Thioacetamide-induced Cataract with Invasive Proliferation of the Lens Epithelium in Rainbow Trout

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Summary

Cataracts developed in 90% of rainbow trout maintained on a thioacetamide diet for prolonged periods of time.

The most striking feature in the histology of these lenses was a massive proliferation of the lens epithelium and its transformation to a pleomorphic cell mass which sometimes replaced a great portion of the anterior lens cortex. The diffuse invasion of the epithelial cells, their disorganization, and the presence of forms similar to Darier cells and epithelial pearls suggest a tumorous growth pattern.

The relation of the epithelial proliferation to fiber damage is not clear nor is the role of the carcinogenic properties of thioacetamide in the development of the lens pathology.

Introduction

Blinding opacities of the crystalline lens were observed in high incidence among rainbow trout (Salmo gairdnerii) fed the hepatic carcinogen, thioacetamide, for a prolonged period. These experiments were conducted in the Western Fish Nutrition Laboratory Station at Hagerman, Idaho, in connection with a comprehensive search for the agent responsible for induction of hepatomas in hatchery trout reared on certain commercial dry diets. Thioacetamide has been found to induce liver cirrhosis, hepatomas, and cholangiocarcinomas when fed to rats (1, 8, 9). There has been no previous report of ocular toxicity of this compound.

The eyes of the trout were subjected to biomicroscopic examination and subsequently to histologic, and cytologic studies. The present report is limited to observations on the crystalline lens, since other eye structures were entirely free of abnormalities.

Materials and Methods

Trout at an initial weight of 0.3 gm were placed on a diet which contained 30 mg of thioacetamide per 100 mg of dry ingredients (Table 1). Dietary exposure was continued for 12 months. Other fish of the same age were fed the reference diet without thioacetamide for the same length of time. A 2nd group of 76 fish was returned to the control diet after 12 months of thioacetamide feeding and was examined and killed 2 months later. The fish were lightly anesthetized with tricaine methanesulfonate to facilitate the biomicroscopic examination and were killed either by an overdose of the anesthetic or by decapitation. One hundred six eyes were fixed in Bouin solution, 28 in formalin, 47 in acetic acid alcohol, and 10 were set aside for enzyme studies which are not reported here. In addition the eyes of 6 control fish were removed and prepared for histologic and cytologic examination.

Results

Bilateral cataracts in various stages of development were seen in 87 of the 106 trout. In 8 fish the lens opacities were present in 1 eye only, and in the remaining 11 trout both lenses were clear. Thus the cataractogenic effect of the experimental diet was observed in 90% of the examined trout. The weight of the treated fish at the termination of thioacetamide feeding averaged 58 gm. The average weight of control fish was 137.5 gm. This 2- to 3-fold difference in growth rate indicated severe impairment of some metabolic processes. Attempts to compare the degree of cataract development with growth inhibition demonstrated no clear correlation between the 2 drug effects. The mean body weight was calculated for each of 4 groups of fish: those with clear lenses, and those with early, advanced, and total cataract. Of the animals which had been fed the control diet for 2 months after the experimental period, those with total cataract weighed significantly less than others of the same series. It is possible that lowered food intake due to interference with vision could account for this depressed growth rate. No weight differences were detected among the other groups.

The livers of fish fed thioacetamide for 20 months were examined histologically by Dr. Laurence Ashley of the Western Fish Nutrition Laboratory. Bile duct proliferation and scattered focal necrosis occurred in variable amount and intensity. In rare instances possibly hepatomatous nodules of microscopic size were observed, but not definite hepatomas or cholangiomas. No other signs of profound toxicity were apparent.

Biomicroscopic Examination

Incipient cataracts, as a rule, involved the anterior aspect of the lens, and frequently consisted of a grey plaque at the anterior pole from which radiated feathery, linear, or netlike opacities. In more advanced cataracts, supranuclear and posterior cortical opacities were also present. Lenses with total cataract often were swollen although, in a few instances, shrinkage of the lens with folding of the capsule was noted. Dense, round, or irregularly shaped anterior polar opacities were recognized in...
approximately 38% of the advanced and total cataracts. On the basis of biomicroscopic examination, there were no differences between the cataractous changes in fish at the end of the 12-month feeding period and those examined after an additional 2 months on the drug-free diet.

**Histologic and Cytologic Examination**

Eyes fixed in acetic acid-alcohol were prepared for cytologic study of flat mounts of the Feulgen-stained lens epithelium. Examination of whole lenses under the dissecting microscope before removal of the capsule and the stained epithelium showed that the plaque opacity observed biomicroscopically near the anterior pole often corresponded to areas in which the epithelial cells were densely packed and possibly multilayered. The shape and location of the regions of accumulated cell nuclei pointed to a relationship to the lens sutures. Small disturbances of the epithelial mosaic consisting of tiny round holes in the regular pattern of the cell layer were also seen.

Of 40 Feulgen-stained lenses, 26 showed unusual changes posteriorly. The epithelium of the normal trout lens covers approximately 1/3 of the spheric surface with the meridional rows and posterior curve of the bow lying well behind the equator. In the lenses of experimental trout the epithelial layer frequently approached the pole more closely. The cell-free area at this site assumed an elliptic or kidney shape, the long axis of which corresponded to the linear suture. In many cases the organization of the meridional rows and bow remained apparently normal despite their backward displacement. However, in some lenses with advanced cataract the architecture of the rows and bow was disrupted by a disorganized migration of epithelial cells toward the posterior pole. Masses of Feulgen-positive nuclear debris were occasionally scattered throughout the posterior lens cortex.

Examination of flat mounts of the epithelium confirmed the changes observed under the dissecting microscope. Areas of multilayered epithelium were located near the anterior pole and were often surrounded by a zone of sparse cellular population. Mitotic figures were absent or not recognized in the areas of proliferated cells but were noted in other regions. Mitotic activity could not be quantitatively determined because of the difficulty in obtaining complete preparations.

Microscopic examination of sections from Bouin-fixed and celloidin-embedded eyes did not disclose abnormalities in any of the ocular structures except for the lens. In the normal trout lens, as seen in eyes of control and experimental fish without biomicroscopically visible opacities, the epithelium spread far behind the equator and covered a broad peripheral zone of the posterior half of the lens. Slender bow nuclei formed an acute angle with the surface epithelium and fanned out anteriorly and posteriorly to a greater extent than observed in lenses of mammalian eyes. The cells were characterized by prominent nucleoli which were particularly large in the meridional rows and in the nuclei of the bow region. The most striking histopathologic changes in the cataractous lenses involved the epithelium and the anterior cortex. The fiber lesions did not differ essentially from those in other forms of cataract, but the epithelial proliferation was unusual with respect to both the magnitude and the nature of the response. The abnormal growth behavior manifested itself in several ways. Frequently, 1 or 2 layers of well-organized epithelium were present beneath the surface layer (Fig. 1). The duplication or triplication of the epithelium usually extended over large areas and occasionally enveloped the entire lens. Cells of the deeper layers either were morphologically similar to normal epithelium or were larger and more lightly stained.

The most common form of epithelial proliferation consisted of diffuse invasion of the lens cortex at the anterior pole or near the equator (Fig. 2). The epithelium became multilayered and formed plugs which broke into the cortex. In cross-section, the invading cell mass frequently assumed a triangular shape with base at the lens surface and apex towards the lens nucleus. From this core of infiltration, cells branched out between fibers in the adjacent cortex (Fig. 3). As a rule cells of the surface layer of the plug of proliferating epithelium were increased in height but maintained a regular arrangement. This cell layer covered several strata of fusiform cells which, in deeper parts, appeared transformed into large cells with a medium-sized nucleus, very prominent nucleoli, and irregularly distributed chromatin. The nuclear-cytoplasmic ratio did not appear to be increased and mitotic figures were rare. Some of the cells resembled Darier's (7) corps ronds (Fig. 4) or cellules à manteau (Fig. 5). In several instances the layers of fusiform cells did not intervene, and the surface epithelial cells retained their original morphology until they reached the depths of the anterior cortex. At the deep border, the cells again became round, assumed a larger size, and seemed to drop off into the surrounding amorphous fiber material (Fig. 6); here, they underwent necrobiotic changes. Satellite lesions composed of nests of large, round cells were also observed. Without serial sections it could not be decided whether these cells were separate from or contiguous with the main bulk of proliferated cells.

The infiltrating cell mass contained fragments of capsule and small and large clumps of engulfed fiber material (Fig. 7). Occasionally, the cells were arranged in concentric layers re-
Sembling epithelial pearls (Fig. 8). The cell plug frequently penetrated, and appeared to cut off, a layer of morphologically normal fibers of the superficial cortex (Fig. 2). Since the piercing of normal fiber layers by proliferating epithelium did not result in typical cataractous damage to the adjacent fibers, it is assumed that the invasion took place at the sutures.

In a 3rd type of epithelial proliferation the cells migrated from the surface deep into the cortex in single-layered sheets or strands which bordered and separated large masses of cataractous or liquefied cortex. The various types of abnormal epithelial growth were often combined in a single lens with advanced cataract.

Swelling and disintegration of fibers were minimal in incipient stages of lens opacification, but liquefaction deep in the anterior cortex in front of the lens nucleus did appear early. Hydrops, decomposition, and liquefaction of fibers were seen throughout the anterior and equatorial cortex in advanced cataracts. Pathologic changes in the posterior cortex were usually confined to the presence of bow nuclei around the posterior pole and to moderate swelling of the fibers. Rarely was the damage of the posterior cortex as extensive as that seen in the anterior or equatorial regions. The persistence of a layer of normal cortex underneath the germinative zone of the epithelium indicated that the differentiation of fibers proceeded normally to some extent despite the havoc of epithelial invasion and fiber destruction. Even in those lenses with unrestrained epithelial proliferation and fiber disorganization, small islands of normal fibers were preserved.

Overproduction and aberrant formation of capsule material was frequent and well shown with periodic acid-Schiff (PAS) staining. Circumscribed thickening of the anterior capsule and finger or club-shaped excrescences protruding into the epithelium between apparently normal neighboring cells were the most common changes. In areas of epithelial proliferation globules of PAS-positive material were enclosed between cells (Fig. 9). At times the thickened capsule split into 2 or more layers with compression and destruction of adjacent cells. The deeper strata of multilayered portions of the epithelium were usually lined by a PAS-positive membrane on the surface next to the cortex. Sheets of capsule material bordered the strands of epithelium which grew into the cortex and segregated portions of cataractous or liquefied fiber material. In areas of extensive cell proliferation formation of capsule-like structures was abundant and gave rise to bizarre networks (Fig. 10) similar to those shown in cataracts of mongoloid idiocy (5), Lowe's disease, and the syndrome characterized by aniridia associated with Wilm's tumor (26).

The capacity to produce these PAS-positive basement membranes was limited to cells of relatively normal morphology. The large, rounded cells in the deeper parts of the proliferating cell mass or in satellite lesions did not form such material. In their transformation the cells had lost this characteristic functional property.

Discussion

Abnormal proliferation of the subcapsular epithelium in the human lens takes place in various types of cataract. Anterior polar cataracts are characterized by localized transformation of the epithelium to spindle-shaped cells with intercellular deposition of fibrillar material. In senile cataract the epithelium may grow subcapsularly all around the lens and give rise to the formation of a "pseudo-epithelium." Moreover, epithelial cells appear to be displaced into the cortex in other forms of cataract. Strands of such cells are depicted by Cogan and Kuwabara (5) within disorganized fiber material in cataract of mongoloid idiocy. Among the experimental cataracts aggregation of cells and development of a multilayered epithelium along the anterior sutures or near the lens equator is not uncommon. These localized proliferations have been observed in galactose (6), alloxan (22), mimosine (23), tryptophane deficiency (25), triparanol (24), tetany (17), and contusion (4) cataracts.

None of these forms of epithelial proliferation resembles the invasive growth behavior of the epithelium in thioacetamide-induced cataract. Here the surface cells proliferate and penetrate layers of normal fibers to form an unorganized cell mass which can occupy a great portion of the anterior cortex to the depth of the lens nucleus. The site of predilection for cellular invasion is the anterior pole, but massive epithelial proliferation also occurred at the equator and, rarely, along the posterior surface. The invasiveness of the epithelial cells and their transformation into a variety of abnormal forms suggest a tumorous growth. The occasional arrangement of cells in whorls similar to the epithelial pearls of squamous cell epithelioma strengthens this contention. Moreover, the large, round cell bodies wrapped in crescent-shaped cells resemble structures described by Darier (7) in the precancerous lesions of Paget's and Bowen's disease and dyskeratosis. Normal growth and differentiation potentialities of the germinative zone were usually preserved, even when the cataractous process was in an advanced stage.

Tumors in the intact lens have never been described despite the recognized potentialities of the continuously growing epithelium for malignant proliferation. Mann (15) documented this capacity by implanting dissected lenses of young mice mixed with the carcinogen, methylcholanthrene, into the flanks of related adult mice. Tumors developed 2 or 3 months after implantation and in several instances appeared to be anaplastic carcinomas. They were composed of large, round cells with voluminous nuclei and prominent nucleoli and contained many mitoses. It was concluded that the tumors derived from the subcapsular epithelium. Metastases were observed in the inguinal lymph nodes.

Rafferty (18) observed invasive and destructive growth of the lens epithelium in Rana pipiens when she had mechanically injured the lens with a 15-gauge needle. The epithelium around the wound proliferated deep into the anterior cortex. The growth was self-limiting and eventually regressed completely. The entire lens epithelium responded to the injury with increased cell division as shown autoradiographically by the uptake of tritiated thymidine (19). Attempts to transplant the "tumor" were unsuccessful. In the past Knapp also reported proliferation of the lens epithelium following stab wounds in frogs (12) and, to a lesser extent, in goldfish (13).

Sachs and Larsen (21) listed certain biologic, metabolic, physical, and chemical factors that might prevent in vivo neoplastic growth of the lens epithelium. Although some of these factors may have been operative in forestalling total destruction of fibers by epithelial infiltration in the trout lenses, they did not check the invasion into considerable portions of the anterior cortex (Fig. 11).
The sequence of pathologic changes in the lens is not clear. In some lenses moderate epithelial proliferation was accompanied by marked destruction of the superficial cortex and may have been elicited by an irritating effect of the fiber decomposition. The cataractous fiber debris frequently seen in the depths of the anterior cortex toward which cells of the invading plug converge may be the source of stimulation that releases the epithelium from factors regulating growth and brings about the profuse proliferation and transformation. In other lenses proliferation of the epithelium was seen without any discernible morphologic changes of adjacent fibers. This observation implies a primary change in the epithelium. It cannot be excluded, however, that a metabolic or chemical alteration of the fiber environment triggered the abnormal growth of cells.

The ectodermal origin of the capsule, that is, as a product of the lens epithelium, is well shown in thioacetamide cataract by the consistent association of epithelial cells and capsule formation, even deep in the body of the lens. Thickening and finger-like extensions of the capsule develop without microscopically visible changes in adjoining epithelial cells. Similarly the clumps of capsule material embedded in the multilayered cell masses are not accompanied by abnormalities of adjacent cells. Brini et al. (2, 3) have studied electron microscopically the proliferation of lens epithelium and neoformation of capsule around small necrotic foci in human senile cataract. He concluded that isolation of the cataractous islands by the 2 layers may result in absorption of disintegrated fiber matter. In thioacetamide cataract both proliferation of epithelium and formation of capsule material, although overwhelming, fail to cope with destruction of fibers.

In recent years the lens capsule has been used as a representative of epithelial basement membranes for comparison of its antigenic properties with those of mesenchymal basement membranes. Krakower and Greenspan (14) found that the different basement membranes have at least 2 types of antigens in common. Therefore, overproduction of capsule deserves consideration in studies of the pathology of vascular basement membranes.

Extensive studies have shown that thioacetamide can induce liver tumors in the rat and that pronounced morphologic changes in the parenchymal cells precede the development of neoplasms. The most striking cytologic effects are doubling of the nuclear volume and a great increase in the size of the nucleoli (20). Similar observations have been made in the kidney (11). Investigation of the fate of \(^{35}\)S-labeled thioacetamide has demonstrated that neither the liver nor kidney concentrate the available drug, although the unaffected bone marrow, thyroid, and adrenals do concentrate it to a considerable degree (16). These observations have led to the hypothesis that some detoxified derivative produced by liver or kidney cells is, in fact, the injurious agent (11, 16). The characteristic cytologic changes produced by thioacetamide in the rat liver and kidney were not seen in the cells of the trout lens epithelium after 12 months of drug administration. The absence of these specific injuries suggests that cataract formation might result from some long-term toxic effect of thioacetamide rather than its carcinogenic property. In view of reported nutritional cataracts in trout (10) the possibility of an indirect effect of the drug on nutritional balance should be considered. Whatever the nature of the abnormal growth induction may be, observations on the thioacetamide-fed trout indicate that the lens epithelium possesses multipotential morphogenetic capacities.

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FIG. 1. Double-layered epithelium with the deeper layer lined by a capsule-like membrane. PAS-hematoxylin, × 640.

FIG. 2. Epithelial plug breaking through normal superficial cortex. The surface epithelium changes to fusiform cells and finally to a mass of disorganized cells. H & E, × 250.

FIG. 3. The invading epithelium preserves its morphology until it reaches the deep cortex. H & E, × 400.


FIG. 5. Darier cell in a satellite lesion. H & E, × 560.

FIG. 6. Dropping off of large, rounded cells in the deep anterior cortex. H & E, × 400.

FIG. 7. Irregular proliferation of the epithelium engulfing clumps of cataractous cortical fibers. H & E, × 400.

FIG. 8. Epithelial cells arranged in concentric layers within the proliferating mass. H & E, × 300.

FIG. 9. Heavy deposition of capsule material in proliferated epithelium and lining its deep surface. PAS-hematoxylin, × 350.

FIG. 10. Stalagmite-like formation of capsule matter at the deep surface of epithelium plug. PAS-hematoxylin, × 250.

FIG. 11. Replacement of large part of the anterior cortex by anaplastic cells. H & E, × 160.
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