THE DEVELOPMENT OF THE RETINA IN AMPHIBIA -- AN EMBRYOLOGICAL AND CYTOLOGICAL STUDY.

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I. Introductory.

(1) Literature

The subject of the development of the vertebrate retina has received attention from many workers at Embryology, and an extensive literature has been the result.

A large amount of research on this structure has been carried out by Kölliker (1 & 2), working chiefly with embryos of the rabbit and also with human embryos. His main conclusions are, that the internal molecular and the ganglionic layers first appear; while the outer and inner nuclear layers are differentiated much later by the interposition of the external molecular layer. He and Babuchin (3) were the first to point out that the rods and cones develop as prolongations from the cells of the external nuclear layer --- a better explanation of the origin of these structures than that adopted by Max Schultze (4) and W. Müller (5), who described them as cuticularisations of the same cells. His also has made several important communications on the development of the retina in the human embryo; but his observations are chiefly confined to the mode of formation of the optic vesicle, optic cup and choroidal fissure. He (6 & 7) and Kölliker (1 & 2) were among the first to show that the optic nerve fibres develop

as axis cylinder processes of the retinal ganglion cells. This has now been accepted as their correct mode of origin, although for a long time it was disputed by many competent authorities, who still clung to the erroneous idea that they were developed from the cells of the optic stalk. Müller (5) has contributed to the literature of the subject an interesting account of the development of the retina in Ammocoetes. Researches on the rabbit embryo were conducted by Löwe (8), but his conclusions differed in some degree from those of Kölliker (above). He described three stages in the differentiation of the retinal layers, and considered the outer limbs of the rods and cones to be derived from metamorphosed cells. This, however, is now known to be a wrong interpretation of the origin of these structures. Regarding the development of the membrana limitans interna there was for some time much doubt. Arnold (9), Löwe (8) and Lieberkuhn (10 & 11) considered it to be formed from the mesoblast; but Kölliker (1) eventually proved, that it was developed from the optic cup. The pigment layer was found by Gunn (12), in the course of a research on the Teleostean retina, to develop from cells which grew in from the deep layer of the cuticular epiblast, and which were distinct from the cells of the optic cup. A general discussion on the development of the Vertebrate retina is given by Cajal (13) at the close of his researches on that structure

in the adult, and he makes a number of references to the work of earlier observers (Kölliker, His &c.). The mode of formation of the optic nerve fibres and choroidal fissure in Amphibia was traced by Assheton (14) who showed that ~~they~~ the former are developed as axis cylinder processes from the retinal ganglion cells.

An interesting paper has been recently published by Cirincione (15), dealing with the embryology of the retina in Reptilia, in which the author treats chiefly of the mode of development of the optic vesicle and cup in this class of Vertebrates.

In 1900, Levi (16) published a paper on the development of the rods and cones in the Urodela; but, unfortunately, I have been unable to consult this memoir.

The latest published account of the embryology of the retina is one by Kerr (17), who gives a brief description of its development in Lepidosiren Paradoxa.

Bernard has made an extensive series of communications (in five parts) on the retina, chiefly of Amphibians (both tadpoles and adult forms); but also of other vertebrate types. These have all been published during the progress of my research. I propose in this paper to record my own independent observations, and do not intend it to be devoted to a criticism of that observer's work. Still, it is necessary for me to refer to his main conclusions. These are as follows:-

(1) The retina consists of a syncytium or cytoplasmic reticulum in which nuclei are suspended. These nuclei are not stationary, but are capable of migrating outwards, ultimately to become rod-nuclei (18).

(2) The rods are prolongations of the cytoplasm of the retinal syncytium; but a great part, if not all, of the fluid, which is first protruded to form the rods, comes from the associated nuclei (19). He also shows that absorption of pigment by the rods occurs during the tadpole stage (20).

(3) The cones in Amphibia are the early stages in the formation of new rods (21).

(4) The fibres of Müller are streams of absorbed pigmentary matter finding its way through the retina. These appear only after the eye has begun to function; no such preformed structures exist (22).

On looking through the above literature it is to be noticed, that most of the descriptions deal chiefly with the mode of formation of the vertebrate optic vesicle and cup, while the study of the development of the various retinal layers from its walls has received much less attention. The embryology of the optic or, (as I prefer to call it), the retinal cup is thus well known; but the mode in which the layers of the retina are formed from its walls is less perfectly understood. This fact was admitted by the late Professor Milnes

Marshall[1] in his text book of Vertebrate Embryology. Professor Schäfer[2] in Quain's Anatomy also states, that the development of the retina from the inner layer of the optic cup has not been fully worked out. An investigation into the subject therefore seemed desirable, and the description contained in this thesis is the result of a research begun about three years ago. The work has been done, partly in the Anatomy Department of the United College, St Andrews University, and, partly in the Laboratory of Professor His at Leipzig. To both Professor Musgrove and Professor His I wish to express my indebtedness for many kind facilities which have been granted to me during the progress of the research. The work was done as a Research-student and later, as a Research-fellow, of St Andrews University. It is necessary to mention, that part of the expenses were paid by the Royal Society through its Government Grant Committee.

(2) Special Methods Employed.

(a) Fixation. In conducting a research on the retina, the greatest difficulty that has to be encountered is the proper fixing of its elements, more especially the rods and cones. These latter structures are so delicate and fragile, that subsequent manipulations easily injure or even completely destroy them, if they

1. page 279. 2. Vol. I. pt. I. page 86.

are imperfectly fixed. After a trial of several agents, I eventually decided to employ the fixative known by the name of Bles' fluid. The formula is as follows:-

70% Alcohol 90 parts
Glacial Acetic Acid 3 parts
Formalin 7 parts.

This has yielded in my hands perfectly satisfactory results. A week's immersion in this fluid was found sufficient to completely fix the tissues.

(b) Dehydration. From this mixture the tissues were directly transferred to 90% alcohol for 24 hours. Subsequently, two baths of absolute alcohol (each of 24 hours' duration) sufficed to complete dehydration.

(c) Clearing. The tissue was then placed in cedar wood oil, which was kept heated in a paraffin stove to a temperature of 55° C., for 24 hours. In the case of very young embryos a shorter time was found sufficient (12 hours).

(d) Embedding. From the cedar wood oil the tissue was transferred to a first bath of melted paraffin and, after one hour, to a second. It was found that the paraffin into which the tissue was placed directly from the cedar wood oil was apt to 'crystallise' when poured into the mould. This was obviated by the second lot of paraffin, in which the tissue was kept for 6 hours, and afterwards used for pouring into the mould.

Paraffin of a M.P. of 51° C. was employed; but in the height of summer at Leipzig, a paraffin of 54° C. had to be resorted to.

(e) Section Cutting. The sections of the frog-tadpoles were cut with a Minot Microtome and those of the toad--tadpoles with a Cambridge Rocking Microtome, each section being of a thickness of 3.3 Microns.

(f) Section Mounting. The sections were fixed on to the slide by a method for which I am indebted to Dr Fraser Harris. The 'ribbons', cut into lengths of suitable size, were placed on the surface of warm water of such a temperature that the paraffin flattened out perfectly, and were floated from this on to the slide. The slides were then placed edgewise in the paraffin stove (top shelf) for 12 hours (overnight). It is quite unnecessary to previously albuminise the slides for not one of the many thousand sections, which I have mounted in this way, has come off during the subsequent staining processes.

(g) Staining. After experimenting with many staining agents, I found that the most satisfactory results were obtained with the Iron-Alum-Haematoxylin stain (Heidenhain). By this the rods and cones are especially well stained throughout their whole extent, even to their tips; but it also brings out in a wonderful way the fibres of Müller and the molecular layers (see Fig. 11 and slide 32). In order that these favourable results

might be obtained, it was necessary to depart somewhat from the ordinary method of using the stain --- as described in the "Microtomist's Vade Mecum."

The method which I employ is as follows:-
The slides are first placed in the iron-alum solution for ten minutes, rinsed in tap water, and then left in the haematoxylin solution until the sections show a bluish black colour, when held between the eye and the shade such as is afforded by the upper margin of a window (if the slide be examined by direct daylight the sections appear black). This tint is usually produced in about 20 minutes in the case of sections which are 3.3 microns thick; but for sections 6.6 microns thick only ten minutes are necessary. The slide is then rinsed in tap water, and again dipped into the iron alum solution, when, almost immediately, the bluish black colour is brightened, so that the sections, when held up to the window as before, appear of an ultramarine-blue tint, the black element of the previously blue-black colour having disappeared. The slide is now placed in a fresh quantity of tap water for 15 minutes, when the colour will be found to be even richer in hue. Overstaining in the haematoxylin prevents successful differentiation, while understaining leads to rapid bleaching in the iron-alum solution. In the latter case the slides should be rinsed once more in tap water and replaced in the haematoxylin.

Old solutions of haematoxylin, even when frequently filtered, are apt to deposit small black specks on the section. When this occurs the solution must be renewed. In the "Microtomist's Vade Mecum" it is recommended to dissolve 5grms. of haematoxylin crystals in 100 c.c. of distilled water. Only a small quantity of this, however, goes into solution, the rest becoming disintegrated to form the deposit mentioned above. Quite as good staining results are obtained when only a crystal or two are used, while the tendency to the formation of deposit is considerably lessened. The iron-alum solution is rather unstable and must be renewed whenever it becomes turbid.

By the above method of staining the nuclei show a brilliant ultramarine-blue colour, and under the high power the intranuclear structure is very well demonstrated.

The other stain employed was Mayer's Haem-Alum, followed by eosine or by Mann's Methyl-blue-eosine. With these the various retinal layers are well shown (see slide 60); but the rods and cones are only faintly stained. It may be observed that, when Mann's solution is used, the eosine stains the outer segments, while the methyl-blue stains the inner segments of the rods and cones. The staining effect on these structures, is, however, very slight compared to that of the iron-alum-haematoxylin.

The Golgi method was also experimented with, but in my hands yielded very unsatisfactory results in the embryonic retina.

(h) Dehydration and Clearing of Sections. After two baths of 90% Alcohol, the sections were completely dehydrated by absolute alcohol, and cleared in exotic oil of lavender. This latter substance, while clearing the tissue perfectly, does not tend to cause shrinkage. The most convenient way to use it is to immerse the slide in the oil contained in a small glass trough, which can be rocked from side to side and from end to end, in order to remove all traces of alcohol. It is advisable to leave the slide in the oil for about five minutes. After draining off the excess of oil, a small piece of dried filter paper is laid over the sections, and the surface of this is gently stroked so as to remove the most of the oil. A drop of Canada balsam is then applied, followed by the cover glass.

(3) The procuring of the embryos.

Two frogs, which had already paired, were placed in a large tank by themselves, and after two days an abundant deposit of spawn was obtained. This was placed in a large dish with water plants, and kept in the laboratory. Under these conditions the ova and resulting tadpoles developed very successfully. Toad-spawn, recognised by the intense black pigment of

the ova, was got at the side of a pool in a disused quarry, and, when found, the ova had practically reached the same stage of development as the frog's ova, when first discovered. The toad-spawn was placed in another dish and allowed to develop under similar conditions. From these dishes embryos were removed every second day for the purpose of examination. The series of frog-embryos is fairly complete, leading to the end of the metamorphosis (94 days). Owing to occasional spells of hot weather in Leipzig, the paraffin cut badly, and some specimens were consequently lost. The series of toad embryos is less complete, the supply of tadpoles having become exhausted by the 76th day (the ages of the embryos are calculated from the date of impregnation of the ova). After the 25th day the tadpoles were kept in the dark for three hours before killing, so as to ensure full retraction of the pigment-cell processes, which were found to become active under the influence of light about that date.

II. Results of the Research.

As already stated, the mode of development of the optic vesicle and cup has been previously very fully described by numerous observers. This research, therefore, deals with the mode of origin of the various retinal layers from the walls of that cup. It is convenient to consider first the inner wall, and note its condition

in the youngest embryo shown (Fig. 29). (It is interesting to observe, that the inner and thicker wall of the optic cup is continuous with the basal portion or floor, while the outer cup-wall is continuous with the lateral wall of the thalamencephalon. At the same time it may be noticed, that the lens is developed from the deeper layer of the epiblast, and is at first solid, the cavity appearing somewhat later (Figs. 2 & 31)). The description which most observers furnish with regard to the structure of the inner retinal wall is, that it is quite uniform --- that is to say --- composed entirely of the same kind of cells. Thus, it is stated in Quain's Anatomy (1) with reference to this inner wall, that "in its earlier stages it closely resembles in structure the wall of the cerebral vesicles, consisting of elongated epithelium -like cells, apparently arranged in several interlocking layers." Somewhat similar statements are made in Balfour's Comparative Embryology, Foster and Balfour's Embryology and Marshall's Vertebrate Embryology. If this wall be minutely examined, however, it will be found to contain all the structures which His (6) has described as being present in the spinal cord of the human embryo, and I propose, that the corresponding structures in the retina receive names in accordance with this view.

1. Vol. I., Pt. I. p. 86.

There are firstly cells (SPONGIOBLASTS)[1], whose processes unite with those of neighbouring spongioblasts to form a network (MYELOSPONGIUM). The outer and inner extremities of this myelospongium-network of the retina form the external and internal limiting membranes respectively. (It may be noted, however, that the external limiting membrane of the retina is next to the original cavity of the optic vesicle, and corresponds, therefore, to the internal limiting membrane of the embryonic spinal cord). In the meshwork of the myelospongium are found two kinds of cells -- (1) GERMINAL CELLS[2], which are always situated immediately underneath the external limiting membrane, and (2) NEUROBLASTS, which are formed by division of the germinal cells. The importance of recognising the presence of these four structures in the inner retinal wall can hardly be overestimated, since the subsequent development of the various layers is much influenced by them, and, undoubtedly, a large amount of the difficulty in the interpretation of the mode of development of the retinal layers has been due to the fact, that the presence of the above structures during the earlier stages has not been fully recognised. Professor His kindly permit-

(1) Certain cells in the inner nuclear layer of the adult retina have been wrongly termed spongioblasts, for they are derived from the neuroblasts of the embryonic retina.

(2) This term will require slight modification, as will be shown later (p.18).

ted me to examine some of his sections of the embryonic human retina, and in these, as well as in some of my own preparations (human), I have been able to demonstrate the presence of the same four structures. I have also studied the retinae of fish-embryos and of the chick, with exactly similar results.

The layer of germinal cells is never a complete one (Figs. 2,3,4,etc.), and, indeed, no karyokinetic figures may be seen at all in some of the sections (Fig. 1). It is interesting to note that the plane of division of the germinal cells is always in one direction namely -- at right angles to the external limiting membrane, so that the two daughter-cells lie at first side by side, and, later, one of them is moved towards the deeper layers of the retina. The first formed neuroblasts are therefore to be found in the site of the future ganglionic layer, while those of a more recent generation are situated at varying distances from the external limiting membrane according to their age -- the newly formed ones being of course placed nearest to the layer of germinal cells. It will be at once understood from this, that those neuroblasts, which become the retinal ganglion-cells, are the first to be formed by the division of the germinal cells, and they are on the whole rather larger than the younger neuroblasts. The germinal cells in the middle of the convexity of the retinal cup cease to divide at

a very early stage of development, and become directly transformed into rod - and cone-cells, and from them the rods and cones develop as processes. They constitute the youngest generation of retinal cells, and no karyokinetic division can be seen to occur in this region after the rods and cones begin to make their appearance (Figs.3 & 4). The germinal cells still persist under the external limiting membrane nearer to the margin of the retinal cup, and by their division the growth of the retina results. The rods and cones thus appear first over the middle of the convexity of the cup, and from this point the development of these structures extends in all directions towards the cup-margin, and they gradually encroach upon the germinal cell-area as development proceeds. The writer has also observed this fact in the retina of Teleostean embryos and of the chick, and Bernard (1) refers to the same point in his paper.

A most striking characteristic which is displayed by the retinal neuroblasts is, that their nuclei all show a progressive diminution in size as development proceeds, and this is well seen on comparing figures of the early with those of the late stages (Compare Figs. 1,2,3&4 with Figs.26,27 & 28, which have all been drawn of exactly the same degree of magnification). Bernard (2) in his paper states, that he has observed certain of the

(1) Quar. Jour. Micr. Sc. Vol.43, p.43.

(2) Quar. Jour. Micr. Sc. Vol.44, p.453.

cone-nuclei in a collapsed condition as if their contents had been forced into the bases of the cones, and I have also certainly noted this phenomenon; but, apart from that, there is a ~~gradual~~ progressive diminution in the size of the nuclei in all the layers, which is so gradual as only to be noticed by comparing figures of early and late stages (in some cases the nuclei are reduced to less than one-fourth of the size which they possess in the early stages). I am forced to abandon the terms "germinal cells" and "neuroblasts", as I have been unable to demonstrate the existence of protoplasm around these structures by the use of cytoplasmic stains. (Tissues fixed in Bles' fluid often do not stain with aniline cytoplasmic stains, such as eosine, so well as tissues which have been fixed in corrossive; but, even after corrossive-fixation, this protoplasmic investment cannot be demonstrated). Other observers state that protoplasm is present, but very scanty in amount, and what there is of it is prolonged into the processes of the neuroblast. After studying these neuroblasts (in the wall of the cerebral vesicles and spinal cord, as well as in the retina) carefully, I have come definitely to the conclusion that this protoplasmic investment is absent and that these processes cannot therefore be derived from it; but, in reality, issue from the substance of the nucleus. His' drawings (1) of these neuroblasts also certainly suggest this conclus-

(1) Figured in Quain's Anat., Vol.I., Pt.I.p.58.

-ion. The processes of the retinal "ganglion-cells" and of the "cells" of the internal nuclear layer first show themselves as protrusions of the nuclear contents, while the rods and cones are at first also protrusions from the nuclei of the external nuclear layer. A clear unstained vesicle can often be seen at one pole of a nucleus of the retinal wall (three such nuclei are shown in Fig.31), and appears to consist of the achromatic nuclear matrix,(1) which is about to be protruded. (I find that this diminution in size of the nuclei is practically universal throughout all embryonic tissues, and is especially evident in striped muscle, where the nuclei, in the earlier stages, are of gigantic size, and, later, some of them give off their contents so suddenly, that they appear quite collapsed. I refer to this fact very briefly here, because it is to form the subject of a future communication).

This appears at first to be rather a bold assertion, rendering necessary a change in many of the views which are at present in vogue regarding the structure of the retina; but the evidence in favour of it is so overwhelming, that I have no hesitation in believing it to be a correct one. This view is further supported by an examination of the germinal cells. It is stated in textbooks, that the nuclei of these cells (in the em-

(1) The Nomenclature given in Quain's Anatomy, Vol.I., Pt.II., p.179., has been adopted.

bryonic spinal cord) are usually in one or other of the stages of karyokinesis, and are surrounded by a zone of "clear protoplasm" -- the term 'clear' being no doubt applied because this material stains only very faintly. After having attempted to demonstrate it by numerous cytoplasmic stains, and having been entirely baffled in this attempt, I am forced to the conclusion that this clear protoplasm is simply the achromatic[1] nuclear matrix, in which the chromatic karyokinetic figures are lying. This achromatin has been set free by the disappearance of the nuclear membrane -- a phenomenon which occurs during karyokinesis. These germinal cells, then, and the neuroblasts to which they give rise, are simply nuclei. Further, the progressive diminution in the size of the nuclei is due to the extrusion of this achromatic nuclear matrix in the form of processes. Hence the difficulty of demonstrating these latter structures, which has been experienced by every observer. The rod- and cone-elements, when they first appear, do so as perfectly clear spherical globules (Figs.1,2,3, 53 & 54)consisting of nothing more than this achromatic nuclear matrix. This fact will be much more fully alluded to when I come to consider the development of these elements. At this early stage of retinal development the only material present, which can be at all

(1) It is to be noted, that the term 'achromatic', which is here employed, is not an absolute one, for this nuclear substance does stain but to a very slight extent.

considered as representing cytoplasm, is the myelospongium-network. The germinal nuclei by their division give origin to all the nuclei of the inner retinal wall, and what appears to happen is, that some of these nuclei become embedded in the 'cytoplasm' to form spongioblasts, while others remain free in the meshwork of the 'cytoplasm' as neuroblasts. This observation regarding the mode of development of these structures does not support the theory advocated by Gaskell (23) namely -- that the inner wall of the vertebrate retina is to be considered as consisting of two parts (Müllerian Fibres and Retinal Ganglion), each with a distinct and separate origin.

The diminution in the size of the retinal nuclei is sufficient to account for the growth of all the processes of the nuclei in the internal nuclear layer, since these are very short;--but on the other hand, it is insufficient to account for all the contents of the rods and cones, as will be explained later when I come to describe the mode of origin of these structures.

The axis-cylinders of the optic nerve arise as processes of the nuclei in the ganglionic layer (being composed for the most part of the achromatic nuclear matrix, and hence the great difficulty of staining the individual fibres); and, therefore, the drain on these nuclei is greatest. This is well demonstrated by the fact that ~~the~~ their relative rate of diminution is the most

rapid; but, as already shown, they are the largest nuclei in the inner retinal wall, and their diminution in the early stages is due to the ingesting of food material by them being insufficient to enable them to retain their previous size, while their contents are being extruded in the form of processes. In the adult frog's retina these nuclei of the ganglionic layer are the only ones which become surrounded by a cytoplasmic investment, and this is undoubtedly excreted from the nucleus.

Miss Huie (25) has shown, that in Drosera the cytoplasm of the gland-cells is produced by the cell-nucleus absorbing food-material, metabolising it, and then excreting it into the body of the cell. So also, in the case of these retinal ganglionic cells, there is ample proof that their protoplasm is excreted from their nuclei. The origin of the axis-cylinders from these nuclei, before they show any trace of surrounding protoplasm, supports the contention of those who insist on the fact that the axis-cylinder process of a nerve-cell can be traced right into the nucleus of that cell.(1)

The newly formed optic vesicle and also the walls of the cerebral vesicles are loaded with yolk-granules; but these disappear at an early stage (by the 20th day),

(1) Quain's Anat. Vol.I.Pt.II.p.318.

and it is quite easy to demonstrate, that they are ingested by the neuroblast-nuclei as food-material; for the nuclei of the inner retinal wall at this period show large irregular masses (which may be termed nucleoli) of deeply staining material. These obscure the intranuclear network, and give to the nuclei a very coarse structure; but this tends to disappear later, so that the intranuclear network again becomes evident (compare Figs.53 & 54 with Fig.56).

In the early stages (e.g., 12 days) yolk-granules may actually be seen in the substance of some of the nuclei -- apparently undergoing the process of digestion (study some of the nuclei in slide 71). This action of these nuclei on the yolk-granules affords ample substantiation of Mann's view (24) that the nucleus has the power of absorbing and metabolising food - a theory which has been ably supported by Miss Huie's researches (25) on the gland-cells of Drosera.

Formation of the Molecular Layers.

The myelospongium-network plays a most important part in the formation of the molecular layers. It can be recognised in the very earliest stages and its general arrangement is as follows:-

(1) The outer ends of the fibres are attached by broad bases to the external limiting membrane, and between these the germinal nuclei are found lying (Fig.57).

(2) Immediately internal to the layer of germinal nuclei each basal portion as a general rule divides into two branches, which unite with their neighbours so as to form a sort of 'arcade', within the arches of which lie the germinal nuclei and, later, the nuclei of the rods and cones. The site of the future 'sense-epithelium'layer is thus mapped off at a very early stage of development.

(3) Between this 'arcade' and the internal limiting membrane, the myelospongium is arranged in the form of an irregular network (within the meshes of which lie the neuroblasts), and its inner extremities divide into two branches which blend to form the internal limiting membrane.

When the internal and external molecular layers develop, then those myelospongium-fibres which run in a radial direction become thicker and, therefore, better marked, and give rise to the fibres of Müller; while the other portions of the network do not tend to become thickened, and now appear as lateral offshoots from the radially directed fibres (these radially directed fibres of the myelospongium may thus be termed the rudiments of the fibres of Müller).

Bernard (see reference 22 in the literature) considers that the fibres of Müller are formed from streams of absorbed pigmentary matter finding their way through the inner retinal wall; and he states that they ap-

pear only after the retina becomes functional, as no such preformed structures exist. I am certainly convinced that the thickening of the radially directed myelospongium-fibres is due to streams of altered pigment passing along them, and this is evident in the retinae of adult frogs, for in these the 'fibres of Müller' have usually a characteristic corkscrew-like course through the internal molecular layer, and this suggests the passage of the streams of altered pigment through that layer. I quite agree with Bernard's conclusion that such streams of absorbed pigmentary matter pass through the retina; but I insist on the fact that the rudiments of the fibres of Müller exist before these streams have made their appearance.

Formation of the Internal Molecular Layer.

When the internal molecular layer first makes its appearance, it is seen to consist of a dense plexus of myelospongium the meshes of which are so small, that cells are unable to remain embedded in it, and are thus pushed to either side. At this early stage the fine structure of the internal molecular layer can be observed to be directly continuous with the myelospongium--fibres -- especially in the toad (Fig.32), where these are of a coarser nature than in the case of the frog (see also Figs.58 & 59).

For an explanation of the mode of origin of the in-

ternal molecular layer it is necessary to study the wall of the cerebral vesicles (the fact that the retina is developed from the wall of the cerebral vesicles must never be lost sight of), where a somewhat analogous change occurs; for, at a very early stage of development -- before the appearance of the internal molecular layer of the retina -- this becomes marked off into an outer portion which is devoid of neuroblasts, and an inner portion which is crowded with neuroblasts. The outer portion of the wall of the cerebral vesicles is seen to consist of a dense mass of myelospongium, and to have exactly the same appearance and staining reactions as the internal molecular layer (see slide 5). In the case of the retina, however, the formation of the internal molecular layer marks off a layer of nuclei(the ganglionic nuclei); but no such phenomenon occurs in the wall of the cerebral vesicle. This can be explained by the fact that these retinal ganglionic nuclei very early give off their axis-cylinders, which at once pass over the choroidal fissure (Fig.1), and thus serve to keep the ganglionic nuclei anchored close to the internal limiting membrane, and the dense plexus of myelospongium is on that account formed to their outer side.

The internal molecular layer always stains well from its very first appearance, and to exactly the same degree of tint as the myelospongium. This layer has

always been understood to be composed for the most part of the arborising processes from the 'cells' on either side of it; but, as already shown, these processes are for the most part achromatic in nature and thus stain very feebly, and could not give the tint of colour which the internal molecular layer shows with staining agents from the date of its very first appearance.

These processes from the nuclei on either side soon begin to grow into this layer, and ramify within it, so that it may be compared to a sort of trellis-work on which the growing nuclear processes are trained, after the fashion of the tendrils of a plant; and this would account for the extreme degree of arborisation which these processes show in the adult retina (Golgi impregnation-method). In the case of the embryonic retina the chief indication of the giving off of these achromatic processes is the diminution in the size of the nuclei, for, as already stated I obtained very unsatisfactory results with the Golgi method in the embryonic retina.

The internal molecular layer first makes its appearance at the centre of the retinal cup, corresponding to the point where the rods and cones first develop, and from here its growth extends in all directions towards the cup margin. If the growing edge of this layer be examined, it is at once observed that it presents a bevelled surface towards the internal nuclear

layer. Thus, it is much thicker in the centre than at the growing margins, and, therefore, presents in sections the shape of a very thin crescent (see slide 32). This characteristic shape suggests to one the hypothesis, that growth must occur on the convex surface of the crescent, and that very little growth occurs on the concave surface (next to the ganglionic layer). This is confirmed by carefully examining those nuclei of the internal nuclear layer, which lie next to the internal molecular layer. Each lies in a sort of cup as if the tissue of the internal molecular layer were attempting to surround it.

Another point to note about these innermost nuclei of the internal nuclear layer is, that they are the first in that layer to give off internal processes into the internal molecular layer. As the latter grows in thickness these nuclear processes become embedded more and more deeply in it; but the processes themselves gradually elongate and this prevents the nuclei ~~themselves~~ from becoming enclosed in that layer.

The nuclei of the internal nuclear layer which lie further out, give off their internal processes somewhat later, and these do not therefore become so deeply embedded in the internal molecular layer. This explains the appearance presented by the internal molecular layer in Golgi preparations of the adult retina, which show, that the internal processes from the nuclei of

the internal nuclear layer reach to varying depths in this layer.

Formation of the External Molecular Layer.

The site of the future external molecular layer is early denoted by the 'arcaded' arrangement of the myelospongium-fibres towards their outer ends, which has been already referred to. In these early stages the centres of the 'arches' are rather pointed, owing to the fact that these fibres join one another to form an acute angle angle (Fig.57). Somewhat later in development the pointed character of the'arch' tends to disappear, and it becomes more and more flattened. The outline of the external molecular layer is therefore at first somewhat sinuous (Fig.58), and when the 'arch' becomes quite flat, then the external molecular layer comes to possess a straight outline (Figs.34 & 59). This rearrangement causes the radial fibres of the myelospongium (Figs.34 & 59) to have a slight lateral bend at the external molecular layer, so that at this stage they do not run an exactly straight course between the external and internal limiting membranes. Somewhat later, however, when the altered pigment, already referred to, begins to pass along these fibres and thus thicken them, they possess a straighter course between the limiting membranes.

It will be understood from this description, that

the myelospongium forms the first ground-work of the external molecular layers just as it forms that of the internal molecular layer. The rod and cone fibres and the external processes of the nuclei in the internal nuclear layer grow into and arborise within the external molecular layer.

This layer does not tend to increase very much in thickness as development proceeds, so that in the adult frog it still forms quite a thin stratum in the inner retinal wall. A slight degree of growth occurs on its outer surface in the form of small projections around the rod- and cone-nuclei, so that the latter structures rest in little cup-like depressions.

The free margin of the external molecular layer extends forwards into the undifferentiated region of the inner retinal wall; but it is a short distance behind the growing margin of the internal molecular layer (slide 32).

Formation of the Ganglionic Layer

The layer of ganglionic nuclei is mapped out very early in development by the internal molecular layer, and, as already stated, the neuroblasts which constitute it are those which are first formed by division of the germinal nuclei. It may be again noted that the nuclei of these neuroblasts are on the whole larger than the nuclei of those which lie nearer to the external limiting membrane.

The ganglionic nuclei are at first arranged quite irregularly, usually in two layers (Fig.6), and their axis-cylinders, which become the fibres of the optic nerve, are found to develop very early (Fig.1), and are the first processes to be given off by them. In the central point of the retinal cup the nuclei of this layer become more regularly arranged so as to form a single layer in this region, by a migration towards the margin of the cup, where the irregular arrangement still holds good. Whenever the ganglionic nuclei become thus arranged in their permanent positions, they give off their external processes, which enter and arborise within the internal molecular layer. These external processes of the ganglionic nuclei anchor them in position, and it is therefore obvious, that their rearrangement so as to form a single layer can only occur previous to the growth of their external processes.

Formation of the Internal Nuclear Layer.

This layer, when first mapped out, consists usually of from three to four layers of closely arranged nuclei. Those which lie next to the internal molecular layer tend to be larger, as a rule, than those lying more externally, and are probably homologous with the amacrine nuclei of the chick's retina. They are the first of the nuclei in this layer to give off processes which enter the internal molecular layer (Fig.4) and arborise there; and their processes usually extend somewhat

more deeply into this layer than those of the more externally placed nuclei. The general rule appears to be, that the further a nucleus of this layer is placed from the internal molecular layer, the later does its process into that layer develop. A large number, however, of the nuclei lying in the outer part of the internal nuclear layer appear to remain for a long time quite free without possessing by means of processes any attachment to the external or internal molecular layer. On account of this fact, one is often disappointed to find in sections, a separation of the internal nuclear layer into an inner portion consisting of nuclei which are anchored by processes to the internal molecular layer, and an outer portion consisting of these loosely arranged nuclei -- and that, even after great care has been exercised in the preparation of the sections.

Another point to be observed with regard to these externally placed nuclei is, that many are found in various stages of migration through the external molecular layer; these are migrating towards the external nuclear layer . Bernard (see reference 18) has also drawn attention to this phenomenon, and he considers that they ultimately become rod nuclei -- an observation which I can with certainty confirm.

Bernard[1] also draws attention to a migration of nuc-

(1) Q.J.M.S. Vol.46,Pt.I.,p.39.

lei in the internal nuclear layer from the undifferentiated retinal rim towards the more central parts of the cup -- noticeable in the early stages of retinal development. According to him, these nuclei have attached to them 'cytoplasmic trailings', which constitute their processes, and successive layers of these become deposited to form the internal and external molecular layers as the nuclei migrate towards the centre of the retinal cup. He considers, then, that the processes of these nuclei are cytoplasmic in nature, and this differs from my view that they emerge from the nuclei themselves. This migration of these nuclei would imply a steady progressive increase in the thickness of the internal nuclear layer as development proceeds.

My observations on this point go to show, that -- both in the frog and toad -- from the 25 day to the beginning of the metamorphosis (Fig.6 to Fig.20) there is a slight diminution in the thickness of the inner nuclear layer; and this is certainly not due to an extensive migration of these nuclei into the external nuclear layer, for the number of rod- and cone-nuclei remains practically the same throughout the tadpole stage (Compare Figs. 6 & 20). There is therefore a slight tendency in the early stages to a migration of the outer nuclei of the internal nuclear layer towards the undifferentiated margin of the cup just as has been already

shown in the case of the ganglionic nuclei (page 29)

On comparing the figure 21 with figures 26,27 & 28 it is at once evident, that, during the metamorphosis and especially in its later stages there is an enormous increase in the number of nuclei of all the layers; but this increase is most marked in the case of the internal nuclear layer. In addition to this, there is a marked enlargement of the surface area of the retina, so that the diameter of the eye is much greater than during the tadpole-stage (slide 66). This is difficult to explain as being entirely due to a migration of nuclei from the marginal portion of the cup, for it would imply a previous accumulation of nuclei on a vast scale in that region. The marginal portion of the retinal wall is certainly somewhat thicker than the more central portion; but this is obviously insufficient to account for the enormous increase both in the surface area and thickness of the retinal wall, which occurs within quite a short period (note the great increase in thickness of the inner retinal wall in Figs.25,26 & 27 - 79th to 88th day).

I was, however, much interested at having observed nuclei, especially in the internal nuclear layer, possessing a constriction in the middle so as to appear dumbbell-shaped. These nuclei are well seen by means of an immersion-lens (one-twelfth), and figure 56 shows such a nucleus with a nucleolus in each segment, and

the whole appearance suggests a process of direct division of these nuclei. The three nuclei represented in figure 56 are taken from the same microscopic field (56th day toad), and they are represented in their relative positions to one another. The one to the right does not possess nucleoli, but it shows a completion of the process of direct division. The one to the left shows a constriction at one margin, and this,no doubt, is the beginning of the process. I have also noticed nuclei in the ganglionic layer and also those nuclei of the external nuclear layer nearest to the external molecular layer, which showed this appearance -- an observation which proves the fact, that direct division occurs in the case of the nuclei of these layers as well. I have, however, been unable to detect it in the rod- and cone-nuclei of the external nuclear layer. Moreover, these evidences of amitotic division have been observed only at the end of the tadpole-stage and throughout the metamorphosis -- the periods during which the great increase in the number of the nuclei of these layers occurs.

This observation is strengthened by noting the size of the nuclei of these layers throughout the whole period of development. Two distinct phases in the process of diminution can be seen to occur. Firstly, in the tadpole-condition there is a rapid rate of diminution in the size of the nuclei during the early

stages (Figs. 1 to 10), and a much slower rate of diminution in the later stages (Figs.10 to 21). The second phase takes place during the stage of metamorphosis; this is especially evident in the internal nuclear layer, and it is here that this process of amitotic division is most active (compare Fig.21 with Fig.28). The first phase in the diminution of the nuclei is entirely due to the giving off of processes, their number remaining unaltered; while the second phase is due in great measure to amitotic division, their number being therefore increased.

Bernard[1] states, that he has observed evidences of 'fragmentation' of these nuclei, and he also refers to figures in a paper by Borysiekiewitz (26) in which that observer represents 'twin-ganglion cells'. I regret, however, that I have been unable to consult this memoir.

This observation regarding direct division appears to me to have a two-fold interest. In the first place, it controverts the opinion which is generally held, that mitotic or karyokinetic division is almost universal throughout the animal kingdom.[2] It is, in the second place, important to note that in the early stages of retinal development all the nuclei are formed by karyokinetic division of the germinal nuclei. During the tadpole-stage karyokinetic division ceases to occur in

(1) Q.J.M.S. Vol.46,Pt.I.p.30.

(2) Quain's Anat. Vol.I.Pt.II.p.183.

those portions of the retina which become actively functioning. It is necessary, however, for the tadpole to pass through all those critical changes which constitute the stage of metamorphosis, in order that it may be raised from its lowly gill-breathing condition to the fully developed lung-breathing Amphibian. In this stage during which important morphological changes take place in many of the organs of the animal -- none are more striking than those which occur in the retina, in order that it may become the well developed structure which exists in the adult. During the tadpole-stage, however, the nuclei of the retinal layers have been in an actively fuctional conditions and it appears as if it were impossible for such nuclei to again undergo karyokinetic division, and hence they multiply in number by direct division.

In the latter part of the tadpole-condition three varieties of nuclei may be recognised in the internal nuclear layer:-

(1) The largest nuclei are found directly in contact with the inner molecular layer and, as already stated (page 29), are probably homologous with the amacrine nuclei of the chick's retina.

(2) Next to the external molecular layer may be seen here and there nuclei having a flattened appearance (Fig.13) with their long axes parallel to the limiting membranes. These are probably homologous with the

horizontal nuclei of the chick's retina, for they are closely attached to the external molecular layer.
(3) Between these nuclei, the rest of the layer is filled up with the bipolar nuclei, which give off external processes into the external molecular layer and internal processes into the internal molecular layer.

The External Nuclear Layer.

The nuclei of the external nuclear layer are those which are last formed by the division of the germinal nuclei, and are, on this account, to be looked upon as the youngest in the retina, just as the ganglionic nuclei are the oldest.

When this layer becomes marked off by the external molecular layer, it consists of two rows of nuclei; but by far the largest number of these are to be found next to the external limiting membrane, and, as the rod- and cone-nuclei have commenced to develop before this period (Figs.3,4 & 34), the rod-nuclei are seen to be protruded to varying extents beyond the external limiting membrane into the bases of the rods. The rod-nuclei therefore lie further out than the cone-nuclei as a rule, and Bernard[1] in his paper points out this fact.

During the metamorphosis (Fig.23) the nuclei of this layer become more numerous, and this is due, partly to migration of nuclei from the internal into the external

(1) Q.J.M.S. Vol.46, Pt.I.p.38.

nuclear layer, and partly to direct division of those nuclei next to the external molecular layer (both these facts have been already referred to).

Those portions of the retina which lie towards the margin of the cup do not at first become differentiated into molecular and nuclear layers; but retain the 'primitive' condition, and therefore show the myelospongium with spongioblasts, and germinal nuclei lying underneath the external limiting membrane. Growth of the retina thus occurs in this position even after the rods and cones are well advanced in development. The margins of the two molecular layers project into this as yet undifferentiated portion of the retina, and, as its growth advances, their free margins also progress in development and are always encroaching more and more upon it.

Development of the Rods and Cones.

The first evidences of the development of the rods and cones are in the form of minute rounded vesicles consisting of a clear transparent non-staining substance (Figs.1,2,53). These appear immediately underneath the external limiting membrane in the central part of the convexity of the retinal cup. Each vesicle will be observed to be interposed between a nucleus of the external nuclear layer and the external limiting membrane; but they soon become protruded so as to form

prominent projections beyond the latter structure. These vesicles are directly attached to the nuclei of the external nuclear layer, and between the two structures a small amount of staining substance may usually be seen. They emerge, therefore, from these nuclei (Bernard (see reference 19) is also of the same opinion), and I have no hesitation in saying that they consist of achromatic nuclear matrix -- as already stated in an earlier part of this thesis (page 17).

Whenever they are protruded beyond the external limiting membrane, they grow with such rapidity, that in about four days (Fig.5) the volume of their contents is about the same as or even greater than the volume of the nuclei from which they arise. The corresponding nucleus has also been diminishing in volume during the same period (compare Figs.1 & 5); but this is not sufficient to account for the great increase in the size of the visual elements. They now also begin to stain very deeply with iron-alum-haematoxylin, and this is in strong contrast to their previous achromatic nature. The achromatic material can, however, still be seen in the form of a clear globule, embedded in the midst of the deeply stained substance (Fig.5). The vesicle has, moreover, much diminished in size -- obviously because part of it has blended with the staining material (Fig. 54 - A). This new staining reaction obviously indicates a great change in the nature of the contents of

the visual elements. Its explanation is found when we examine the pigment cells, for it will be noticed that their processes develop simultaneously with the rod- and cone-elements; and there must therefore be a sort of attraction (or POSITIVE CHEMIOTAXIS, as I would term it) between the two sets of structures (compare Figs. 53 & 54). What occurs is, that the vesicles, after their protrusion beyond the external limiting membrane, become embedded in the developing processes of the retin-al pigment-cells, and ingest the pigment, and transform it into the above-mentioned deeply stained material. This is supported by the fact that pigment-granules may be seen adhering to the developing rod- and cone--elements, while particles of chromatic material are also seen suspended in the midst of many of the clear globules (examine some of the globules in slide 16 with the high power). The deeply staining material of the newly formed visual elements forms first on the outer aspect of the vesicle -- that is to say -- next to the pigment-processes (Fig.54 -B); later on, the staining matter tends to surround the vesicle (Fig.54 -C), and, still later, part of the vesicle blends with the staining material, leaving only the small globule (Fig.54 -A). Probably the digesting portion of the vesicle blends with the visual element, while the non-digesting portion remains to form the globule. It is possible, that some of the nuclear chromatin as well may be ex-

-truded into the visual element; but this is obviously difficult to demonstrate.

This phenomenon illustrates a very interesting point, for it shows that the achromatic nuclear matrix of the vesicles contains a substance, which has the power of absorbing and digesting the pigment; and this effect throws further light upon the functions of the nucleus. It was pointed out in a previous part of this thesis, that the retinal nuclei ingested the yolk-granules, and this was shown by an increase in the amount of the nuclear chromatic substance. To this may now be added the fact, that it is the achromatic nuclear matrix which has the power of digesting the absorbed food material, which is then stored up as chromatic substance. The nuclear chromatin is, however, apparently capable of being converted into achromatin, and given off from the nucleus as processes; for it was also shown (page 21), that in addition to the diminution in the size of the retinal nuclei there was also a diminution in the amount of their chromatin. Miss Huie at the close of her paper (25) expresses doubt as to which of the nuclear contents has the digestive action, and the above observation appears to furnish an answer. Moreover, this digestive action must be of a peculiarly selective character, for the pigment which is acted upon, is known to resist the influence of ordinary digestive ferments.

About the 23rd or 24th day (Figs.5 & 34) two kinds of elements become differentiated:-

(1) The first consists of markedly cone-shaped structures, their basal part being attached to the nucleus, while their apical portion tapers off to a very fine point (Fig.6). Strangely enough, these are the rudiments of the rods, and in their basal portion the first protruded vesicle still persists as a clear spherical globule, which stands out in marked contrast to the surrounding deeply stained material.

At this stage of development there also appears in these rod-rudiments another structure of the shape of a biconvex lens, which develops on the distal side of the globule, and contains an achromatic substance; it is on that account sharply marked off from the neighbouring chromatic material. This second structure becomes a very striking object in rods which have been stained with iron-alum-haematoxylin, for it is not in the least affected by this stain; while the segments of the rods on its outer and inner aspects stain very deeply, and thus bring it into prominence. Its nature would seem to be the same as that of the spherical body, from which it is at least partly derived, for it appears when the globular body is beginning to disappear. As already stated, it is usually shaped like a section of a biconvex lens; but its outer margin may be plane instead of convex. A day or two later a very deeply

stained body appears by its side, and abuts against its inner surface (Figs.9 & 37). This new body is concavo-convex -- the concave surface being in contact with the inner convex surface of the earlier structure. These two objects together constitute the rod-ellipsoid, and they resemble the structures which Schultze[1] has described in the rods of the newt. The iron-alum-haematoxylin stain is to my mind the most perfect for demonstrating the rod-ellipsoid at this early stage, for by it this is shown to consist of two parts -- a non-staining outer part and a deeply staining inner part. When I first noticed the outer non-staining portion, I thought that it might be due to a plane of cleavage at that spot, but, on further study, it was found to be so remarkably constant in position and in shape throughout the whole period of development, that its presence was evidently not due to any mere mechanical effect of cleavage. Planes of cleavage can be seen in those portions of the rods distal to the ellipsoid, and these are irregular both in position and shape. Although this outer part of the rod-ellipsoid is not due to mechanical cleavage, still the outer segment is apt to separate from the inner segment at this point, which therefore denotes a weakness here.

The remains of the first protruded vesicle are to be

(1) See Ref.4 in Lit.,"Arch. für Micr. Anat"Bd.V. 1869.

seen in the inner segments of the rods until about the 30th day, and instead of being central in position in that segment are found irregularly placed. The last remnant is visible in the inner deeply staining portion of the rod-ellipsoid, so that it probably terminates its existence by blending with the outer achromatic portion, or it may pass beyond this, and enter the outer rod-segment.

The inner rod-segment at the end of the metamorphosis stains much less deeply, as also does the inner portion of the ellipsoid (Fig.28). This is easily accounted for by the fact that the rod-nucleus still continues extruding some of its achromatic matrix into the rod; this is especially evident in the toad (note four such rods in Fig.39), where this material is in the form of globular masses in the inner rod-segment, which blend later with the material of the rod (the diminution of the rod- and cone-nuclei occurs not only inthe early tadpole stage, but during the whole period of development also; fig.11 illustrates this point beautifully, for the nuclei in the central part of the cup are much smaller than those near the cup margin, which are just beginning to give off processes). Probably some of this freshly protruded achromatic nuclear matrix also passes into the outer segment; but it is obviously impossible to demonstrate the passage of this through the outer non-staining portion of the ellipsoid.

A proof is, however, furnished by the fact that the volume of the inner segment does not increase, for the distance of the rod-ellipsoid from the external limiting membrane remains practically the same as it was at its first appearance, while the thickness of this segment of the rod tends to diminish somewhat towards the end of metamorphosis (Fig.28).

In the early stages the rod is, as already stated, distinctly cone-shaped, but after the development of the ellipsoid the outer limb increases markedly in length, while the point becomes very fine (in the case of the frog), so that the whole element becomes rather needle-shaped and strikingly elegant in appearance (Fig.11 & slide 32). (In the toad-tadpole the rod tends to be thicker and with a rather blunted extremity, and altogether looks a much clumsier and coarser structure (Fig.44)) This needle-shaped character persists throughout the greater part of the tadpole stage; but at the beginning of the metamorphosis the apex becomes more blunt and rounded, and the margins more or less parallel to one another. The diameter of the inner limb next to the nucleus also diminishes, so that its margins too become parallel, and the characteristic rod-shape is produced. The rods have now a striking resemblance to an organ-pipe -- the mouth being represented by the biconvex clear portion of the ellipsoid (Fig.27 & slide 66). During the later stages the

outer segment is, therefore, the only part which shows an increase in size, and this is due most certainly to the ingestion of more pigment from the pigment cells, for it stains as intensely as ever with iron-alum-haematoxylin.

It is interesting to note now, that the rhodopsin - producing function of the rods, which is so characteristic a feature of these elements throughout life, is manifested in the earliest stages of their formation; for the substance in the outer segments which stains so deeply with iron-alum-haematoxylin is probably rhodopsin, or a body closely allied to it. Kühne's observations (27) on the relation of the pigment to the visual purple in the adult frog thus receive confirmation at a very early period of development. Further, the rods owe their increase in growth to the ingestion of this pigment -- the nucleus only supplying the necessary material by which this process of ingestion is carried out. This is well demonstrated by the appearance of the vesicles after they become protruded beyond the external limiting membrane, for many of them have been shown to contain particles of chromatic material in their midst. They may, on the other hand, stain of a diffuse blue colour; but some of the vesicles are perfectly clear and transparent, and this points to the fact that the pigment granules may be first of all transformed into a colourless achromatic

substance, and then secondly into the chromatic substance. In the rhodopsin-producing function of the fully developed rods an intermediate substance called rhodophyllin is first of all formed, so that the colourless achromatic substance mentioned in the last sentence may represent it. At this point it may be noted, that stained material appears between the rod- and cone--nuclei during the later stages of development, and is probably the altered pigment matter from the visual elements which has been shown to pass inwards along the fibres of Müller.

The nuclei of the rods tend to be protruded to varying degrees beyond the external limiting membrane, and this is perhaps explained by the fact that they are occasionally giving off more and more of their achromatic substance into the rods. This close interaction between the nucleus and the rod is probably the cause of the nucleus itself being drawn into the basal portion of the rod. It is difficult to explain this phenomenon on any other possible grounds.

(2) The second kind of visual element is characterised by a gradual narrowing and lengthening of its inner segment, so that the whole structure becomes markedly spindle-shaped, and is attached to the corresponding nucleus by the attenuated inner segment, which is very variable in length and forms a sort of connecting stalk. In the centre of the spindle are the globular remains

of the first protruded vesicle (which forms the so-called 'oil globule'[1]), while distal to this body there is in most cases a clear achromatic area which, like the outer part of the rod-ellipsoid, is usually somewhat biconvex in shape; and between the two is a portion which stains usually very deeply with iron-alum-haematoxylin, and probably corresponds to the deeply staining portion of the rod-ellipsoid (these two structures thus form the cone-ellipsoid).

This second type of visual element[2], which has been rather infelicitously termed 'cone', does not tend to increase much in size during the tadpole stage; this deficiency of growth may be explained by the fact, that no further supplies of achromatin substance can be seen to pass along the finely attenuated stalk towards the body of the cone. The necessary addition of fresh ingesting material from the nucleus is thus cut off, and it would therefore appear, that the ingestive power of the first protruded vesicle of achromatic nuclear substance must become exhausted and prevent the occurrence of further growth. In the case of the rods, however, the necessary supply of fresh nuclear achromatin is kept up and accounts for the progressively rapid growth

(1) I will show later, that I can find no evidence of the 'oily' nature of this globule.

(2) I have been unable to discover any trace of the terminal vesicle which Bernard describes at the tip of this visual element (Q.J.M.S., Vol.43, p.25.).

of these elements. There is, therefore, not such a close interaction between the cone-nucleus and the cone, as there is between the rod -nucleus and the rod. The cone-nuclei thus do not tend to become protruded into the inner segments of the cone; but remain in the external nuclear layer near to the external molecular layer. In the introduction to this thesis I have referred to the statement of Bernard's (21), that the cones of the Amphibia are converted into rods. I have been able to trace the mode of formation of Schwalbe's rods (those with narrow inner segments) from this spindle-shaped structure. The transformation appears to take place during the stage of metamorphosis; and consists in a lengthening of the outer part of the spindle, and a narrowing of the body of the structure (Fig.28), so that the walls of the outer segment become more or less parallel to one another. (The 'oil' globule blends with the biconvex-shaped area on its outer side to form the rod-ellipsoid). This outer segment remains attached to the cone-nucleus by the still narrow stalk of the originally spindle-shaped structure (Fig.55). The term 'cone' is therefore quite a misnomer in the case of the Amphibia, as Bernard has already shown; for the developing Amphibian rod has more the shape of a cone than the spindle-shaped structure which receives this title.

In the early stages of retinal development these

are the only two types of visual elements to be found; but about the 35th day another type of element suddenly makes its appearance. This new structure consists of a basal portion which is distended with achromatic nuclear substance, and a fine pointed extremity. The origin of this element from the 'cone' is quite easy to demonstrate, for it is simply produced by the narrow stalk of the 'cone' becoming distended by the sudden discharge into it of a large amount of nuclear achromatin. This great loss of the nuclear contents is usually shown by a marked condition of collapse of the corresponding nucleus (~~refer~~ referred to on page 16 of this thesis). In figure 11 - opposite the letter A the stalk of a 'cone' is shown becoming distended with this nuclear achromatin.

When two spindle-shaped 'cones' develop side by side, and the stalk of one becomes distended in this way, then the structure known as a double 'cone' is produced (as has been shown by Bernard)(1). This suddenly extruded mass of fluid may blend with the globular remains of the primary vesicle, or the latter may still remain distinct and separate. (The fact that these two bodies are capable of blending readily with one another, is sufficient to render untenable the idea, that the remnant of the first protruded vesicle in the 'cone' is 'oily' in nature). As a result of this fresh supply

(1) Q.J.M.S., Vol.43, p.33

of nuclear achromatin the second type of 'cone' takes on a new phase of growth. The distal limb becomes elongated and filled with deeply stained material, just as in the case of the rods; but the achromatic substance tends to remain at first in its basal portion, and does not pass into the outer segment until later. The basal vesicle which at its first extrusion is quite clear, shows particles of chromatic substance embedded in it, just as has been already shown in the case of the primary vesicle; and also, no doubt, implies a process of ingestion of pigment by it.

I have been able to trace signs of transformation of this type of element into rods. During the metamorphosis the basal vesicle tends to diminish in size. This appears to be due to a gradual passage ot its contents into the outer segment, and there they blend with the deeply stained material. The outer segment thus increases in size, and the whole structure becomes gradually transformed into a rod -- some of the contents of the basal vesicle persisting as the clear part of the ellipsoid.

It occurred to me to test the appearance of these globules of the rods and cones as seen through the micro-polariscope (for the loan of which I owe my best thanks to Dr Fraser Harris). These globules were studied in this way at all stages of development, and were found to be singly refractive, for they appeared dark

in the field of the polarising microscope, when the two Nicol's prisms were at right angles to one another. It was, however, thought that the stain might have the effect of altering their refractivity; I mounted an unstained preparation (slide 126), and examined it as before, and found the globules still singly refractive. (The pigment-granules showed bright in the dark field, and are on this account to be considered as doubly refractive.) I cannot find any previous allusion to the examination of the refractivity of these globules, and the above observations are therefore worthy of being recorded.

The globule in the 'cones' which I have spoken of as being the remains of the primary vesicle, and which consists, therefore, of nuclear achromatin, is described in textbooks as the 'refractive oil globule'; but I can find no evidence of its 'oily' nature.

The tadpole-tissues from which all the specimens have been obtained, were fixed in Bles' fluid, dehydrated in alcohol, and then exposed to heat for some time in the paraffin-stove. I resolved to test the refractivity of the fat globules of milk under similar conditions (the fat globules of milk are of about the same size as these globules of the rods and 'cones').

The milk was first of all examined under the micro-polariscope, when its fat-globules were found to be doubly refractive (it has just been shown that the 'oil'

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globule of the 'cone' is singly refractive). To another drop of milk a small quantity of Bles' fluid was added. On examining this, it was observed that the fat-globules had run into clumps; but their power of doubly refracting was, if anything, increased under the influence of this fluid.

Absolute alcohol was added to another drop of milk; this was similarly examined, and the double-refractivity was found unaltered.

A small quantity of milk was exposed to a temperature of about 55° C for some time and then examined as before; its double refractivity was still found unchanged.

This series of experiments showed that the various processes, through which the retinal tissues had to pass would not tend to alter the doubly refracting power of these globules. It was found impossible to cut sections of the fresh tadpole-retinae for the purposes of such examination, as the delicate globules were invariably destroyed by this procedure.

Development of the Retinal Pigment-Cells.

The outer wall of the retinal cup becomes pigmented very early (almost immediately after its formation), and this is very evident in the earliest shown figures (1 & 29).

The processes of these pigment-cells also develop at a very early stage -- simultaneously with the appearance of the rods and 'cones'. The attractive in-

fluence (positive chemiotaxis [1]) which exists between these structures, has been already alluded to. Figure 11 illustrates this well, for it shows a complete section of the retina of a 35 day frog-tadpole. The animal, from which this section was prepared, remained in a totally dark chamber for about three hours before killing, so as to ensure the full retraction of the pigment processes; and thus the influence of light on these structures was completely eliminated. The rods and 'cones' in the central portion of the retinal convexity are in the most advanced stage of development, as are also the pigment-processes which are situated opposite them. Towards the margins of the retinal cup, however, it will be observed, that the rods and 'cones' and also the pigment-processes are less well developed. Still further forwards, where the visual elements are in the primary vesicular stage, the pigment-processes are also just beginning to show. Finally, where the rod-and-cone-layer ceases, there also, the pigment-layer becomes devoid of processes.

About the 23rd day the pigment-cell processes begin to be influenced by light, for after that date it was found necessary to keep the tadpoles in the dark for three hours before killing them, so as to ensure complete retraction of the former.

The writer (28) has shown that the rate of their protrusion under the influence of light is about the

(1) The accumulation of the pigment along the free margins of the retinal pigment-cells is also, no doubt, due to this positive chemiotaxis.

same as that of the pseudopodia of white blood corpuscles; and that their movements are to be looked upon as being amoeboid in character. In the same paper it is also pointed out, that the pigment-granules do not move backwards and forwards within the processes; but are borne along with the movements of the protoplasm in which they lie.

The pigment-cell processes may be observed to be arranged in groups corresponding to the individual pigment-cells; but this is not always evident.

Summary and Concluding Remarks.

Many of the statements contained in this thesis with regard to the mode of development and the structure of the Amphibian retina are of a somewhat revolutionary nature; but the evidence in support of them is of a convincing character. Their acceptance necessitates a complete change in many of the current beliefs and opinions which are in vogue concerning the ontogeny of the retina.

(1) It is first of all necessary to mention, that the value of Bles' fluid as a fixative for the retina cannot be too higly praised; while the use of the iron-alum-haematoxylin (Heidenhain) stain for the retina, as applied by the method which is described in the introduction to this thesis, has given most excellent results (Page 5 et seq.).

(2) It is important to again emphasise the great value of recognizing in the inner wall of the retinal cup four groups of structures namely:-- GERMINAL NUCLEI, NEUROBLASTS, MYELOSPONGIUM and SPONGIOBLASTS; for these all play a most significant part in the development of the various layers of the retina (Page 13).

(3) One of the most important results which has been attained in this research is with regard to the origin of the processes of the 'cells' in the inner retinal wall. It has been shown that the processes of the 'cells' in the ganglionic and internal nuclear layers emerge from the nuclei,and not from any surrounding cytoplasm, for the presence of the latter cannot be demonstrated in the embryonic retina. Moreover, the first rudiments of the rods and'cones' are in the form of protrusions from the nuclei of the external nuclear layer. These processes have been shown to consist for the most part of nuclear achromatin, for they stain very feebly with the materials which are usually employed for this purpose. This extrusion of these peocesses is evidenced by a progressive diminution in the size of the corresponding nuclei, which takes place throughout development (Page 15 et seq.).

(4) The nuclei of the embryonic inner retinal wall (except the nuclei in the fibres of Muller) do not possess any surrounding cytoplasm, and, in the case of the adult retina, the ganglionic nuclei are the only ones which

come to possess such an investment, and this, like the processes, is excreted from the nucleus (Page 19 et seq.).

(5) Mitotic division is usually believed to be almost universal in the case of animal cells; but in the developing retina there is abundant proof of the occurrence of amitotic or direct division of nuclei. In the early tadpole-stage, before the retinal nuclei begin to function, growth takes place by mitotic division of the germinal nuclei which appear to be set apart for this purpose. After the retinal nuclei have become actively functional, they lose the power of undergoing mitotic division, and now multiply by amitotic division (Page 32 et seq.).

(6) The mode of development of the rods and'cones' is also of great interest, for the first rudiments of these structures have been shown to be vesicles of nuclear achromatin extruded from the nuclei of the external nuclear layer. These by their growth and protrusion beyond the external limiting membrane exert a POSITIVE CHEMIOTAXIS on the retinal pigment -cells, which causes the processes of the latter to develop simultaneously with them. Moreover, what is more important still, the rod- and cone-rudiments actually ingest this pigment, and metabolise it into a substance (rhodopsin ?) which stains very intensely with iron-alum-haematoxylin. This substance appears to act as food material to the rods and 'cones', for these grow with great rapidity

after becoming protruded beyond the external limiting membrane. This phenomenon therefore points strongly to the fact, that the nuclear achromatic substance (of the embryonic nucleus at least) is the portion of that structure, which has the power of absorbing and metabolising food-material (Page 37 et seq.).

The results which are embodied in this research thus not only form an addition to the literature on the embryology of the Vertebrate retina; but also throw a large amount of light on the properties and functions of the cell-nucleus.

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List of authors referred to in this thesis.

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(1) Kölliker, A., "Entwickelungsgeschichte des Menschen und der höheren Thiere", Leipzig, 1879.

(2) Kölliker, A., "Zur Entwicklung des Auges und Geruchsorgans menschlicher Embryonen." Zum Jubiläum der Univ. Zurich, Würzburg, 1883.

(3) Babuchin, A., "Beiträge zur Entwickelungsgeschichte des Auges," Würzburger naturwissenschaftliche Zeitschrift, Bd. 8.

(4) Schultze, Max., Arch. für Microscop. Anat. Bd. 3, 1867, and Bd. 5, 1869.

(5) Müller, W., "Ueber die Stammesentwickelung des Sehorgans der Wirbelthiere." Festgabe Carl Ludwig. Leipzig, 1874.

(6) His, W., "Anatomie Menschlicher Embryonen," Leipzig, from 1880 onwards.

(7) His, W., "Histogenese und Zusammenhang der Nervenelemente," Archiv für Anat. und Physiol., 1890, Supplement.

(8) Löwe, L., "Die Histogenese der Retina," Archiv für Micros. Anat., Bd. 15, 1878.

(9) Arnold, J., "Beiträge zur Entwickelungsgeschichte des Auges," Heidelberg, 1874.

(10) Lieberkühn, N., "Beiträge zur Anat. des embryon. Auges," Archiv für Anat. und Physiol., 1879.

(11) Lieberkühn, N., "Ueber das Auge des Wirbelthierembryo," Cassel, 1872.

(12) Gunn, R. Marcus., "The Embryology of the Retina of Teleosteans," The Annals and Magazine of Natural History, Series, 6, Vol. 2.

(13) Ramón y Cajal,S., "La Retine des Vertebres," La Cellule, IX., 1893.

(14) Assheton, R., "On the Development of the Optic Nerve of Vertebrates and the Choroidal Fissure of embryonic life," Q.J.M.S.,Vol.34.

(15) Cirincione, G., "The development of the retina of reptiles," published in Italian at Palermo.

(16) Levi, "Osservazioni sullo svilluppo dei coni e bastoncini della retina degli Urodeli," Lo Sperimentale, anno liv., 1900.

(17) Kerr,J.Graham., "The Development of Lepidosiren Paradoxa," Q.J.M.S., Vol.46.

(18) Bernard,H.M., "Studies in the Retina," Quar. Jour.Micr.Sc., Vol.44,p.452.

(19) Bernard,H.M., Op. Cit., Q.J.M.S.,Vol.44,p.453.

(20) Bernard,H.M., Op. Cit.,Q.J.M.S.,Vol.44,p.457.

(21) Bernard,H.M., Op.Cit.,Q.J.M.S.,Vol.46.p.69.

(22) Bernard,H.M., Op.Cit.,Q.J.M.S.,Vol.46.p.59.

(23) Gaskell, W. H., "On the Origin of Vertebrates," Jour.of Anat. & Phys. Vol.35.

(24) Mann, G., "The Functions, Staining Reactions and Structure of Nuclei," Proc.Brit.Assoc., 1892 Section D.

(25 Huie, Lily, "Changes in the Cell-organs of Drosera Rotundifolia produced by Feeding with Egg-albumen, Q.J.M.S., Vol.39.Also Vol.42.

(26) Borysiekiewitz, "Untersuch. über den feineren Bau der Netzhaut",p.19, 1887.

(27) Kühne, "Untersuch. aus dem physiol. Inst. der Univ. Heidelberg", 1878-1882.

(28) Cameron, John, "The Movements of the Processes of the Retinal Pigment-cells", Proc. of the Scott. Micros. Soc., 1901-1902.

Description of Figures.

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Illustrating Mr John Cameron's thesis on

"The Development of the Retina in Amphibia."

List of reference letters.

C.,	cone.
$C.^2$,	second type of cone.
C.E.,	cone-ellipsoid.
C.G.,	cone-globule.
C.N.,	cone-nucleus.
C.O.V.,	cavity of optic vesicle.
C.R.,	rim of retinal cup.
E.L.M.,	external limiting membrane.
E.M.L.,	external molecular layer.
E.N.L.,	external nuclear layer.
F.M.,	fibre of Müller.
G.N.	germinal nucleus.
Ga.L.,	ganglionic layer.
Ga.N.,	ganglionic nucleus.
I.L.M.,	internal limiting membrane.
I.M.L.,	internal molecular layer.
I.N.L.,	internal nuclear layer.
L.,	lens.
N.,	neuroblast.
R.,	rod.
R.E.,	rod-ellipsoid.
R.G.,	rod-globule.
R.N.	rod-nucleus.
R.P.,	margin of retinal pigment-cells.
R.P.C.,	retinal pigment-cells.
V.E.,	visual elements.
V.P.,	primary vesicle of the visual elements.

All the figures, except 57,58 & 59, have been drawn with the aid of Zeiss' camera lucida and special drawing table. Zeiss' D lens and No.3 ocular were used for figures 1 to 52, and the table was raised so that the paper was five inches distant from the centre of the camera lucida mirror. Leitz's oil-immersion lens(one-twelfth) and Zeiss' No.3 ocular were employed

for the production of figures 53,54,55 & 56. In the case of figures 53,54 & 56, the paper was 11½ inches, and in the case of figure 55 the paper was five inches distant from the centre of the camera lucida mirror.

Figs.57,58 & 59 are purely diagrammatic.

All the sections,from which the figures have been drawn, were stained with iron-alum-haematoxylin, except those from which figures 1,2,4,9,53 & 54 have been taken. The latter sections were stained with haem-alum, followed by eosine.

Fig.1. - A vertical section of the retina of a 15th day frog-tadpole (7.5 m.m. long), passing through the choroidal fissure. The outer retinal wall is already pigmented, while the rudiments of the visual elements are just appearing. The myelospongium network and neuroblasts are well seen (slide 5).

Fig.2. - 17th day frog-tadpole (8.5 m.m.long). One of the vesicular rudiments of the visual elements has become protruded beyond the external limiting membrane. Numerous germinal nuclei are seen. It may also be noted, that the lens now possesses a central cavity. The retina represented in this figure is much smaller than that of figure 1 - apparently due to differences of nutrition (slide 7).

Fig.3 - 19th day frog-tadpole (11 m.m.long). The visual elements are now protruded beyond the external limiting membrane, and the margin of the retinal pigment layer (which has separated slightly) is becoming irregular in outline, due to the positive chemiotaxis exerted on it by the developing visual elements. The internal and external molecular layers have appeared, and hence also the internal and external nuclear and ganglionic layers. The rudiments of the fibres of Müller are well seen (slide 10).

Fig.4 shows a further stage in the development of the visual elements, and also in the formation of the pigment cell processes. 21st day frog-tadpole - 12 m.m.long. (slide 13).

Fig.5 shows a great advance in the development both of the visual elements and pigment cell-processes. The rods and cones are becoming differentiated, and the remains of the primary vesicles are seen in both kinds of elements. The rod-ellipsoid has also appeared. 23rd day frog-tadpole - 14 m.m.long (slide 16).

Fig.6 - Note the markedly cone-shaped character of the developing rods. 25th day frog-tadpole - 15 m.m.long. (slide 18).

Figs.7 to 10 show the rapid growth of the visual elements. The rods and cones are now well differentiated. At about the 31st day the deeply stained portion of the rod-ellipsoid becomes evident. 27 to 33 days. Slides 20, 26,27 & 30.

Figs.11 & 12 are drawn from the same section. Fig.11 shows the rods and cones in all stages of formation - the more fully developed ones being near the centre of the retinal cup. Opposite the letter A a cone will be seen becoming transformed into the second type of this structure by a distending of its stalk with more extruded achromatin from the cone-nucleus. The needle-shaped character of the more fully developed rods is well marked. (slide 32).

Figs.13 to 21 show the condition of the retina and visual elements in the later part of the tadpole-stage. 37 to 53 days. Slides 34,36,38, 40,42,43,45,48 & 50.

Figs.22 to 28 represent the appearance of the retinal wall during the stage of metamorphosis. The rods gradually assume their fully developed condition. A great increase in the number of nuclei of all the layers in the inner retinal wall is also evident, especially in the later stages. 67 to 94 days. Slides 53,56,57,61, 64,66 & 68.

Figs.29 to 52 represent the stages in the development of the retina of toad-tadpoles.

Figs.29,30 & 31 show the early stage of development of the optic cup. Fig.31 shows the first appearance of a visual element, while in the deeper layers of the inner wall in the same figure are seen two nuclei with a globule of achromatin at one pole, apparently about to be protruded. Toad-tadpoles - 12 to 16 days. Slides 71,73 & 75.

Figs.32 and 33 - Some of the visual elements have become protruded beyond the external limiting membrane, and the processes of the retinal pigment-cells are also manifesting themselves. The internal molecular layer is just beginning to appear, and the rudiments of the fibres of Müller are well represented. 18th and 20th day toad-tadpoles. Slides 78 & 80.

Figs.34 & 35 show further stages in the development of the visual elements and pigment-cell processes. Both the molecular layers have appeared. 22nd and 24th day toad-tadpoles. Slides 82 & 83.

Fig.36 - The rod- and cone-elements have become differentiated. The rod-ellipsoid is also seen; as also are the globular remains of the primary vesicle. 28th day toad-tadpole. 15 m.m. Slide 87.

Figs.37 to 52 show the progress of development during the later tadpole stages of the toad (32nd to 76th days), and it is seen to be practically the same as in the case of the frog-tadpole. Slides 89,91,92,94,96,98,101,~~103~~,105,109,111, 112,116,117,120,122 &124.

Fig.53 gives the relation of the primary vesicles of the visual elements to the nuclei of the external nuclear layer. As yet no pigment-processes are evident. 15th day frog-tadpole - 7.5m.m. Slide 5.

Fig.54 - The visual elements are now protruded beyond the external limiting membrane, and have exerted their positive chemiotaxis on the retinal pigment-cells. B shows the deeply stained material lying on the outer aspect of the vesicle. C shows the primary vesicle practically surrounded with stained matter. A shows the diminution in size of the vesicle, due to its blending with the stained material. The nuclei in figures 53 & 54 are seen to be loaded with irregular masses of deeply stained material, which obscure the intranuclear network. 2ist day frog-tadpole - 12.m.m. Slide 13.

Fig.55 - A Schwalbe's rod which has been developed from the first type of cone. 73rd day frog-tadpole. Slide 57.

Fig.56 represents three nuclei from the internal nuclear layer of the retina of a 56th day toad-tadpole, showing evidences of amitotic or direct division. The nucleus in the middle of the figure is seen to be dumb-bell shaped, with a nucleolus in each segment. The nucleus on the right of the figure shows the completion of the process, while the one on the left shows the commencement of the process. The intranuclear network is well seen (Compare with figures 53 & 54). Slide 112.

Figs.57,58 & 59 are diagrams to show the relation of the myelospongium to the two molecular layers. Figure 57 represents the 'arcaded' arrangement of the outer ends of the myelospongium fibres. In figure 58 the 'arch' is becoming somewhat flatter, and this gives the external molecular layer a sinuous outline. In figure 59 the flattening of the 'arch' is complete, and the outline of the external molecular layer is now straight, while the fibres of Müller have a slight lateral bend in this region.

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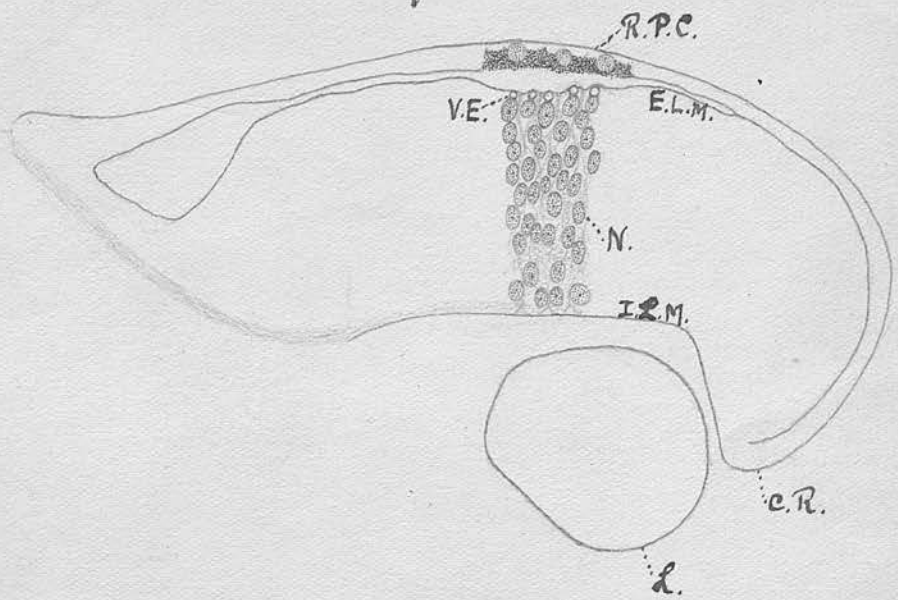
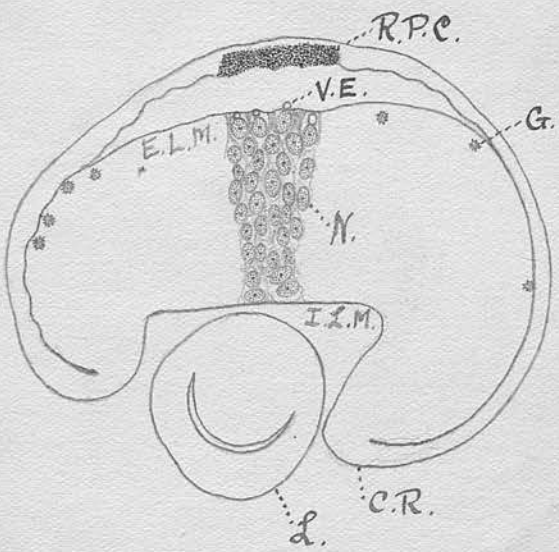
Cam. Lucida drawing. Fig. 1. Slide 5.



Frog

Tadpole 15 days. 7.5 m.m. long. Haem-Al. Eosine

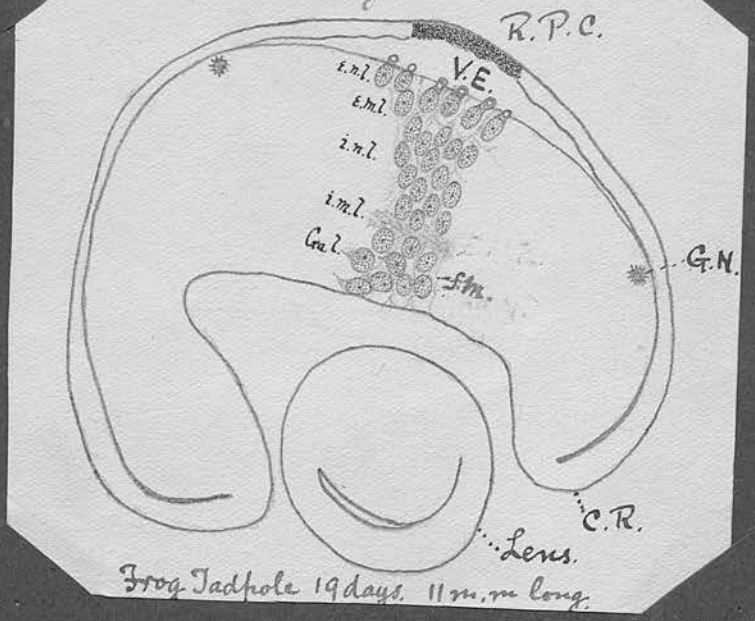
Cam. Luc. drawing. Fig. 2 slide 7.



Frog.

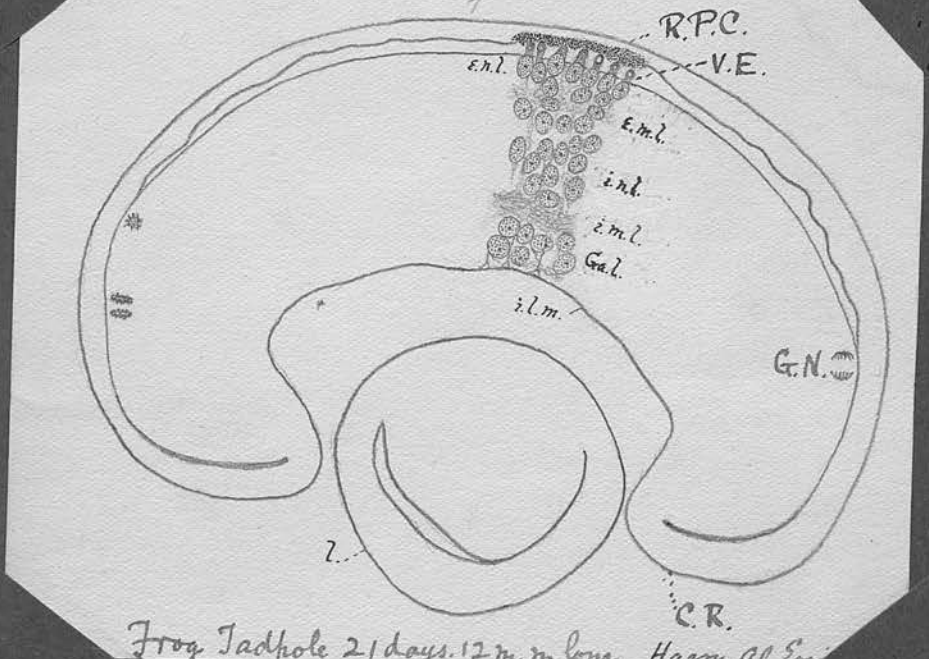
Tadpole 17 days. 8.5 m.m long. Haem-Al. Eos

Cam. Luc. drawing Fig. 3. slide 10.



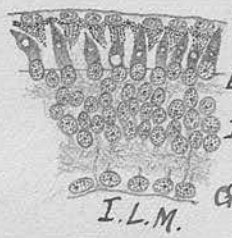
Frog Tadpole 19 days. 11 m.m. long.

Cam. Luc. drawing. Fig 4 slide 13.



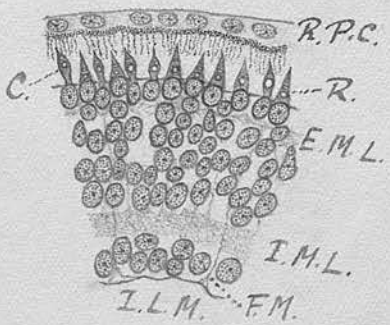
Frog Tadpole 21 days. 12 m.m. long. Haem. Al. Eos

Cam. Luc. drawing. Fig 5 slide 16.



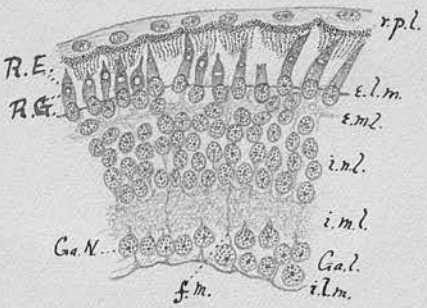
Frog Tadpole 23 days.
1/4 m.m. long.

Cam. Luc. drawing. Fig. 6 slide 18.



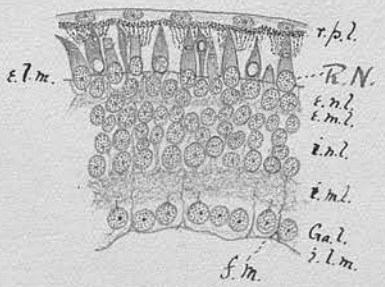
Frog Tadpole 25 days
1/5 m.m.

Cam. Luc. drawing. Fig. 7 slide 20.



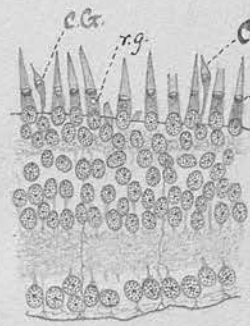
Frog Tadpole 27 days
16 m.m.

Cam. Luc. drawing. Fig. 8. slide 26



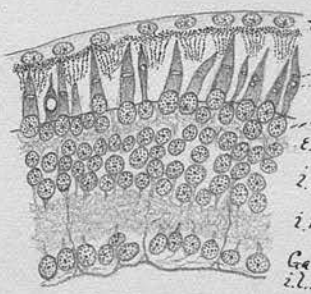
Frog Tadpole 29 days.
16.5 m.m.

Cam. Luc. drawing. Fig. 9 slide 27.



Frog Tadpole. 17 m.m. long
31 days. H.Al. Eos

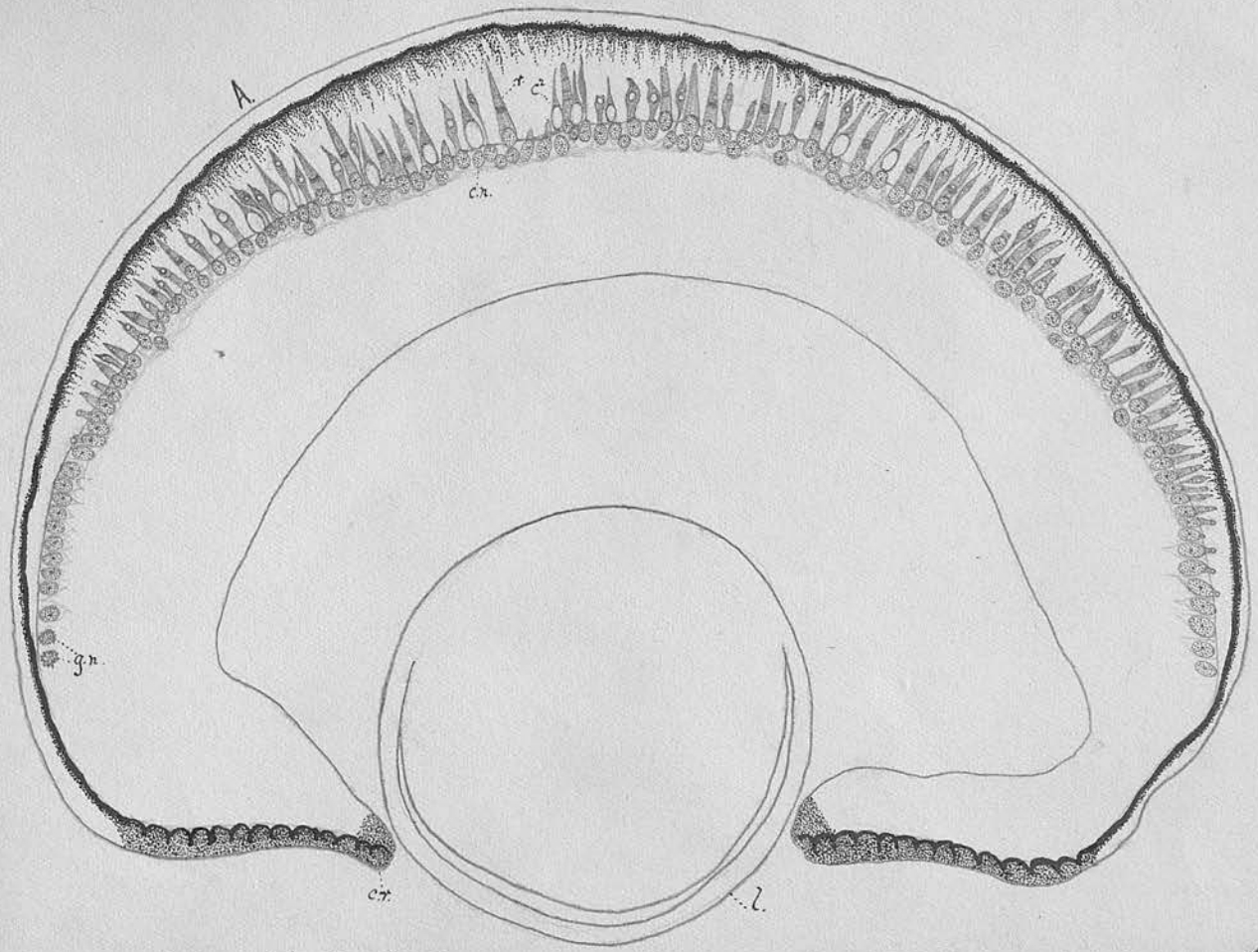
Cam. Luc. drawing Fig 10. slide 30.



Frog Tadpole 33 days.
18 m.m.

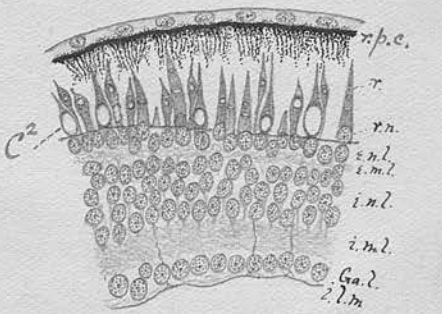
Cam. Luc. drawing.

Fig. 11 slide 32.



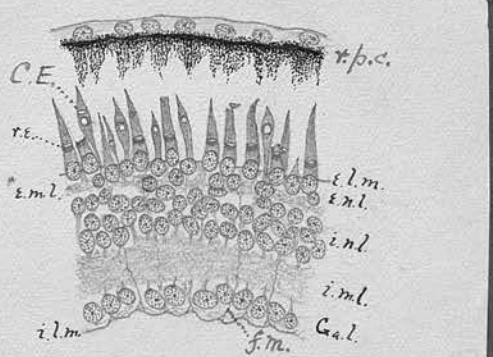
Frog Tadpole. 35 days. 19.5 m.m Long.

Cam. Luc. drawing. Fig. 12 slide 32.



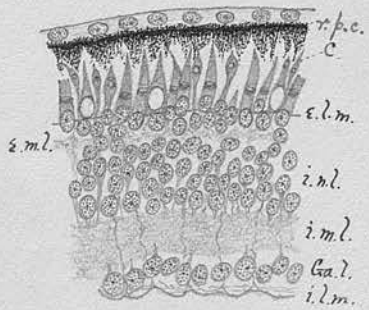
Frog Tadpole 35 days.
19·5 m.m long.

Cam Luc. drawing. Fig. 13 slide 34.



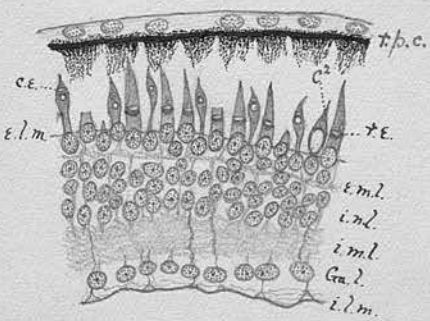
Frog Tadpole 37 days.
21·5 m.m long.

Cam. Luc. drawing. Fig. 14 slide 36.



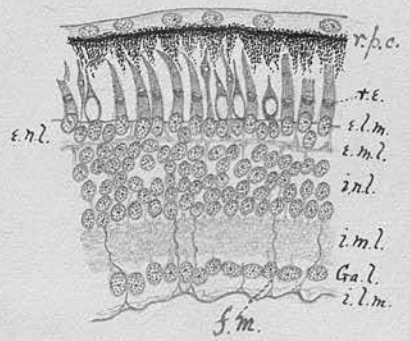
Frog Tadpole 39 days.
2 3/4 m.m. long.

Cam. Luc. drawing. Fig. 15 slide 38.



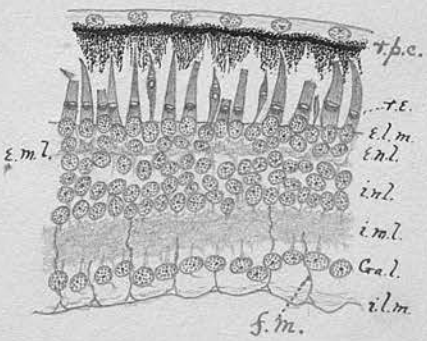
Frog Tadpole. 41 days.
25 m.m. long.

Cam. Luc. drawing. Fig. 16 slide 40.



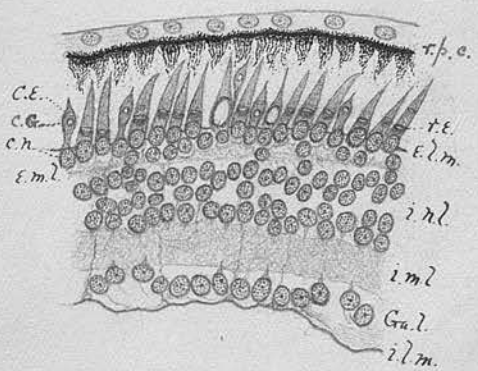
Frog Tadpole 43 days.
24 m.m. long.

Cam. Luc. drawing. Fig. 17 slide 42.



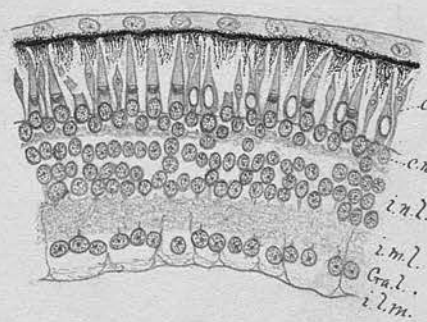
Frog Tadpole 45 days
28 m.m. long.

Cam. Luc. drawing. Fig. 18 slide 43.



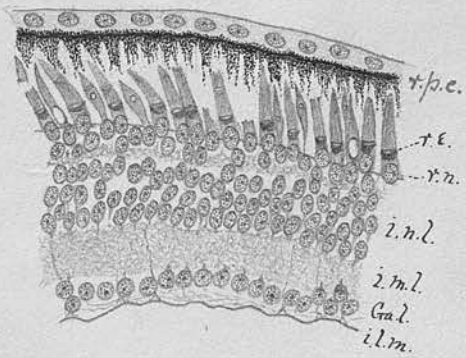
Frog Tadpole 47 days
29 m.m. long

Cam. Luc. drawing. Fig. 19 slide 45



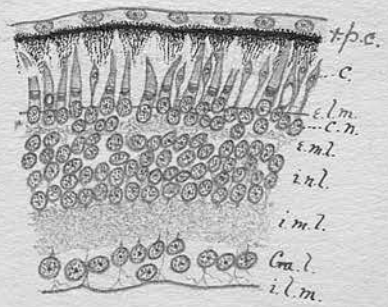
Frog Tadpole. 49 days.
30 m.m. long

Cam. Luc. drawing. Fig. 20. slide 48.



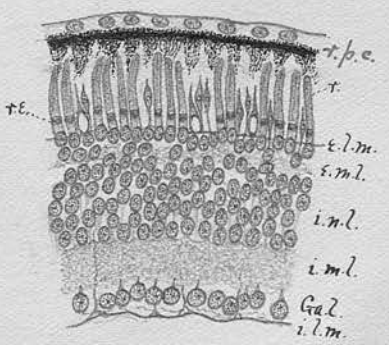
Frog Tadpole 51 days.
30 m. m. long.

Cam. Luc. drawing. Fig. 21 slide 50.



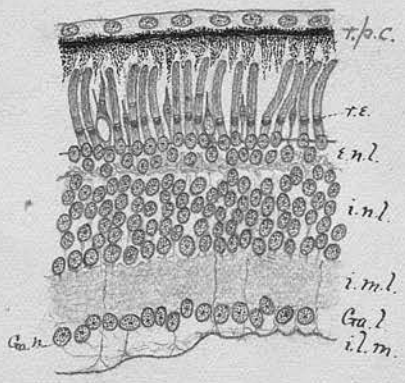
Frog Tadpole 53 days.
30 m. m. long.

Cam. Luc. drawing Fig. 22. Slide 53.



Frog Tadpole 67 days.
28 m.m. long.

Cam. Luc. drawing. Fig. 23 Slide 56

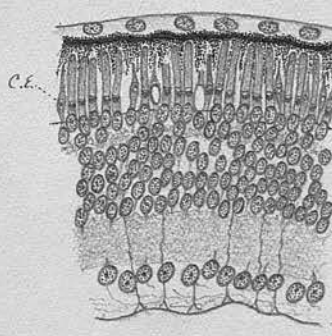


Frog Tadpole 70 days.
35 m.m. long.

Cam. Luc. drawing. Fig. 24 slide 54

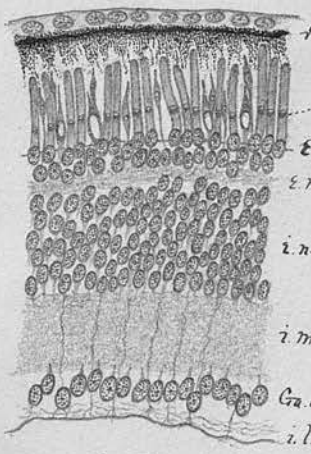
Frog Tadpole 73 days.
32 m.m. long.

Cam. Luc. drawing Fig. 25. slide 61.



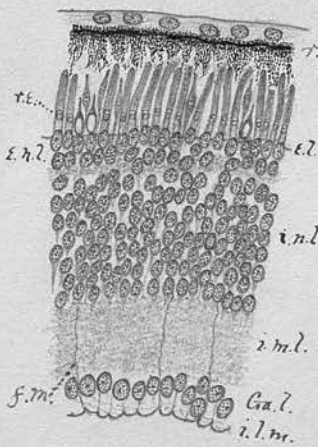
Frog Tadpole 79 days
33 m.m. long.

Cam. Luc. drawing. Fig. 26 - slide C 4



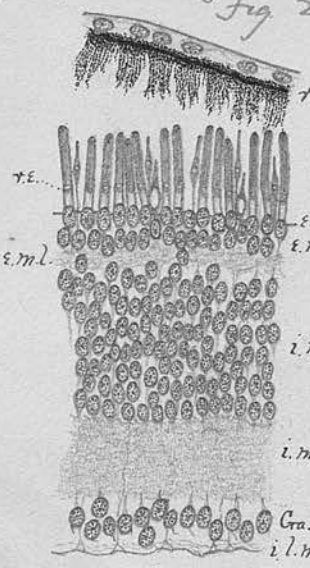
Frog Tadpole 85 days
½ of tail absorbed

Cam. Luc. drawing. Fig. 27 slide 66.



Frog 58 days. Tail stump

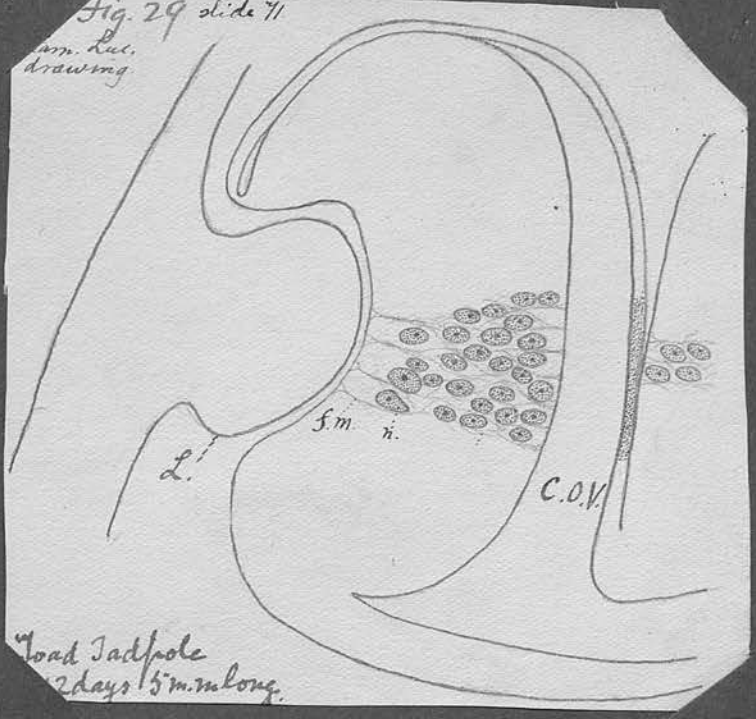
Cam. Luc. drawing. Fig 28. slide 68.



Frog 94 days. Tail absorb

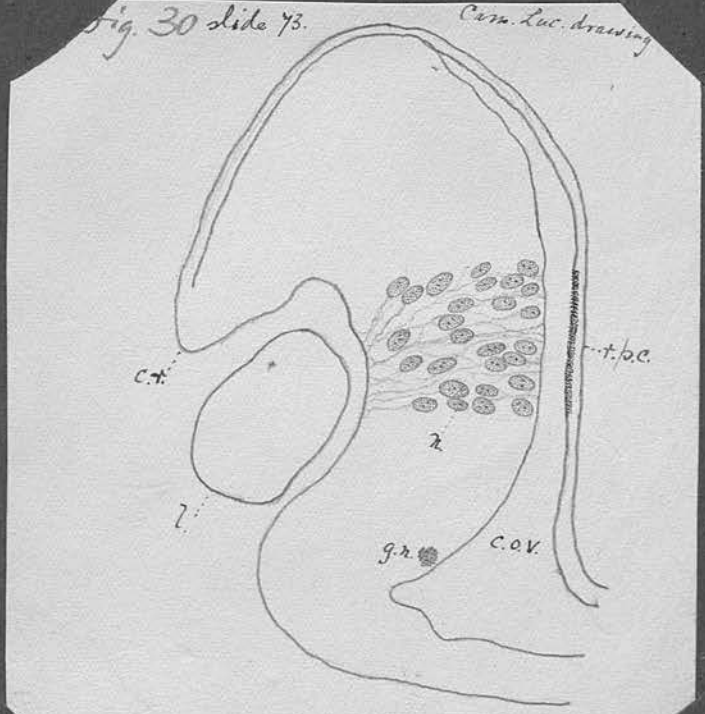
Fig. 29 slide 71

Cam. Luc. drawing



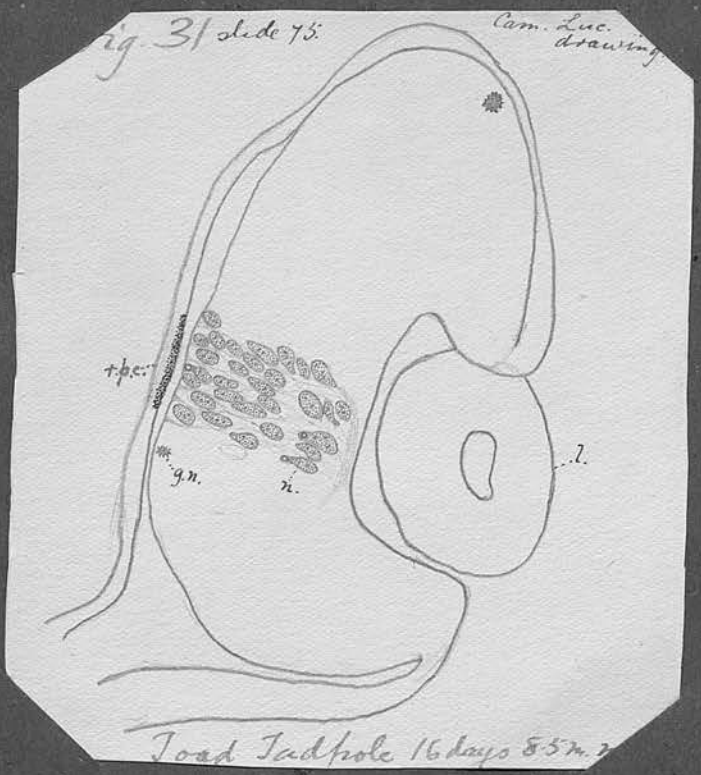
Toad Tadpole
2 days 5 m.m. long.

Fig. 30 slide 73. Cam. Luc. drawing



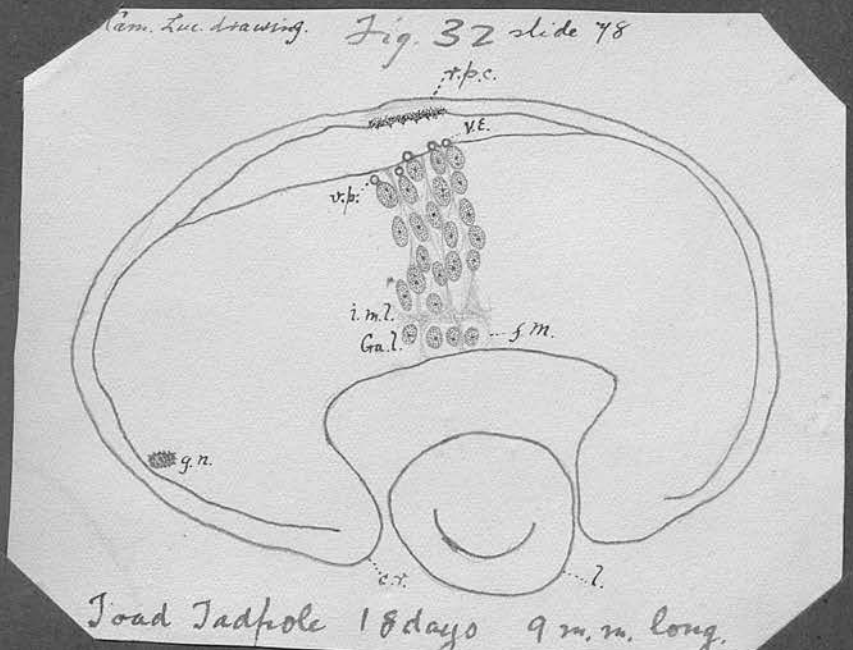
Toad Tadpole 14 days. 7 m.m. long

Fig 31 slide 75. Cam. Luc. drawing.



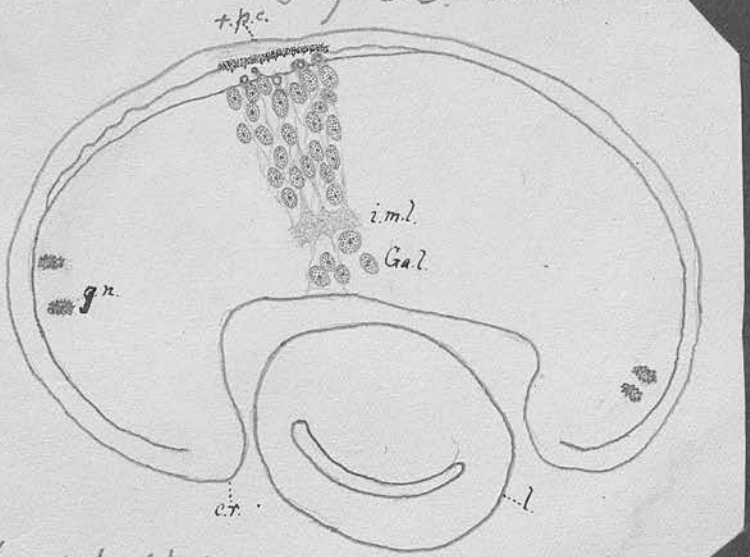
Toad Tadpole 16 days 8.5 m. m.

Cam. Luc. drawing. Fig. 32 slide 78



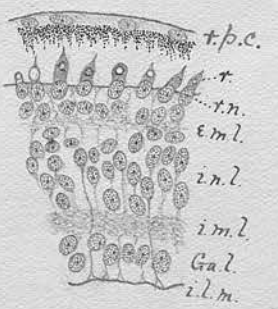
Toad Tadpole 18 days 9 m. m. long.

Cam. Luc. drawing Fig. 33. slide 80



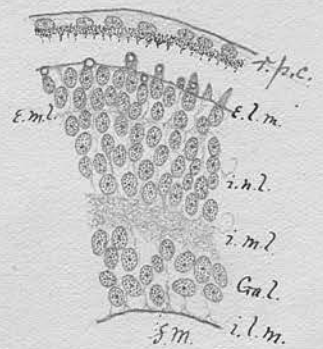
Toad tadpole. 20 days. 10 m. m. long

Cam. Luc. drawing. Fig 34 slide 82.



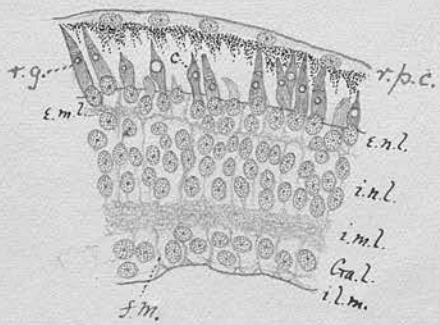
Toad Tadpole 22 days.
11.5 m. m. long

Cam. Luc. drawing Fig. 35 slide 83.



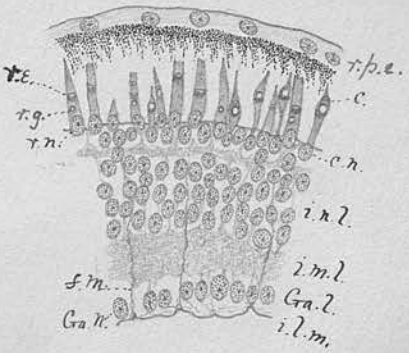
Toad Tadpole 24 days
11 m.m. long.

Cam. Luc. drawing. Fig. 36 slide 84.



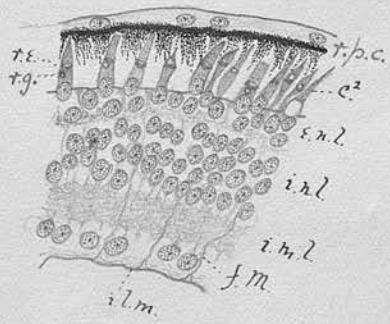
Toad Tadpole 2 5 days
15 m.m. long

Cam. Luc. drawing. Fig. 37 slide 89.



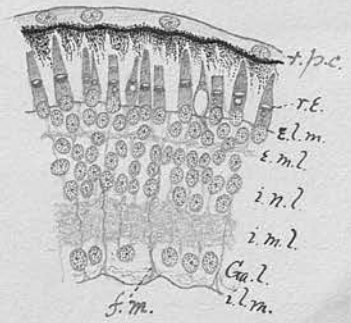
Toad Tadpole 32 days
21 m.m. long.

Cam. Luc. drawing. Fig. 38 slide 91.



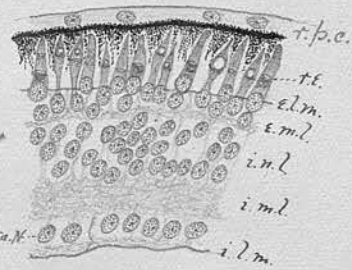
Toad Tadpole 34 days
20 m.m. long.

Cam. Luc. drawing. Fig 39 slide 92.



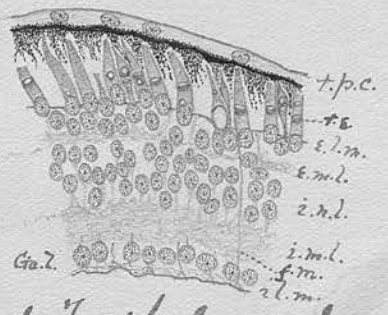
Toad Tadpole 36 days
21 m.m. long

Cam. Luc. drawing. Fig 40 slide 94.



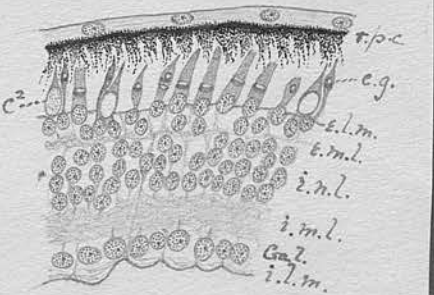
Toad Tadpole 38 days
23 m.m. long.

Cam. Luc. drawing. Fig. 41 slide 96.



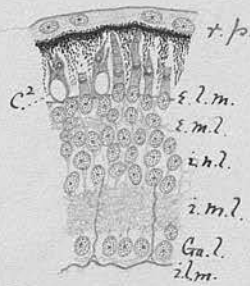
Toad Tadpole 40 days
23 m.m. long

Cam. Luc. drawing. Fig. 42 slide 98.



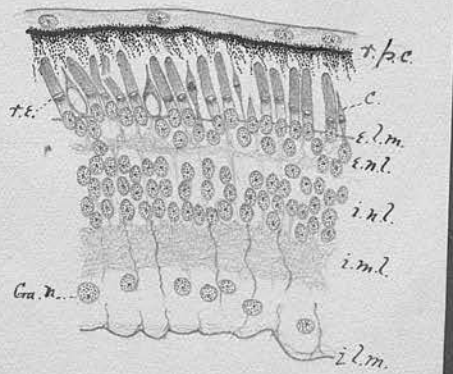
Toad Tadpole 42 days
25 m.m. long

Cam. Luc. drawing. Fig. 43 slide 101



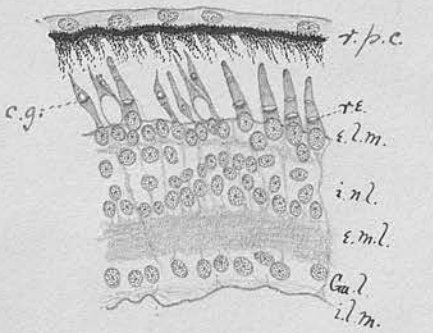
Toad Tadpole 44 days.
25 m.m. long.

Cam. Luc. drawing. Fig. 44 slide 103.



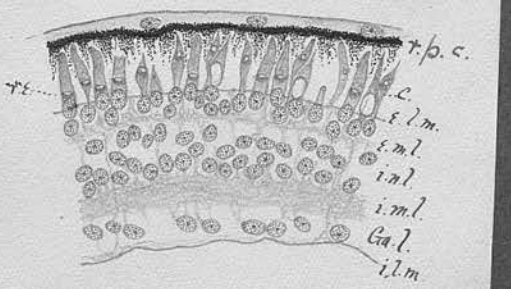
Toad Tadpole 50 days.
26 m.m. long.

Cam. Luc. drawing. Fig. 45 slide 109.



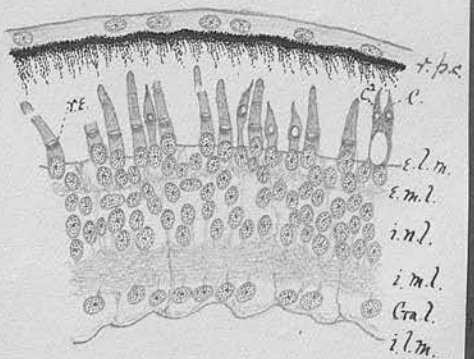
Toad Tadpole 52 days
24 m.m. long.

Cam. Luc. drawing. Fig. 46 slide 111.



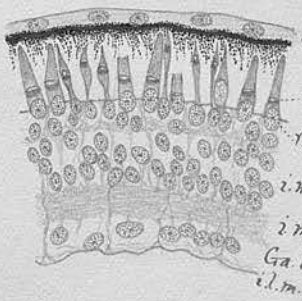
Toad Tadpole 54 days
30 m.m. long.

Cam. Luc. drawing. Fig. 47. slide 112.



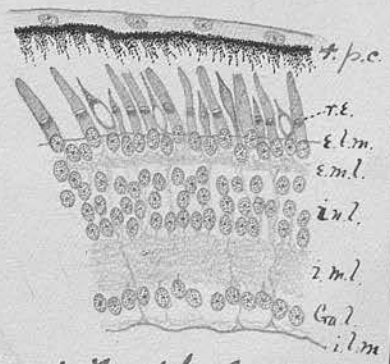
Toad Tadpole 56 days.
26 m. m. long.

Cam. Luc. drawing. Fig. 48. slide 11



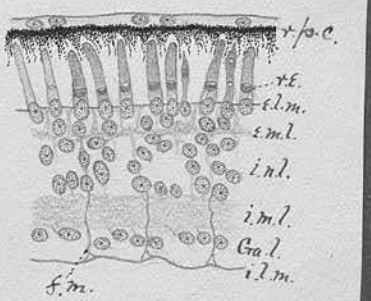
Toad Tadpole 58 days.
29 m. m. long.

cam. Luc. drawing. Fig. 49 slide 117.



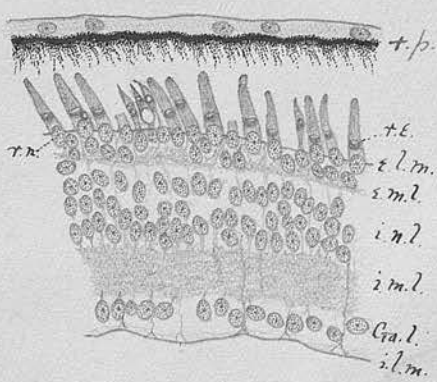
Toad Tadpole 66 days.
26 m.m. long

cam. Luc. drawing. Fig. 50 slide 120



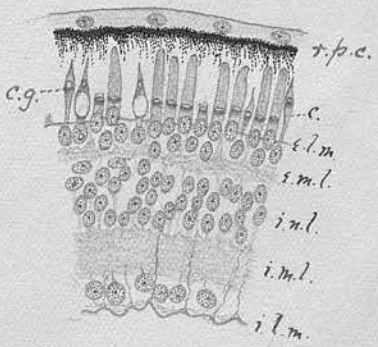
Toad Tadpole 68 days.
30 m.m. long

Cam. Luc. drawing. Fig. 51 slide 122.



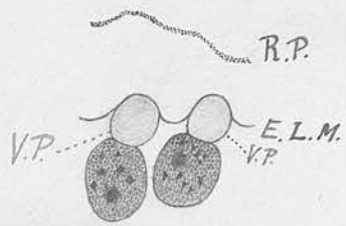
Toad Tadpole. 72 days
27 m.m. long.

Cam. Luc. drawing. Fig. 52 slide 124.



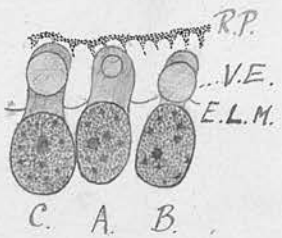
Toad Tadpole 76 days
30 m.m. long

Cam. Lucida. Fig. 53. slide 5.



Frog Tadpole 4.5 m. m Long. H&E. Eos
15 days.

Cam. Lucida. Fig. 54 slide 13.

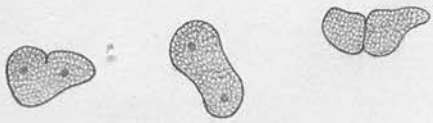


Frog Tadpole 21 days. 12 m. m. Long.
Haem-Al. Eos.

Cam. Luc. Fig. 55 slide 54.

43rd day frog tadpole

Cam. Lucida. Fig. 56 slide 112.



56th day toad-tadpole.

Fig. 57.

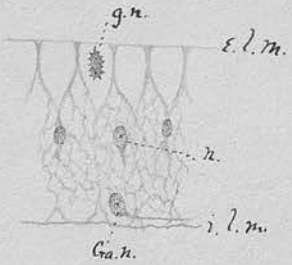


Fig. 58

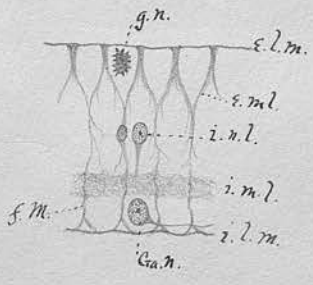


Fig. 59.