The First Appearance of Specific Antigens during the Induction of the Lens

by JAN LANGMAN

From the Department of Anatomy, McGill University, Montreal

INTRODUCTION

The formation of the lens in the chick embryo is known to depend upon 'inductive' influences from the eye-cup (Alexander, 1937; Van Deth, 1940; Waddington & Cohen, 1936). A period of direct contact between eye-cup and presumptive lens ectoderm from the 9- to the 20-somite stage is essential for the induction (Weiss, 1947; McKeehan, 1951; Langman, 1956). At the beginning of this period (9–12-somite stage), the cytoplasm of the presumptive lens ectoderm cells is vacuolated and the nuclei have a random distribution, as in the ectodermal epithelium of the head region. During subsequent development (13–16-somite stage) the intracellular vacuoles disappear from the presumptive lens ectoderm and the nuclei become gradually displaced toward the base of the cells in contact with the retinal surface (McKeehan, 1951). At the 16–19-somite stage the cells become more and more columnar (so-called palisading phenomenon) and the nuclei elongated perpendicularly to the basement membrane (lens placode formation). According to Weiss (1947) this nuclear orientation suggests molecular orientation (perhaps as a result of the fixation of a protein molecule from the optic vesicle to the basal surface of the presumptive lens ectoderm).

After the lens placode has been formed, its invagination begins (20–22-somite stage, 44–50 hours of incubation) and leads gradually to the formation of a lens vesicle, which is separated from the ectoderm at the 31–34-somite stage (63–72 hours of incubation). Soon thereafter the cells in the posterior wall of the lens vesicle become elongated and form the first lens fibres (34–41-somite stage, 72–96 hours of incubation). At the end of the 5th day a group of tall cylindrical cells found at the equator of the lens turn 130 degrees and form new lens fibres, which join peripherally the central fibres. This marginal zone formation reaches completion at the 8th day.

To determine at what stage of morphological development chemical substances characteristic for the adult lens appear, Burke et al. (1944) applied the

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2 Author's address: Department of Anatomy, Medical Building, McGill University, Montreal, Canada.

complement-fixation technique and were able to detect the presence of lens antigens in 146-hour embryos, that is, at a time when the first marginal lens fibres have been formed, but not earlier. Ten Cate & Van Doorenmaalen (1950), applying microprecipitin tests, found lens antigens as early as the 30-somite stage, when the lens vesicle is still open to the surface. Langman, Schalekamp, Kuijken, & Veen (1957) searched for lens antigens in chick embryos from 7 to 20 somites by explanting presumptive lens ectoderm with the associated eye-cup into a medium which contained lens antibodies. Degeneration of presumptive lens ectoderm was found in explants cultured in this medium, but not in controls.

In the present work, these experiments were repeated under different experimental conditions using presumptive lens ectoderm not only with, but also without the associated eye-cup. The latter procedure made it possible to avoid the inductive influence of the eye-cup during the explantation period and to examine whether the first lens antigens appear before or during the contact period. In addition, these experiments gave information on the relationship between chemical and morphological differentiation in presumptive lens ectoderm cells.

MATERIAL AND METHODS

In a first series of experiments, eye-cup material covered with presumptive lens ectoderm obtained from White Leghorn chick embryos of 5–20 somites was explanted in a fluid medium composed of equal parts of 8½-day chick embryo extract, Tyrode solution, and rabbit serum containing antibodies against adult lenses. The antiserum was prepared by injecting chicken lens extract (10 per cent.) into healthy rabbits, using an injection schedule devised by Ten Cate & Van Doorenmaalen (1950). In the control series the anti-lens serum was replaced either by serum of normal healthy rabbits (normal serum) or by serum of rabbits injected with the myosin fraction of frog muscle (DeHaan, 1954).

In the second series of experiments, presumptive lens ectoderm tissue from 7–20-somite chick embryos was dissected free from the eye-cup. The first attempts to do so resulted in damage to the presumptive lens ectoderm due to difficulties in separating the tightly bound ectoderm and eye-cup. Success was achieved after dipping the heads of the embryos in trypsin solution (1:10,000) for 1 minute and rinsing in Tyrode solution. It was then possible to dissect the presumptive lens ectoderm without damaging the cells. The ectoderm cells were explanted on a clot which had the same constituents as the experimental medium mentioned above, but to which an equal amount of chicken plasma was added in order to obtain a coagulum. In the control series the anti-lens serum was replaced by ‘normal’ serum.

The titre of the lens antiserum used in these experiments was determined by Boyd's method and found to vary from 1:6,400 to 1:16,000. To determine the specificity of the lens antiserum it was tested with extracts from a variety of organs and tissues of adult chickens. No precipitin reaction was found with most
RESULTS

Presumptive lens ectoderm with associated eye-cup cultured in 'normal' and 'anti-myosin' medium for 72 hours gave rise to lens formation in most cases (Text-fig. 1). The percentage of lens-forming explants was found to increase with the age of the embryo from which the tissue is obtained. Failure of lens formation in some of the explants cultured in the control media may be due to mechanical and thermal damage during dissection and heterologous constituents in the culture medium (Langman, 1953a, b). Microscopic study of the explants cultured in control media showed a normal, although delayed, morphological development of the lens in comparison with in vivo development. Furthermore, the explants cultured in 'anti-myosin' medium showed almost no mesenchyme between the ectodermal derivatives, or only some necrotic cellular material. Lens, optic cup, and surface ectoderm were normal.

The explants cultured in 'anti-lens' medium for 72 hours behaved differently. In studying these tissues during the explantation period under the dissecting microscope, it was observed that 10–20 hours after the beginning of the tissue-culture period the explants showed a white, degenerating area in the presumptive lens ectoderm. This area became larger during the next few hours and often
broke off from the explant. Thus in some of the cultures the uncovered optic vesicle protruded through a collar of non-presumptive lens ectoderm, whereas the necrotic presumptive lens area dropped to the bottom of the container (Text-fig. 2). However, it may be seen from Text-fig. 1 that this pattern was not followed by all explants. All those obtained from 5–16-somite embryos were unable to form a lens in anti-lens medium, those from 16 to 17 somites developed a lens in 24 per cent. of the cases, while the explants from embryos of 18 and more somites behaved like the controls and formed a lens.

![Text-fig. 2. Diagram of changes in explants of optic vesicle and presumptive lens ectoderm from 10-somite embryos cultured in 'anti-lens' medium. On the left, at explantation. Middle, degenerating presumptive lens area after 10 hours. On the right, degenerate lens area lost, optic vesicle protruding through ectoderm.](image)

This experiment, which exactly confirms under our present laboratory conditions a series of data previously obtained (Langman et al., 1957), indicates that ‘normal’ and ‘anti-myosin’ media do not interfere with lens formation in vitro; ‘anti-lens’ medium, however, prevents lens development in explants obtained from chick embryos of 5–17 somites. Presumably the lens antibodies present in the culture medium are able to react with constituents of the presumptive lens ectoderm cells in such a manner that the cells are unable to survive.

This experiment did not decisively show whether lens antigens arise during or before the induction period (9–20 somites). It was possible that lens antigens were not yet present in the presumptive lens ectoderm cells of a 7-somite embryo but arose under the influence of the associated eye-cup at some time during the explantation period. The result of a 72-hour explantation period in an ‘anti-lens’ medium would then be the same, namely, degeneration of the presumptive lens ectoderm. Presumptive lens ectoderm from 7–20-somite embryos was therefore dissected free from the eye-cup and explanted on a clot. By doing so it was possible to exclude the inductive influence of the eye-cup during the 72-hour culture period.

*Presumptive lens ectoderm explants without associated eye-cup* obtained
from embryos of 7–20 somites were studied under the microscope during the explantation period. Twelve to twenty-four hours after the beginning of the culture period explants on 'normal' medium showed migration and outgrowth in the form of a membrane in or on the medium. At the end of the explantation period 80–100 per cent. of the explants showed outgrowth, whatever the age of the donors, that is to say, regardless of whether or not the ectoderm had been exposed to the inductive influence of the eye-cup prior to explantation. However, although the cells looked healthy and migrated, no lenses were formed.

The explants grown on 'anti-lens' medium behaved differently according to the age of the embryos. Presumptive lens ectoderm obtained from 7–10-somite embryos showed outgrowth as explants in normal medium. Cultures obtained from 11–12-somite embryos showed outgrowth only in about 30 per cent. of the cases and those from 13–16-somite embryos in none. In the latter, disintegration and death of the cells were observed in all cases 12–24 hours after the beginning of the culture period. Fragments obtained from embryos beyond the 17-somite stage again showed outgrowth and migration just as the control explants did (Text-fig. 3; Table 1).

From this experiment it can be concluded that, shortly after the 9–10-somite stage, when direct contact between eye-cup and ectoderm is established and induction is thus elicited, the cells lose their ability to survive in 'anti-lens' medium.
A new component somehow appears in the presumptive lens ectoderm cells and reacts with the lens antibodies in the medium in such a way that the cells are unable to survive. Toward the end of the induction period (18 somites), when adhesion between eye-cup and ectoderm is diminishing, the presumptive lens cells are no longer killed by lens antibodies.

**Table 1**

*Influence of 'normal' and 'anti-lens' medium on presumptive lens ectoderm*

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<tr>
<th>Number of somites</th>
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**Discussion**

Antibodies produced by injecting specific substances—antigens—into rabbits have the capacity to react selectively with these antigens and with closely related substances. This reaction, which is based upon the formation of an antibody-antigen complex, can become visible as a flocculation, agglutination, precipitate, cell degeneration, &c. By testing extracts of unknown composition with antibodies prepared against a well-defined organ, it is possible to detect whether or not antigens specific for this organ are present in the unknown extracts. Applying this immunological detection method in embryology, it was found that antibodies prepared with antigens of adult organs and tissues may react with antigenically active materials in embryos—a fact indicating that embryonic organs and tissues may possess antigenic substances which are identical or closely related to adult antigens. Some of these embryonic substances persist up to the adult stage, while others are transitory and disappear during development (Ebert, 1953; Tyler, 1955; Nace, 1955).
7–10-somite stage

By culturing presumptive lens ectoderm without the associated eye-cup on a medium containing lens antibodies it was found that explants obtained from 7–10-somite embryos showed the same active outgrowth and migration as the controls (Text-fig. 3). Thus at this stage of development no antigenic substances capable of reacting with lens antibodies were detectable. Examination of presumptive lens ectoderm at this stage using the agar gel technique of Ouchterlony (1948) showed no lens antigens either (unpublished data). Although it is known that the eye-cup has started exerting its inductive influence upon the presumptive lens ectoderm cells at the 9–10-somite stage these cells do not yet contain any chemicals related to lens proteins. Since there is no morphological distinction from the surrounding head ectoderm either, it may be concluded that no detectable chemical or morphological differentiation has yet taken place.

11–17-somite stage

As shown in the introduction, the cells of the presumptive lens ectoderm start morphological differentiation at the 13-somite stage. Explantation of presumptive lens ectoderm with or without the associated eye-cup from 11–17-somite embryos in a medium containing lens antibodies produced degeneration of the cells (Text-figs. 1 and 3). This result means that substances able to react with lens antibodies arose in the presumptive lens ectoderm at the 11-somite stage, that is, shortly before the cells become distinguishable from the surrounding head ectoderm cells. Hence the first lens antigens seemed to be formed shortly before the first features of morphological differentiation appeared (13 somites).

The lens antisera used in our experiments were prepared with ‘adult’ lens material. Does this mean that the antigens detected in presumptive lens cells by use of these antisera were the same as in the adult? In testing the presence of lens antigen in chick embryos by means of the precipitin method, Ten Cate & Van Doorenmaalen (1950) concluded that ‘adult’ lens antigen was present at the 30-somite stage. However, since Landsteiner (1947) found that the specificity of antigenic structures may be represented by relatively small chemical groups on a large molecule rather than by the molecule as a whole, the presumptive lens cells need not necessarily have identical antigens, but merely identical chemical groups.

The fact that morphological lens differentiation was preceded by the appearance of specific chemical units suggests that, under the inductive influence of the eye-cup, some components of the indifferent multi-potent head ectoderm cells acquired the determining groups characteristic of lens antigens. These antigens appearing at the 11-somite stage (first-stage antigens) may give rise to a number of antigenic structures, each preceding the appearance of a new morphological structure as the placode transforms through lens vesicle to adult lens. François et al. (1956) showed by immunochemical analysis of extract of adult lens material that at least eight different antigens are present at the adult stage.
A possible mechanism by which antibodies kill presumptive lens ectoderm cells during the 11–17-somite stage may be a cell surface reaction. This antigen-antibody surface reaction may interfere with exchange processes between the cell and its environment, or cause some other change in surface properties (surface effect). Another possibility is the passage of antibodies through the cell membrane and the combination with substances within the cell (intracellular effect). The antibody-antigen binding may block the function of a cell substance essential for cell growth and survival, or the formed complex may be toxic and thus kill the cell. The entry of the antibody into the cell presumed to occur during the 11–17-somite stage may be an indication of a high permeability of the cell membrane.

18- and more than 18-somite stage

At this stage of development, when the ‘first stage’ lens antigens have been formed, the differentiation of the lens cells can proceed without any further need for the eye-cup. This was demonstrated by McKeehan (1954), who transplanted placode cells of a 21-somite embryo into the coelomic cavity of another chick embryo. This transplant showed the capacity to form a small lens, indicating independent self-differentiation at this stage of development. Similarly, it appeared from our experiments that at the 18-somite stage the future lens cells are no longer sensitive to lens antibodies (Text-figs. 1 and 3). A possible explanation was that the ‘first stage’ lens antigens were transitional antigens. This was unlikely since, using the same antilens serum but a different technique (Ouchterlony, 1948) we demonstrated the presence of lens antigens at the 18–22-somite stage (unpublished).

An alternative explanation is that the ‘surface effect’ no longer takes place, perhaps because of a change in the cell membrane. That there are indeed changes in the cell membrane is suggested by the fact that at the 18-somite stage the presumptive lens cells can be separated from the eye-cup without treatment by trypsin solution. This change in the cell membrane may also prevent the access of antibodies into the cells and thus avoid their ‘intracellular effect’. If, however, the intracellular reaction is not blocked, it may be that the neutralized antigens are no longer toxic to cell life or, if the original antigens were essential, they can now be replaced by other substances.

From these experiments it may be concluded that lens antibodies applied in tissue culture can act as sensitive indicators of the formation or presence of specific substances (lens antigens) in embryonic cells. The formation of an antigen-antibody complex leads to the death of the presumptive lens ectoderm cells only during the 11–17-somite stage. Before and after this stage the cells are not visibly affected by the presence of lens antibodies, indicating either that the reacting antigens are not present or that the formation of an antigen-antibody complex is prevented. The most likely mode of antibody action is considered to
be the ‘intracellular effect’, the cell membrane, however, having an important function in admitting the antibodies into the cells.

SUMMARY

1. Presumptive lens ectoderm with associated eye-cup from 5-17-somite chick embryos was explanted in ‘normal’ medium, ‘anti-myosin’ medium, and a medium containing lens antibodies. The control explants in ‘normal’ and ‘anti-myosin’ medium showed lens formation in 80-100 per cent. of the cases (Text-fig. 1); the explants in ‘anti-lens’ medium showed degeneration and necrosis of the future lens area, indicating the presence of substances (lens antigens) capable of reacting with lens antibodies (Text-fig. 1). Explants from embryos with 18 or more somites in ‘anti-lens’ medium formed lenses as the controls.

2. Presumptive lens ectoderm without the associated eye-cup from 7-20-somite embryos was cultured on a ‘normal’ and ‘anti-lens’ medium. The controls showed epithelial membranous outgrowth in 80-100 per cent. of the explants (Text-fig. 3; Table 1). Explants on ‘anti-lens’ medium showed outgrowth similar to controls when obtained from embryos younger than 11 and older than 17 somites, but showed degeneration when obtained from 11- to 17-somite embryos, indicating the formation of the first reacting substances (lens antigens) at the 11-somite stage (Text-fig. 3; Table 1).

3. The ‘first-stage’ lens antigens are formed at the 11-somite stage, shortly after the adhesion between eye-cup and presumptive lens ectoderm has been established (9 somites) and shortly before the morphological changes characterized by loss of vacuolization, nuclear orientation, and palisade phenomenon appear (13-19 somites).

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REFERENCES


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