The first sign of lens development in the vertebrate embryo is the appearance of a thickening of the head ectoderm in the area of contact between the ectoderm and the eye vesicle (lens placode).

The cytoplasm of the placode cells in the chicken embryo loses its vacuolization, the cells acquire a cylindrical form, the nuclei arrange themselves perpendicularly to the contact surface and move to the base of the cells (McKeehan, 1951).

At about the same time chemical changes take place in the placode cells. Ten Cate and Van Doorenmaalen (1950), using serological methods, were able to demonstrate the presence of specific lens proteins in the placodal stage of the lens in axolotl and chicken embryos. They could not entirely exclude the possibility of the presence of these proteins in the ectoderm at earlier stages, but with their sensitive method they got no indication of them before the placodal stage.

At present it is, therefore, impossible to decide whether the appearance of this early protein specificity must be regarded as a pre-morphological cell differentiation or whether it occurs synchronously with or after the morphological changes described by McKeehan.

One is inclined, however, to assume that processes of chemodifferentiation by which the typical building-blocks of the cells are produced will precede visible morphological changes.

It is generally accepted that the changes in the head ectoderm are due to an influence which the eye vesicle exercises upon that ectoderm at the contact surface (embryonic induction). One might consequently assume that the production of specific lens proteins takes place under the influence of an inductive action of the eye vesicle. To investigate this assumption the following experiments were done.

Saline extracts of presumptive lens ectoderm of axolotl neurulae (before the appearance of a lens placode) were mixed with rabbit anti-serum against lens proteins. No precipitin reaction could be demonstrated. Neither could a precipitin reaction be found when saline extracts of young eye vesicles (freed from head

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ectoderm) were mixed with the anti-serum. When, however, ectoderm extract and eye vesicle extract were mixed and incubated at 37° C. for 24 hours the mixture, when tested with the anti-serum, showed a precipitin reaction whereas the separate components of the mixture did not contain antigens after the same incubation (Woerdeman, 1950). Thus it seems permissible to assume that the production of specific lens proteins must be ascribed to an interaction between head ectoderm and the eye vesicle.

It is, of course, probable that this protein production takes place in the ectodermal cells normally as a very slow reaction and is only accelerated by substances passing from the eye vesicle into the ectoderm, but it is also imaginable that it would not occur at all without the influence of the eye vesicle and that enzymes of the cytoplasm of the ectodermal cells have to be activated or coenzymes to be delivered by the eye vesicle. Substances may also pass into the ectoderm which together with other ectodermal substances may be combined to form lens proteins. Still another possibility is that lens proteins may be present in the ectoderm in a masked form and be unmasked. It seems anyhow to be proved that their demonstrability in the lens placode of amphibians is a result of an action of the eye vesicle on the surface ectoderm.

The objection can be raised that in some amphibians (e.g. Rana esculenta, Spemann, 1912; Xenopus laevis, Balinsky, 1951) the lens can apparently develop independently when the eye vesicle is absent. In Rana esculenta, however, it is very probable that lens induction occurs at a very early stage (open neural plate) and takes only a very short time (Woerdeman, 1952). The same may be the case in Xenopus. I see therefore no reason to admit that some amphibians must be excepted from the general rule that lens formation is produced by an induction process.

When lens placodes of young frog neurulae are extirpated and grafted under the ectoderm of the abdominal wall or when they are explanted into saline solutions, they develop into small undifferentiated vesicles. In some cases, however, some of their cells show a transformation into lens fibres, but this happens only when somewhat older donors are used (Woerdeman, 1941).

When undifferentiated lens vesicles are transplanted in the manner mentioned above, they develop into small lenses with an irregular mass of lens fibres. There are indications to show the presence of a very short period during which no difference in fibre-forming potency exists between the exterior and the interior wall of the lens vesicle, but very soon fibre formation originates only from the interior wall which has been in contact with the presumptive nervous layer of the retina.

These observations, together with many data from the literature on lens development, indicate that for a normal differentiation of the lens a lasting influence of the eye cup on the lens anlage is necessary.

Most of my observations mentioned above were made on larvae of Rana esculenta, and here again the objection may be raised that in Spemann's paper
of 1912 well-differentiated lenses are to be seen which have developed independently of an eye cup.

In Spemann's laboratory the eggs were often kept at a low temperature before the operation. Ten Cate (1946) showed that under these circumstances the chemodifferentiation is less retarded than the morphological processes. Before the cooled embryos reach the stage of operation, induction may have taken place during a much longer time than under normal conditions, and chemodifferentiation may have proceeded almost normally. This may explain why, after extirpation of the eye anlage in cooled neurula stages, a well-differentiated lens develops, whereas it fails to develop if the same operation is carried out on neurulae kept at room temperature. If this explanation is valid Rana esculenta would develop a lens in accordance with what has been shown for other amphibians and no discordance would exist between the result of my experiments on Rana esculenta (at room temperature) and the results of Spemann.

Our conclusion must therefore be that the induction process in amphibians is a process of long duration. It takes only a short time to start the formation of specific lens proteins in the ectoderm, and the development of a placode (the first visible morphogenetic phenomenon). When the inductive action continues, the placode acquires the potency of vesicle formation and of separating itself from the surface ectoderm. In a following phase a change takes place in the interior wall which enables its cells to form fibres (development of a medio-lateral polarity).

It is still questionable if all these differentiation steps are caused by one inductive factor. It might be assumed that there is a whole series of factors, each of which is responsible for a certain phase of lens development. We must bear in mind that during lens development the chemodifferentiation and morphogenetic processes in the eye vesicle and eye cup also proceed, and that consequently their inductive influence may be changing continually. I need only mention the gradual disappearance of glycogen in the cells of that part of the eye vesicle which becomes invaginated (Woerdeman, 1933).

On the other hand, our experiments have shown that eye vesicles and eye cups of various phases of development transplanted under undifferentiated ectoderm will always induce the same sequence of morphogenetic processes, i.e. formation of a placode, transformation into a vesicle, separation of the vesicle from the surface ectoderm, and differentiation of one of its walls into lens fibres. When an old eye cup is transplanted under indifferent ectoderm, it never induces the direct production of lens fibres in the surface ectoderm.

The various steps of differentiation seem to be dependent one on the other, and this phenomenon indicates another possibility, namely, that the inducing agent remains unchanged but that its effect depends on the preceding changes which have occurred in the reacting ectoderm.

Various experiments have demonstrated that the eye cup preserves the capacity of inducing lenses for a long time, much longer than is needed for the normal
lens induction (e.g. Woerdeman, 1939). Accepting the hypothesis of a continually changing inductive influence, we should have to admit that the various phases of induction overlap each other considerably. Accepting the hypothesis of the existence of one and the same inductive action of the eye cup throughout lens development, one has to consider this action as the stimulus which keeps going a certain fundamental process (e.g. formation of lens proteins) which is the indispensable source of modifications in the lens anlage leading to its final differentiation.

In some of the experiments cited above in which eye cups of various ages were transplanted under undifferentiated ectoderm, I also used material of old blastulae or very young gastrulae and made it react with old eye cups. I observed the development from this material of lens vesicles which showed a marked difference from normal lens vesicles. Whereas the cells of the latter do not possess vitelline platelets in their cytoplasm, the cells of the former contained a large quantity of vitelline platelets (Woerdeman, 1938). It seems possible, therefore, to induce lens formation in cells which have not yet passed through some of the processes of chemodifferentiation, although normally these processes are passed before lens formation starts. Since I killed my operated animals rather soon after the experiment I have not been able to observe the older stages of differentiation of these 'too young' lens vesicles. It might be interesting to repeat the experiments and to follow the fate of the induced lens vesicles (using also histochemical methods) to see if certain morphogenetic processes are dependent on or independent of preceding developmental processes in the cells.

The only conclusion which I wish to draw at present is that the competence for placode and vesicle formation is already established in the ectodermal cells at an early stage of development. They react, however, only after having made contact with the eye vesicle.

Another observation may be included here. When in frog embryos the lens vesicle which has separated from the surface ectoderm is removed through a small incision in the ectoderm, the wound closes and the regenerating ectoderm is able to form a new lens. This shows that shortly after lens formation the ectoderm has not yet lost its competence to be induced to lens development (Woerdeman, 1952).

Returning now to the further differentiation of the crystalline lens I must mention another influence of the eye cup on fibre-formation.

In the amphibian lens the growth of the fibres is directed in such a way that their ends meet in two lens sutures, an anterior one coinciding with the plane of the foetal fissure of the eye cup and a posterior one perpendicular to the anterior. By a series of experiments which will not be described in detail, I tried to investigate if there exists a causal connexion between the bilaterality of the eye vesicle (expressing itself in the formation of the foetal fissure) and the direction of growth of the lens fibres (resulting in the formation of two sutures, one of which coincides with the plane of the foetal fissure).
These experiments consisted in excision of the presumptive lens ectoderm, rotation through 90° and reimplantation, or in rotation of the eye vesicle or of the presumptive group of eye cells in the neural plate through 90° leaving the presumptive lens ectoderm in its place and normal orientation.

From these experiments it was concluded: (a) that the extent of the fibre-forming area of the interior wall of the lens vesicle depends on the area of contact with the eye vesicle, (b) that abnormally invaginated eye cups which have more than one area of contact with the lens vesicle induce more than one cone of fibres, and (c) that the direction of the growth of the lens fibres depends on the direction in which the eye vesicle invaginates (Woerdeman, 1932, 1934).

There must be assumed to be differences in various parts of the wall of the eye vesicle which later invaginates and which makes contact with the interior wall of the lens vesicle. These differences are manifested in the number of mitoses, content of glycogen and ribonucleic acid, &c. The pattern of these differences in the exterior wall of the eye vesicle seems to be responsible for the way in which the lens fibres start growing. I cannot exclude, however, that regulative forces acting within the lens vesicle also play a role in the establishment of the final structure.

Hitherto I have restricted my description to the influence which the eye vesicle and eye cup exercise on lens development. On the other hand, there is no doubt that the lens anlage influences the differentiation of the eye cup. I have only to mention the suppression of the lens-forming capacity of the border of the iris by the presence of a lens in some amphibia. But I will not enter into a description of this aspect of the interaction between eye cup and lens.

It was my intention to show by some examples that the crystalline lens is a most favourable object of study for one of the topics of our symposium: intercellular relations in differentiation.

REFERENCES


For a survey of the author's work on lens development, see *Année biol.* 26, 699 (1950).