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DEVELOPMENT AND DIFFERENTIATION OF THE TADPOLE EYE

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IN an earlier paper (Glücksmann 1930) it was shown that it is possible to explain the visible changes in form which take place during the development of organs of epithelial origin, in terms of certain cell processes. Thus, it was found that morphogenetic processes such as the invagination and folding of the epithelial tissues which form the optic cup and lens of the mammalian eye are the result of a co-ordinated series of cell movements, cell degenerations and orientated cell divisions. The question now arises as to how these different manifestations of cellular activity are, in fact, co-ordinated so as to ensure that an organ shall develop satisfactorily. Further, there is the problem of how the growth of such an organ is affected by the differentiation and mitosis of its cells and further of how such a regulation of cell processes can be brought about.

The answer to some of these questions was found by studying the early development of the eye of *Rana temporaria*. The tadpole eye is a particularly interesting subject for this type of investigation since the whole process of differentiation from the stage of the primary eye vesicle to that of the fully functioning organ is

accomplished so quickly. Whereas in the higher vertebrates the eye is not ready to function until near the end of the growth period, in the tadpole the differentiated parts have to function while the eye is still developing.

In the present paper the morphological changes which occur during the development of the tadpole eye will first of all be described. Later the problem of the regulation of the various cell processes which occur during morphogenesis and differentiation will be discussed.

Material and technique.—A lump of the fertilised frog spawn of *Rana temporaria* was taken from a neighbouring pond and kept in an aerated tank at room temperature. Batches of animals were killed at fixed time intervals over a period of ten days. In the period just after fertilisation a selection of eggs was taken every 5 hours but as the tadpoles got older the interval was increased to 17 and later to 24 hours.

Most of the material was fixed (direct from the tank) in either Zenker's or Susa fluid. A few specimens were fixed in formol and a few in Bouin's mixture. The embryos were embedded in paraffin and serial sections through the head were cut at 10 μ . The sections were stained by Feulgen's method (counterstained with a mixture of light-green and naphthol green) by Wilder's method or with haematoxylin and eosin. The material to be considered in the present paper consists of 224 tadpoles from the spawn of one individual and therefore presumably all fertilised at the same time, fixed at 13 different times during the ten days following removal from the pond. The number of embryos used for each fixation varied from 6 to 45. In addition some 80 tadpoles fixed directly from the pond in previous seasons were examined; the results do not differ in any way from those obtained with this year's material (1938).

Stages in the development of the tadpole eye.—In order to facilitate the presentation of the results the development of the eye, and more particularly of the retina, has been divided into 12 stages.

Stage 1. The stage of the primary optic vesicle. The cells contain both yolk and pigment, the latter accumulated on the internal surface of the vesicle. Two layers of dividing cells are found at this stage, one bordering the central surface of the eye vesicle while the other is situated between this and a layer of non-dividing cells at the other surface. (Plate 1, Fig. 1.)

Stage 2. The primary optic vesicle is elongated in a dorsal direction. The proximal wall of the vesicle which will later form the outer part of the optic cup appears stretched and thinner than the distal wall. There is no change in the distribution of mitotic cells or of pigment within the cells. (Fig. 2.)

Stage 3. The ectoderm covering the optic vesicle is thickened and begins to form the lens plate. The optic vesicle itself is further stretched and there is an increased thinning of the proximal wall. The distal wall has lost its spherical outline and has become flattened while its mitotic cells are now confined to one layer only, bordering the central surface of the vesicle. This layer will later become the outer layer of the developing retina (Fig. 3). Mitoses are present in the pigment epithelium throughout the period of development.

Stage 4. The lens appears as a shallow groove and the ectoderm composing it consists of three layers: (1) the unpigmented inner layer (the lens plate), (2) an intermediate layer of irregularly arranged pigmented cells, and (3) the pigmented outer or peridermal layer.

The transformation of the optic vesicle into the optic cup has begun, the proximal wall of the vesicle becoming the pigment epithelium and the distal wall the retina proper. The proximal wall has become further stretched so that the thickness of the pigment layer is only about one-fifth of that of the retina. The retinal cells still contain yolk and pigment but the latter now tends to accumulate at the inner surface of the retina. (Fig. 4.) The cells have become more conical in shape, the base of the cone in each case being on the outer surface and the apex pointing towards the inner surface of the retina.

Stage 5. The lens pit is deeper and the optic cup is further elongated towards the dorsal side, it has also become deeper changing its form from that of a plate to that of a bowl. The stretched pigment epithelium now adheres closely to the retina and is composed of only one layer of very flat cells. The amount of yolk in the individual cells steadily decreases during development and at this stage is greatly reduced as compared with stage 1. The pigment in the retinal cells is still accumulated at the inner surface of the retina (Fig. 5).

Stage 6. The lens is now connected to the epidermis by a stalk only. The eye has become more cup-like by a further dorsal elongation and a stretching of the pigment epithelium which is still in close contact with the retina. The retinal cells are irregularly arranged and many of the dividing cells appear pycnotic and degenerate. (Fig. 13.) These dying cells are confined to the central area of the retina and the mitotic cells in the periphery appear quite healthy. The retinal cells still contain yolk which can also be seen in the dividing cells both normal and pycnotic. Pigment is present in the retina but there is less in the central than in the peripheral parts, and it still tends to accumulate at the inner surface. (Fig. 6.)

Stage 7. In this stage the lens is separated from the epidermis.

In the central part of the retina the ganglion cells (recognisable by the larger size and fainter staining of their nuclei) can now be seen in a layer along the inner surface. Mitotic cells have completely disappeared from the outer region of the central part of the retina but are still to be seen in the periphery. These dividing cells are all quite healthy; in fact, practically no degenerate cells can be found in the specimens at this stage. The central part of the retina has also become denuded of pigment though there is still plenty at the inner surface in the periphery. The extreme periphery of the retina has begun to grow in towards the lens. (Fig. 7.)

Stage 8. The lens has now become a proper vesicle. The cells of the pigment epithelium are rather more cubical in shape than in the preceding stages and the layer as a whole has begun to be separated from the retina especially in the centre of the eye. A few fibres can just be seen separating the ganglion cells from the outer part of the retina. This is the first sign of the development of the inner fibre layer. Yolk remains in the retinal cells even in the differentiated ganglion cells but the amount of yolk present per cell is still decreasing. Very few degenerate cells occur but there is plenty of cell division in the periphery. The retinal pigment is confined to the extreme periphery at this stage. (Fig. 8.)

Stage 9. The development of the lens fibres has begun in the posterior wall of the lens vesicle. The pigment epithelial cells have become even more cubical than in stage 8 and the layer which contains a few degenerate cells is markedly detached from the retina. In the retina the inner fibre layer is now well marked and it is possible to distinguish inner nuclear cells outside it. In this region there are a great many degenerate cells which are restricted to the middle layers of the central retina, they are not found either in the ganglion cell layer or on the outer surface. (Fig. 14.) The extreme periphery is still characterised by the presence of pigment and of dividing cells neither of which occur anywhere else in the retina in this or the succeeding stages. (Fig. 9.)

Stage 10. The lens fibres have developed until they nearly fill the lumen of the lens vesicle. The central part of the pigment epithelium is still detached from the retina; the outline of the surface facing the retina has become irregular foreshadowing the development of the cell processes which later carry the pigment to and fro over the visual cells as the eye is light or dark adapted. In the centre of the retina the first few fibres of the outer fibre layer separating the inner nuclear from the outer nuclear layer can be seen. The outer nuclear layer is only one cell thick and the inner limbs of the visual elements have begun to differentiate

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in a few places, although the cells are not yet regularly arranged. A few degenerate cells occur at the border of differentiation just on the inner side of the outer nuclear layer. In the inner nuclear layer a few spindle-shaped cells are present associated with fibres running through the retina towards its inner surface—these are the beginnings of Müller's fibres and supporting cells. At this stage the retina is clearly divided into a central differentiated and a peripheral undifferentiated area. The latter is further subdivided into the germinative zone containing dividing cells and a pigmented zone in the extreme periphery. The undifferentiated area is clearly marked by the occurrence of mitosis, the absence of visual cells and the smooth outline of the cells of the pigment epithelium. All the retinal cells are now practically free from yolk.

The pigmented part in the extreme periphery of the retina has turned further in towards the lens, preparatory to forming the iris. (Fig. 10.)

Stage 11. In the cornea the pigment has disappeared from the inner epidermal layer and is much decreased in the peridermal layer.

Fibres have now begun to form at the equator of the lens and the total number of lens fibres is much greater than before.

In the central area the cells of the pigment epithelium are drawn out into short pigmented processes pointing towards the retina.

In the retina itself the central area is by now almost fully differentiated. The nuclei of the visual cells (the outer nuclear layer) are arranged more regularly and the vacuoles of the visual elements are easily recognisable, while in the very centre short outer limbs are already visible. The outer fibre layer is well differentiated and the number of Müller's cells and fibres in the inner nuclear layer has increased. At the border of the differentiated and undifferentiated areas of the retina, degenerate cells may be seen in both the outer and inner nuclear layers where the two layers meet in the germinative zone.

The iris is much more pronounced at this stage. (Fig. 11.)

Stage 12. The differentiation of the tadpole eye is completed at this stage, and until metamorphosis the only further change is enlargement caused by cellular multiplication.

Pigment disappears completely from the cornea and in the retina the outer limbs of the visual cells are at last fully differentiated. These outer limbs are separated from one another by the cell processes of the pigment epithelium. The division of the retina into a central differentiated zone, an intermediate germinative zone and the peripheral pigmented zone of the iris still persists. Since mitosis is confined to the germinative zone

and since the number of cells in the central area and particularly in the inner nuclear layer continues to increase it is obvious that cells must migrate into this region from the germinative zone. Until metamorphosis there is a progressive change in the general shape of the eyeball. This can be seen in diagram 1 in which camera lucida drawings of the outlines of the eye at various stages have been superimposed on one another. An idea of the increase in size of the different layers of the retina is also given by the diagram.

TABLE I

Time	Stages												Total	Stage	Average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII			
0 hrs.	4	7	—	—	—	—	—	—	—	—	—	—	11	1'63	± '13
25 ..	—	—	4	17	7	13	4	—	—	—	—	—	45	4'9	± '17
48 ..	—	—	—	—	—	27	6	4	—	—	—	—	37	6'37	± '10
53 ..	—	—	—	—	—	—	—	6	5	—	—	—	11	8'45	± '13
72 ..	—	—	—	—	—	1	—	—	19	8	—	—	28	9'17	± '13
77 ..	—	—	—	—	—	—	—	—	10	6	—	—	16	9'37	± '10
96 ..	—	—	—	—	—	—	—	1	12	16	—	—	29	9'51	± '10
101 ..	—	—	—	—	—	—	—	—	2	10	—	—	12	9'83	± '08
120 ..	—	—	—	—	—	—	—	—	—	4	5	—	9	10'5	± '15
144 ..	—	—	—	—	—	—	—	—	—	—	—	5	5	12'0	—
168 ..	—	—	—	—	—	—	—	—	—	—	1	5	6	11'83	± '13
192 ..	—	—	—	—	—	—	—	—	—	—	1	6	7	11'86	± '10
240 ..	—	—	—	—	—	—	—	—	—	—	—	8	8	12'0	—

In calculating the standard deviation of the mean stage at each age (last column) Shepherd's correction for grouping was applied, since only integers were used in assigning stage numbers to the individual animals.

The time relations of the stages of development.—Table I shows the number of specimens in each stage of development which was found at each fixation time. From these figures the stage average reached at any given time interval after the first fixation of material has been calculated and an average figure for the duration of each stage obtained. These figures are plotted in Graph I where the solid line represents the average stage achieved at any given time and the two dotted lines the most advanced and the most backward stage observed. It is evident that, in general, the variation in the stage of development reached at any given time is relatively small. There is one interval, however, for which this generalisation does not hold. At 25 hours there is a considerable variation in the development since all stages from 3 to 7 were found at this time. Of the tadpoles fixed at this interval some were hatched and some were not, and it was found that the hatched material tended to be more advanced than the

GRAPH 1.

Showing the average stage (continuous line) in eye development reached at any given time interval after the first fixation of material. The two dotted lines represent the most advanced and the most backward stage observed.

unhatched. Thus, 25 hours after the first fixation of material the stage average for unhatched animals is 3.7 ± 0.15 while that for the hatched animals is 5.2 ± 0.16 . After this period the variation in the stage found at any given time is again reduced so that one must assume that the process of hatching has some effect on the rate of development, in other words, those tadpoles which are late in hatching are also late in development and have to make up leeway after they are hatched.

From Graph I the average time taken before each stage is reached can be read and from this one can calculate the average duration of each stage. It will be seen that there is a distinct flattening of the curve between stages 5 and 7 and between stages 8 and 10, in other words stages 6 and 9 are reached later than one would expect from the general shape of the curve. It seems significant that this retardation in the rate of development should occur at the stages in which an unusual number of degenerate cells are present.

Cell size and the area of the retina.—The volume of the eye can be calculated by area measurements taken from photographs at a constant magnification of $\times 175$ of eyes at different stages. Such area measurements for stages 1, 2, 3, 8 and 9 are given in Table II. During the period of invagination there is little increase in the

total volume of the tadpole eye. In mammals, on the other hand, there is a considerable increase in volume during this period. Data given in an earlier paper (Glücksmann 1930, Figs. 2, 5, 24) show that in the mouse eye the area increases by about $\times 2.3$ while the area of the human eye (over a shorter developmental period) increases by $\times 1.5$. If the layer of marginal fibres is ignored in the area measurements for the human eye the increase in area amounts to $\times 2$ in comparable stages.

TABLE II
TADPOLES

Stage	Area	Total Cell Number	Average Area	Average Cell Number	Proportion Area to Cell Number
I	556	67			
II	994	82	775	74.5	10.4
III	600	103			5.8
VIII	730	225			
IX	784	307	757	226	2.9

Area measurements in square millimetres at a magnification of $\times 175$.

MICE

Figure	Area in square millimetres at a magnification of $\times 350$
2	397
5	473
24	914

Figure 2 is comparable to Tadpole Stage II
,, 24 ,, ,, ,, IX

In both the mammalian eyes investigated (mouse and human) there was no appreciable change in the size of individual cells and the increase in the volume of the eyeball was due to an increase in the number of cells contained in it. In the tadpole the number of cells also increases during development but in spite of the fact that there are 3.5 times more cells present in the retina at stages 8-9 than there are in stages 1-2, the volume of the eye remains practically unaltered. This is possible because the increase in cell number is associated with a decrease in cell size so that at the later stages the size of a cell is only about $\frac{1}{3}$ of that

of a cell at the earlier stage. The decrease is largely due to the reduction of the yolk content of the individual cells. In other words, the slowly decreasing amount of yolk available is distributed among a greater number of cells and each cell therefore contains less yolk and is smaller in consequence.

The invagination of the optic cup.—The decrease in cell size due to the diminution of yolk content in amphibian embryos has a profound effect on the mechanism of the invagination of the optic vesicle to form the optic cup. In the mammal this invagination is largely the result of a combination of morphogenetic degenerations which leave room for cell movements and the cell divisions which produce an increase in area and volume. In the amphibian embryo, on the other hand, the decrease in cell size allows the cells to move freely in an area which hardly increases at all. This can be seen if the cell pattern of the undeveloped retina of the mammal is compared with that of the tadpole. In the former the cells of the outer part of the retina are arranged as a regular columnar epithelium with their long axes and nuclei orientated at right angles to the free surface. In the latter, at least in the earlier stages of development, there is no such regular pattern. The cells are more or less round in shape and their nuclei only show a slight orientation of their long axes, although later as invagination progresses and the cells decrease in size and lose their yolk, they become shaped like cones arranged in a regular pattern with their bases on the outer surface of the retina.

Another indication of the difference between the structure of the two types of retina during development is to be found in the different distribution of the mitotic cells. In the mammal dividing cells are confined to the outer surface of the retina (Glücksmann 1930) while in the tadpole another layer of mitotic cells is present up to stage 3. This layer is situated in the middle of the eye wall and here, as on the outer surface, the cells in meta- and early anaphase are spherical. Also the separation of the daughter cells in telophase is achieved as easily in this position as on the free outer surface which is good evidence that cell movements in the tadpole retina are relatively unrestricted, at least in the early stages of development. Here again the decrease in size and yolk content is accompanied by a more regular arrangement of the cells and by the restriction of mitosis to the free surface of the retina.

It is evident, therefore, that because of the initial yolk content and subsequent decrease in cell size, the space for the cell movements required for changes in the form of the eye is available in the tadpole without the necessity for morphogenetic degenerations. There is another factor which restricts free movements at least in the human retina, which is absent from the tadpole. In the human embryo there is a network of fibres in an area free from cell nuclei

at the inner surface of the retina—the so-called marginal layer. This net-work of fibres which must obviously emphasise the rigid arrangement of the cells and restrict their movements is not present in the tadpole eye during its invagination.

The actual process of invagination is also modified in the amphibian as compared to the mammalian eye. This is especially noticeable in the rapid disappearance of the primary lumen of the optic vesicle and in the configuration of the pigment epithelium in the tadpole. The optic vesicle stretches considerably in the dorsal direction before invagination thus narrowing the lumen,

In Graphs 2 and 3, the absolute and percentage counts respectively of mitotic and degenerate cells are plotted at a scale ten times that of the natal and resting cell counts respectively.

GRAPH 2.

Showing the absolute counts of total, resting, dividing and degenerate cells with their standard deviations in different stages of eye development.

——— total number of cells. ——— resting cells.
 - - - - - dividing cells, ——— calculated number
 of dividing cells. Stippled area — degenerate cells.

while at the same time the pigment epithelium is thinned until it becomes a single layer of flat cells. The transformation into the optic cup is then achieved not so much by an actual bending of the retinal wall as by the elongation of its peripheral parts. In the human embryo, on the other hand, the lumen of the primary optic vesicle is only slowly reduced by the inward folding of the retina while the pigment layer retains its thickness. This process in the human is bound up with a considerable increase in the total size of the eye whereas in the tadpole there is no appreciable change in volume at this stage.

**Quantitative Analysis of the Cell Processes Involved
in the Early Development of the Tadpole Eye**

For this purpose some typical specimens at each stage of development was selected and the number of resting, dividing and degenerating cells was counted in two central sections through each eye. The sections chosen were those through the papilla and the choroid fissure. The means and standard deviations of the counts for each stage were determined and the results plotted (Graphs 2 and 3). Graph 2 shows the absolute counts of total,

GRAPH 3.

Showing the absolute total cell count, the degenerative index and the calculated and observed mitotic indices.

— total cell count. — — — observed mitotic index.
— — — calculated mitotic index. Stippled area — degenerative index.

dividing and degenerate cells with their standard deviations while the mitotic and degenerative indices for each stage are plotted in Graph 3. The mitotic index is given by the number of mitotic cells divided by the total number of cells while the degenerative index is, similarly, the number of degenerate cells divided by the total cell count. As can be seen from the graphs there is a steady increase in the total number of cells and also in the number of resting cells present from stage 1 to stage 6 and again from stage 9 onwards. This increase amounts to about 50 cells in 10 hours for the first period and to about 40 cells in 10 hours for the later period. Between stages 6 and 7 the increase in the total cell count is slowed down and this is followed by a considerable acceleration from stage 7 to 8, which, again, falls off slightly between stages 8 and 9.

The number of mitotic cells remains fairly constant throughout the development of the retina except in stages 5, 6, 8 and 9 where significant variations are observed. The mitotic cell count is low in stage 6 and high in stages 5, 8 and 9. The reduction in the rate of increase of the total cell count in stage 6 seems to be due to the small number of mitotic cells present at this stage but it is not always possible to correlate a high rate of total cell increase with a high mitotic count. For instance, the mitotic counts found in stages 8 and 9 are no higher than those found between stages 9 and 10 but the increase in the total number of cells present is considerably greater during the earlier period. The explanation of this discrepancy is to be found in a consideration of the degenerate cells present. The degenerate cells observed in stage 9, which are included in the total cell count, have disappeared by stage 10 and their absence accounts for the failure to reach the expected higher total cell count for this stage. Similarly the high mitotic index at stage 5 decreases the reduction in the total cell count for stage 6 which should follow from the large number of cells which have degenerated at this stage. On the other hand it is doubtful whether the slight increase in the number of mitoses observed from stage 6 to stage 7 is sufficient to account for the high rate of increase in the total cell count during this period of development. This discrepancy might be explained by the assumption that there is a speeding up of the process of cell division and a shortening of the interkinetic interval over this period.

While the total number of cells increases steadily and the number of mitotic cells remains fairly constant during development, the degenerate cell count rises in three distinct peaks. Only the first of these waves of degeneration, that which occurs at stage 6, is associated with a marked decrease in the mitotic cell count. The second peak (stage 9) coincides with a period of relatively

high mitotic activity and the third is not associated with any change in the number of mitotic cells.

Some additional information is given by Graph 3. It shows, for instance, that the first wave of degeneration in stage 6 is the highest in proportion to the total cell count. Further it indicates that at stage 6 there is a significant difference between the mitotic index calculated for a steady increase in total cell count and that which is actually obtained. For the rest of the developmental period there is a fairly good correspondence between these two curves. The calculation of the mitotic index is based on the assumption that of the two daughter cells resulting from one cell division one divides again while the other becomes a resting or differentiating cell. The fact that the actual number of mitotic cells remains so constant throughout seems to justify this assumption. The statistical analysis of results given in Graph 3, with the exception of those for stage 6, also points to this being the true interpretation.

As described above, mitotic cells are fairly evenly distributed over both the central and peripheral regions of the retina during development from stage 1 to stage 6. In stage 6 the dividing cells of the central retina become pycnotic and degenerate and from stage 7 onwards this area remains free from mitoses. In spite of the fact that a large percentage of the dividing cells degenerates in stage 6, the mitotic cell count for the whole retina is able eventually to return to normal and therefore it is obvious that both the daughter cells of those divisions which are successful at this stage must divide again. In addition the interkinetic interval may be shortened, that is cells might begin to divide sooner after their last mitosis and therefore during a period when they would normally be resting. In this way cells from the peripheral retina are made available to replace those which have been lost from the centre. Thus there is a complete reorganisation of the dividing cells in stage 6.

Cell degeneration.—The first wave of degeneration begins at the outer surface of the retina among the dividing cells and is first detectable by a pycnosis of the chromatin material which usually appears during meta- or anaphase. As the degeneration proceeds to chromatolysis these cells move towards the inner retinal surface where they finally disappear, either as a result of intracellular absorption by neighbouring cells or by complete dissolution. The details of the degenerative process need not be described here as they have already been dealt with in earlier papers (Glücksmann 1930; Spear and Glücksmann 1938). During the later phases of stage 6 some ganglion cells, conspicuous on account of their larger size and the fainter staining of their nuclei, can be recognised for the first time among the degenerate

cells on the inner surface of the retina. This process of degeneration and migration of degenerating cells is confined to the central area of the retina.

The second wave of degeneration affects the cells of the middle zone of the central retina in the position which will later be occupied by the inner nuclear layer. The degenerative process starts during the resting phase of these cells—there are no mitotic cells in this layer—and passes through the usual stages of chromatopycnosis, hyperchromatosis and chromatolysis (see Spear and Glücksmann, 1938). Sometimes this process is associated with pigment formation and this is very conspicuous since the pigment found in the retina during early development has already disappeared from the central area. There seems to be a considerable individual variation in the number of cells which degenerate at this stage. In some eyes there are so many that they cause a rupture of the outer surface of the retina towards the pigment epithelium and cells are extruded into the lumen of the optic vesicle. With the resorption of these cells, which occurs during the later phases of stage 9, the retina recovers its orderly pattern and only a few pigmented patches remain to bear witness to the disorganisation that was caused by the presence of excessive numbers of degenerate cells. The rupture of the retina which often follows the accumulation of large numbers of degenerate cells is a result of the tendency of these cells to congregate in the same place (Glücksmann, 1930; Glücksmann and Tansley, 1936). Finally the pigmented remnants also disappear although traces of them can sometimes be found in stage 10 and even as late as stage 11.

Simultaneously with the appearance of the second degeneration wave in the retina, degenerate cells can be found in the pigment epithelium and here again they are restricted to the central area. The dead cells of both the first and second degeneration waves contain roughly the same amount of yolk as is present in the normal cells at these stages of development.

The central parts of the retina are not involved in the third wave of degeneration which occurs in stage 11. The cells concerned are restricted to the border between the central, differentiated retina and the germinative zone and in this area they are found in the outer nuclear layer and the outer part of the inner nuclear layer. The degeneration of these cells also begins in the resting phase but does not result in pigment formation. Mitotic cells are rarely involved.

Cell Division.—It has already been pointed out that the number of mitotic cells remains almost constant throughout the period of development under consideration and that, except during stage 6,

it is very probable that only one daughter cell divides again. During the first stages of development there are two layers of dividing cells in the retina but later on these are restricted to the outer surface. In the earlier period pigment and yolk are regularly found in the dividing cells to much the same extent as in the resting cells.

Since only one of the daughter cells of a mitosis divides again the other has to migrate into the differentiated parts of the retina which in the later stages of development usually means into the inner nuclear layer. That the other daughter cell also leaves the outer surface is shown by the fact that this area contains only mitotic cells. The second daughter cell must, however, return to the outer surface for its own division later.

It is known that the mitotic cells of the developing retina tend to show a definite orientation of their axes (Glücksman, 1930). In the tadpole the distribution of position in stage 12 was found to be as follows: 82.2 per cent. lay with the axis of mitosis parallel to the outer surface of the retina, and 17.8 per cent. with their axes at right angles to the outer surface. There is, therefore, a marked preference for a plane parallel to the outer surface of the retina in which both daughter cells lie at the free surface of the epithelium. In only 17.8 per cent. of all cell divisions does one daughter cell lie at the free surface and the other nearer the inner surface of the retina.

Cell movement.—The shape of a cell and its movements are closely associated. Thus, changes in its external form are caused by movements of its protoplasm and at the same time movements of the cell as a whole are brought about by suitable changes in its shape; in other words the cell is capable of movement by changes in its form. The reality of movements within the cell is shown by the changes in the distribution of pigment during the early stages of the development of these retinæ. In the very young eye the retinal cells are ellipsoid in shape and the pigment is concentrated on the outer surface of the retina but when, during development, the cells become conical and their apices point inwards the pigment changes its position and collects on the inner surface of the retina. Whenever there is any change in the external form of a cell the pigment within it tends to accumulate at the narrowest part.

The appearances by which the movement of a cell may be recognised by the shape and staining power of its nucleus have already been described (Glücksman, 1930) and it is not necessary to consider the subject in detail again here. Briefly it was found that if there is no obstruction by external factors, the nucleus becomes stream-lined in the direction of movement and the

anterior pole can be recognised by the faintness with which it stains.

In addition to the movements of resting cells there are migrations of dividing cells, both before and after mitosis, and also of degenerate cells. The movements of mitotic cells have already been dealt with to some extent earlier in this paper but those of the degenerate cells must now be described.

Degenerate cells always show a tendency to congregate; in stage 6 for instance, they become concentrated on the inner surface of the retina while in stage 9 they mostly occur near the outer surface. In stage 6 all the cells in the early stages of degeneration are situated at the outer surface (they originate as dividing cells) and only those in the terminal stages of degeneration are to be seen at the inner surface. It is clear, therefore, that these cells must move inwards through the retina while they are in the process of degenerating. In some cases the movement is really accomplished by normal resting cells which pick up the degenerating remnants and carry them to the inner surface but there are also free remnants present which must somehow have made the journey by themselves. These degenerate cells are round and liquified and seem to float rather than to move actively. They may either be dissolved intercellularly or be resorbed by neighbouring cells when they reach the inner surface.

In stage 9 the degenerating cells show a tendency to congregate on the outer border of the inner nuclear layer and as stated above this may lead to bulging and even to rupture of the outer surface of the retina. Thus, the degenerating cells of stage 9 move in the opposite direction to those of stage 6.

During early development in the tadpole there is a rapid change in the form of the eye and the arrangement of the retinal cells is very irregular. Before stage 7, when a regular epithelial pattern of cells first appears in the centre, the cells of the retina, although showing signs of orientation in the radial direction, tend to lie at all angles to each other and exhibit the characteristic signs of movement. The direction of these movements differs in different parts of the retina and depends on the changes in form of the eye. Thus, in the central retina at stage 6 and also early in stage 7 there is a noticeable movement of cells towards the inner surface.

It is not until stage 12 is reached that the cell movements really become regulated in relation to the whole retina. At this stage the retina is divided into the iris, the germinative zone to which the dividing cells are confined and the central differentiated area. Under these conditions the differentiated parts of the retina are dependent on the germinative zone for their supply of new cells. Since the number of cells in the central retina continues to increase and since this area contains no mitoses there must be a movement

of cells into the area from the germinative zone. There is also morphological evidence for such migrations into the central retina. On the border between the germinative and differentiated zones cells may be found in the outer part of the inner nuclear layer lying at right angles to the main radial axis, and such cells can be traced right into the central parts of the retina where they are present throughout the inner nuclear layer. Some of these cells then migrate through the outer fibre layer to become visual cells. All the stages of this journey from the germinative zone to the outer surface of the central retina are easily recognisable and the appearance of the cells as they make their way through the outer fibre layer is very similar to that observed in an earlier investigation on the olfactory epithelium (Glücksman, 1930). These migrating cells of the retina are affected in the fasting experiments already reported by Spear and Glücksman (1939).

It should be relatively easy for the cells situated at the outer part of the edge of the differentiated region to migrate directly into the outer nuclear layer and for those at the inner surface to move into the ganglion cell layer. Both these migrations do, in fact, occur and the moving cells are easily recognised by their shape and also by the staining of their nuclei. Towards the periphery the cells of all three nuclear layers are much more irregular in their arrangement and the layers themselves are thicker than in the centre; this difference is especially marked in the earlier periods of development before metamorphosis. In the amphibian eye the inner nuclear layer always remains thicker than the outer nuclear layer because it harbours not only its own ganglion and supporting cells but also acts as a store house for future visual cells.

Diagram 1 shows that the change in general form of the eye also involves the differentiated central retina and especially the outer nuclear layer which elongates considerably during development. This increase in length of the outer nuclear layer is due to an increase in the number of its cells and it has already been shown that this is achieved partly by the simple addition of cells at the peripheral border from the germinative zone and partly by the acquisition of cells which also originate in the germinative zone but reach their destination in the centre via the inner nuclear layer and then across the outer fibre layer. The ganglion cell layer also increases in length but there is no evidence that this increase is due to additions to its cell population from the inner nuclear layer. It is, in fact, impossible that this layer should acquire many new cells during development since its increase in length is accompanied by an increase in the distance between individual ganglion cells. There is, however, some addition of cells to the periphery direct from the germinative zone.

DIAGRAM 1.

Camera lucida drawings of the outlines of the eye have been superimposed to show the progressive change in the general shape of the eye.

During development the inner nuclear layer not only provides a path along its outer part for cells moving from the peripheral to the central retina but also acts as a storehouse for undifferentiated cells.

Discussion

The possible part played in the morphogenesis of organs by such cell activities as mitosis, movement and degeneration, has attracted little attention. In 1914 Gurwitsch showed that cell division and cell movement may be responsible for changes in the form of an organ and both he and his pupils have since provided further evidence to support this idea. On the other hand Glaser (1914) in a much quoted paper arrived at the conclusion that morphological changes are not dependent on such cell processes but rather on differences in the absorption of water. He based his conclusions on an analysis of the folding of the neural tube in *Cryptobranchus alleghiensis* and also on observations of the changes of acidity which occur during the early development of the eggs and embryos of *Rana pipiens* and *Amblystoma punctatum*. The case of *Cryptobranchus* is exceptional since in this species there is an increase both in total volume and cell size without any increase in the number of cells present and it is

possible therefore that water absorption does in fact play some part in this instance. The changes in acidity which occur in *Rana pipiens* and *Amblystoma punctatum* were thought by Glaser to explain the mechanism of water absorption by causing surface effects but in this case he ignored the significance of the decrease in cell size which takes place during the development of these species. It should be noted that Glaser apparently overlooked the importance of considering mitosis and cell degeneration in any investigation of the development of tissues. He did not in fact examine any histological preparations of his material from *Rana pipiens* and *Amblystoma*, nor does he record whether cell divisions or degenerations were present in the sections of *Cryptobranchus* which he investigated.

In 1930 the present author showed that in mammalian organs which increase in volume and cell number while the cell size remains constant, there is a very definite relationship between changes of form and the cell processes of mitosis, degeneration and movement, and it was established that such cell activities always precede the change in form. The present investigation is concerned with an organ which does not change in volume during development but in which the cell number increases while the cell size decreases. Although, in the present case, a correlation can be found between cell movements and division and morphological changes in the eye, the absence of true morphogenetic degeneration is very evident. This difference between the mechanism of morphogenesis in mammals and in amphibia is undoubtedly due to the presence of yolk in the amphibian cells. It has already been pointed out that the presence of yolk and its progressive dilution by distribution among an increasing number of cells makes a freer epithelial arrangement possible in the amphibian retina. In this eye, also, the cells can move in a relatively unrestricted way whereas in the mammalian eye with its more rigid epithelial pattern such movements become possible only if some of the cells degenerate. Thus, the yolk content of developing amphibian cells is not only responsible for the different forms of gastrulation characteristic of these animals (Rabl, 1897) and for the different degree of differentiation of the muscular system (Glücksman, 1934) but also for the different mechanism of such morphogenetic processes as folding, bending and invagination.

It has been clearly demonstrated above (and in the case of mammals in an earlier paper (Glücksman, 1930)) that mitotic cells do not lie about haphazard but that they are very definitely orientated. This orientation both of the position of dividing cells and of cell movements must be under some sort of directing influence if the orderly development of the organ is to be safeguarded, and we have now to discover, if possible, how all these

processes are regulated and correlated one with another. The suggestion that cells may act independently of one another according to a preconceived harmony, probably transmitted by their genes—a possible explanation of self-differentiation can be dismissed as highly improbable. On the other hand, if the cells of an organ depend on each other and are all directed from outside, the question arises as to what influences are responsible, how they act and where they originate. That one organ may, in fact, be dependent on such influences from another is well known from the recent work on induction and organisers.

Lewis (1907/8) has shown that transplantation of the amphibian eye into other parts of the body does not prevent its normal development even when the removal is effected before the eye has reached the first stage described in this paper. Thus, even at this very early stage the amphibian eye appears to be capable of self differentiation, so that one must look for the factors which determine its development in the eye itself. This does not mean, however, that all the parts of the eye are already determined at this stage since most of these exhibit great powers of regeneration (Stone and others, 1937).

In our inquiry into the nature of these influences the three waves of degeneration which appear during the development of the retina provide a useful clue. Each of these waves precedes the differentiation of a new retinal layer and corresponds in its localisation to that differentiation. Thus, the first wave is followed by the appearance of the ganglion cells, the second by that of the inner nuclear cells and the third by that of the visual cells. The first degenerations affect mitotic cells only and after this wave has passed no more cell divisions are to be found in the area concerned. It seems probable that the factor or factors responsible for the differentiation of this part of the retina also exert an inhibitory effect on cell division and, further, that those cells which have already begun to divide when these influences are first exerted are destroyed. It is possible to explain the other two degeneration waves in a similar manner, only in these it is differentiating instead of dividing cells that are affected. For instance, the cells which degenerate during the second wave are those which are in the process of becoming ganglion cells and are unable to survive the influence which determines the differentiation of inner nuclear cells. In the same way the third wave of degeneration occurs because those cells which have now begun to develop into inner nuclear cells are overtaken by the new impulse to differentiate into visual cells, and die. The coincidence of each wave of degeneration with the beginning of a new type of differentiation seems significant enough to justify the

hypothesis just outlined. Also, as soon as one process of differentiation is thoroughly established there is no more degeneration until the next type of differentiation begins and this again indicates that the cells degenerate because they are unable to withstand the conflict between two different impulses to differentiate. When, later, one or other of the impulses predominates the cells are no longer faced with this unendurable conflict and are able to differentiate in the required manner.

We now have to inquire into the origin of these differentiation impulses. It is obvious that they are present within the eye and must, therefore, be intrinsic in the retinal cells. It is known that failure to differentiate in the normal fashion can be genetically controlled and it seems reasonable, therefore, to assume that genetic factors may be responsible for differentiation and that these factors may come into play only at suitable periods of development. (Goldschmidt, 1938.) If this assumption is correct the gene or gene complex which is normally bound to the chromatic material of the nucleus must also be able to exert its influence on neighbouring cells thus producing a field of differentiation.

Since size as well as differentiation is known to be genetically controlled the same mental picture can be used to explain the early stages of development when only multiplication of cells occurs. Here the regulating process seems to be relatively simple and to result in only one of the daughter cells of a mitosis being able to divide again. The differentiation of the central retina in stage 6 upsets this arrangement but it is soon re-established in the periphery in stage 7. To explain the orientation of cell movements and of the axes of the mitotic cells one may perhaps assume that this is due to the influence of the ultra-structure as postulated by Weiss (1933). Since it has been shown, however, that even morphological form is genetically controlled it may be that such a control is also responsible for the orientation of the ultra-structure.

Summary

1. The development of the tadpole eye and especially of the retina is described from the stage of the primary optic vesicle to that of the fully differentiated organ. For convenience the developmental history is arbitrarily divided into twelve morphological stages. The duration of each of these stages is calculated.
2. Counts have been made of the number of resting, dividing and degenerating cells in the retina at each stage of development,

and the inter-relation and significance of such cell activities is discussed.

3. The decrease in yolk content and consequent decrease in cell size during retinal development is described and the effect on the process of development of the amphibian eye is compared with its development in the mammal where the cells do not contain yolk.

4. Except in one stage of development the number of mitotic cells in the tadpole retina remains remarkably constant while the total cell count steadily increases. This suggests that only one of the daughter cells resulting from a mitosis can divide again. During the earlier development, dividing cells are distributed along the free outer surface of the whole retina but later they are restricted to the outer surface of the germinative zone. This change is marked by a transient fall in the total number of mitoses caused by the degeneration of the dividing cells of the central retina.

5. Three distinct waves of cell degeneration are observed during the development of the retina. The first is due to the death of the dividing cells of the central retina and is followed by the appearance of ganglion cells in this area. The second wave involves the resting cells of the future inner nuclear layer and precedes the differentiation of this layer, and the third is caused by the death of resting cells of the outer nuclear layer and occurs immediately before its appearance. The cells concerned in these waves of degeneration seem, therefore, to be those which are caught by the new differentiation impulse after they have already begun to obey the last.

6. Owing to the progressive decrease in cell size consequent on the decreasing yolk content, cell movements are unrestricted during the early development in the tadpole. After the division of the retina into a central differentiated area and a peripheral germinative zone, cell movements are mostly confined to migration from the germinative zone into the inner nuclear layer and from this into the outer nuclear layer.

7. The importance of these cell activities in determining the course of the morphogenetic changes which occur during the development of the amphibian retina is discussed and an attempt is made to determine the origin of the factors which are responsible for the co-ordination of all cell activities into an orderly pattern which will ensure the proper development of the eye as a whole.

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LIST OF ABBREVIATIONS.

d	dorsal	m	mitosis
dc	degenerate cells	ogc	optic ganglion cells
gz	germinative zone	ofl	outer fibre layer
i	iris	onl	outer nuclear layer
ifl	inner fibre layer	osr	outer surface of the retina
inl	inner nuclear layer	p	pigment epithelium
isr	inner surface of the retina	pw	proximal wall of the optic vesicle
lp	lens plate	ve	visual elements

DESCRIPTION OF PLATES

All the figures are photomicrographs. Figures 13 and 14 are taken from areas outlined in Figures 6 and 9 respectively. A magnification of x 175 was used for Figures 1–12 and of x 775 for Figures 13 and 14.

Cross sections of the tadpole are cut at 10μ thickness, stained by Feulgen's method and counterstained with a mixture of light green and naphtholgreen.

FIG. 1. Stage of the primary optic vesicle. Note the two layers of mitotic cells (m) and the accumulation of pigment on the internal surface of the vesicle.

FIG. 2. The primary optic vesicle is elongated in a dorsal direction (d). The proximal wall (pw) of the vesicle is thinner than the distal wall.

FIG. 3. The optic vesicle is further stretched and the thinning of the proximal wall (pw) increased. The lens plate (lp) is visible as a thickening of the ectoderm.

FIG. 4. The transformation of the optic vesicle into the optic cup has begun. In the retina the pigment tends to accumulate on the inner surface (isr). The lens plate (lp) is transformed into a shallow groove.

FIG. 5. The transformation of the optic vesicle into the optic cup is completed. The pigment epithelium (p) adheres closely to the retina. The decrease in yolk content of the retina is indicated by the closer packing of the nuclei. The lens pit is deepened.

FIG. 6. The lens is connected to the epidermis by a stalk only. The eye has become more cup-like by a further dorsal (d) elongation. Pigment in the retina is accumulated at the inner surface (isr). Cf. Fig. 13.

FIG. 7. In the central part of the retina ganglion cells (ogc) are seen in a layer along the inner surface (isr). In this central zone pigment and dividing cells have disappeared. Mitosis and pigment, however, is present in the peripheral parts of the retina. The pigment epithelium (p) consists of a thin layer of flat cells.

FIG. 8. The lens has now become a proper vesicle. The cells of the pigment epithelium (p) are more cubical in shape. This layer as a whole is detached from the central parts of the retina. The differentiation of the optic ganglion cell layer (ogc) has progressed.

FIG. 9. The development of lens fibres has begun. In the central parts of the retina the inner fibre layer (ifl) is visible. Cf. Fig. 14.

FIG. 10. The pigment content in the central part of the cornea is decreased. The central part of the retina is differentiated into the optic ganglion cell layer (ogc), the inner nuclear layer (inl) and the outer nuclear layer (onl). The outer fibre layer (off) is now marked. The inner limbs of the visual elements (ve) have begun to differentiate. The outline of the inner surface of the pigment epithelium (p) has become irregular foreshadowing the development of cell processes.

FIG. 11. In the cornea the pigment has disappeared from the inner epidermal layer and much decreased in the peridermal layer. In the centre of the retina the visual cells are more regularly arranged; the vacuoles and short outer limbs of the visual elements (ve) are now visible. At the border of the differentiated and undifferentiated areas of the retina degenerate cells (dc) are found. The peripheral parts of the retina are subdivided into a germinative zone (gz) and the iris (i).

FIG. 12. The cornea is free from pigment. The central part of the retina is fully differentiated. In the pigment epithelial cell processes corresponding to the visual elements have appeared.

FIG. 13. Cf. Fig. 6. Note the pycnotic dividing cells (dc) on the outer surface of the retina (osr).

FIG. 14. Cf. Fig. 9. Note the degenerate nuclei (dc) in the future inner nuclear layer.

* For reproduction the plates were reduced to x0.55 of the original size.

PLATE I

PLATE II