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THE PROCESS OF DIFFERENTIATION OF THE
RETINAL LAYERS IN VERTEBRATES*

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THE development of the various nuclear and fibrillar layers of the adult retina from the undifferentiated neural ectoderm of the walls of the optic cup is a complicated process. Nevertheless a study of its stages in animals representative of the various classes of vertebrates brings out the interesting fact that a basal plan exists for the entire phylum. Modifications, usually in the form of abbreviation or slurring over of stages, occur from time to time, but in all the animals examined the principle of the differentiative process remained unchanged and was exemplified in the most primitive as well as in the most specialised types. Superimposed on this basal arrangement there was also apparent a slow evolutionary increase in efficiency, brought about rather by alteration in size and appearance than by modification of arrangement of the anatomical elements involved.

It is proposed to deduce by examination of various species both the fundamental plan and the mechanism of evolutionary improvement on it.

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The earliest period of development which concerns us is that immediately after the invagination of the primary optic vesicle to form the optic cup. The changes to be described involve principally the inner wall of the cup, which, at its inception, is composed of a neuro-epithelium throughout the entire thickness of which are scattered oval nuclei, no cell boundaries being distinguishable. The outer wall of the optic cup is composed of a thinner neuro-epithelium containing only a single row of nuclei. It remains in this condition throughout the entire life of the individual. The only changes which occur in it beyond increase in area are connected with its pigmentation. The nuclei of the inner wall, on the other hand, must become as it were sorted out and re-arranged in definite layers divided by fibrillar intervals before they can resemble the adult condition. The nuclei themselves must also undergo differentiation, which is always signalled by a change of outline from oval to circular, while in addition the protoplasm of the cell body must become distinct from the original mass.

It may be stated as a general rule that differentiation of nuclei begins in those nearest to the inner surface of the optic cup and spreads gradually outwards. This differentiation is accompanied at first by migration of the nuclei towards the inner surface, so that, soon after the beginning of the process, two nuclear layers can be recognised, an inner composed of round nuclei and an outer composed of oval nuclei. These will be referred to as the *inner* and *outer neuroblastic layers* respectively. From them the layers of the adult retina are developed. Since differentiation spreads from the inner surface of the retina outwards it follows that the conducting and supporting mechanism is developed in advance of the receptor itself. This order is of common occurrence throughout the embryo, in which as a general rule development progresses from less specialised to more specialised organs and tissues.

Cyclostomes

The Cyclostomes represent the lowest known type of vertebrate. They are usually classed with the fish, but differ from them (and indeed from the rest of the vertebrates) in certain important particulars, for example in the absence of a branchial skeleton and in the persistence of the notochord. *Petromyzon fluviatilis* (the lamprey) may be taken as an example. In the larva (known as *Ammocoetes*) the optic cups are present as outgrowths from the forebrain, but are not yet in direct contact with the surface ectoderm. During the third year metamorphosis takes place, the eye reaches the surface and a lens is developed. Long before this,

however, the process of differentiation has begun in the wall of the optic cup and the retinal mechanism is complete before the end of the larval stage.

Fig. 1 shows the beginnings of retinal differentiation. The optic cup is seen lying empty and folded against the side of the fore-brain, embedded in the myotomic muscles. The portion of the cup lying above the insertion of the optic stalk shows commencing

FIG. 1.

Developing eye of *Petromyzon fluviatilis*. Early stage.

- a* = pigment layer.
- b* = outer neuroblastic layer.
- c* = inner neuroblastic layer.
- d* = marginal layer.

differentiation. The cells of the outer layer contain pigment granules, while those of the inner layer, which is about three and a half times as thick as the outer, are beginning to show typical changes. This inner layer can be divided into a nucleus-free inner zone and an outer zone containing nuclei of two kinds. The inner zone is known as the marginal layer. It is comparable with the marginal layer which appears throughout the developing central nervous system and reaches its acme in the embryonic spinal cord. (The marginal layer, wherever it occurs, seems to

act as a nidus for the reception of cells formed by division of nuclei in the layers deep to it and of the nerve fibres developed from these cells.) The innermost nuclei of the nuclear zone are circular in outline (*i.e.*, they are already specialised for function rather than

FIG. 2.

Later stage of developing eye of *Petromyzon fluviatilis*.

- a* = outer nuclear layer.
- b* = outer molecular layer.
- c* = inner nuclear layer.
- d* = ganglion cells and nerve fibres.
- e* = optic stalk.

division.) They form a more or less irregular single row encroaching on the marginal layer and constitute the *inner neuroblastic layer*. The two rows of undifferentiated oval nuclei lying outside this constitute the *outer neuroblastic layer*. Those abutting on the pigment layer undergo karyokinesis and by their division add both to the size of the eye and to the number of nuclei internal to themselves. They are the last to differentiate. Before they do so, the inner neuroblastic layer has migrated further into the marginal

layer and its cells have given rise to axons which, running in the marginal layer parallel to the surface, constitute the nerve fibre layer. The most internal of the cells of the outer neuroblastic layer form bipolar cells, while the outermost undergo a very specialised development and give rise to the percipient elements themselves. These changes can be seen in progress in Fig. 2. This represents a considerable advance on the condition seen in Fig. 1, though the optic cup is still folded and empty and there is no lens. (The section does not pass through the pupil, and the upper and lower parts of the cup appear in continuity above, though below there is a gap, the foetal fissure, leading into the interior of the cup, and occupied by a wedge of vascular mesoderm). The region of the posterior pole just above the insertion of the optic stalk can now be seen to present a more complicated picture than the corresponding region in Fig. 1. The marginal layer as such has almost disappeared, being filled up with circular nuclei (derived from the inner neuroblastic layer) and with nerve fibres. The nuclei are those of the ganglion cells. The nerve fibres run downwards and, entering the optic stalk, pass backwards to the brain. They appear, as in all the animals examined, coincidentally with the ganglion cells and in many instances can be seen developing as actual processes of these. Their early advent exemplifies the general rule mentioned above of appearance of conducting layers before percipient.

Deep to the ganglion cells and nerve fibres are two or three rows of irregularly arranged circular nuclei, the most superficial of which appear to lie within the original marginal layer, while the more deeply placed ones correspond in position with the innermost nuclei of the outer neuroblastic layer of the previous stage. These round nuclei constitute the inner nuclear layer and are separated by a narrow interval, the outer molecular layer, from a single row of nuclei connected with the developing percipient elements. These latter are all of one type, very large and separated from each other by wide intervals. Various stages in their formation can be recognised in the figure. (We are not concerned here with details of structure, but only with the mechanism of differentiation of layers, so that the individual elements need not be described further.)

We can, therefore, recognise at this stage the typical layering of the adult retina. Of these the nerve fibre and ganglion cell layers have developed from the *inner* neuroblastic layer of the previous stage, the inner nuclear layer appears to contain elements from both *inner* and *outer* neuroblastic layers (though they cannot be easily distinguished apart) and the outer nuclear layer (or nuclei of the percipient elements) has formed by differentiation of the outermost (proliferating) row of nuclei of the *outer* neuro-

blastic layer. This may be represented simply by a scheme, thus :—

It is to be noted that, although the layering in Fig. 2 is that of the highest vertebrates, it is not possible to distinguish so many elements as in their retinae. Fibres of Müller, amacrine cells and horizontal cells cannot be recognised definitely at this the corresponding stage in *Petromyzon* to that at which they are evident in higher forms.

Fish

As an example of a primitive fish, the Ganoid, *Amia calva* (American bowfin) has been chosen. The early stages of growth of the optic vesicle and the invagination of it to form the optic cup follow the usual vertebrate lines. Pigmentation begins in the outer layer of the optic cup at a time when the inner layer has still the character of a simple neuro-epithelium with ten to twelve rows of oval nuclei. The differentiation of the pigment layer progresses a stage further than it does in *Petromyzon*, since the cells develop flame-shaped processes from their inner surfaces, which processes pass up between the outer limbs of the rods when these appear. This condition is common in most of the vertebrates below the mammals.

With regard to the inner layer, we see in Fig. 3 that the first sign of differentiation is (as in *Petromyzon*) a change of shape of the most internal nuclei from oval to circular. There is not, in fish, at any time a well-marked nucleus-free marginal layer. This, occurring as it does in *Petromyzon*, and also in other species and in other parts of the central nervous system, may be looked on as a primitive stage which is slurred over or abbreviated in the fish. This abbreviation may be correlated with the relatively short

larval stage of fish as compared with Cyclostomes and is probably merely a means of ensuring rapidity of development. In Fig. 3 we can recognise therefore the commencement of the formation of the inner neuroblastic layer from the innermost cells of the neuro-epithelium. These first cells to differentiate will become ganglion cells and give rise to nerve fibres long before the outermost cells have begun to specialise. In Fig. 3 these outer cells can be seen

FIG. 3.

Early stage of differentiation of the retina of the Ganoid fish, *Amia calva*.

- a* = beginning of inner neuroblastic layer.
- b* = undifferentiated neuro-epithelium.
- c* = proliferating layer.
- d* = pigment layer.

undergoing mitosis, and forming a proliferating layer next the basement membrane.

The inner neuroblastic layer increases in thickness by increment of cells external to it. Nerve fibres arise from its innermost cells and form a nerve-fibre layer on its inner surface. Fig. 4 shows an eye in which this is occurring. The layers at the posterior pole here consist of an innermost nerve fibre layer, an inner neuroblastic layer of round nuclei, and an outer neuroblastic layer of oval nuclei, those in the outermost row of all being in a state of active proliferation. After this stage the inner neuroblastic layer increases in thickness and becomes divided into an inner and an

outer band by a fibrillar layer, the inner molecular layer. The cells of the outer band are separated from those of the outer neuroblastic layer by a very narrow interval (the "transient fibre layer" described by Chiewitz* in human embryos), but are as well easily distinguishable from them by containing slightly less chromatin

FIG. 4.

Developing eye of *Amia calva*.

- a* = pigment layer.
- b* = proliferating layer.
- c* = outer neuroblastic layer.
- d* = inner neuroblastic layer.
- e* = nerve fibre layer.

in the nucleus, and in consequence staining less deeply. Soon after the appearance of the inner molecular layer the specialisation of nuclei begins to involve the outer neuroblastic layer as well. Its nuclei become in turn spherical and separated into two bands, an outer and an inner, by a fibrillar layer, the outer molecular layer. When this has occurred the retina consists of four bands of nuclei, separated from each other by three fibrillar layers, of which the centre one, or transient fibre layer of Chiewitz is very narrow.

*Chiewitz, J. H.—*Intern. Zeitschr. f. Anat. u. Physiol.*, Vol. IV, p. 201, 1887, and *Anat. Anzeig. Jena*, III, 579, 1888.

Fig. 5 shows this stage. The four nuclear layers from within outwards can be recognised as the ganglion cell layer, a layer of spherical nuclei which later form amacrine cells, a layer of more darkly staining bi-polar nuclei, and a layer of nuclei, still oval, in connection with which the percipient elements are just beginning to develop. The transient layer of Chiewitz disappears very

FIG. 5.

Developing retina of *Amia Calva*.

<i>a</i> = nerve fibre layer.	<i>f</i> = bi-polar layer.
<i>b</i> = ganglion cell layer.	<i>g</i> = outer molecular layer.
<i>c</i> = inner molecular layer.	<i>h</i> = rod and cone nuclei.
<i>d</i> = amacrine cell layer.	<i>i</i> = developing rods and cones.
<i>e</i> = transient fibre layer.	<i>j</i> = pigment layer.

soon and the second and third nuclear layers then fuse to form the definitive inner nuclear layer, from the outermost elements of which horizontal cells are formed during the next stage. We thus see that the complexity of the inner nuclear layer foreshadowed in *Petromyzon* is now clear. It contains three types of cell :—

1. amacrine cells, derived from the inner neuroblastic layer,
2. bi-polar cells, and
3. horizontal cells, which two latter types are derived from the outer neuroblastic layer.

Fig. 6 shows the end result of the disappearance of the transient fibre layer and the formation of the horizontal cells. In this, the retina of an advanced embryonic stage of *Amia*, one can recognise three bands of cells; the innermost is the ganglion cell layer. The

FIG. 6.

Retina of advanced embryo of *Amia Calva*.

<i>a</i> = nerve fibre layer.	<i>f</i> = outer molecular layer.
<i>b</i> = ganglion cells.	<i>g</i> = rod nuclei.
<i>c</i> = inner molecular layer.	<i>h</i> = cone nuclei.
<i>d</i> = amacrine and bi-polar nuclei.	<i>i</i> = rods and cones.
<i>e</i> = horizontal cells.	<i>j</i> = pigment layer.

outlines of the cell bodies are here visible. Nerve fibres are forming a thin layer internal to them. Deep to the ganglion cell layer is the inner molecular layer. This is relatively very wide, a condition characteristic of vertebrates below the mammals. The next cell layer is the inner nuclear layer. It contains amacrine and bi-polar cell nuclei, now not to be distinguished from each other, and is bounded externally by a single row of horizontal cells. Outside it lies the outer molecular layer and outside this the nuclei of the percipient elements. These latter are clearly of two kinds, rods

and cones. The nuclei of the rods are small and lie internal to the larger cone nuclei. The pigment epithelium shows flame-shaped processes.

The differentiation of the retina of *Amia* is therefore more complete than that of *Petromyzon*, though in principle it is similar. It may be reduced to a scheme thus:—

It is to be noted that as yet the retina contains no demonstrable supporting tissue. Fibres of Müller cannot be recognised as such though it is probable that some sort of undifferentiated glial scaffolding does exist. This paucity of recognisable supporting tissue (in stages corresponding to those in which it is clearly recognisable in higher forms) may perhaps be looked on as merely part of the low grade of development of such tissues throughout the body, *e.g.*, the major part of the skeleton in fish never reaches the stage of ossification and the connective tissues generally often show a less degree of condensation than they do in higher animals.

A further example may be cited among fish, namely the Elasmobranch, *Squalus acanthias*, the dogfish. In this animal the same abbreviation of early stages is apparent, and there is moreover greater difficulty in distinguishing the various types of cell than there is in *Amia*. The main plan of differentiation is similar, but the details are not so clear cut. There is the same paucity of glial scaffolding as in *Amia*. Fig. 7 shows an early stage of the primary optic vesicle in which the entire wall consists of undifferentiated neuro-epithelium. Fig. 8 shows the eye of an older dogfish. It represents a stage when invagination is complete and the distinction between the thin outer and thick inner layer of the cup is well marked. The inner layer shows a very narrow marginal layer, which soon becomes filled up with nerve fibres arising from the cells immediately subjacent to it. These ganglion cells appear to be relatively late in separating from the underlying

FIG. 7.
Optic vesicle and forebrain of dogfish embryo.

FIG. 8.
Section through optic cup of dogfish embryo.
a = marginal layer.
b = nerve fibres.

layers. Fig. 9 shows a portion of the retina of a young dogfish in what is generally known as the "pup" stage. The nerve fibre layer is thick, but the inner molecular layer is still narrow, and the ganglion cell nuclei are arranged in three rows. There is no distinction between amacrine, bi-polar and horizontal nuclei; together they form an inner nuclear layer of seven rows deep. The rods and

FIG. 9.

Retina of dogfish ("pup" stage).

a = nerve fibre layer.	e = outer molecular layer
b = ganglion cells.	f = outer nuclear layer.
c = inner molecular layer.	g = rods and cones.
d = inner nuclear layer.	h = pigment layer.

cones are present and their nuclei are arranged in four rows. The pigment epithelium is a dense black. Processes from it are not well marked.

The true fish thus shows an advance on the Cyclostome in that a greater variety of cells can be recognised, though the main arrangement is the same in both types.

Amphibians

In the Amphibia the same principles of differentiation appear to hold good, but the process can be followed one stage further, namely, to the formation of a distinct glial system with well-formed Müllerian fibres. The retina of the frog tadpole seen in

FIG. 10.

Retina of frog tadpole.

<i>a</i> = nerve fibre layer.	<i>f</i> = outer molecular layer.
<i>b</i> = ganglion cells.	<i>g</i> = outer nuclear layer.
<i>c</i> = inner molecular layer.	<i>h</i> = rods and cones.
<i>d</i> = amacrine cells and fibres of Müller.	<i>i</i> = pigment epithelium.
<i>e</i> = bi-polar cells.	

FIG. 11.

Developing eye of Necturus.

- a* = amacrine cells.
b = layer of Chiewitz.
c = bi-polar cells.

Fig. 10 shows these fibres running from the internal limiting membrane to the outer molecular layer. Their nuclei (which lie in the inner nuclear layer) are not easy to distinguish. Amacrine cells occur on the inner surface of the inner nuclear layer, but horizontal cells are not apparent, a difference from the condition found

FIG 12.

Retina of frog.

<i>a</i> = nerve fibre layer.	<i>g</i> = rod nuclei.
<i>b</i> = ganglion cells.	<i>h</i> = cone nuclei.
<i>c</i> = inner molecular layer.	<i>i</i> = rods and cones.
<i>d</i> = amacrine and bi-polar nuclei.	<i>j</i> = pigment layer.
<i>f</i> = outer molecular layer.	

in *Amia*. It is not possible in *Amphibia* to make out for certain the layer from which come the nuclei of the Müllerian fibres. Their appearance at this stage is, however, interesting. It may possibly be correlated with the more generally advanced supporting tissues of these animals. That the amacrine cells in *Amphibia* as well as in other orders come from the outer neuroblastic layer, and secondarily fuse with the layer of bi-polar nuclei to form the inner nuclear layer can be seen from the stage of *Necturus* seen in Fig. 11.

Here the amacrine nuclei are arranged in two rows separated from the ganglion cell nuclei by the inner molecular layer and from the bi-polar nuclei by an interval which from its position is the transient fibre layer of Chiewitz. Subsequent fusion of these two nuclear layers (amacrines and bi-polars) produces the definitive inner nuclear layer. Fig. 12 shows the adult condition in the frog. It differs from that of *Amia* (Fig. 6) in the presence of fibres of Müller and the absence of recognisable horizontal cells. Otherwise the two are exactly similar.

Reptiles

The developing reptilian retina is in some ways more primitive than that of the fish, in that it does not show abbreviation of stages. This leads to a relatively slower rate of differentiation, and hence the successive changes are clear cut and can be more easily followed. Like that of the Cyclostome, *Petromyzon*, the reptilian retina passes through a stage in which the inner wall of the optic cup consists of a neuro-epithelium having a nucleus free marginal layer on its inner surface, and deep to this a mantle-layer of oval nuclei, the outermost row of which is in active mitosis and forms the proliferating layer. At this stage the appearance of the wall of the cup is very similar to that of the spinal cord, which, in early stages, differentiates in exactly the same way. From the nuclei of the mantle layer are formed the inner and outer neuroblastic layers. The inner neuroblastic layer arises by the migration of the innermost nuclei of the mantle layer into the marginal layer. At the same time these nuclei change their outline from oval to circular. They can be seen in Fig. 13 (which shows the eye of a *Chrysemys marginata* embryo) as a single row (containing only five cells) lying in the marginal layer. This migration continues, and very soon processes develop from the innermost of the migrating cells, which run in the marginal layer at right angles to its surface, and, passing towards the region of insertion of the optic stalk, turn into the inner layer of this to form the nerve fibres of the optic nerve. The first of the retinal cells to differentiate are therefore the ganglion cells (as in all the other species examined). They can be seen in Fig. 14, which also shows their axons running into the optic stalk.

The inner neuroblastic layer thus formed increases in thickness. Its innermost cells form ganglion cells as we have seen, but its deeper cells undergo differentiation in another way. Like the ganglion cells their nuclei become circular, but they do not send axons into the nerve fibre layer. Instead they become separated from the ganglion cells by an interval (the inner molecular layer) and form a more or less distinct row (recognisable as the amacrine layer) separated from the outer neuroblastic layer by a fibrillar

FIG. 13.

Section through the optic cup of a young *Chrysemys marginata* embryo. *a* = cells of inner neuroblastic layer migrating into marginal layer.

FIG. 14.

Slightly older stage of *Chrysemys marginata*.
a = nerve fibres appearing in marginal layer.

interval of varying thickness. This is the transient fibre layer of Chiewitz. It can be seen in Fig. 15, which shows a few amacrine cells in process of separation from the ganglion cells. This continues until they form a considerable band, the transient fibre layer diminishing in thickness meanwhile. This disappears finally and they fuse with the outer neuroblastic layer to form the final inner nuclear layer, though they can long be recognised as

FIG. 15.

Section through the eye of a *Chrysemys marginata* embryo.

a = amacrine cell, separating from ganglion cell layer internal to it.

b = transient fibre layer.

distinct from this. Fig. 16 shows them forming a layer on the surface of the outer neuroblastic layer, which has meanwhile divided into an inner bi-polar layer and an outer layer of rod and cone nuclei. These are separated from each other by the outer molecular layer, and the definitive condition is reached merely by thinning of nuclear and thickening of fibrillar layers as the eye enlarges.

The reptiles show definite fibres of Müller (Fig. 16). Their nuclei are not easy to see, but appear to lie among the amacrine cells and to be developed with them from the outer neuroblastic layer.

The reptilian retina therefore shows the stages of differentiation very clearly, and they may be reduced to a scheme which will be

Above the Reptilia phylogenetic progress appears to travel along two slightly diverging lines, leading on the one hand to the birds, and on the other, to the higher mammals. Both these possess retinæ of a very high grade of functional efficiency, and, in both, the embryonic stages of formation of the retinal layers follow very closely the primitive plan. It is as well to consider the birds first, though in many ways they show a higher grade of retinal differentiation than do the mammals.

Birds

These show abbreviation of early stages such as occurred in the fish. The marginal layer is not well developed and ganglion cells and nerve fibres begin to appear on the inner surface at a very early stage. Fig. 17 shows the earliest stage at which ganglion cells can be recognised. They lie on the surface of the neuro-epithelium. Their nuclei are round and they do not as yet show any axons.

They differentiate rapidly, as can be seen in Fig. 18. At this stage the following layers can be recognised from within outwards.

FIG. 17.

Retina of chick embryo of 3 days.

a = early ganglion cells.

b = proliferating layer.

c = outer wall of optic cup.

1. An internal limiting membrane forming by fusion of the ends of the Müllerian fibres.
2. A narrow marginal layer becoming filled up with axons of ganglion cells.
3. A layer of nuclei representing the inner neuroblastic layer. The cells in this layer are of two sorts, namely ganglion cells and supporting cells. The ganglion cells are easily distinguished.

FIG. 18.

Retina of chick. For numbering of layers see text.

They are multipolar and their processes can often be traced some little distance. The axon enters the nerve fibre layer. The supporting cells have oval undifferentiated nuclei and an elongated cell body arranged at right angles to the direction of the layer. This is prolonged into an inner and an outer process, the former running through the nerve fibre layer to its inner surface and representing the inner limb of a Müllerian fibre, and the latter passing outwards to be lost among the cells of the outer neuroblastic layer.

4. A narrow interval (the transient fibre layer) separating inner from outer neuroblastic layers.

5. The outer neuroblastic layer, composed of many superimposed rows of undifferentiated oval nuclei, the deepest row of which is undergoing mitosis.

6. The pigment layer (outer wall of optic cup). This has no processes as yet.

This stage shows very clearly the origin of the Müllerian fibres from the outer neuroblastic layer.

FIG. 19.

Retina of chick. For numbering of layers see text.

The next stage of interest is that seen in Fig. 19.

The structures seen will be considered *seriatim* as before:—

1. An internal limiting membrane.
2. A nerve fibre layer.
3. A layer of ganglion cells and Müllerian fibre nuclei (the latter not so easily seen now, as they are farther apart). The outermost row of the ganglion cell nuclei are becoming separated from their fellows by a very narrow interval (3a in the figure), which is the forerunner of the inner molecular layer. This single row of cells (3b) is that of the future amacrine cells, just beginning to separate out from the rest of the inner neuroblastic layer.

4. The transient fibre layer, wider and free from nuclei.
5. A thick band of nuclei, the inner rows of which are differentiating and are round, while the outer ones are still oval. This band represents the major part of the outer neuroblastic layer. The cells of it will form bi-polar cells later.
6. A narrow interval, the outer molecular layer.

FIG. 20.

Retina of chick. Numbering of layers as in Fig. 19 and text.

7. Two rows of nuclei derived directly from the proliferating cells of the previous stage and destined to form the rod and cone nuclei.
8. An external limiting membrane, showing small projections, the beginnings of the visual elements themselves.
9. The pigment layer.

After this stage the row of amacrine cells and also the Müllerian fibre nuclei become separated from the ganglion cells and begin to fuse with the bi-polar layer, thus bringing about a widening of the inner molecular layer and a narrowing and final disappearance of the transient fibre layer of Chiewitz. Fig. 20 shows this change

in progress. In it the amacrine cells form a distinctly separate layer and the layer of Chiewitz already contains a few nuclei in transit. The layers are indicated in the figure by the same

FIG. 21.

Retina of hen.

1 = internal limiting membrane.	6 = outer molecular layer.
2 = nerve fibre layer.	7 = outer nuclear layer.
3 = ganglion cells.	8 = rods and cones.
4 = inner molecular layer.	9 = pigment epithelium.
5 = inner nuclear layer.	

numbers as in Fig. 19. Finally the layer of Chiewitz completely disappears and the adult condition is reached as in other species by the narrowing of the nuclear layers and the widening of the fibrillar zones consequent on the general enlargement of the eye. Fig. 21 shows the process complete, even to the development of flame-shaped processes in the pigment layer.

Mammals

We have seen in birds an even clearer demonstration of the derivation of the various cell layers according to the basal vertebrate plan. They furnish by far the most convincing material for the study of the amacrine cells and the formation of the transient fibre layer, even though there is abbreviation of early stages. In the mammals, on the other hand, the early stages are very clear

FIG. 22.

Section through both walls of the optic cup of a human embryo of 5 mm.

a = marginal layer.
b = capillary.

cut, but the later stages tend to become somewhat slurred. In addition another process becomes apparent, namely, a transient vascularisation of the retina in early stages. In all the animals below the mammals the wall of the optic cup is completely avascular throughout the period of cellular rearrangement which we have been studying. In some mammals*, however, the beginning of differentiation seems to be accompanied and possibly to some extent initiated by a penetration of small blood-capillaries into the inner wall of the optic cup from its inner surface. This is merely part of a general capillary vascularisation of the wall of the nerve tube at this stage and is in no sense peculiar to the region of the optic vesicle. It is extremely transient, the capillaries (which

* Man and the mouse only have been available for investigation, so that nothing definite can be said of other species.

only involve at their maximum development the lower part of the invaginating optic vesicle) disappearing as soon as the distinction between inner and outer neuroblastic layers is established. This initial peculiarity of the developing mammalian retina is probably to be looked on as an expression of the great vascularity of the eye

FIG. 23.

Section through the retina of a human embryo of 21 mm.

- a* = fibres of Müller forming internal limiting membrane.
- b* = nerve fibre layer.
- c* = ganglion cells
- d* = amacrine cells } inner neuroblastic layer.
- e* = transient fibre layer.
- f* = outer neuroblastic layer.
- g* = pigment layer.

region throughout the whole of the organo-genetic period, which is one of the distinguishing features of the mammalian embryo.

In other respects the appearance of the retinal layers in the mammals follows the vertebrate plan so far seen to hold good, with modifications, for representatives of all the other classes. We can consider briefly some of the more important stages as seen in human embryos.

Fig. 22 shows the stage of vascularisation described above and also emphasises the fact that there is no abbreviation of early

stages. A well-formed marginal layer is present before the distinction between inner and outer neuroblastic layers has begun. The formation of the inner neuroblastic layer by migration of the innermost nuclei into the marginal layer is in man exactly similar to that seen in the reptile *Chrysemys marginata* (Fig. 13) and need not be figured. The derivation of the amacrine cells from the inner neuroblastic layer is seen in Fig. 23. It will be noted that

FIG. 24.

Section through the retina of a five and a half months human foetus.

1 = ganglion cells.	4 = bi-polar and Müllerian fibre nuclei.
2 = inner molecular layer.	5 = outer molecular layer.
3 = amacrine cells.	6 = outer nuclear layer.

fibres of Müller are well marked and that the distinction between the ganglion cells and the amacrine cells is marked by difference of staining and of position, though together they constitute a single layer. The position of the transient fibre layer of Chiewitz is apparent, but it is not a true layer as in birds. It is of note, however, that, in the human macular region, the layer of Chiewitz attains a development equal to that seen in birds and can be studied as easily. Over the rest of the human retina it is only present as a line of separation, not as a true layer.

Fig. 24 shows the formation of the inner molecular layer by fusion of the amacrine cells and nuclei of fibres of Müller with the

percipient end-organ of the retina. This alone will go a long way to account for the high grade of efficiency of so primitive an organ as we have seen the human retina to be. It need not be dealt with further here.

The second factor is the slow but definite and steady tendency throughout phylogeny towards an increase in the number of percipient elements in a given area. This of course allows of increase in visual acuity since an increasing number of points on an object can be perceived as distinct. The process involves not only a greater concentration of percipient elements in a given area, but also to a less extent a diminution in the size of the individual elements themselves. For example, if we look at the cones in *Petromyzon* we see that each cone measures approximately 0.006 mm. across, and that there is a gap of 0.004 mm. between the adjacent cones. Therefore, in a strip of retina 1 mm. long and 0.01 mm. wide, there would be only 100 cones. In the amphibian retina, a decrease both in size of visual elements and in the gaps between them is apparent. The approximate measurements in the retina of a fully grown frog were :—

Average thickness of individual elements	=	0.005 mm.
Gap between adjacent elements	=	0.003 mm.
Total number of elements in a strip of retina 1 mm. $\times$ 0.01 mm.	=	125

In the *Sauropsida* the decrease in size of the elements and of the gaps between them is further apparent: thus, in a hen's retina, near the posterior pole, the measurements were :—

Average thickness of individual element	=	0.003 mm.
Gaps between adjacent elements	=	0.0025 mm.
Total number of elements in a strip of retina as above	=	327

In the macular region in man the increase in number is brought about by still further reduction of the gaps, so that the adjacent cones are in apposition, the size of the cone remaining little changed, giving the following result :—

Diameter of cone at the macula	=	0.004 mm.
Gaps between cones	=	nil.
Total number of cones in strip as above	=	652

The third factor in the phylogenetic improvement of the eye is the tendency towards specialisation of areas of acute vision, which begins to appear above the reptiles, and which makes possible on the one hand the elaborate arrangements of birds, and on the other, the macula lutea of man. These areas of acute vision are very varied in size and shape. In some of the lower mammals they are

merely represented by broad areas having a slightly higher functional activity than the rest of the fundus. In birds they are still further elaborated, reaching a stage which surpasses that seen in man. The shape, size and number of maculae present in an avian eye varies with the species, but in many instances there are both nasal and temporal maculae in each eye, and in some cases these are connected by a specialised band-like area, which presumably gives clear definition over a continuous strip of the field of 90° or more. In man the structure of the macula is well known. It is worthy of note, however, that its differentiation resembles that found in the avian retina rather more than does the differentiation of the more peripheral parts of the human retina. There seems some ground for supposing that the clear cut developmental differentiation of the amacrine cells which accompanies a high degree of development of the transient fibre layer of Chiewitz is associated with a final high grade of functional activity. It is also suggestive to note that the amacrine cells are developmentally more important and more closely linked with the ganglion cells than would be thought from their study in the adult only.

Conclusions

From the foregoing examination of the process of differentiation of the layers of the vertebrate retina we can distinguish an underlying principle which remains unchanged, as well as an evolutionary scale of modifications superimposed on this.

From a study of the general principle it is apparent in every case :—

- (a) that the ganglion cells are the first to differentiate;
- (b) that the amacrine cells are intimately associated with the ganglion cells and only secondarily separated from them;
- (c) that the inner nuclear layer is a complex layer containing elements derived from both the primitive retinal layers (*i.e.*, the inner and the outer neuroblastic layers);
- (d) that the percipient elements themselves are the last to differentiate.

From a study of the secondary modifications we see :—

- (a) that supporting tissue differentiates relatively late in phylogeny.
- (b) that abbreviation of stages can occur without modification of the general plan;
- (c) that throughout phylogeny there is a tendency to improvement of function by
 1. crowding together of percipient elements,
 2. development of special areas of acute vision, the differentiation of which, however, always follows the original general plan.