Cytotoxicity of lens antisera to dissociated chick neural retina cells in tissue culture

By MAX BRAVERMAN¹, CARL COHEN² & ARTHUR KATOH³

Immunoprecipitation techniques have shown that characteristic lens proteins can be found in many tissues of the chick eye. Langman & Prescott (1959), Maisel & Langman (1961a), Maisel (1962) and Maisel & Harmison (1963), among others, have demonstrated antigens cross-reacting with adult chick lens antisera in iris, pigmented retina, cornea and aqueous and vitreous humour. Maisel (1963) suggested that lens antigens are present in neural retina, but the presence of lens antigens in this tissue has not been firmly established, and a number of investigators reporting lens antigens in other ocular tissues have not found them in the neuro-retina. [For reviews of immunological investigations on the development and ubiquity of lens proteins see Langman (1959a, b), Maisel & Langman (1961b), Rabaey (1962), Woedereman (1961), Zwaan (1963), Ikeda & Zwaan (1966, 1967), Zwaan & Ikeda (1968) and Clayton, Campbell & Truman (1968).]

Chick embryos developing in the presence of lens specific antiserum do, however, exhibit defects of the neural retina. Burke, Sullivan, Petersen & Weed (1944), Langman (1960), Fowler & Clarke (1960), Langman & Maisel (1962) and Langman, Maisel & Squires (1962) found that embryos developing in the presence of antisera against lens crystallins evidence defects not only of the lens, but of the optic cup and often of the brain as well. Langman et al. (1962) found that when 0.1 ml of lens antiserum was dropped on chick embryos in ovo at 24 or 32 h of incubation ‘the lens was less frequently affected than the nervous retina’. Clarke & Fowler (1960) explanted optic vesicles of 4–7 somite embryos to medium containing lens antiserum, after removing the overlying ectoderm (presumptive lens). The tissues were removed from the treatment medium and wrapped in untreated ectoderm competent to form lens. These sandwiches were allowed to develop, and sectioned. Whereas 50% of the

¹ Author’s address: Cellular and Radiation Biology Laboratories, Clinical Radiation Therapy Research Center, Dept. of Radiology, Allegheny General Hospital, Pittsburgh, Pa. 15212, U.S.A.
² Author’s address: Department of Biology, Western Reserve University, Cleveland, Ohio, 44106, U.S.A.
³ Author’s address: Cancer Research Laboratories, Mercy Hospital, Pittsburgh, Pa. 15219, U.S.A.
sandwiches containing untreated eye cups formed lenses, only 3% of those containing treated explants did.

These developmental assays indicate that the antigen or antigens in question, although not present as soluble cell products, exist in cells of the neural retina, and they exist in such a state that combining the antigen or antigen precursor with antibody results in cell death or rechannelling of developmental proclivities.

This interpretation, if substantiated, implies an intermediary stage of gene regulation in which a protein normally soluble is not demonstrable as a soluble protein but may be present either as a precursor or in extremely small quantities, or in a bound state, and in this state is associated with cell viability in a way not known to be true of soluble proteins (Pressman, 1958).

Because of the importance of this hypothesis to our understanding of gene regulation during development, it is necessary to find out whether it is the neural retina or the adjacent pigmented retina (known to contain soluble lens antigens) that is directly responsive to lens antiserum.

The optic cup is a two-layered structure formed by the invagination of the optic vesicle. This structure begins to invaginate around stage 15 (50 h) (O'Rahilly & Meyer, 1959), and at about that time differences between the inner and outer layers become apparent. Subsequently the internal lamina of the cup thickens and becomes the neural retina; the external lamina acquires pigment and develops into the pigmented retina. The dependence of these two layers upon each other has been noted by Uhlenhuth (1916). Because of their origin in the same structure, the optic vesicle, it might be the pigmented retina (known to contain soluble lens antigens) which is directly affected by lens antisera, the neuro-retina being affected only indirectly as a result of this damage. Langman & Maisel's (1962) finding that at 42 h (at which time the two laminae are forming from the optic vesicle precursor) the embryo is no longer sensitive to lens antiserum encourages this interpretation, that it is not the neural retina which is sensitive to lens antibodies, but the precursor optic vesicle, in which divergent differentiation of these two tissues may not yet have taken place.

In order to determine if isolated neural retina is sensitive to lens antiserum we have dissociated the 7-day chick embryo neural retina and treated the cells with dilute concentrations of homologous and heterologous lens antiserum.

The resulting reaggregates were analysed histologically for indications of an effect of the sera upon cell viability and cell differentiation.

**METHODS**

*Culture media.* 'M': Eagle's Minimum Essential Medium (Microbiological Associates, Inc., Bethesda) plus 2% EE50, 100 units/ml streptomycin, 100 units/ml penicillin and 1% glutamine.
Neuro-retinal cells in lens antiserum

'PBS': NaCl 4 g, KCl 0·100 g, Na₂HPO₄ 0·10 g, phenol red 0·06 ml (1 % solution in H₂O), 500 ml H₂O, 100 units/ml penicillin and streptomycin.

'EE50': Equal volumes of PBS and 7-day chick embryos (minus the eyes). The embryos are expressed through a syringe into the PBS, frozen and thawed two times, then centrifuged at 500/g for 20 min and the supernatant is frozen for later use.

'T': 0·25 % 1:250 Difco Trypsin in PBS.

Preparation of dissociated cell suspensions. Seven-day-old chick embryos are removed aseptically from their eggs to M. The eyes are dissected free. Neural retinae are dissected from the eyes (Coulombre, 1955) and collected in M. These are washed in PBS, then washed once with T, then incubated with shaking at 37 °C in about 10 ml T. After 5–7 min, when cells can be seen coming off the retinae, about 1 ml of EE50 is added. The solution is removed, and the retinae resuspended in M. They are gently pipetted with coarse, then fine tipped, pipettes, centrifuged at 500 g for 5 min, washed twice in M, then resuspended in 10 ml of M. An aliquot is removed, diluted twenty times, and counted in a hemocytometer. The cells are diluted to a concentration of 4 × 10⁷ cells/ml with M. Heat inactivated fetal calf serum is added to a concentration of 10 %. This stock solution of cells is then diluted with the appropriate media to give the following concentrations: 4 × 10⁶ cells/ml, 10 % heat inactivated fetal calf serum, 2 % EE50. Three ml of this solution is dispensed into 15 or 25 ml Erlenmeyer flasks, gassed if necessary to a pH of 6·8–7·0, sealed with gum rubber stoppers and incubated with rotary shaking at 37 °C.

One or two solid reaggregates form in these cultures after about 24 h. In one series of experiments, however, a large number of small spherical reaggregates formed, similar to the usual reaggregates in every respect except their size.

Histology. Reaggregates are transferred to small conical test tubes and centrifuged a few minutes at about 250 × g, washed once with PBS, then fixed in acid alcohol. They are then pipetted free from the bottom of the test tube and removed with a wide mouth pipette to a microscope slide. When the alcohol has almost evaporated, warm 1½ % agar in PBS is pipetted on the slide. The portion containing the reaggregate is cut from the solid agar and put in acid alcohol fixative. The agar piece is routinely processed, sectioned at 6 μ and stained in hematoxylin and eosin.

Immunological techniques. Antisera for incorporation into culture media are prepared with freshly collected chick, beef or sheep lenses. The mammalian eyes are removed from the carcasses no more than 10 min post mortem and collected on ice. Lenses are dissected from the eyes within four hours, washed in 0·85 % NaCl solution and homogenized in a mini-blender at a concentration of 100 mg wet weight/ml with 0·85 % NaCl solution, and frozen immediately. Eyes are dissected from 10-day-old chick embryos, collected and dissected in M, washed and extracted in salt solution as above.

Rabbits are immunized with two injections of the whole lens brei containing
0·5 ml of lens extract, and 0·5 ml of complete Freund's adjuvant, given 1 week apart, and injected intramuscularly. They are bled 3 weeks after the last injection.
Sera to be incorporated into culture media are unfrozen shortly before use, and diluted with M to the appropriate concentration. The resultant solution is filtered through a Millipore filter (0.45 μm pore size). Calf serum is added to the final culture medium in such volume as to make a 10% total of all sera, fetal calf plus rabbit. Immune sera were used at concentrations of 1:10 to 1:200 volume of immune sera to total culture volume.

Culture material is prepared for immunoprecipitation by centrifuging reaggregates or cells at low speeds, washing once in PBS, then homogenizing in 2 ml of 0.85% NaCl solution and freezing. This gives an approximate concentration of about 10 mg wet weight of cells/ml (assuming 10⁶ cells to weigh about 1 g). The thawed homogenate is centrifuged at about 600 g and the supernatant used for immunodiffusion experiments. Micro-immunoelectrophoresis and micro-Ouchterlony analyses were carried out using the standard agar techniques reported in Crowle (1961). Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) using Bovine Serum Albumin Standard (Pentex).

RESULTS

Cells of the 7-day chick neural retina, dissociated and cultured as described above, reaggregate one or two large tissue masses containing cells organized in a manner reminiscent of the tissue of origin (Moscona & Moscona, 1952; Moscona, 1960). The neuro-retinal reaggregates obtained with our preparations in the absence of anti-lens antibody were similar, but not identical, to those described by Moscona (1957). The 72 h reaggregate (Fig. 1A) is grossly differentiated into a central area of cellular degeneration and a periphery demonstrating characteristic neuro-epithelial organization. Pseudo-rosettes (Fig. 1C) retinal rosettes (Fig. 1B) and what Moscona (1957) calls lentoids (Fig. 1D) are present.

**Neuro-retinal reaggregates cultured in lens antiserum.** When neuro-retinal cell suspensions were dispersed in medium containing 1:50 (v/v) dilutions of rabbit antiserum to 10-day chick embryo lens, and cultured in the standard manner for 48 h, very few cells survived the treatment. Instead of the few large reaggregates obtained in control cultures, very small cell clumps only could be seen in treated material. Histological examination of these cultures revealed (Fig. 2) a small

Fig. 1. The bar is 50 μ long.

A. Cross-section through entire reaggregate of 7-day chick neuro-retinal cells.
B. Characteristic retinal rosette with an inner delimiting membrane found in reaggregates of 7-day chick neuro-retina.
C. Pseudorosette from 7-day chick neuro-retina reaggregate. Nuclei tend to orient with their long axes normal to the center. Pseudorosettes frequently contain a small number of pale nuclei at their centers.
D. Characteristic lentoid from a 7-day chick neuro-retinal reaggregate.
number of cells resembling lentoids surrounded by loosely aggregated cells with densely staining, pycnotic nuclei. Thirty-five chick neuro-retinal cultures treated with concentrations of 10-day chick lens antiserum ranging from 1:10 to 1:50 dilution gave similar results. Some contained only the loosely associated cells with pycnotic nuclei, but most were as shown.

Serum controls. To determine if the cytotoxic effect of 10-day chick lens antiserum is due to species or tissue specific components, and to rule out the possibility that some normal constituent of rabbit serum is responsible for the cyto toxicity, cells were cultured in pre-immunization rabbit serum, antiserum against human red blood cells, and antiserum against calf and against sheep lens. Cultures grown in unimmunized rabbit serum produced normal reaggregates. Cells distributed into medium containing a high titer human red blood cell antiserum at 1:25, 1:50 and 1:100 dilutions formed normal reaggregates. When suspensions of neuro-retinal cells were inoculated into medium containing either antiserum against calf lens (two cultures) or antiserum against sheep lens (Fig. 3D) (2 cultures, 1:25, 1:50) the same destructive effects as in anti-chick lens serum occurred.

Neither unimmunized rabbit serum in itself, nor serum from rabbits immunized against human red blood cells, and thus containing much \( \gamma \)-globulin, has an effect upon the viability of neuro-retinal cells or the differentiation of the neuro-retinal reaggregates. Since both anti-sheep lens and anti-calf lens sera evoke the same cytotoxic response from chick neuro-retinal cells as does anti-chick lens serum, the possibility that cytotoxicity is being caused by species-specific antibodies is ruled out.

Antiserum concentration studies. Four experiments investigating the concentration dependence of the cytotoxic effect gave the same results. Cultures grown in 1:200 dilutions of the antiserum were essentially normal (Fig. 3A). Those grown at 1:100 tended to form reaggregates of somewhat smaller size (Fig. 3B). In some 1:100 cultures (not shown) the reaggregates consisted for the most part

![Fig. 2. Reaggregates of 7-day chick neuro-retinal cells cultured in the presence of 1:50 (v/v) dilution of antiserum to 10-day chick lens. The bar is 50 \( \mu \) long.](image)
Neuro-retinal cells in lens antiserum

of undifferentiated neuro-retinal cells, not demonstrating characteristic reaggregate differentiation. Cells cultured in 1:50, 1:25, or 1:10 dilutions of antiserum (Fig. 3C) were affected as described above. In a single experiment testing concentration dependence with sheep lens antiserum, the cut-off point was also 1:50; cells grown at this concentration of antiserum were affected, those grown at 1:100 were not (Fig. 3D).

Fig. 3. Reaggregates formed in medium containing lens antiserum at different dilutions. The bar is 50 \( \mu \) long.

A. 1:200 anti-10-day chick lens.
B. 1:100 anti-10-day chick lens.
C. 1:50 anti-10-day chick lens.
D. 1:50 anti-sheep lens.

Tissue controls. Is only neural retina sensitive to anti-lens serum, or are all embryonic cells sensitized by dissociation similarly affected by lens antibodies? To test this 7-day embryos were decapitated, and the entire body portions dissociated and cultured in 10-day chick lens serum, and sheep lens serum. In both cases reaggregates formed of normal size containing no unusual evidence of cell death (Fig. 4). Although we can conclude from this experiment that lens antiserum is not toxic to all chick tissues, it would be inappropriate to conclude that only nervous retina is sensitive to lens antiserum. To draw the latter conclusion, one would need to compare the numbers of each cell type, and the nature of differentiation in treated and control cultures. The limited conclusion
does permit us to say that it is not some common element of avian and mammalian cells, such as mitochondria, or ubiquitous enzymes, that is affected directly.

Fig. 4. Reaggregation of dissociated cells of entire (decapitated) 7-day chick embryos. The bar is 50 μ long.
A. Cultured in antiserum against 10-day chick lens. Antiserum dilution 1:10
B. Cultured in antiserum against sheep lens. Antiserum dilution 1:10.

Time of antiserum addition. Cultures were established in the usual manner and anti-chick lens serum was added at a 1:10 concentration 12, 24, 36, or 48 h later. The effect of antiserum on cultures which had already begun to reaggre-
Neuro-retinal cells in lens antiserum

Gate was being tested. Time-course studies (M. Braverman, unpublished) indicate that at 8 h the culture consists of a number of small reaggregates which are in the process of fusing and forming a large agglomerate. The cultures, to which antiserum had been added at various times after inoculation, were all harvested after a total time in culture of 72 h, and analysed histologically.

A consistent variation in the severity of defect with the time of addition of antiserum could not be seen. There was, however, a clear-cut difference between cultures treated at the time of inoculation, and those to which antiserum was added subsequently. Figure 5 shows two different cultures to which antiserum was added at 36 h (Fig. 5B, C), and a control culture not treated with antiserum (Fig. 5A). These 36 h addition cultures are characteristic of all cultures treated after the culture was established. When antiserum is added 12–48 h after the culture is initiated, the majority of the cells survive, and form a small number of large reaggregates. These consistently differ from untreated controls (Fig. 5A), in that around the exterior of the treated reaggregates are numbers of loosely associated cells with dense, pycnotic nuclei (Fig. 5C). In some, but not all cultures treated after a delay, cells with apparently empty nuclei are incorporated into the reaggregate among the majority of characteristic neuroepithelial cells (Fig. 5B).

It is not possible to tell from the appearance of the reaggregate whether the pycnotic cells of the periphery did not adhere to the reaggregate until after the antiserum was added, and thus were present as single cells in the antiserum, or if they were part of the reaggregate when antiserum was added, and were affected because, being on the periphery, they were exposed to the antiserum, whereas other, interior, cells were not.

Assay for lens antigen in reaggregates and in neuro-retina. Despite the lack of evidence in the literature for the presence of lens antigens in adult chick neuro-retina, the possibility remained that 7-day embryo neuro-retina contained soluble lens antigens, and that the presence of these antigens accounted for the otherwise paradoxical effect of lens antiserum. Accordingly, 7-day neuro-retina was assayed for the presence of soluble lens antigens, by means of micro-immunodiffusion and immunoelectrophoresis, with the same chick, sheep and beef antisera as had been introduced into culture. The antisera cross-reacted to the original lens antigens (80 µg protein/ml), but no cross-reaction to a soluble extract of neuro-retina (190 µg protein/ml) could be seen.

Although freshly isolated neuro-retina appears to contain no soluble lens antigens, it is possible that such soluble antigens are synthesized during the course of culture. Samples were obtained from neuro-retinal cultures at 0, 8, 16, 24, 36, 48, 72 and 96 h, and from cultures established at concentrations ranging from $2 \times 10^5$ to $8 \times 10^6$ cells/ml, at 48 h. These were tested in micro-immunodiffusion plates for evidence of cross-reaction to the homologous and heterologous sera. No evidence for such cross-reaction was found. Although these sera killed dissociated neuro-retinal cells when the cells were cultured in
media containing small concentrations of the antisera, no cross-reaction between the antisera and the neural retina, dissociated neuro-retinal cells, or any stage of the neuro-retinal reaggregate could be demonstrated.

**DISCUSSION**

We have shown that antiserum against chick, sheep or beef lens is toxic to dissociated cells of the 7-day chick neural retina. This toxicity might conceivably result from a number of trivial factors such as general serum toxicity, a species-specific antiserum component, or an antiserum against an ubiquitous chick cell component. But we believe that our controls have ruled out such trivial factors, and that this result confirms the indications of earlier experiments that the chick nervous retina is sensitive to lens antiserum.

Although emphasized previously, one point requires further clarification. The dissociated whole embryo treated with lens antiserum gives rise to large, grossly normal, reaggregates containing no dead cells. It is possible, however, that entire cell species are wiped out by the antiserum, or that differentiation of some cell types is rechannelled. We did not analyse the reaggregates to determine the kinds of tissues they included.

Dissociation appears to render tissues sensitive to antiserum. We have demonstrated that tissues unaffected by antiserum at 2 days are affected in the dissociated state at seven. Of course, the cytotoxicity might depend upon the normal reacquisition of sensitivity at the later period, but the failure of antiserum added after reaggregation has begun to affect the cells appears to rule out that possibility.

It does not now seem possible rigorously to interpret the significance of our findings that cells of a tissue not containing lens antigens are destroyed by lens antiserum. We know, after all, virtually nothing of how antiserum affects developing tissues, or the cellular location of this effect (Eds, 1958). A number of observations lead to the suggestion that it is not a combination of antibodies with antigens which are being synthesized and released as soluble proteins that leads to cell death. Langman, Maisel & Squires (1962) observe that it was more often the optic cup than the lens which was affected by lens antiserum; Ehrlich, Halbert & Manski (1962) report that rabbit antiserum to rabbit lens has no effect upon cultured lens epithelium. Fowler & Clarke (1960) note that in embryos of 20–25 somites that are treated with lens antiserum it is the optic cup which is defective, and the lens is normal, although embryos treated at an

---

Fig. 5. The effect of delayed addition of lens antiserum on the reaggregation of chick neuro-retinal cells. The bar is 50 μ long.

A. Control; no antiserum. The controls in this series tended to form less solid reaggregates.

B, C. Chick lens antiserum added at 36 h of culture, harvested after 72 h. Antiserum dilution 1:10.
earlier time do show defects of the lens; and Pressman (1958) finds that cytotoxicity does not immediately follow the localizing of anti-kidney serum in the kidney of rats.

The unexpressed developmental alternatives of a cell or tissue of an embryo can be thought of as all those 'genes' not represented in the cytoplasm by their protein analogs. With regard to those proteins known to be soluble: if they are present, we would assume that the appropriate DNA was being transcribed and its messenger RNA translated, and the proteins so specified released from the messenger; if they were absent, it would be reasonable to assume that the appropriate DNA was not being transcribed (although another alternative has been demonstrated in oocytes; see Tyler, 1967).

The experiments we report here suggest that a third alternative state of proteins can be recognized with simple techniques. A protein not present in a tissue as a soluble cell product can be recognized by the effect of the antiserum to it on the cell. It would be premature to speculate on the position in the chain of protein regulation of this third alternative. It does seem reasonable, however, that proteins so identified are a different class of unexpressed developmental alternatives than those neither present in the cell nor recognizeable by the cytotoxic assay, and are obviously different from those present as soluble cell products.

Speculations on the significance of bound antigens must, however, be formulated with caution in view of findings which identify an apparently ubiquitous basement membrane antigen in the lens capsule, in ocular blood vessels, the ciliary body and iris (Roberts, 1957; Nozaki, Foster & Sery, 1963). It does not seem possible, however, that antiserum to this constituent of the eye lens capsule is responsible for cytotoxicity to the neural retina, inasmuch as immunofluorescence studies (Roberts, 1957; Nozaki, Foster & Sery, 1963) and defect studies in adult guinea pigs (T. W. Sery, personal communication), although recording effects on other ocular components, present no evidence for the presence of this basement membrane antigen in the nervous retina.

Ideas about the uniqueness or the ubiquity of lens proteins are now in a state of flux. On the one hand Zwaan (1963) and Ikeda & Zwaan (1966, 1967) claim that the crystallins of the lens are unique to the lens and that previous workers who have reported otherwise were misled by artifacts. On the other, Clayton, Campbell & Truman (1968) report that the use of hyper-immune lens sera permits the demonstration of cross-reacting antigens in all tissues tested. (Unfortunately, they do not differentiate between neural and pigmented retina in their report.) Zwaan's contention that some antigens are found in lens and only in lens is true; this has clearly been demonstrated by Yamada and his co-workers (Takata, Albright and Yamada, 1964) who have used such an antigen as a marker as they studied the transformation of newt iris cells to lens. It also seems to be a truism that there are constituents of lens cells that are found in all other tissues of the animal, for so are many enzymes and morpho-
logically recognizable structures. We believe it would be a mistake, however, to blur the distinction between ubiquitous antigens and those held in common by lens and by other ocular tissues only, for it is by studying the distribution and the expression of such antigens that the embryologist can obtain insight into the strategy of development.

**SUMMARY**

1. When dissociated cells of the 7-day chick embryo neural retina are cultured in low concentrations of rabbit antiserum to whole chick lens, few cells survive; those that do form small reaggregates resembling lentoids.

2. Dissociated cells of the neural retina cultured in pre-immunization serum, or rabbit antiserum to human red blood cells form the same characteristic reaggregates as do cells in medium with no rabbit serum, indicating that no non-specific toxic element in either rabbit serum or serum high in γ-globulin content is involved.

3. Cells cultured in antisera prepared against beef or sheep lenses demonstrate the same cytotoxicity as do those cultured in chick lens antiserum, indicating that the cytotoxicity occurs in response to a tissue-specific, not a species-specific protein.

4. Dissociated cells of whole 7-day chick embryos, cultured in the same antisera against chick or sheep lens, form large normal appearing reaggregates showing that not all chick tissues are destroyed by lens antiserum.

5. The same antisera causing death in cultures of cells of the nervous retina gave no evidence, in immunoprecipitation tests, of cross-reacting antigens in 7-day nervous retina, or cultures of nervous retina sampled at various times after inoculation.

6. Antiserum added to the culture after reaggregation has begun (12 h) has little effect on the reaggregating cells, suggesting that it is dissociation which renders the tissue sensitive to the antiserum.

7. We conclude that an antibody to a tissue-specific lens constituent is responsible for the death of dissociated neuro-retinal cells cultured in antiserum against lens, and that this antigen is not common to all 7-day chick cells. From the effect of lens antiserum on an antigen not demonstrable as a soluble cell constituent, we infer an intermediary level of protein regulation. This antigen seems to be an unexpressed developmental alternative regulated elsewhere than at the level of DNA transcription.

**RÉSUMÉ**

*Effet cytoxique de sérums anti-cristallis sur les cellules dissociées de rétine nerveuse de Poulet en culture de tissu*

1. Des cellules dissociées de rétine nerveuse d'embryon de Poulet de sept jours cultivées en présence de faibles concentrations de sérum de lapin anti-
crystallin de poulet, survivent en petit nombre; cells qui survivent forment de petits réagréats ressemblant à des ‘lentoides’.

2. Des cellules dissociées de rétine nerveuse cultivées dans un sérum prélevé avant immunisation, ou un sérum anti-érythrocytes humains, forment les mêmes réagréats caractéristiques que si elles sont cultivées dans un milieu dépourvu de sérum de lapin; ceci indique qu’aucun facteur toxique non spécifique ne peut être mis en cause.

3. Des cellules cultivées dans des sérums anti-crystallin de bœuf ou de mouton montrent le même réaction cytotoxique que si elles sont cultivées en présence de sérum anti-crystallin de poulet; ceci montre que la cytotoxicité observée est due à la présence d’une protéine spécifique de tissu et non spécifique d’espèce.

4. Des cellules dissociées d’embryons entiers de poulet de 7 jours, cultivées dans les mêmes sérums anti-crystallins de poulet ou de mouton, forment de très grands réagréats normaux; ceci montre que tous les tissus de poulet ne sont pas détruits par un sérum anti-crystallin.

5. En présence des mêmes antisérum qui causent la mort des cellules de rétine nerveuse en culture, les tests d’immunoprécipitation n’ont pas permis de mettre en évidence de réaction de type croisé, ni dans la rétine nerveuse de 7 jours, ni dans les cellules de rétine nerveuse en culture, prélevées à des temps variés après l’inoculation.

6. Un antisérum ajouté à la culture après le début de la réagréation (12 heures) a peu d’effet sur les cellules; ceci suggère que la dissociation rend les cellules sensibles à l’antisérum.

7. Nous concluons qu’un anticorps réagissant spécifiquement avec un constituant du crystallin est responsable de la mort des cellules de rétine nerveuse dissociées et cultivées en présence d’un sérum anti-crystallin; cet antigène n’est pas commun à toutes les cellules de l’embryon de poulet de 7 jours. L’effet du sérum anti-crystallin sur un antigène dont on ne peut démontrer qu’il est un constituant soluble de la cellule, nous fait supposer l’existence d’un niveau intermédiaire de régulation protéique. Cet antigène semble représenter une alternative non exprimée du développement, qui serait régulée à un niveau autre que la transcription de DNA.

We thank Professor Boris Ephrussi for the use of his laboratory facilities and for his stimulating discussions. Parts of this research were done during the tenure of a research associateship (MB) at the Developmental Biology Center of Western Reserve University, for which the investigator is grateful to Professor Howard Schneiderman.

Mrs Robert (Joan) Davis provided excellent technical assistance.

Parts of this research were supported by grants AT (30-1) 3825 (AEC) to A. K. and GM 104444 (N.I.H.) to C. C. and PHS 1Po2Ca10438 NCI. to Allegheny genl. Hosp.

REFERENCES


(Manuscript received 6 August 1968)