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A Tissue Culture Study of Inherited Dystrophy of the Retina in Mice*

By

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With 15 Figures in the Text

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Inherited retinal dystrophy in certain strains of rats and mice resembles in many respects retinitis pigmentosa in man being characterized by a progressive and selective loss of visual cells at a certain age (KEELER; NOELL; LUCAS). Studies in vitro on dystrophic retinae have previously been performed only for short term experiments in organ cultures (LUCAS; SIDMAN). The results obtained usually showed a partial and occasionally a complete degeneration of the visual cells comparable to the changes occuring in vivo. There was no evidence indicating the occurence of a dystrophy promoting extrinsic factor.

However, the total time sequence of changes in the visual receptors from the earliest signs of degeneration to the final destruction of these cells can only be followed in long term tissue cultures using time lapse cine phase microscopy. In tissue cultures it is possible to exclude the influence of extrinsic factors on the retinal cells and also to study the effects of tissue pieces of different origins on the explants from mice with inherited retinal dystrophy. The results had to be carefully compared with those of normal retinal explants under identical conditions.

Materials and methods

Approximately 75 mice of an inbred pigmented C3H strain were used for tissue culture experiments. The animals were all fully afflicted with the typical degeneration of cells of the outer nuclear layer of the retina as determined by histological examination of sections of 68 eyes from C3H mice of different ages. The age of the mice used for tissue culture studies ranged from newborn to three weeks old. The C3H mice showed a high frequency of tumors of the mammary glands as previously described by BITTNER. The stock was fed on commercial pellets and water ad libitum. For comparison, explants were prepared from inbred CBA mice, inbred Swiss albino mice and Sprague-Dawley rats.

Culture technique. The tissue cultures were prepared as previously described in detail by HANSSON and SOURANDER. Briefly, small pieces of retinal tissue, varying from a few cells to whole retinae, were explanted in plasma clots, on collagen-coated cover glasses in Gey chambers, in Leighton tubes or in roller tubes. In some experimental series the explants were allowed to settle and grow on clean cover slides. The nutrient medium concisted of a salt solution with high glucose concentration, calf serum and human umbilical cord serum, with addition of penicillin and streptomycin. A total of about 700 growing retinal cultures, each comprising at least three explants, from inbred C3H mice have been used. For comparison, cultures of retinal tissue from inbred CBA and Swiss albino mice and of cerebellum, heart, kidney, striated muscle and subcutaneous tissue from the three strains of mice have been studied. In a few experiments retinal tissue from both C3H mice and Swiss albino mice or Sprague-Dawley rats have been explanted in close connection or intermixed in the same

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culture chambers. In a few instances the retinal tissue was treated with trypsin, despersed mechanically by drawing it repeatedly into a pipette before being cultured or picked out by free-hand dissection by means of a stainless steel needle under stereomicroscopic observation (HYDÉN).

Observation technique. The cultures were observed daily in inverted microscopes. The neuroectodermal cells usually appeared structurally unchanged and active for about six weeks before marked degeneration was discernable. The longest observation period was for about three months. Suitable structures in living cultures were photographed with phase contrast objectives with a microflash as a light source. A Zeiss microcine-camera with phase contrast and time lapse technique was used for long term studies of the time sequences of cellular changes in living cultures. In order to identify Nissl substance a series of cultures were grown on quartz glass and observed before and after enzymatic digestion by ribonuclease (Worthington Biochemical Co., 0,4 mg/ml at pH 7,6 for 60 minutes at room temperature) using a Zeiss UV II microscope.

Suitable cultures were fixed for histochemical staining (ROMEIS; BARKA and ANDERSSON).

The nerve cells of the inner nuclear layer of the retina were selectively indentified in a fluorescence microscope after retinal cultures had been pretreated with M-223, 10 (2-diethyl-aminoethyl)-9-acridone hydrochloride (Mallinckrodt Chemical Co) at a concentration of 5 to 50 μg per ml nutrient medium, for 5 seconds to 15 minutes either freshly without further treatments or after being frozen and freeze-dried. Living and fixed cultures have also been examined after treatment with acridine orange.

Results

Histological examination of dystrophic and normal retinae

All eyes examined of the inbred C3H mice showed the typical degeneration of the cells of the outer nuclear layer of the retina. The process was characterized by nuclear pyknosis and cell disintegration and was remarkably uniform among the animals. The degeneration of the cells was first apparent on the 11th day after birth, reached a maximum during the 14th to 16th days and was complete within ten days after the onset of the initial changes in agreement with previous reports (DUNN; LUCAS and NEWHOUSE; NOELL; LUCAS). Discrete nuclear changes could, however, be recognized as early as on the 10th day in the central region of the retina. In eyes from CBA mice, Swiss albino mice and Sprague-Dawley rats there has not been observed any animal with partial or complete dystrophy of the retinae. Mast cells have never been recognized in the retinae from C3H of the strain used either in serial sections or in flat preparations.

The general characteristics of the retinal cultures

The general pattern of development of the retinal cultures from C3H mice is essentially similar to that previously described (HANSSON and SOURANDER). It is characterized by a rapid centrifugal outgrowth of mesenchymal cells forming a monolayer around the explants. Processes from nerve cells and neuroglial cells are observed extending far from the tissue pieces. No obvious differences have been seen in the pattern of outgrowth, maturation and survival between cultures growing in plasma clots, on collagen surfaces or directly on clean cover slides.

However, excellent outgrowth and differentiation is much more difficult to obtain with dystrophic retinal tissue than with normal retinal tissue. The tendency of neuroectodermal cells to migrate peripherally and thus to flatten the explant is diminished and this reduces the possibilities for high power observations on living cultures. The outgrowth characteristics of the retinal cultures changed with the age of the animals used for the experiments.

Rosettes were observed in almost every explant in cultures of retinal tissue of C3H mice from birth up to four days old. They consisted of visual cells enclosing a central lumen separated from the cell bodies by a distinct membrane. The lumen was usually bordered by short, filamentous rod-like processes. The nuclei of the living cells, which were arranged perpendicularly to the membrane, were oval and contained several chromatin granules, which formed large aggregates during the progressive maturation process. The rosettes were surrounded by bipolar nerve cells, neuroglial cells and sometimes also by ganglion cells. However, these neuroectodermal cells were often observed to be intermingled among the organized rods.

In explants from C3H mice from five to eight days old, the tendency to form rosettes was progressively diminished and these structures were incomplete. No rosettes or rosette-like structures have been observed in cultures of retinal tissue more than nine days old. Retinal explants have a tendency to curl into a roll with the ganglion cell layer on the inside and the outer nuclear layer on the outside. The rods from C3H mice more than five to six days old showed very little if any migratory activity as judged from cine records.

In retinal cultures from two day old C3H mice there was a rapidly increasing tendency of the visual cells forming the rosettes to degenerate spontaneously after about eight to ten days in vitro. Initially the degenerating cells are irregularly distributed. The limiting membrane of the rosettes were disrupted when the visual cells were destroyed. Two days after the onset of this process only a few photoreceptor cells could be observed scattered throughout the culture and after a few further days no such cells remained. In some cultures, in which the cells from the outer nuclear layer seemed to be less mature, the degeneration process in vitro was initiated one to three days later. The nerve cells, neuroglial cells and mesenchymal cells were unaffected. There was only a slight increase in the number of phagocytes in and around the explants. The time factor was of the same order, i. e. 3 to 24 hours, for the disintegration of visual cells from dystrophic retinae and for those rods which have lost their contacts with the neuroglial or mesenchymal cells.

Rosettes were often formed also in explants consisting of a small number of cells picked from the outer layer of the retina by free hand dissection with a thin steel needle under stereomicroscopic observation. The rosettes were also sometimes found in explants from cells isolated into clumps of about ten cells by pipetting of small tissue pieces prior to the explantation.

By comparison, rosettes were almost always observed in retinal cultures of explants from CBA mice and Swiss albino mice more than five days old. There was no tendency in such cultures for sudden, spontaneous disintegration of the visual cells forming the rosettes. The rod-like processes bordering the lumen in these rosettes of retinal tissue from normal mice were much longer than the rudimentary ones in those from the C3H mice, which sometimes showed no processes at all.

After about two weeks in vitro a few nerve cells and neuroglial cells were recognized in cultures of C3H mice growing on the surface of the monolayer of cells of mesenchymal origin. In cultures from control mice and rats there were a large number of rods in addition to the nerve cells and neuroglial cells.

Cells of neuroectodermal origin

1. Nerve cells. The nerve cells, which include ganglion cells, bipolar nerve cells, amacrine cells and horizontal cells, grew on the surface of a monolayer of cells of mesenchymal origin. There were no apparent differences between nerve cells from normal mice and rats and C3H mice in vitro. The characteristic features of this group of cells of different size and shape included the occurence of Nissl substance, of neurofibrils and of a nucleus containing one nucleolus or a few nucleoli. Nuclear infoldnings, which changed their shape and size quite rapidly, were often observed in nerve cells as well as in rods, neuroglial cells and cells of mesenchymal origin. They also showed cyclic rhythmic contractions, fusion of dendrites by elongation of cytoplasmic protrusions, rarely by simple approximation of nerve cell dendrites, and other signs of dynamic activity in agreement with the results previously described (HANSSON and SOURANDER).

The occurence of ribonucleoproteins in nerve cells has been demonstrated by comparing the ultraviolet absorption of nerve cells at 2570 Å in explants on quartz glass before and after treatment with ribonuclease. The ribonucleoprotein was found to be finely granular both in the cytoplasma and the proximal parts of the dendrites. No quantitative measurements have yet been performed. The nerve cells from the inner nuclear layer have been further identified by their ability to show a very intensive nuclear fluorescence when treated with the drug M-223 in vitro not only after freeze-drying but also in living condition. A faint fluorescence was sometimes observed in the cytoplasm of ganglion cells and in rods in the explants.

2. Visual cells. In mice and rats the visual cells from the outer nuclear layer of the retina consist mainly of rods. The rods have been found to show the same pattern of development as previously described for rods of rats (HANSSON and SOURANDER). The cells from the outer nuclear layer of C3H mice up to four day old formed rosettes during the first day in vitro. This was true both for large explants and for small pieces consisting only of about ten cells which had been picked out from the outer nuclear layer. During the first days in vitro the rods of both dystrophic and normal retina from mice showed signs of progressive development. The nuclei were at first oval with finely granular chromatin which later formed aggregates of increasing size. These large chromatin aggregates formed a smaller number, usually two to six, of increasingly stained conglomerates, at the same time as the size of the nucleus diminished and the shape became spherical, sometimes with one or more infoldings of the nuclear membrane. The rod-like processes from the visual cells lining the rosettes in explants from C3H mice were frequently lacking, if present, shorter than those from control mice. The visual cells from C3H mice were about as mature as those of normal mice and rats as judged by the morphology and size of the cytoplasm and the nucleus. An increasing number of rods in retinal tissue from two day old C3H mice revealed small vesicles in their cytoplasm after eight to ten days in vitro. The oval or spherical nuclei with a few large chromatin granules became irregular in outline and there was a clumping and later on dispersion of the chromatin. The cytoplasmic vacuoles increased in size and the nuclei showed signs of progressive pyknosis. Finally the vacuoles fused, the nucleus and the cytoplasm swelled and the cells disintegrated leaving depression in the cytoplasm of the

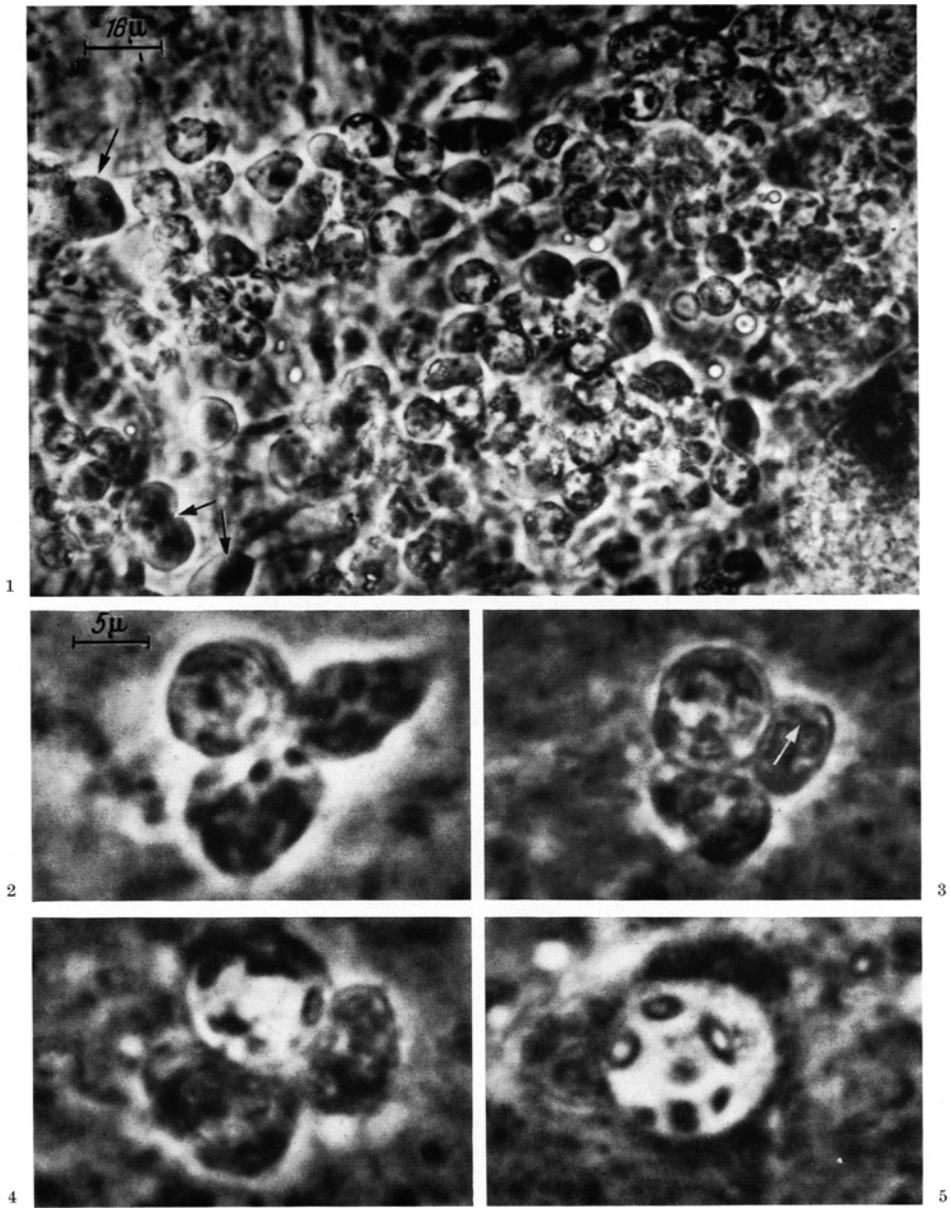
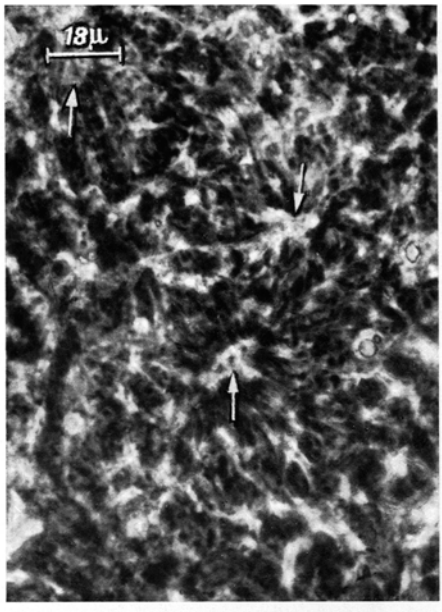


Fig. 1. Rods in different stages of degeneration from a living 10 day old culture from a 3 day old C3H mouse. The homogenous cells in the center of the field are rods which have not disintegrated. In the left margin are nerve cells (arrows). The magnification is indicated by a bar

Figs. 2—5. Selected frames from a phase contrast cine record of a retinal culture after 12 days in vitro from a 1 day old C3H mouse. The intervals are 45, 4 and 17 minutes respectively. Three rods are shown with increasing signs of degeneration including terminal swelling of the central cell. Note the nuclear infolding in Fig. 3 (arrow)

mesenchymal cells, where the rods have been. These events occured whether cells from the pigment epithelium layer were present or not. Time lapse cinematography during the first days in vitro revealed rhythmic pulsatile activity by the



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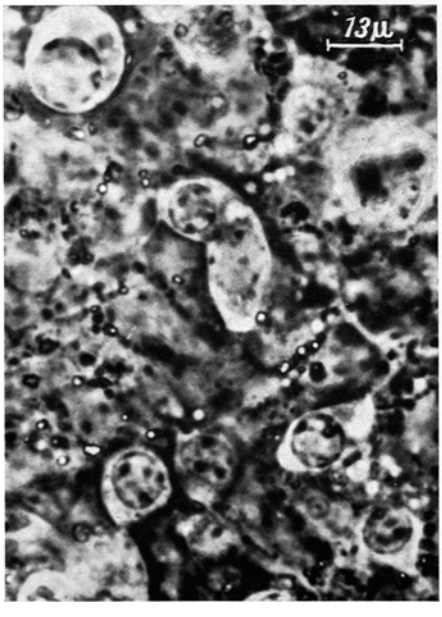

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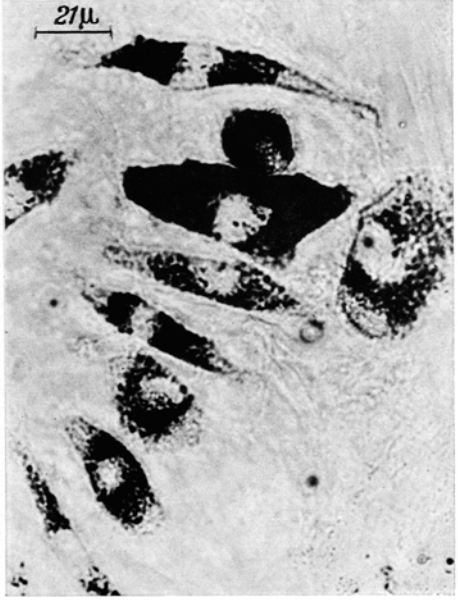

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Fig. 6. Rosette with a central lumen lined by a membranous structure with an inner border of rod-like processes (arrow). Living 6 day old retinal culture from an 1 day old C3H mouse

Fig. 7. Two small multipolar nerve cells in close contact with a degenerating rod (arrow) on a monolayer of mesenchymal cells. Phase contrast of living culture after 11 days in vitro from an 1 day old C3H mouse

Fig. 8. Degenerating swollen rods on the mesenchymal monolayer from a 14 day old living culture from an 1 day old C3H mouse; phase contrast

Fig. 9. Pigment epithelium cells in the center of the field. Living, unstained retinal culture after 14 days in vitro from an 1 day old C3H mouse. Brightfield

rods as well as by the other neuroectodermal cells. Finally, the visual cells ceased this activity less than 24 hours before other signs of degeneration occured.

Retinal tissue from C3H mice five to ten days old were more difficult to culture and the rods did not reveal any apparent pulsatile activity. There were no prominent tendency to form rosettes or rosette-like structures.

3. Neuroglial cells. The neuroglial cells consisted mainly of fibrous multipolar astrocytes of the same type as previously described (HANSSON and SOURANDER).

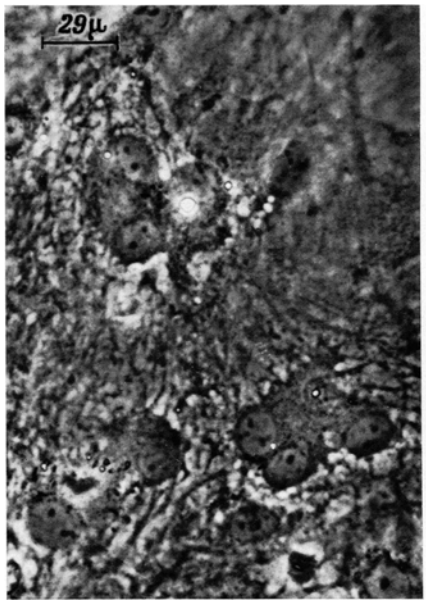


Fig. 10

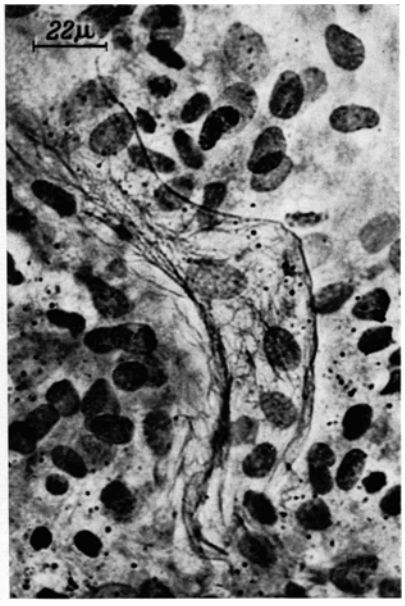


Fig. 11

Fig. 10. Large protoplasmic, multinucleated astrocytes with many short irregular processes on a monolayer of cells of mesenchymal origin. Living 14 day old retinal culture from an 1 day old C3H mouse; phase contrast

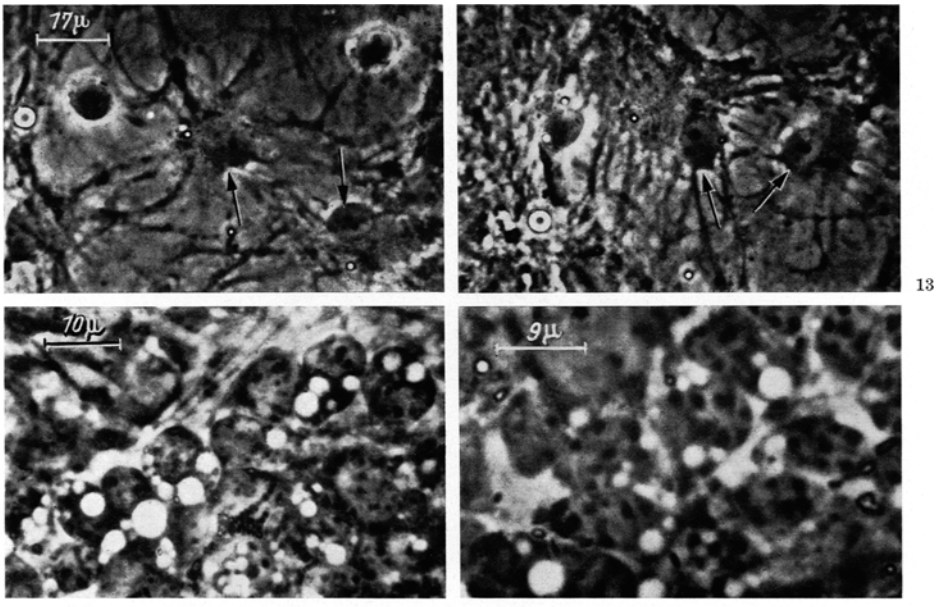
Fig. 11. A network of precollagen on a monolayer of mesenchymal cells, which have light nuclei. The neuroectodermal cells have smaller, dark nuclei. Fixed 8 day old retinal culture from a 2 day old C3H mouse. Gomori stain

The richly dividing processes were of varying thickness and often revealed webbing at the points of branching. The nucleus was well marked, usually oval, with a few quite small diffusely bordered nucleoli and a fine-grained chromatin structure. In older cultures these cells were often observed intermingled with mesenchymal cells in the monolayer.

Another type of neuroglial cell has been observed only in cultures of retinal tissue from C3H mice after at least a week in vitro. This cell type was characterized by a wide, thin layer of cytoplasm. Sometimes there was only one eccentrically situated nucleus, but usually two or three nuclei were present. The numerous processes were much shorter and thicker than those of fibrous astrocytes. The processes also showed a tortuous, irregular course. The cytoplasm was finely granulated, not only in the perinuclear area as generally observed in cells of mesenchymal origin but also throughout the cytoplasm. These cells migrated slowly and pinocytotic vacuoles were formed only at the tips of the broad,

irregular processes. No rhythmic, cyclic pulsations have been definitely observed on cine records. Amitotic nuclear divisions in neuroglial cells of both types have been observed a few times.

4. Cells from the retinal pigment epithelium layer. The cells from the retinal pigment epithelium layer were observed in some cultures as a part of the monolayer, although no pigment cells generally accompanied the explants. However, with a little experience it was possible to pick these cells from their quite firm attachments to the choroid layer. The pigments cells were fairly large cells, often polar,



Figs. 12 and 13. Two large protoplasmic astrocytes with short clumpsy processes (arrows). Phase contrast cine records with about 10 hours interval. There is one more astrocyte in Fig. 12 in the lower left corner. Living 14 day old retinal culture from a 2 day old C3H mouse

Figs. 14 and 15. Degenerating rods with cytoplasmic vesicles. Fig. 14 is a living 10 day old culture from a 2 day old C3H mouse. Fig. 15 shows a retinal culture after 13 days in vitro from an 1 day old C3H mouse. Living cultures, phase contrast

with dark brown, rod-shaped, nearly uniform melanin granules, which strongly absorbed UV-light at 2570 Å. The granules were distributed regularly in the central parts of the cytoplasm but not in a narrow perinuclear zone. The nuclei were usually oval with several distinct nucleoli and smaller than the nuclei of phagocytes and other mesenchymal cells. Like the nerve cells and rods, they revealed only a slight migratory ability in contrast to the other cell types in the cultures. Mesenchymal cells have shown phagocytosis of pigment granules from disrupted pigment cells. This material was soon located in a restricted perinuclear area and then the granules underwent changes of size and shape. The number of cells from the retinal pigment epithelium layer seemed to increase slightly with time in vitro. Mitoses have not been observed in cells from the retinal pigment epithelium layer although they have been observed in other cells with pigments.

Cells of mesenchymal origin

The cells of mesenchymal origin from the retinal vessels grew out to form the thin membranous monolayer. The neuroectodermal cells described above were growing on the surface of this monolayer. Granulated mesenchymal cells of different size and form, which both changed with time, were observed both in the explants and surrounding them. The mesenchymal cells were usually spindle-shaped in the central parts of explants and polygonal more peripherally. The central nucleus, usually single, had several nucleoli of irregular contour. Mitoses were often observed and amitotic nuclear divisions were sometimes also seen, especially in older cultures. Mesenchymal cells in the monolayer formed pre-collagen as demonstrated with the silver-impregnation method of GOMORI.

The number of granulated cells and the number of degenerating cells varied with the general condition of cultures. Their shape and size showed considerable variations from polar to spherical. Most of the granulated cells were characterized by a high motility and very active phagocytosis. The granules of these cells showed even at early stages of differentiation a high activity as compared to other mesenchymal cells in vitro when assayed for acid phosphatases, unspecific esterases and leucylaminopeptidase. A few of these granulated cells, especially the largest spherical ones, showed a metachromasia when stained with toluidine blue at pH 4,5 and dyebinding of Astra blue. These latter cells differentiated from cells of the mesenchymal monolayer, and were interpreted to be mast cells.

Trypsin digestion of freshly isolated retina prior to the explantation destroyed most of the neuroectodermal cells. The cells of the blood vessels were more resistant and grew out forming monolayers often devoid of neuroectodermal cells.

Discussion

The general pattern of development of retinal cultures from C3H mice is in agreement with that previously described for different mammals, especially the rat (HANSSON and SOURANDER). Nerve cells of different types and size show signs of progressive differentiation and grow out on the surface of a monolayer of cells of mesenchym origin. There is no apparent difference in vitro between explants from dystrophic and normal retina during the first days in vitro as judged by their staining properties and behaviour as observed in cine records. However, cultures of tissue from C3H mice are much more difficult to handle in all respects and do not flatten out to the same extent as do normal cultures, presumably because of less cellular migration of neuroectodermal cells.

The rods develop in vitro and form rosettes. In explants from C3H mice less than five days old, the visual cells showed a slight migratory activity during the first days in vitro and the pattern of movements of intracellular organelles was the same as that observed in cultures from normal animals. However, the rod-like processes from the photoreceptor cells, if present, are shorter in C3H strain cultures than those in the control cultures.

The first signs of degeneration are observed in a few irregularly distributed cells from the outer nuclear layer of retinal explants from one day old C3H mice after about ten days in vitro (Figs. 14 und 15). Small vacuoles are formed and increase in size and ultimately seem to fuse into one or two vacuoles while the nucleus becomes pyknotic. Ultimately the rods swell, the cytoplasm resembles a

large vacuole in the monolayer and the nucleus is swollen and hyperchromatic (Fig. 8). Within four or five days all of the visual cells disintegrate into eosinophilic debris. This mode of degeneration agrees in general with that described for in vivo conditions (TANSLEY; DUNN; NOELL; THEILER and CAGIANUT), and in organ cultures (LUCAS; SIDMAN). However, the vacuolization and the final swelling of the rods observed in this study have not previously been reported. The formation of vesicular material of increasing size and number in both the outer and inner segments has been documented in electron microscopic studies on dystrophic retina of C3H mice (LASANSKY and DE ROBERTIS) as well as of rats (DOWLING and SIDMAN). A disorganized growth of rod sacs was observed as well as direct communications from the outer segments into the inner segments and also a very extensive alteration including vacuolization of mitochondria in the inner segments. It thus seems likely that the vacuolization of the visual cells observed in vitro with phase contrast microscopy corresponds to the electron microscopic observations. The differences in the methods used are probably responsible for the fact that these events have not been observed in organ cultures of dystrophic retina.

LUCAS and NEWHOUSE studying retinal dystrophy in mice have pointed out that starvation and n-propylthiouracil retarded the degeneration of the outer nuclear layer while thyroxin accelerated it. An incomplete degeneration was in general observed in eyes in organ cultures (LUCAS; SIDMAN). In this study the rods in the cell cultures started to degenerate at or before the expected time and the last visual cells disintegrated in about five days in well differentiated explants. Subtracting the time for the initial lag phase in vitro, the whole process of disintegration seems to proceed at approximately the same or faster rate as in vivo. The reason for these differences in time course may possibly reflect different time factors for changes in the development of metabolic processes, which are known to occur in dystrophic retina of mice and which are assumed to be the biochemical background for the degeneration of the photoreceptor cells (for discussion see LUCAS). However, it may also reflect changes in the metabolic pattern established in the rods by the cell culture conditions which do not occur in vivo. In any case, the rods appear to be roughly as well differentiated in cell cultures as in vivo.

It has not been possible to influence the timing of events in the degeneration pattern by explantation of normal and dystrophic retina in close connection nor by intermixing, nor by introducing pieces of other organs from normal or diseased animals into the cultures. SIDMAN reported that in organ cultures of eyes, cis-11-retinene influenced the pattern of development in vitro of normal visual cells but not dystrophic ones. Thus, the conclusion that the rod disintegration is independent of external influences (LUCAS and NEWHOUSE; LUCAS; SIDMAN) has been further supported by the results of this study. Probably the degeneration of the rods depends upon some metabolic error with genetic basis. This contrasts to the tumors of the mammary glands caused by virus in the inbred strain of C3H mice used.

In the present investigation the rosettes were established within the first day in vitro in explants both from C3H mice less than five days old and from the normal mice and rats of corresponding age. Rosette-like structures seldom formed in retinal cultures from C3H mice from five to ten days old and there was no

apparent evidens indicating migration and pulsations of the rods or formation of processes despite signs of nuclear maturation. The activity of the diseased rods from up to four or five day old C3H mice diminished about a day before the first apparent signs of degeneration. In rat retina with inherited dystrophy the protein synthesis is reduced six to eight days before the occurence of morphologically changes in histological sections as judged by labelled amino acids (READING and SORSBY). The diminution of cellular activities of the rods in cultures from C3H mice probably reflect such early metabolic changes. A further important conclusion is that it seems to be an essential requirement for the formation of rosettes with normal activity of the rods as such organized structures were not formed by living visual cells lacking visable movements. The rosettes dissolved when the rods degenerated.

Retinal cells isolated into clumps of from a few to ten cells by repeated pipetting prior to the explantation sometimes formed rosettes or rosette-like structures as did a small number of cells mainly from the outer layer of neonatal C3H mice picked out and grown in vitro. Retinal cells isolated enzymatically have been shown to have a strong tendency to clump together in vitro forming highly organized rosettes (MOSCONA; STEINBERG and ROTH). The three last treatments of the tissue prior to the explantation all have in common the elimination of binding forces between the different parts of the retina. The available evidence seems to indicate that the rosettes are mainly formed in vitro by active redistribution of the visual cells in explanted retinal tissue pieces in agreement with results reported previously (HANSSON and SOURANDER). This is not in agreement with ARAKI, who interpreted these rosette-like structures as pseudo-rosettes passively occuring as a result of "curling up" of the retina because of the difference in tension between the outer and inner layers, i. e. with a cell distribution the reverse of that in the rosettes in vitro.

Because the membranous border of the rosettes dissolves at the same time as the rods degenerate, leaving no trace of structures forming the limiting membrane, it seems very likely that it consists of terminal bars between the rods and thus corresponds in vitro to the outer limiting membrane of the retina in vivo. This has also been shown to be the condition in vivo in an electron microscopic study (FINE).

In cell cultures from C3H mice two main types of astrocytes have been observed. The most common one supposed to correspond to Müller's cell is fibrous and of the same appearance as those in cell cultures from CBA mice, albino mice, inbred hooded rats and from albino Sprague-Dawley rats (HANSSON and SOURANDER). The other type is a mostly bi or trinucleated cell with an extensive, thin membranous cytoplasm with many broad, short expansions. It has not been observed before the second week in vitro. This cell type closely resembles the large reactive astrocytes often seen in histological sections in cases of gliosis (SPIELMEYER; PENFIELD; WOLTER; HOGAN and ZIMMERMAN). Similar cells have been observed in explants of cerebral tissue from chick embryos (KERSTING). Amitotic nuclear divisions of neuroglial cells have been observed previously in cine records of rat retinal cell cultures (HANSSON and SOURANDER) and in this study in explants from C3H mice. It seems likely, therefore, that this type of large, often multinuclear protoplasmic cell is formed in vitro by amitotic divisions and modification

from the common fibrous astrocytes. There is no evidence indicating that these protoplasmic cells form from cells of mesenchymal origin.

In retinal cell cultures it is sometimes difficult to distinguish multipolar ganglion cells from the fibrous astrocytes, both of which have many long, slender processes. The nerve cell processes are of more uniform thickness, denser and often longer than the neuroglial ones. The former have no signs of webbing, i. e. thin, delicate membranous expansions at the branching points. Both types of processes often show quite rapidly moving varicosities. However, the staining reactions of these two cell types are different with Nissl stains and acridine orange. The Nissl substance, which consists of ribonucleoproteins, has also been identified by observations of retinal cell cultures on quartz slides before and after treatment with ribonuclease at 2570 Å, 2750 Å and 3000 Å (DEITCH and MOSES). The different types of nerve cells, as recognized by phase contrast microscopy, have all been found to contain ribonucleasesensitive material absorbing at 2570 Å. In contrast, no difference of the absorption before and after ribonuclease treatment could be established by the method used in rods, astrocytes or mesenchymal cells. Thus, this cytochemical test confirms the results of phase contrast microscopy and staining reactions.

The unique distribution of M-223, as demonstrated by MAYER and BAIN, in only the nuclear structures of cells of the inner nuclear layer of freeze-dried retinae is quite remarkable. MURRAY, PETERSON and LOESER, studying cell cultures of spinal ganglion and cerebellum, could not observe any specific binding of the drug to the cells examined in living cultures. However, in the present study the drug seems to bind specifically to nerve cells of the inner nuclear layer in both living and in frozen, freeze-dried retinal cultures. Thus the distribution of these nerve cells in the explants, especially in the rosettes can be clearly distinguished. The stained nerve cells are often observed irregularly distributed in the explants indicating active redistribution of cells in the retinal cultures. The reason for these special staining properties are unknown.

In a previous study evidence has been presented indicating that the monolayer, on the surface of which neuroectodermal cells are growing, consists mainly of cells of mesenchymal origin (HANSSON and SOURANDER). Present support for such an interpretation includes the observation of synthesis of precollagen fibers by the cells of the monolayer. Important further evidence is the fact that cells fulfilling the criteria of mast cells have been observed differentiating from cells of the monolayer. Mast cells have been obtained also in cultures of trypsinized retinal tissue which consisted mainly of disrupted vessels. Mast cells, however, are formed and differentiated in the tissue cultures; no such cells have been observed during the first days in vitro. In a recent report further evidence has been presented indicating the possibility to induce formation of a large number of mast cells by means of biochemical and physical methods causing destruction of neuroectodermal cells in tissue cultures of nervous origin (OLSSON, HANSSON and SOURANDER). The results of the present study strongly support the conclusion that the monolayer consists mainly of cells of mesenchymal origin from vascular tissue structures and not of neuroectodermal cells (HANSSON and SOURANDER). In contrast HERTHA MEYER, in her study on retinal tissue from young chick embryos, stated that in older cultures nerve cells dedifferentiate to form

the monolayer. In cultures of brain tissue the monolayer has been interpreted as formed by cells of neuroectodermal origin (BAUER; POMERAT and COSTERO; NAKAI and OKAMOTO). However, there is no evidence indicating either of these processes in the present study.

This study has proved that the only cell type undergoing a primary degeneration in retinal tissue cultures from C3H mice are the rods. The decrease in the number of bipolar nerve cells and ganglion cells observed in histological sections from dystrophic retinae in vivo has been interpreted as a secondary phenomen (TANSLEY; NOELL; THEILER and CAGIANUT). Thus, this finding from retinal tissue cultures supports the conclusion based on the study of histological sections from dystrophic retinae, that the numerical reduction of the bipolar nerve cells and the ganglion cells in contrast to the degeneration of the rods is not of primary, genetically determined origin.

Summary

Inherited dystrophy of the retina in young inbred C3H mice, which were all fully afflicted, has been studied in tissue cultures in order to examine the pathogenesis and to observe the pattern of development of the disease. Explants of retinal tissue both from C3H and normal mice grew in vitro to become monolayer cultures consisting of differentiated nerve cells, visual cells, neuroglial cells and pigment epithelium cells on the surface of a monolayer of cells of mesenchymal origin. Evidence has been obtained indicating that the progressive degeneration of the rods depends upon a primary disturbance of the cell metabolism. The visual cells from young C3H mice formed rosettes with a central lumen bordered by a membranous structure and rod-like process shorter than those in cultures from normal mice. All the rods started to degenerate at about the same time in vitro and in vivo although the progress to complete destruction proceeded faster in tissue cultures than in vivo. The nerve cells, neuroglial cells, pigment epithelium cells and cells of mesenchymal origin from C3H mice showed no signs of such a sudden spontaneous degeneration, and no other differences as compared to the retinal cultures from normal mice. There was only a slight increase in the number of phagocytes and mast cells during the degeneration of the rods from C3H mice. Large, multipolar cells, resembling hypertrophic, reactive astrocytes, have been observed only in cultures from C3H mice with disintegrated rods.

Untersuchung der erblichen Dystrophie der Retina von Mäusen in der Gewebekultur

Zusammenfassung

Explantate von Netzhaut sowohl der kranken C3H-Mäuse wie normaler Mäuse wachsen in vitro über einer flächenhaften Lage (monolayer) von mesenchymalen Zellen in Form einer flachen Zellage, die aus differenzierten Nervenzellen, Sehzellen, Neuroglialzellen, Pigmentepithelien und Zellen mesenchymaler Herkunft besteht. Die dabei zu erhebenden Befunde weisen darauf hin, daß die fortschreitende Degeneration der Stäbchen bei den erkrankten Tieren abhängig ist von einer primären Störung des Zellstoffwechsels. Die Sehzellen von jungen C3H-Mäusen bilden Rosetten mit einer zentralen Lichtung, die von einer membranartigen Struktur und Stäbchenfortsätzen begrenzt ist; diese letzteren sind aber

kürzer als diejenigen normaler Mäuse. Alle Stäbchen beginnen etwa zur selben Zeit in vivo und in vitro zu degenerieren, allerdings läuft dieser Vorgang in der Gewebekultur schneller ab als am lebenden Tier. Die Nervenzellen, Neurogliazellen, Pigmentepithelien und Zellen mesenchymaler Herkunft der C3H-Mäuse zeigen keine Zeichen einer solchen plötzlichen spontanen Degeneration und auch keine anderen Unterschiede gegenüber den Netzhautkulturen normaler Mäuse. Während der Degeneration der Stäbchen bei den C3H-Mäusen findet sich eine leichte Vermehrung der Zahl der Phagocyten und Mastzellen. Große multipolare Zellen, welche hypertrophischen reaktiven Astrocyten gleichen, konnten nur in den Kulturen von C3H-Mäusen mit zerfallenden Stäbchen beobachtet werden.

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