer to the fixing action of the fluids employed.

In *Ichthyophis* the process of synapsis can be easily followed as the seriation of the different stages is very clear. The crowded threads of the early leptotene become condensed and each thread appears more or less distinctly like a string of beads, all the threads pointing towards the pole. A typical leptotene bouquet is shown in fig. 6 and it will be seen that in the distal region the threads still present a networklike appearance on account of the repeated crossing and intercrossing of the threads. It

will also be noticed that the beaded nature of the threads is not so regular in the rest of the nucleus as it is at the proximal pole.

It is just about this time that the process of side-by-side union of the threads occurs. The conjugation begins at the pole and first affects the two beads of the apposing threads nearest the nuclear membrane and travels down the threads. In fig. 7 the fusion of the threads has already advanced some way down them though the doubleness of each thick thread at the pole is very clear. It corresponds to the typical amphitene stage shown by JANSSENS (1905) in his fig. 36 and in fact is even earlier, for the doubleness of the threads at the pole is far more clear in my figure than in JANSSENS's. There is hardly any doubt that the thick thread at the proximal pole is formed by the fusion of two thinner threads into which, in fact, the former can be traced. The course of the latter can be followed to a considerable distance into the nucleus and it will be noticed also that the threads have become cleared up in this region in fig. 7 as compared with that in fig. 6, which represents the typical leptotene nucleus and where the interior was still a mass of intercrossing threads.

Fig. 8. A typical pachytene bouquet. All trace of a duality of the threads is lost. × 2400.

In *Ichthyophis* the polarisation of the threads continues to be evident for a long time, i.e., even when the condensation and conjugation of the threads has advanced so far as to mask all duality of the threads that have resulted from conjugation (fig. 8), so that while a pachytene bouquet is not seen in forms like *Bufo arenarum* (SAEZ and others, 1936), it is clearly in evidence in *Ichthyophis*.

Pachytene. The duality of the pachytene threads appears to vary in different animals and so far as is known, in the Amphibia, the duality disappears sooner or later in the pachytene (*Batrachoseps*, JANSSENS and DUMEZ, 1903; *Bufo arenarum*, SAEZ and others, 1936); in some cases, the duality disappearing even in the amphitene stage so that the stem of the Y in the amphitene already shows a more or less complete fusion of the threads that entered into the association. The case of *Ichthyophis* shows that while in the amphitene stage, the stem of the Y shows a very clear longitudinal cleft (fig. 7) the pachytene loops do not show any (fig. 8). But cases may sometimes occur where a faint indication of such a cleft is seen even in late pachytene. It is more than probable that as WILSON (1928) says, the question of the fusion of the apposing threads is largely bound up with that of the type of technique employed and probably "the seeming fusion of the conjugants is deceptive and that in internal structure the bivalents are all double structures" (p. 555).

While the leptotene loops do not lend themselves to counting the loops of the pachytene stage can be counted. In very thin sections passing

through the polar region of the pachytene nucleus horizontally, the ends of the thick threads directed towards the pole appear as dots and can be counted with ease. Fig. 9 shows such a view from a 3 microns section and 42 such dots can be counted. Such countings have become very popular since the work of the SCHREINERS (1905) on *Tomopteris* and offer one of the best methods of making a count of the pachytene loops, assuming that all the loops direct both their ends towards the pole. There is hardly any doubt about this latter fact, for in addition to the findings in many animals (Orthoptera) that even short rod-shaped chromosomes elongate at the leptotene and pachytene stages and become V shaped loops directing the two limbs towards the pole, there is the fact that in the pachytene stage, the nucleus is clearer towards the distal pole (only the longer loops extending as far as this) and does not show the ends of any complete threads at this region.

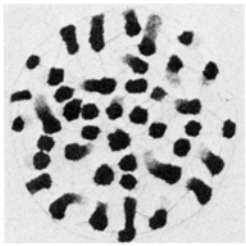

Fig. 9. Polar view of a typical pachytene nucleus showing the 42 ends of the 21 pachytene loops, all directed towards the pole. × 2400.

The pachytene stage is probably of long duration, — longer than either the leptotene or the amphitene, — as, in a section of a testis locule, many groups of cells are seen in this stage, which can be distinguished even under the low power of the microscope by the characteristic parallel arrangement of the darkly staining filaments pointing towards the proximal pole.

Much has been written about the role and fate of the nucleolus (EISEN, 1900; JANSSENS, 1905; CHAMPY, 1913; CARRICK, 1934) in the Amphibia. In *Ichthyophis* the nucleolus is clearly seen in the leptotene as a smooth body but it does not occupy any definite position inside the nucleus as indicated in the work of JANSSENS (1905) on *Batrachoseps* and of CHAMPY (1924) on *Discoglossus* where the nucleolus occupies the distal pole of the leptotene nucleus. In *Ichthyophis* the leptotene nucleoli may be multiple or there may be a single nucleolus occupying any position in the nucleus. There is some evidence to show that it may take part in the formation of the filaments but it is seen that as prophase advances the nucleolus breaks up into small bodies adhering to the nuclear membrane in the form of small hemispherical bodies. How these bodies disappear is not known and after all it is likely that as CHAMPY observes, a change in reaction results in the loss of the nucleolus to the view.

A contraction and condensation of the threads results in a closer drawing together of the successive double row of granules composing the pachytene threads which is the essential feature of the pachytene processes.

The next stage is not heralded till the conjugation of the leptotene filaments is complete throughout their extent and till the condensation is very marked. The most significant change that occurs now is a loss of the polar orientation of the threads. This is brought about by a widening

out of the two limbs of each thread (fig. 10). Whether the peripherally placed loops widen out first, the central ones following soon after, is a point I have not been able to determine. It is probable that this widening out is a quick process affecting all the loops almost simultaneously, with the peripherally placed loops initiating the process. However, the result of this widening out is a loss of the characteristic polar orientation and a scattering of the threads in the nuclear cavity spanning it from end to end (fig. 11). The breaking up of the polar orientation is, as I said, a quick process, for in the same group of cells one can see both types of

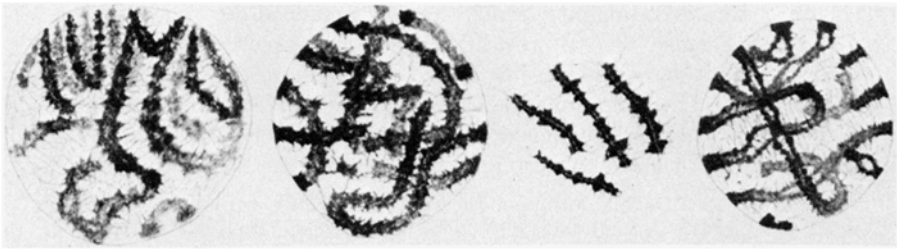

Fig. 10. Fig. 11. Fig. 12. Fig. 13.

Fig. 10. Nucleus showing the beginnings of the loss of polar orientation. Many of the loops are widening out. A reappearance of duality is seen in some of the threads, especially distally, where the condensation is less than at the pole. × 2400.

Fig. 11. Nucleus showing complete loss of polar orientation and the loops distributed inside the nucleus without any regular arrangement. The duality of the threads is clear. × 2400.

Fig. 12. Four chromosome threads from a nucleus which is just losing polar orientation. × 2400.

Fig. 13. A nucleus in early diplotene. The splitting of the threads is clear. × 2400.

nuclei, — those where the polar disposition is clear and those where this direction is lost.

Diplotene. Associated with the loss of polar orientation is the very important process of the separation of the two filaments that compose each pachytene thread. In good preparations the doubleness of the threads makes its appearance while the threads are exhibiting the first signs of breaking away from the pole and very thin sections will reveal the clearly dual nature of the granules that make up each thread. Four such threads are shown in fig. 12. It will be noticed that the space between the two filaments is very clear though not very wide. But it is to be observed that the filaments themselves have not diverged from each other though many of the granules have. A distinction between the filament and the granules that compose it is becoming increasingly important (see DARLINGTON, 1932; p. 291 and 302), and the separation first affects the granules and later on, the filaments. The separation of the filaments themselves occurs when the polar orientation is almost completely lost. The breaking apart of the exconjugants can be seen in fig. 13. It will be noticed that the separation does not take place all

along the filament at the same time so that a heterogeneous appearance is presented by the threads at this stage, — at some parts clearly double and the filaments separated by a space, at others still attached, though the granules show clear duality.

It is interesting to note that while the conjugation of the leptotene filaments begins at their ends, their separation after the pachytene stage begins interstitially and is never terminal as revealed in fig. 13.

The splitting of the threads indicates the beginning of the diplotene stage. It must be mentioned that the splitting does not completely separate the exconjugants which remain attached at various points along their length. The interest attached to the diplotene in *Ichthyophis* is more than a mere passing one, for very soon changes begin to occur in the nucleus. The exconjugants become pulled apart (except at places where they still retain connection with each other) and associated with this is a loss of staining power on the part of the filaments.

WILSON (1923) in his outline of the meiotic processes in plants and animals refers to a "diffuse or confused stage" following the separation of the exconjugants, which, though not of universal occurrence is nevertheless pronounced in some animals (Insects) and whose important feature is a diminished basophily of the nucleus. In the majority of animals however, the diplotene passes more or less clearly into the next stage, — Diakinesis, — without the chromosomes being obscured in any manner. The Amphibia are an instance in point. The work of MEVES (1897) on *Salamandra,* of MACGREGOR (1899) on *Amphiuma,* of JANSSENS (1901) on *Triton,* of KINGSBURY (1902) on *Desmognathus,* of KING (1907) on *Bufo lentiginosus,* of JANSSENS and WILLEMS (1909) on *Alytes,* of CHAMPY (1913, 1924) on a number of Amphibia, of SNOOK and LONG (1914) on *Aneides.* of STIEVE (1920) on *Proteus,* of WITSCHI (1924) on *Rana temporaria,* of SATO (1932) on *Diemyctylus* and of SAEZ and others (1936) on *Bufo arenarum* all show that the pachytene chromosomes become those of diakinesis with little or no change in their appearance (except great condensation). SAEZ and others (1936) report a slight loss of staining power in the nucleus but no diffuse stage as such has been noticed by them. The only work in which such a stage occurs is, I believe, that of JANSSENS and DUMEZ (1903) on *Batrachoseps.* These authors have figured and described a condition which makes the nearest approach to it. Their photographs 16 and 17 recall to my mind stages described in insects. They call this the stage of nuclear tension and in *Batrachoseps* it is of very short duration because this stage is met with only occasionally. On careful examination, it is seen that in this stage of nuclear tension, many of the individual chromosomes can be made out clearly though some are obscure and lose themselves in an indecipherable deeply staining knot.

My observations on *Ichthyophis* are very interesting in this respect. An examination of the testis under low power will show groups of large rounded nuclei showing all the characters of a resting stage. No trace of any chromosomes can be observed, though the chromatin is disposed in the form of irregular angular blocks with faint strands running between them, and on the whole the entire nucleus presents the appearance of a faintly staining network. Seeing that one encounters many nuclei in this stage it must be one of long duration.

Now, the diffuse stage, wherever it occurs, presents various degrees of diffusion of chromatin. In the Amphibia generally, where the pachytene or diplotene chromosomes are never lost to view, there can hardly be said to be any diffusion of the nucleus. Even in *Batrachoseps*, where probably the nearest approach to diffusion of all the Amphibia is seen, most of the chromosome complex can be made out. In the spermatocytes of some insects on the other hand (certain Hemiptera), a very pronounced diffuse stage is noticed. In many cases, it has been noticed that even in the greatest diffusion, the chromosomes are distinctly double, though widely separated and though staining very feebly and WILSON (1928) concludes that "probably therefore the diffuse stage should be regarded as a highly modified diplotene in which the duality of the early diplotene however it may be obscured, in some manner persists throughout" (p. 545).

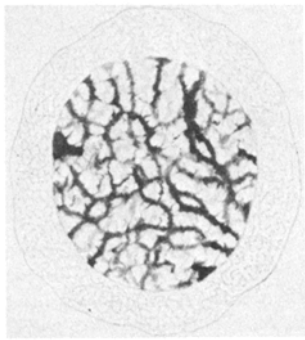

Fig. 14. Nucleus showing the beginning of diffusion of chromatin. Though some of the widely separated bivalents can be made out, the chromosomes on the whole appear faintly staining and irregularly placed in the nucleus. × 2400.

The diffusion of chromatin in the spermatocytes of *Ichthyophis* is probably the most pronounced in all Amphibia and presents in its greatest development all the appearance of a true resting stage. Fig. 16 shows such a nucleus. It will be noticed that the large angular blocks of chromatin send off fine processes which span the nuclear cavity. No trace of either the chromosomes of the diplotene that preceded it or those of diakinesis that follows it is seen. There is no doubt that in this stage, the individuality of the chromosomes becomes masked and the whole nucleus presents the appearance of a network.

While the nucleus in its greatest diffusion shows these features, an earlier stage than this shown in fig. 14 gives us the clue to the meaning and significance of the diffuse stage. In thick sections the nucleus shows all the characters of a feebly staining network but the basophily being not yet so feeble the strands composing the network are more uniform and the outlines of some of the chromosomes may still be seen. The correct interpretation of this stage can be understood if thin sections of the nucleus are examined. In 5 microns sections of the testis, in a group of cells in this stage, some nuclei appear thick while in others the razor has cut the nucleus into thin sections some of them including only a few strands of the nuclear network. Figs. 14 and 15 are from the same group of cells revealing the same stage of development. And while in the former, the nucleus is cut into fairly thick sections, in the latter only a few of the chromosomes are seen. The difference in appearance presented

by these two figures is very great. While in the thicker sections as figured in 14 it is difficult to make out the outlines of the individual chromosomes, in the thinner, as shown in fig. 15, they are clear. It will be noticed in the latter, that the two exconjugants which separated at the diplotene are still clearly visible though they are placed wide apart. Three such bivalents are seen and in each the components are clear. It is possible that the identity of the individual chromosomes becomes obscured by the superimposition of a large number of these feebly staining and widely separated exconjugants which can be seen clearly in sections of the nuclei where only a few of the peripheral strands are included.

Fig. 15.

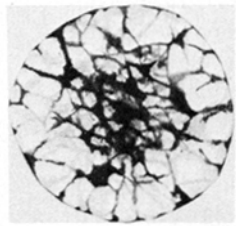

Fig. 16.

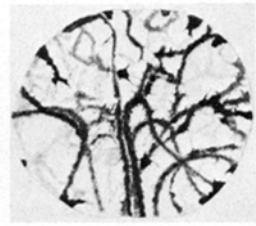

Fig. 17.

Fig. 15. Three of the bivalents from a nucleus showing the first signs of diffusion. The nucleus was cut in such a manner that these three chromosomes were separated from the others. The exconjugants have moved wide apart and show a diminished basophily. × 2400.

Fig. 16. The "Diffuse Stage" in its typical development. × 2400.

Fig. 17. Nucleus emerging from the diffuse condition. The filaments are becoming clarified. × 2400.

It is probable that the condition of diffusion found in *Batrachoseps* by JANSSENS and DUMEZ (1903) corresponds to what I have just described. In both cases, there is a certain amount of diffusion and diminished basophily but in both cases, many of the chromosomes can be made out though some lose themselves in the tangle and do not lend themselves to interpretation. But while in *Batrachoseps* the stage of diffusion stops here and later gives place to the strepsinema, in *Ichthyophis,* on the other hand, it is carried farther along the road of diffusion till, even in thin sections, the individuality of the different chromosomes becomes completely lost.

Incidentally I may mention that I have examined the testis of *Uraeotyphlus menoni,* another Caecilian obtainable in India and find such a stage in the spermatocytes of this animal also, so that it is not unlikely that the diffuse stage characterises the spermatocytes of the Apoda.

The emergence of the bivalents from this faintly staining mass is a process which is the obvious reverse of that which converted the diplotene into the diffuse stage, — a regaining of basophily and a gradual condensation of the chromosomes. The manner in which the threads emerge can be studied in the same group of cells where usually all stages from the diffuse condition to early diakinesis are seen. It is probable that this emergence therefore, is a rapid process occupying not a long time. Fig. 17 shows what is probably the first stage in the unravelling of the network. The individual chromosomal threads are clearly visible

and take a deeper stain than before and the associated pairs lie more or less close together, though certain deeply staining masses are still unable to be classified and identified. Probably they are, like JANSSENS's figures, caused by faults in the technique and it is likely that in the living condition and in perfect preparations these blobs do not occur.

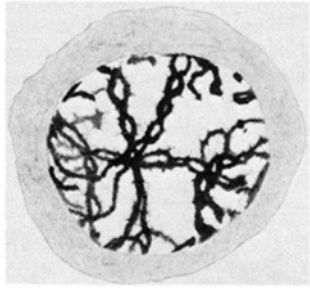

Fig 18. Later stage. The filaments are clearer and many of them tend to meet at the centre. Clear indications of a twisting are seen. × 2400.

The emergence of the chromosomes from the diffuse condition is often associated with the appearance, in the centre of the nucleus of a strange knot at which most of the chromosomes, at least the longer ones, meet. This has been noticed among the Amphibia in *Batrachoseps* and *Alytes* and has been carefully examined by JANSSENS and his collaborators (1905 and 1909). Often, according to them, this knot is closely associated with the chromoplast or its remains (*Batrachoseps*; JANSSENS, 1905). I have found such a grouping of the chromosomes around a central mass in some cases in *Ichthyophis* (fig. 18) but I have not noticed in relation with this knot any remains of the chromoplast. The view has been held by some authors that the orientation of the chromosomes in the early strepsitene around a central knot, in structure often obscure and indistinguishable, is in the nature of a second contraction or synizesis. But JANSSENS criticies this view and feels that no such importance can be attributed to it. The grouping of the chromosomes around the central knot is never very pronounced in *Ichthyophis*, and in all cases the chromosomes being more or less clear, it cannot be said that it is in the nature of a contraction figure.

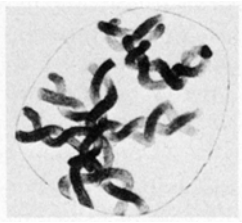

Fig. 19. A typical strepsinema. Only a few of the chromosomes are shown. × 2400.

Strepsitene. The chromosomes now exhibit signs of twisting about one another and indicate the unmistakable appearance of what is known as the strepsitene. Clear strepsitene stages are figured in a number of Amphibia, by JANSSENS and his collaborators in *Triton* (1901), *Batrachoseps* (1905) and *Alytes* (1909), by SATO (1932) in *Diemyctylus,* by CHAMPY (1913) in a number of Amphibia and by CARRICK (1934) in *Amblystoma.* The twisted appearance of the chromosomes becomes more conspicuous in *Ichthyophis* where the chromosomes become more condensed and their contours become more even (fig. 19). It has not been possible for me to determine the number of chiasmata in each chromosome in *Ichthyophis,* but in the larger ones the chiasmata may be as many as 6 or 7. As diakinesis progresses, their number becomes reduced till in late diakinesis, it is at a minimum.

Generally, a longitudinal split appears in each of the two chromosomes of the bivalent about the end of the diplotene or the beginning of diakinesis. WITSCHI (1924) has noticed this split in *Rana temporaria* and SAEZ and others (1936) appear to have seen it in *Bufo arenarum* at the beginning of diakinesis but I do not think that this split is noticed clearly in any other amphibian till very much later, very often, until the anaphase of the first division. Amphibia, as a rule, do not form such advantageous material as, for instance, insects do, and though recent researches have brought to light the important fact that this split does occur, it is very probable that in many amphibians, at any rate, it must be taken more for granted than observed, due to the inadequacy of our staining methods or the inherent constitution of the chromosomal material itself. I have with great diligence and anxiety looked for this split in each dyad of *Ichthyophis* at this stage but have not found it till the anaphase of the first division.

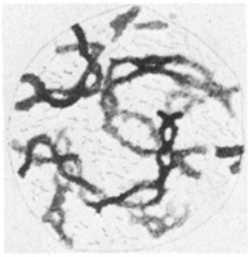

Fig. 20.
Strepsinema showing chromosomes with large number of chiasmata.
× 2400.

Diakinesis. WILSON (1928) distinguishes three different phases during diakinesis and is of opinion that the beginning of the first phase is to be reckoned from the moment the chromosomes are emerging from out of the confused stage. The essential features of diakinesis are a consolidation and contraction of the tetrads so that they come to lie evenly distributed in the nucleus, and a reduction in the total number of chiasmata in the chromosomes.

When the chromosomes are emerging from the diffuse stage they are thin, long and have a large number of chiasmata. On account of the large number of chromosomes it has not been possible for me to determine the number in each bivalent at different stages in *Ichthyophis* as it has been done in a large number of plants and some insects but one of the easiest things is to satisfy oneself about the fact that in early diakinesis, the chromosomes are longer and the chiasmata more numerous than in the later stages. It is also probable that the chiasmata, as diakinesis advances, become gradually terminal till, just when the chromosomes are getting on to the spindle, the terminal chiasmata are most numerous while the total number of chiasmata has become reduced.

The chromosomes of early diakinesis present an irregular beaded appearance caused by an alternation between light and dark areas along them. The condensation and contraction of the chromosomes can be followed gradually in the same group of cells and at the end of diakinesis the tetrads are darkly staining, greatly contracted bodies whose components cannot be made out easily (except in case of the large chromosomes) and which are arranged along the periphery of the nucleus (figs. 21, 22, 23). It is in the middle diakinesis that the characteristic forms of tetrads can be made out. Rods, rings and crosses are in great numbers, formed from the smaller chromosomes while the larger ones form compound rings (figs. 21, 22, 23).

The spermatogonial chromosomes, as shown by me (1936) are 42 in number and an examination of the metaphase plate of a primary spermatogonium shows that they can be arranged in two series. The large ones are six in number, of which four are V shaped and the other two are elongated rods. Of the remaining 36 chromosomes, six are small V shaped chromosomes. They also occupy the periphery of the plate like the large chromosomes and are quite as conspicuous. The rest of the complex, consisting of 30 chromosomes is made up of either small rods or dots occupying for the most part, the centre of the plate.

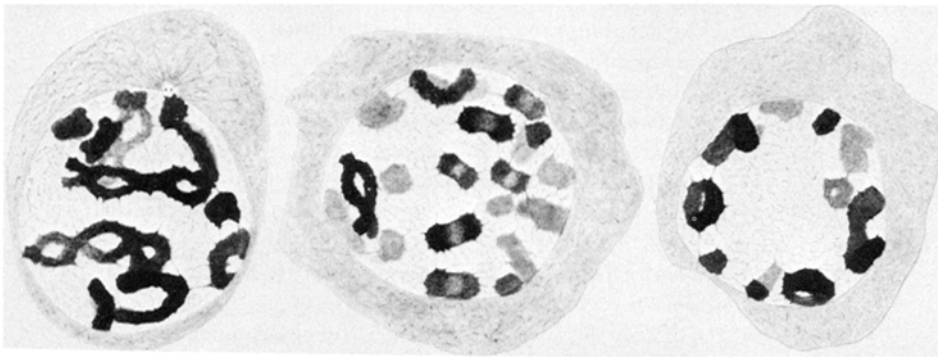

Fig. 21. Fig. 22. Fig. 23.

Fig. 21. Early diakinesis showing some tetrads. Simple and compound rings and rod tetrads are seen. × 2400.

Fig. 22. A cell showing the nucleus in diakinesis. × 2400.

Fig. 23. Primary spermatocyte showing nucleus in diakinesis. The tetrads are disposed peripherally. × 2400.

So far as the chromosome number is concerned, *Ichthyophis* occupies a unique position in the Amphibia. Among the Anura, the diploid chromosome number, so far as is known, tends to vary between 22 (species of *Bufo;* STOHLER, 1928; MAKINO, 1930; MINOUCHI and IRIKI, 1932; WITSCHI, 1933; SAEZ and others 1936) and 26 (species of *Rana;* SWINGLE, 1917; WITSCHI, 1924; IRIKI, 1932; MAKINO, 1932; SATO, 1933, and *Rhacophorus,* MAKINO, 1932); *Alytes* (JANSSENS and WILLEMS, 1909) being the only known example where the number is the highest among the Anura, — 32. The Urodela, on the other hand, appear to fall into two very definite series: species of *Salamandra* (FLEMMING, 1887; VOM RATH, 1893; MEVES, 1897; A. and K. E. SCHREINER, 1906; CHAMPY, 1913); *Triton* (JANSSENS, 1901); *Batrachoseps* (JANSSENS, 1905); *Amblystoma* (CARRICK, 1934); *Amphiuma* (MACGREGOR, 1899); *Aneides* (SNOOK and LONG, 1914); *Desmognathus* (KINGSBURY, 1902); *Diemyctylus* (SATO, 1932); *Geotriton* (TERNI, 1911); *Plethodon* (JANSSENS and DUMEZ, 1903) all have chromosome numbers which vary between 24 and 28, while forms like *Hynobius* (MAKINO, 1932 and 1934; SATO, 1936); *Cryptobranchus* (MAKINO, 1935); *Megalobatrachus* (IRIKI, 1931); *Salamandrella* (MAKINO, 1932) and *Pachypalaminus* (SATO, 1936) have a very large number of chromosomes, varying between 40 of *Hynobius retardatus* (MAKINO, 1932) and 64 of *Megalobatrachus japonicus* (IRIKI, 1931), the latter being the largest number reported among the Amphibia.

Recently attempts have been made for the Amphibia (MAKINO, 1932; SATO, 1936) on the lines done in Reptiles (MATTHEY, 1931) to homologise the chromosome

numbers in different species by assuming that the small rod and dot shaped chromosomes result from a fragmentation of the large V shaped chromosomes so that a certain uniformity in the chromosome number can be arrived at by treating two rod-shaped chromosomes as equivalent to a single V shaped chromosome, for purposes of counting. If this is accepted, the facts offered by the chromosomes of *Ichthyophis glutinosus* are very interesting; of the 42 chromosomes, ten are V shaped chromosomes and 32 are rods and dots. If the latter are treated as equivalent to 16 V shaped chromosomes, the total number of V's arrived at is 26, a figure strikingly near that of many Urodela and Anura where the chromosomes are all either large or small V shaped ones. In this way the apparent chromosome differences between the Anura and Urodela on the one hand and an example of the Apoda on the other can be bridged.

The later diakinesis is marked by a great condensation of the tetrads and their distribution along the periphery of the nucleus. The split between the two bivalents which compose each tetrad can be made out in the longer tetrads while in the smaller ones the fusion of the bivalents is so close and intimate that the split cannot be seen. The condensation in case of the smaller tetrads takes place in different ways according to the kind of tetrad we are dealing with. In case of small rings, the space enclosed by the ring becomes gradually smaller as in the case of the smaller chromosomes of *Bufo arenarum* investigated by SAEZ and others (1936) till no split is in evidence. In the earlier stages, light areas can be recognised (fig. 22) in the position in which the space enclosed by the ring was present. Gradually this space becomes smaller, by the ring condensing and closing up till the whole tetrad looks like a single deeply staining round or quadrilateral body. In the case of the crosses, the condensation is essentially a process by which the arms of the cross become shortened and drawn to the centre till in the final stages the condensation is so great that no arms can be distinguished and the whole tetrad assumes the appearance of a block of chromosome material. In the case of the rod tetrads, the approximation of the parallel pairs gradually becomes so close that the components becomes hardly distinguishable. This followed by a linear contraction of the tetrad results in an apparent final shape not different from that derived from the rings or crosses. In the case of the larger pair of rod-shaped chromosomes, the tetrad is a linear body wherein even in the final state, four chiasmata can be distinguished, the loops of the successive chiasmata lying at right angles to one another. The attachment is terminal in this case and the tetrad lies at the periphery of the spindle in metaphase (fig. 29). The four large V shaped chromosomes seen in the spermatogonium give rise to two similar compound tetrads and in both cases the spindle attachment is median. Of the three smaller tetrads formed by the six smaller V shaped chromosomes of the spermatogonium, I believe one has a slightly submedian attachment while the other two have median attachments. In the case of the small chromosomes the spindle attachment is, in some, median and in others, terminal.

I believe that some at any rate, of the smaller tetrads form single rings with a median attachment, reminding us of figures found in certain insects (Hemiptera). I have not noticed such tetrads figured by any worker on amphibian spermatogenesis. The reason for this is that in many Amphibia rod and dot shaped chromosomes are not seen in the spermatogonial complexes. Many Urodela and Anura have V shaped chromosomes with atelometic attachments. In some Urodeles, however, with large chromosome number like species of *Hynobius, Salamandrella, Pachypalaminus, Cryptobranchus* and *Megalobatrachus* (IRIKI, 1932; MAKINO, 1932, 1934, 1935; SATO, 1936); dot like chromosomes appear in the centre of the spermatogonial metaphase plates. But none of the above authors has described the details of spermatogenesis or the behaviour of the small rod tetrads in Anaphase I. It is probable that such single ringed tetrads with median spindle attachments are found in these animals also. Future work on these interesting urodeles will show this.

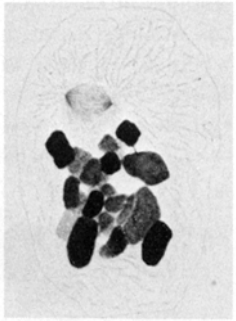

Fig. 24. Spindle formation. The nuclear membrane has disappeared and the tetrads are free in the cell and show a massing together. × 2400.

The growth of the nucleus has reached a maximum now with a diameter of 15 microns. The diameter of the nucleus in different stages of spermatogenesis is given below:

Primary spermatocytes at rest . .	10.0	to 10.5	microns
,, ,, in the pachytene	11.0	to 12.5	,,
,, ,, in diffuse stage .	13.0	to 14.0	,,
,, ,, in diakinesis . .	14.0	to 15.0	,,
Early secondary spermatocytes . .	10.0	to 10.5	,,
Late secondary spermatocytes . .	10.5	to 11.0	,,
Early spermatids	7.0	to 7.5	,,

The complete condensation of the chromosomes marks the end of diakinesis and the beginning of the dissolution of the nuclear membrane. I cannot say if the nuclear membrane breaks down first at the pole near the sphere as CARRICK (1934) appears to have noticed in *Amblystoma*, but even if it does, it is a fairly rapid process affecting the entire nuclear wall almost simultaneously.

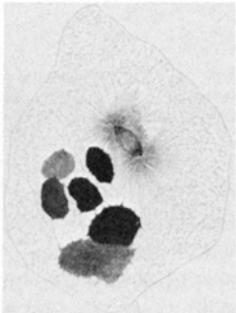

Fig. 25. Same as Fig. 24. The spindle probably takes its origin inside the sphere substance which envelops it. A few of the smaller tetrads are seen. × 2400.

The chromosomes appear irregularly distributed in the cell at the time the nuclear membrane disappears (fig. 24). I have also noticed a condensation of the chromosomes to form a more or less indistinguishable knot in which the individual tetrads cannot be identified. Whether this is an artefact or whether it is in the nature of a normal occurrence in spermatogenesis, I cannot say. It is noteworthy, however, that this great contraction and condensation are seen in material prepared by a variety of fixatives, — Bouin's fluid, Corrosive sublimate-acetic, Carnoy's fluid and others. It is also seen that a few of the smaller tetrads may appear clear and separate from the condensed knot (fig. 25). It is therefore impossible to determine the definite forms

of the tetrads at this stage but in favourable preparations, the characteristic shapes of the larger tetrads can be seen.

The centrioles, it will be noticed, remain in a position they occupied at the beginning of spermatogenesis, — close to each other, but distinct enough to be made out clearly. Till the beginning of the dissolution of the nuclear membrane the centrioles remain in this condition and only when the nuclear membrane is about to disappear, the centrioles move apart. The migration of the centrioles and the formation of the central spindle are processes which take place very rapidly and in a single group of cells, every stage from the middle diakinesis to the metaphase can be seen.

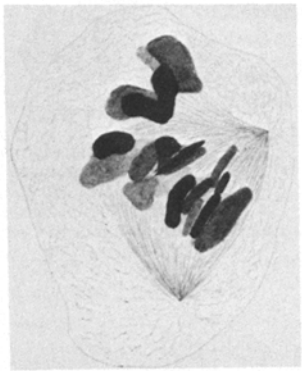

Fig. 26. Figure showing the inclusion of the tetrads on the spindle. The smaller chromosomes have already taken their places on it. × 2400.

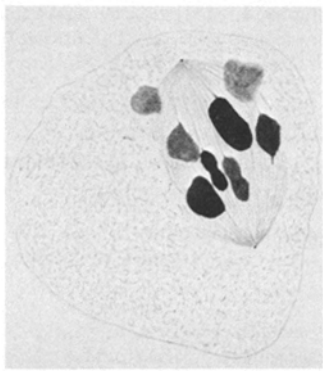

Fig. 27. Same as Fig. 26, showing an irregular distribution of the tetrads on the spindle before their being arranged to form a regular equatorial plate. × 2400.

The formation of the spindle in the primary spermatocytes follows the same plan outlined for the spermatogonia but a clear space about which the beginnings of the spindle are laid, is probably not present. The chromosomes get on to the spindle very rapidly and the elongation of the spindle as well as the inclusion of the chromosomes in it take place simultaneously. The smaller tetrads take their position on the spindle earlier than the larger ones (fig. 26) and that is probably how they come to occupy a more or less central position on the plate. By the time the spindle is fully formed with the centrioles at the opposite ends, the arrangement of the chromosomes on it is complete. At first the tetrads are irregularly placed on the spindle (fig. 27) and only later take up the perfectly equatorial position seen in metaphase.

Metaphase. The metaphase is a stage of fairly long duration and in favourable preparations, the individual tetrads remain distinct. FLEMMING, HERMANN and LINDSAY-JOHNSON preparations usually reveal very clear figures while in Bouin sections the tetrads tend to clump together obscuring the distinctness of the individual tetrads. But I have obtained

a number of preparations where the chromosomes in metaphase are exceedingly clear and lend themselves to counting with ease.

Likewise, the effect of fixatives on the appearance of the spindle is considerable. In preparations where the contraction of the chromosomes in metaphase is so intense that they form an indistinguishable knot, the length of the spindle also is small, probably brought about by the same process of contraction, while in good preparations where the chromosomes are distinct, this contraction of the spindle is not noticeable. In this connexion it should be remembered that a time of fixation which preserves the metaphase plates with clearness is often different from that which preserves the rest of the stages of spermatogenesis

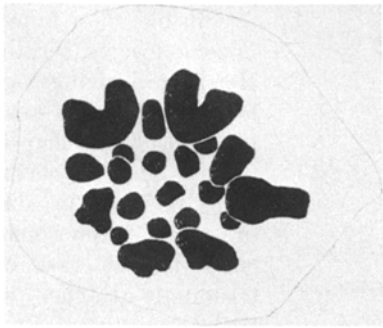

Fig. 28. Polar view of a metaphase plate of the primary spermatocyte showing the 21 tetrads. × 2400.

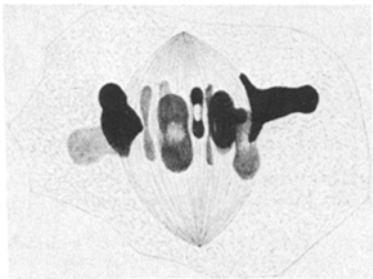

Fig. 29. Side view of the spindle in early anaphase. The terminally attached rod tetrad is seen to the right. × 2400.

with the same amount of clearness. This is especially so with fixatives which act rapidly, like Carnoy's fluid and Bouin's fluid.

A split in the tetrad indicating the two dyads which compose it, is not clear at this stage, though in many other Amphibia, this split never disappears and is always visible (Carrick, 1934). The arrangement of the chromosomes in the metaphase is such that, as already noticed, the larger chromosomes lie in the periphery while the smaller ones are placed in the centre. Fig. 28 illustrates a polar view of the metaphase plate of a primary spermatocyte. The two large V shaped tetrads, one large rodshaped tetrad and three smaller V shaped tetrads occur in the periphery while the 15 smaller tetrads derived from the small rods and dots of the spermatogonial complex occupy the centre. It is also noticed that amongst themselves the tetrads do not occur in any regular fashion on the plate so that any two metaphase plates differ from each other so far as the arrangement of the chromosomes are concerned. This feature has been noticed by Witschi (1924) in *Rana temporaria* and more recently by Saez and others (1936) in *Bufo arenarum* who have published a number of figures of metaphase plates of the first division.

Anaphase (figs. 29 and 30). The beginning of the anaphase is marked by the reappearance of the split in the tetrads and it is also now that

we notice the spindle attachments of the chromosomes. As already indicated, the large rodshaped tetrad shows a terminal attachment and the two large V shaped tetrads have a median attachment. Of the three small V shaped tetrads, I believe that in the case of two, the attachment is median and in the third, it is submedian. With regard to the smaller tetrads, I believe some have a terminal attachment while others have a median one, the latter giving rise to forms of tetrads like those found in some insects (certain Hemiptera), giving single ring figures in anaphase. Dumbell-shaped figures, long or short, according to the size of the chromosome, occur in the case of terminally attached tetrads.

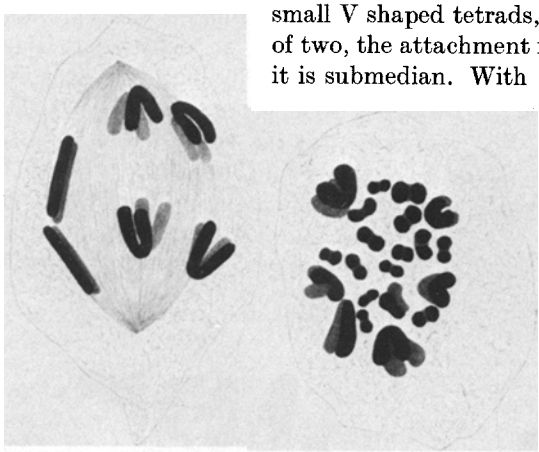

Fig. 30. Fig. 31.

Fig. 30. Later anaphase showing the division of the three largest tetrads. × 2400.

Fig. 31. Anaphase plate showing 21 tetrads, the doubleness of each of which is cear. × 2400.

The first indications of the secondary split separating the two monads composing each dyad make their appearance at the beginning of the anaphase and as the two dyads move away from each other towards the poles, the split becomes greatly accentuated, till in late anaphase, the two monads have become completely separate (fig. 30). Necessarily, this can be seen better in the larger tetrads than in the smaller ones but in the anaphase plate at each pole (fig. 31), a stage slightly in advance of that figured in 30, the dual nature of each dyad is very clear and reminds us of the condition figured by MATTHEY (1936) in certain mammals. In the case of the large chromosomes the two monads lie close together in different planes, while in the case of the smaller ones, a light area indicates the split between the two monads.

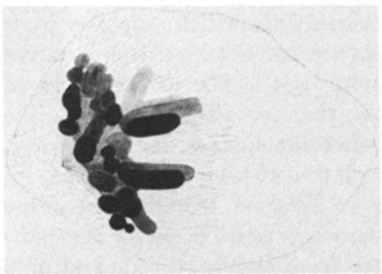

Fig. 32. Side view of later stage showing the beginning of the massing of the chromosomes. × 2400.

Telophase. Once more, a severe contraction of the chromosomes takes place involving a massing together of the dyads and resulting in an indistinguishable knot from which the ends of only the larger chromosomes protrude (fig. 32). This massing of the chromosomes in early

telophase probably does occur and is not solely the result of the contraction brought about by the fixative, for it has been observed by me in preparations made in a variety of ways and also it has been reported in a number of animals. The constriction of the spindle fibres in the middle to form a midbody, the origin of

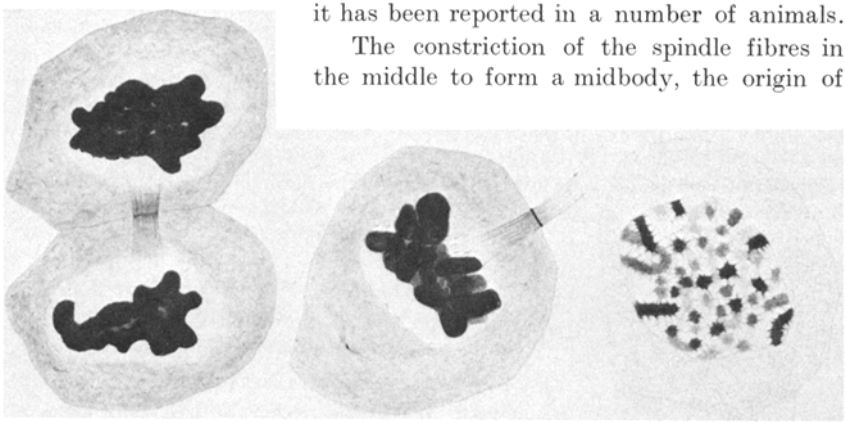

Fig. 33. Fig. 34. Fig. 35.

Fig. 33. Telophase I. Granules appear along the spindle fibres in the centre. A clear space surrounds the telophase mass. × 2400.

Fig. 34. Later stage. The bridge body is in the form of a plate. × 2400.

Fig. 35. Nuclear membrane is formed and the chromosomes are discrete. Filamentous projections are seen between the chromosomes. × 2400.

a space around the telophase mass and the final separation of the two daughter cells are similar to what I have described in the spermatogonia and to what other authors have noticed (figs. 33, 34, 35). The bridge-body makes its appearance at first in the form of a number of discrete granules (fig. 33) which, as the constriction advances and as the daughter cells separate, unite to form a deeply staining plate (fig. 34). Later the bridge-body with a conical remnant of spindle fibres attached to each side is cast off and probably degenerates.

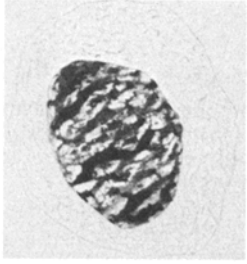

Fig. 36. Telophase metamorphosis. Nucleus showing a resolution of the telophase chromosomes. × 2400.

The picture one sees of the nucleus immediately after the nuclear membrane is formed clearly shows all the chromosomes inside the membrane sending off fine processes connecting one another, — picture strikingly similar to what has been figured in *Salamandra maculosa* (MEVES, 1897) and *Geotriton fuscus* (TERNI, 1911). Such a nucleus is shown in fig. 35 where the individual chromosomes are seen putting out processes. The telophase metamorphosis is rapid and a vacuolation of the chromosomes (fig. 36) results in a diffusely staining network which characterises the main stage of interkinesis (fig. 37).

Interkinesis.

In the Amphibia the length of duration of interkinesis varies in different species and probably also in the same species and even in the same individual. In forms like *Desmognathus* (KINGSBURY, 1902), *Salamandra* (MEVES, 1897), *Bufo lentiginosus* (KING, 1907) and *Bufo arenarum* (SAEZ and others, 1936), the chromosomes of the telophase of the first division enter on the spindle of the second division without any marked alteration. In others like *Amphiuma* (MACGREGOR, 1899) and *Amblystoma* (CARRICK, 1934), a diffuse network results from a branching of the chromosomes though in these two animals this stage of diffusion is not of any considerable duration. Probably the extreme in this respect is seen in *Rana*, *Alytes* and *Bombinator* (CHAMPY, 1913) where the chromosomes become greatly branched, lose most of their staining capacity and remind us of the resting nucleus of the primary spermatogonium. Nucleoli are also seen.

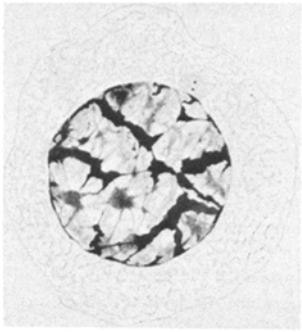

Fig. 37. A nucleus in typical interkinesis. × 2400.

The condition in *Ichthyophis* resembles the third kind describedabove and I am sure a stage of great diffusion of the chromosomes, resembling remarkably the resting stage, intervenes between the telophase of the first division and the prophase of the second. Such a stage is shown in fig. 37. It can also be seen that it is of long duration as the nuclei of a group of cells are all in this stage and also a number of cysts in this stage are encountered in a preparation of the testis.

The importance once attached to this stage is now lessened after the realisation of the important fact that the chromosomes of metaphase II are essentially the same as those of Anaphase I. Further, a vast body of evidence has accumulated to show that every transition between cases where the chromosomes after one division enter on to a second without any change and those where extreme diffusion takes place, occurs in both plants and animals.

Secondary spermatocytes.

The history of prophase II is the same as that of prophase I from the time of emergence of the chromosomes from the "diffuse stage". The nucleus regains basophily which had been temporarily lost during interkinesis and filaments become organised. At first they are irregular and uneven in their appearance but gradually they become uniform and show a peripheral distribution in the nucleus (fig. 38). They are all distinct but no duality can be seen at this stage. The resolution of the chromosomes, the disappearance of the nuclear membrane and the placing of the chromosomes on the spindle are processes which take place rapidly, for stages indicating every one of these processes occur in the same group of cells.

Some authors describe the formation of a filament directed towards the pole in the secondary spermatocytes emerging out of the diffuse interkinesis, very much like that seen in the primary spermatocytes, though not so pronounced (*Bombinator:* CHAMPY, 1913). I have never noticed such a condition in *Ichthyophis* and I believe it does not exist.

A stage similar to the knotting of the chromosomes into a mass during spindle formation, observed in the primary spermatocytes is seen in the

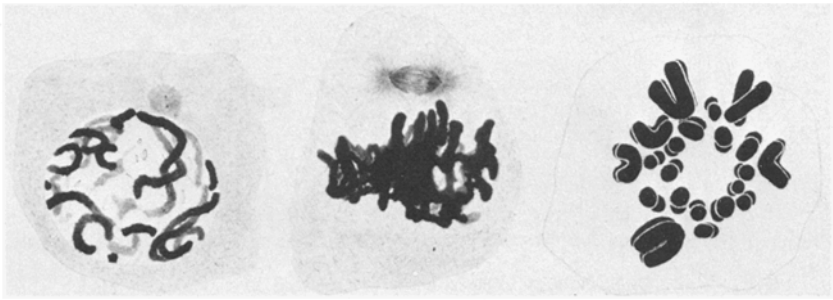

Fig. 38. Fig. 39. Fig. 40.

Fig. 38. Prophase II. The chromosomes havee merged from the network and are clear. × 2400.
Fig. 39. Spindle formation. The nuclear membrane is lost and the chromosomes have again massed together. × 2400.
Fig. 40. Polar view of a metaphase plate II. 21 dyads, each double, are seen. × 2400.

secondary spermatocytes also. In this mass (fig. 39) no details can be seen and the individual chromosomes cannot be made out. The dyads can be observed clearly only in metaphase when they are arranged

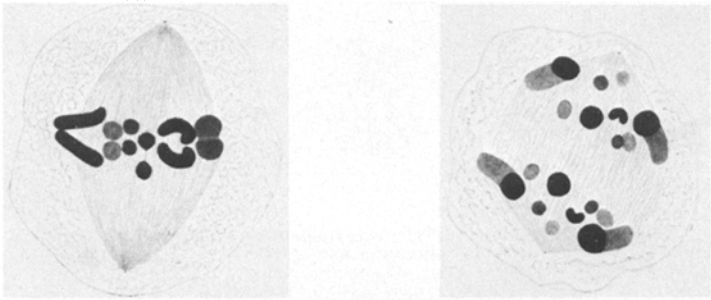

Fig. 41. Early anaphase II. × 2400. Fig. 42. Later anaphase II. × 2400.

equatorially on the spindle. A figure of this stage is depicted in 40. It will be noticed that in a polar view 21 dyads can be seen and the doubleness of each dyad is also clear. The distribution of the chromosomes in metaphase is the same as that observed in spermatogonia and primary spermatocytes, with the larger chromosomes occupying the periphery and the smaller ones placed in the centre. The different chromosomes can also be recognised, one large rodshaped chromosome, two large V shaped chromosomes, three smaller V shaped chromosomes and the rest, rods

and dots. Here also no regularity in the arrangement of the chromosomes with reference to one another can be noticed.

The separation of the dyads into the constituent monads takes place in Anaphase II. Figs. 41, 42, 43 illustrate the successive stages in the

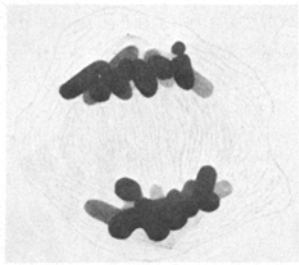

Fig. 43.

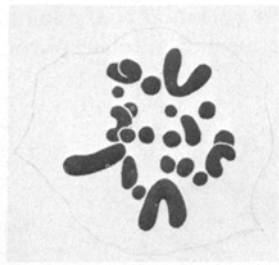

Fig. 44.

Fig. 43. Stage later than that illustrated in fig. 42. Already a massing of the chromosomes is in evidence. × 2400.

Fig. 44. Early anaphase plate of second division showing 21 monads. × 2400.

process and at the end of division each daughter cell has 21 monads. In early anaphase pictures, the 21 monads can be counted with ease (fig. 44), while in later anaphase (fig. 43) and in telophase (fig. 45) the same massing of the chromosomes seen in the first division is noticed.

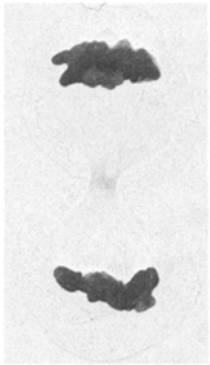

Fig. 45. Telophase; Side view. × 2400.

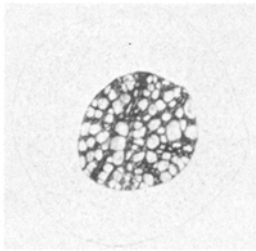

Fig. 46. Telophase vacuolation of the chromosomes. × 2400.

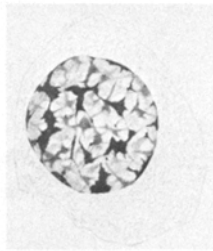

Fig. 47. An early spermatid nucleus. × 2400.

The telophase changes in the chromosomes of the two daughter nuclei after division II consist essentially of the same processes as those described for spermatogonia and primary spermatocytes consisting of a vacuolation of the chromosomes (fig. 46) and their branching to give rise ultimately to a diffuse network characteristic of the early spermatid (fig. 47).

Summary.

The primary spermatocytes which are the products of division of the spermatogonia embark on the prophase of the first division after a brief period of rest. The leptotene filaments are formed soon after and

the polar orientation of the filaments results in a leptotene bouquet. Parallel conjugation of the leptotene filaments gives rise to the thicker pachytene threads, the conjugation beginning at the proximal pole. When the fusion between the apposing filaments is more or less intimate, the polarisation is lost and immediately after, splits appear along the threads at intervals, giving rise to the diplotene stage. The diplotene is followed by a pronounced and conspicuous diffuse stage where all individuality of the chromosomes is temporarily lost, the nucleus itself becoming a faintly staining reticulum, in its greatest development. Soon, the chromosomes emerge from the diffuse mass and the bivalents which now appear more or less clear, twist about each other giving rise to the strepsinema. A condensation and contraction of the chromosomes give rise to the diakinesis where 21 deeply staining tetrads of different forms are seen arranged peripherally inside the nuclear membrane. The spindle is formed between the centrioles, the nuclear membrane is lost and the tetrads take their place on the spindle. 21 tetrads can be clearly seen in the metaphase plates of the division, the four components of each tetrad being seen clearly for the first time in anaphase. A conspicuous interkinesis separates the first and second divisions, when the nucleus resumes the appearance of a faintly staining network. The second division follows quickly separating each dyad into two monads. 21 dyads are seen in the metaphase plates of this division while 21 monads can be counted in the anaphase plates. The resulting cells are the spermatids.

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