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HISTOPATHOLOGICAL ANALYSES OF TISSUE SECTIONS OF THE EYES OF INDIGENOUS SPECIES OF MARINE/ESTUARINE AND ESTUARINE FISH

A Report to the

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by

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Histopathological Analysis of Tissue Sections of Eyes of Indigenous Species of Marine/Estuarine and Estuarine Fish

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FOREWORD

The Elizabeth River is the most heavily settled and industrialized major subestuary in Virginia's Chesapeake System. Under increasing use and development since around 1610, its' waters have been exposed to all types of domestic, agricultural, military and industrial contaminants. Sewage treatment plant and industrial outfalls, land drainage, subsurface leaching, dredging and dredge spoil operations, and aeolean transport combine to contribute the hundreds of inorganic and organic chemicals involved (cf. Neilson and Sturm 1978). Its sediments are contaminated by heavy metals, PAHs and all other introduced materials that accumulate and are stored there, with or without chemical transformation. In certain heavily contaminated sites the concentrations of Polynucleated Aromatic Hydrocarbons are probably the highest on the east coast (Huggett, Bender and Unger, In Press and Bieri et al., 1986). Hargis, Roberts and Zwerner (1984) reported

PAH levels as high as 3900 ppm found during analyses of sediment samples taken by Smith-McIntyre Grab from Station 7 (Green Navigation Marker 9) in the Elizabeth and placed in their experimental tanks. A number of specific PAH molecules known for their biological activity, including carcinogenicity, found in those sediments were reported in the same paper (Table I).

If effects of contaminants on individuals and populations cannot be detected in such an hostile environment it would be naive to expect to do so in less-affected estuarine systems. This reasoning and a need to explore further the condition of the sediments, waters and biota of the Elizabeth caused us to undertake a study of pathological conditions found in certain finfish captured there. The work also was prompted by suspicions of possible contamination of nearby estuarine (Hampton Roads and the lower Chesapeake Bay) and coastal oceanic waters (offshore dump sites) by effluents from the Elizabeth and by resuspended and relocated sediments (dredge-spoil) resulting from maintenance and improvement dredging of the ship channel and associated transportation and disposal of the spoil.

Collection of fish began in the Elizabeth River in the summer of 1982. Later the nearby Nansemond River was added as a source of reference collections from a less-contaminated "Control" subestuary. Laboratory experiments designed to investigate the effects of exposure to contaminated sediments and sediment-influenced water under controlled conditions were undertaken in the same year. This was done to see if the effects observed in feral populations could be duplicated in the laboratory and to lay the groundwork for further experimental work on them.

Early field and laboratory observations were directed at discovering the range of effects of exposure to Elizabeth River contaminants on feral

TABLE 1
Concentrations of 20 Selected PAHs
(ppm dry weight of sediment)

PAH species	*23 August*		*7 October*	
	Control[1]	*Experimental*[2]	*Control*[1]	*Experimental*[2]
Benzothiophene	0·014	0	0·000	0
2-Methylnaphthalene	0·025	15	0·009	20
1-Methylnaphthalene	0·037	25	0·015	47
Biphenyl	0·016	8	0·008	16
Fluorene	0·079	75	0·032	137
Dibenzothiophene	0·016	23	0·007	51
Phenanthrene	0·140	268	0·082	468
Anthracene	0·020	85	0·008	125
Fluoranthracene	0·054	230	0·048	324
Pyrene	0·040	155	0·035	226
Benzo[*a*]fluorene	0·007	65	0·010	86
Benzo[*b*]fluorene	0·007	63	0·009	82
Benz[*a*]anthracene	0·008	60	0·009	82
Chrysene	0·024	78	0·028	105
Benzofluoranthrene	0·025	73	0·034	94
Benzo[*e*]pyrene	0·011	33	0·017	35
Benzo[*a*]pyrene	0·008	35	0·009	43
Perylene	0·006	10	0·004	12
Indeno[1,2,3-*cd*]pyrene	0·004	13	0·005	20
Benzo[*ghi*]perylene	0·006	13	0·007	16

1. "Control" aquaria contained York River sediments
2. "Experimental" aquaria contained heavily-contaminated Elizabeth River sediments from Station 7 (217/218).

From Hargis, Roberts, and Zwerner (1984).

fish. As experience and knowledge grew, field sampling and controlled experiments were refined and directed at specific elements, such as prevalence of the different disease responses in fish populations in the Elizabeth and Nansemond.

At first relatively crude examinations of acute toxic and rapidly-developing pathological effects, our laboratory experiments have been increasingly refined to answer such questions as 1) the dosages required to produce chronic disturbances only and not deaths, and 2) the possible influence of ambient laboratory light and sunlight upon the development of cataracts in fish held in aquaria containing PAH-contaminated sediments, among others.

Others at the Institute have studied chemical, immunological and toxicological aspects of finfish responses and even the distribution and abundance of benthic infauna in relation to sediment contamination. Our efforts have been directed at the pathological responses, gross and histological. We have concentrated upon several marine/estuarine and estuarine species whose distribution, abundance, regular availability, ease of capture and marked responses to toxification made them especially useful. These are the estuarine-endemic, bottom-dwellers of restricted distribution (Hogchoker -- Trinectes maculatus and Oyster Toadfish -- Opsanus tau); the marine/estuarine, bottom-tending Spot (Leiostomus xanthurus) and its relatives the Atlantic Croaker (Micropogonias undulatus) and the mesopelagic predator -- the Weakfish (Cynoscion regalis), both of which, like Spot, spawn in the ocean and grow up in the estuaries (hence marine/estuarine). While several other species have been captured, examined and remarked from time-to-time, these five have predominated the field studies. Experimental efforts employed the hardy and easily-handled Spot.

This report is directed primarily at some of the histopathological effects observed thus far and draws upon both laboratory and field observations. It begins with the general responses and then narrows to concentrate upon specific lesions observed, or induced, in the eyes of the several species listed above.

The research continues and many samples are not completely processed or analyzed. Undoubtedly, later findings will cause modification of present concepts; however, certain findings which have been made warrant reporting at this time. This report must be regarded as a forerunner or preliminary document.

INTRODUCTION

In the fall of 1982 70 Spot (L. xanthurus) were exposed to heavily contaminated (by PAHs and other chemicals) Elizabeth River sediments obtained from ER Station No. 7 (ER 217/218) in a flow-through aquarium arrangement. A like number of control fish were similarly exposed to relatively uncontaminated York River sediments. Smaller tanks, receiving only the overflow of sediment-exposed water (contaminated and uncontaminated) from both main tanks, held other Spot. Animals in the contaminated-sediment tank rapidly developed (beginning at day 8) externally-visible disturbances including hyperaemia and petechiae, severe fin erosion and fulminating ulcerations. Many began to die at the same time. Those in the contaminated-sediment overflow tank developed opacities of the lenses (cataracts) of the eyes visible to the naked eye, as well as ulcerations. These results are reported in Hargis, Roberts and Zwerner (1984).

Wild Elizabeth River fish also showed external (gross) pathological disturbances such as hyperaemia and fin erosion. Histopathological examinations revealed skin, gill and liver (including hepatopancreatic) anomalies. However, the full extent of possible pathological effects in feral populations were not recognized until the fall of 1983 when early trial samples and then larger collections from the Elizabeth displayed widespread and well-developed lesions, including not only hyperaemia and severe fin erosion but also cataracts, and to a lesser degree, marked integumental ulcerations. The three nektonic sciaenids (Spot, Atlantic Croaker and Weakfish) and the two benthic species (Toadfish and Hogchokers) were most heavily involved, though cataracts were not seen in the last two species. (Hogchoker eyes generally are too small to examine for these features grossly and gross examinations of Toadfish eyes have been neglected at times.) Intensive sampling followed and the extent (prevalence) of these pathological conditions in these five (and several other) species is deployed in Table II, which includes observations made from 1982 to 1985. Most were collected in 10 months of intensive sampling in 1984. Species other than those shown in Table II were captured and some, such as the Summer Flounder (*Paralichthys dentatus*) and White Perch (*Morone americana*), have been processed and examined but are not specifically mentioned in the results portion of this report since those examined to date have been negative for the lesions of primary interest.[1]

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[1] Over 74,000 individuals have been examined grossly for cataracts and other externally-visible lesions. Not all are reported here.

Table II

Lesions Observed versus Total Individuals
(Elizabeth River Collections)

1. Fin Erosion

Species	Total Examined	No. w. Lesions	% w. Lesions
Spot	42,561	6	0.01
Atlantic Croaker	8,039	20	0.25
Weakfish	5,905	18	0.30
Hogchoker	10,216	182	1.78
Oyster Toadfish*	618	35	5.66

2. Cataracts

Species	Total Examined	No. w. Lesions	% w. Lesions
Spot	42,561	1247	2.93
Atlantic Croaker	8,039	399	4.96
Weakfish	5,905	183	3.10
Spotted Hake	2,983	47	1.58
Gizzard Shad	37	2	5.40

3. Ulcerations**

Species	Total Examined	No. w. Lesions	% w. Lesions
Spotted Hake	2,983	23	0.77
Red Hake	27	1	3.70

* Toadfish erosion involved mostly the pelvic fins.

** Ulcerations have been observed in other species. Tabulations of all lesions are not yet complete.

Totals are less than those cited in the text above since only individuals from the 10 months of 1983 and 1984 in which all eleven stations were covered are included. This allows direct comparison between all sampling periods.

As Table II shows, 70,386 individuals of 8 species were collected, examined and recorded during the period. Also, of the 8 species reported representatives of each bore one or two of the lesions mentioned. Additional samples collected in 1986, but not yet tabulated, appear to confirm the results.

Many investigators have reported hyperaemia and fin erosion in fishes taken from contaminated waters. A number have treated nutritionally-induced cataracts in hatchery and pond-reared fish. But few have commented upon lesions of the eye, especially cataracts, in wild fishes[2]. The possibility that this easily recognized, enumerated and recorded eye lesion might be more specific than other disturbances as indicators of PAH-contamination in feral populations prompted special interest in more detailed research on cataracts in wild and confined fishes. Interest has been piqued further by the possible use of cataracts, along with other externally visible lesions, as bioassay indicators which could show existence of unfavorable environmental conditions even before chemical analyses were able (or available) to do so (Hargis and Colvocoresses, In Press). Consequently, special attention has been focused upon gross and histopathological lesions of the eyes of those susceptible and readily available species

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2 Just recently a report of cataracts observed in collections of the sciaenid Micropogonias furnieri from the coast of Brazil has come to my attention. Apparently the authors, who are using electrophoresis of the lens proteins to investigate populations (Vazzoler and Phan, 1981) have attempted to associate these affected fish with a specific estuary in the region. Further discussion of their findings must await translation of the Brazilian text. It is especially interesting that this report involves another croaker (M. furnieri), a close relative of one of our cataract-susceptible fish, M. undulatus. This aspect and its ramifications deserve further investigation.

exhibiting cataracts in the wild (i.e. Spot, Atlantic Croaker and Weakfish). The bottom-dwellers, Hogchokers and Toadfish, were included in the histological processing, despite the fact that their eyes are not easily observed, because we wished to learn from histological preparations if these endemic estuarine species developed cataracts also. Several other species have been collected and processed as the opportunity arose to broaden our coverage of available marine and estuarine animals. They will be reported elsewhere.

Eyes of individual Spot deliberately exposed to contaminated Elizabeth River sediments and sediment-associated water under laboratory conditions for periods of from 66 to 90+ days also have been examined for cataracts and other ocular effects as well as for hyperaemia, fin erosion and ulceration. Samples from each of these two efforts, field and laboratory, along with preserved and processed larval and juvenile Spot, form the basis of this report.

MATERIALS AND METHODS

Fish from nature were captured by standard tows of a 30-foot semi-balloon trawl with a 1/2 inch stretch-mesh liner. Those from laboratory experiments were taken by dip net. All fish were kept alive until the time of necropsy. Fish obtained from the experiments or the wild were necropsied as soon as possible. Thus, all materials noted were "fresh", taken from animals whose tissues were still alive. Eyes were excised carefully and as quickly as possible. In many instances they still were able to rotate in efforts at orientation even after the head had been separated from the rest of the body.

During necropsy, data such as lengths (SL, FL, TL), weight, sex, estimated age (some), hematocrits, presence or absence of other lesions (i.e. fin erosion, ulcerations) and large external, gill cavity and buccal parasites, general appearance, presence or absence of food and others were secured. Blood smears were made and samples of liver, kidney, intestine and gills were taken and preserved for later processing and examination. Condition of internal organs of many individuals was noted and obvious external and internal lesions were excised and preserved.

In several instances whole, live samples have been taken for immunological studies by Dr . Weeks and Mr. Warriner and their associates. Also, eyeballs, bile and other tissues such as muscle have been taken for microchemical analyses. Results of the latter are not available.

While a great many other tissues and processed materials and analyses have been accumulated and other lesions are being examined, this report is concentrated on the eyes and the ocular materials processed thus far. A great many more eyes have been taken than have been processed and analyzed.

Usually only one "cataractous" eye, the worst, was taken for preservation and processing. Occasionally, where bilateral cataracts existed (with or without differences) or some other interesting condition and microscopic analysis of each seemed useful in diagnosis, both eyes were taken. Samples were then turned over to the histological laboratories at VIMS and (later) NEI/NIH for processing. Processed eyes were transferred to the pathologists for examination.

Dietrich's and Bouin's (mostly the former) fixatives were employed for tissues processed at VIMS. In most cases duplicate samples were fixed and preserved in NBF. Paraffin-embedded materials were sectioned and processed into slides for histological examinations now underway as part of another phase of this research program. A few of the samples were of lenses alone which were excised, fixed in Dietrich's fixative, processed into paraffin, sectioned and stained with hematoxylin and eosin (H&E). A larger number of entire eyeballs were excised and processed in like fashion. Though yielding useful preparations, these early attempts in our (VIMS') histological laboratory were not entirely successful. Specimens were brittle, lenses shattered and tissues were distorted, especially in sectioning (cf. Figures I and J).

On the advice of specialists at the National Eye Institute of the National Institutes of Health (NEI/NIH) later samples of eyeballs were excised whole, killed and fixed in a chemical series involving a glutyraldehyde mixture as a fixative, a cacodylate rinse, immersion in NBF for 24 hours at room temperature and preservation in chilled NBF. They were then transferred to NEI. At NEI specimens were placed in a plastic embedding medium and sectioned on a special microtome. All specimens had been examined for cataracts and other lesions at the time of necropsy, prior to excision of the eyeballs. Observations were recorded. In many cases the presence or absence of cataracts detected upon gross examination was confirmed by stereomicroscopic examination of the intact eye at the time of necropsy.

In our (VIMS') analyses any opacity visible to the naked eye in the lens, from minute pinpoints to general cloudiness, is termed a cataract. Since we are unable to determine whether all of these opacities actually interfere significantly with the passage of light through the affected lens and cause optical distortions, this diagnosis is morphological and not functional. In like fashion any abnormal disturbance visible under brightfield microscopy in the various components of the lens such as vacuolation of the cortex (whether equatorial or poleward) and nucleus, distortion of growth of the lens fibers, or aberrant epithelial growth is considered a cataract in our diagnostic procedures. Abnormality is based upon morphological differences in those features of lenses and other eye tissues seen in the majority of "Control" individuals (i.e. those from relatively clean waters which appear "normal" upon gross examination and whose tissues are histologically "clean") of any species in question. Examination of the photographs in Figures C-K should yield an idea of the appearance of normal and abnormal tissues (Figures C through E represent the "normals" and F through K, the "abnormals").

A total of 319 slides bearing 1156 sections (most slides of adult tissues have 4 sections each) have been produced from the preserved lenses or eyes (or whole fish in the case of the larvae and juveniles). It is obvious that most of the ocular samples are actually represented by very few sections. In only a few cases have more than 4 sections (ca. 2-6 μ in thickness) been cut and mounted per eyeball. Since the fish eyes under study regularly exceed 8 to 10 mm in the anterior-posterior dimension (length) these sections do not represent much of the entire eyeball or even of the lenses, which may themselves, be as much as 3-4 mm in diameter or more. Hence, small eyeball and lens lesions may be lacking in the sections and not available to the microscopist and the results correspondingly limited, representing a minimum. In other words, an histological negative may not actually prove that the lens of the individual in question did not have the "cataract" (as defined above) which was observed and recorded during gross examination at the time of necropsy! This disparity in extent of the actual material observed may account for some of the differences between the numbers obtained at the time of the gross observations (whole eyes) and those obtained from reading the sections (actually representing very small portions of the eyes and lenses).

After processing, the slides were read under the brightfield microscope and the results recorded. These results are described and detailed below in the Results and Discussion section, including the similarities and differences between the observers conducting the gross necropsy and those reading the slides (i.e. the NEI/NIH opthalmologist and the VIMS pathologist). And, as shown in Tables III and IV, there are differences.

The early eye preparations processed at VIMS, mentioned above, were examined at VIMS (by Hargis and D. E. Zwerner) and then by Dr. Ronald C. Riis, Opthalmologist in the Veterinary Program at Cornell University. The NEI-processed slides were studied by opthalmological specialists there and then transferred, along with the records of their findings, to VIMS for further examination. Consequently, all slides have been examined by two pathologists, one an opthalmological scientist. Thus, there have been three points of observation on each individual fish, the gross examination at necropsy and two microscopic examinations of each section made by at least two different diagnosticians.

Differences between the results of the diagnoses by NEI/NIH personnel and those of VIMS and the possible reasons for them are discussed as the results of the tabulations are presented below. There are some technical reasons which may contribute to uncertainty in diagnosis which should be treated here. First, in some sections, lenses or parts of lenses are missing. These may have been treated differently by the diagnosticians. Second, in <u>all</u> of the eyes sectioned at NEI after the first group, the epithelia and capsules are separated from the underlying cortex of the lens.

Diagnoses may have been affected by this phenomenon since it is difficult to distinguish vacuolation and other disturbances of the outer cortex and the epithelium produced by the process of "pulling-away", tearing or separation of the epithelium and the capsule from the fibers of the cortex (and from each other) from those produced by intoxication. Third, a possible technical (procedural) reason for the variances between the diagnoses of the two groups is a difference between conditions under which the diagnosticians (NEI) operated when reading the slides. The NEI operators had a summary detailing the species identities and place of capture (i.e. uncontaminated or contaminated stations (for the ferals) and aquaria (for the experimentals) available to them at the time the diagnoses were made and/or the data were recorded. The VIMS pathologist read his slides and recorded his data in the blind, knowing only the NEI processing number. He did not know the original VIMS necropsy number at this point. Thus, he knew neither the species identity nor the site of capture nor the condition of feral or experimental exposure (i.e. whether exposed to sediment-borne contaminants or not)! It is possible that the NEI opthalmologist had the species identity and the station of capture or experimental source readily accessible while reading the slides and/or recording the results and that such background knowledge assisted him in deciding between alternative diagnoses. Whether this possibility resulted in some of the diagnostic differences shown in Tables III and IV and discussed below is not known. Procedural and technical differences and their effects on the data must be settled by comparative review involving all diagnosticians, who participated.

The slide collection undergirding the greatest bulk of this report (i.e. except for the portions referring to the larvae and juveniles) consists of ocular materials from three separate groups as follows: 1) a controlled experiment designed to study the lesions induced by exposure of Spot to PAH-contaminated ER sediments and sediment-exposed water, 2) feral individuals captured at 4 stations in the Elizabeth River [these were the heavily-contaminated ER Station No. 7 (or 217/218) and two stations relatively close by (ER Station Nos. 9 and 10 just upstream of Station 7) and, one station far downstream (ER Station 3, which is not heavily contaminated by PAHs)] and 3 stations in the baseline or reference Nansemond; and, 3) laboratory-reared and feral larval and juvenile Spot (for background and developmental studies of their eyes). The results from each of the sample groups are reported below. The data from the laboratory-exposed fish are presented in Table III and those from the wild specimens (from the Elizabeth and Nansemond Rivers) are in Table IV.

In the presentations of feral fish (exposed in the wild) animals from ER Station 7 and 10 are considered to have been exposed to the heavily-contaminated sediments while those from ER Station 3 are not. Hence individuals from ER Sta. 7 and 10 are grouped together (Table IV) as "Contaminateds". Those from ER Sta. 3 and the Nansemond are grouped as "Uncontaminateds" and have at times been considered as "Field Controls" as well, especially those from the Nansemond, the reference subestuary nearby. Experimental exposures, presented in Table III are more straightforward. Animals exposed in the laboratory to PAH-contaminated ER sediments (from ER Station 7) are the "Contaminateds". Those exposed to relatively PAH free York River sediments are the "Controls".

The research effort described above has concentrated upon young-of-the-year and older fish, those sampled by our trawl. Since the fish may well have been exposed at earlier stages we have determined to examine post-larval and juvenile materials. To begin laying the groundwork for later studies in the development of cataracts and other eye disorders and to establish normal "baseline" conditions in young fish a series of larval and juvenile (wild and laboratory-reared) Spot (*L. xanthurus*) have been taken under study. These slides, prepared by Dr. John J. Govoni (of NMFS, Beaufort) and transferred to us for this study are under examination here. Thus far, some 72 slides of several individual Spot larvae and juveniles have been examined and are reported below. Many more have yet to be read.

Diagrammatic representations of the generalized teleost eye and lens, including the orientation and nomenclature of the fish lens developed for the study are presented in Figures A and B to aid in comparative studies of the microphotographs presented in the rest of the figures as well as the text. Figure B was especially developed to deal with the fact that fish eyes are usually not oriented forward but laterally. This makes it necessary to utilize different terms for the axes and other orientational nomenclature for fish eyes from those commonly employed for upright-standing, binocular-visioned humans.

RESULTS AND DISCUSSION

Results of Experimental Exposures

Introduction

The experimental populations involve several individuals from groups of wild-collected, laboratory-acclimated Spot. Four batches (2 "Controls" exposed to "clean" York River sediments and 2 "Contaminateds" exposed to

PAH-contaminated Elizabeth River sediments from ER Sta. 7) have been processed thus far.

The "Uncontaminateds" or "Controls"

According to Table III, among the 40 slides representing the individuals reported here from the group that was exposed to flowing water in the Uncontaminated-Sediment tanks over the period of 66 to over 90 days (i.e. the "Uncontaminateds" or "Controls") none of the individuals were recorded as displaying cataracts to the gross examiner at the time of necropsy. Only one was recorded as a "Questionable", ("?")! Review of the original necropsy records revealed that both eyes of the fish involved, presumably the corneas of that individual, were cloudy. This evidently prevented the observer from being certain about a diagnosis regarding cataracts.

The gross necropsy numbers of "No Cataracts" and those of the NIH examiners numbers compare very closely. Those of the VIMS examiner diverge somewhat. For example, the NEI specialist reported that all of the slides of this group of animals (100%) were negative for cataracts among those sections containing lenses, including the individual with the "cloudy eyes". On the other hand, the VIMS examiner recorded 33 (86.8%) negatives and 5 (13.2%) Questionables, "?", among the 38 (of an original 40) whose lenses remained intact after sectioning. [Of the 2 eyes whose lenses were missing one was lost in processing and the processed eye of the other individual was aphakic (without a lens) when sampled[3]. In this particular individual fish the other eye did have a lens which showed no cataract at necropsy and was

3 A phakia is a condition observed occasionally in samples from "clean" as well as "contaminated" waters. Its significance, if there is any besides being a teratogenic or ontogenetic abnormality, is not known.

Table III

Results of Experimental Exposures (ERXIV)

Group	Slides	Sects.	Sex ♂♂	Sex ♀♀	Sex ?	Gross. Exam. Cats	Gross. Exam. ?	Gross. Exam. No Cats	NEI/NIH Cats	NEI/NIH ?	NEI/NIH No Cats	VIMS Cats	VIMS ?	VIMS No Cats
YR A	25	99	11	14	0	0	1	24	0	1[1]	24	0	3[1]	22
YR B	15	60	6	7	2	0	1[2]	14	0	1[3]	14	0	4[3]	11
Uncontam. Sed.	40[5]	159	17	21	2	0	2	38	0	2	38	0	7	33
% Total			42.5	52.5	5.0	0	5.0	95.0	0	5.0	95.0	0	17.5	82.5
ER A	13	52	6	7	0	0	0	13	0	2[3]	11	1	6[3,4]	6
ER B	15	58	7	8	0	0	4[2]	11	0	0	15	1	3	11
Contam. Sed.	28[5]	110	13	15	0	0	4	24	0	2	26	2	9	17
% Total			46.4	53.6	0	0	14.3	85.7	0	7.1	92.2	7.1	32.1	60.7

1 Lens missing. One eye aphakic, which was the only eye sectioned in this exercise.
2 No comment on cataract on necropsy sheet, probably "No Cat". Included as "Probable or Possible" however.
3 One or more lenses lost in processing. Included as "?" -- "Questionable" or "Possible".
4 Possible cataracts, individuals whose lenses or parts of lens remain in the slides.
5 No. of individual fish involved coincides with the no. of slides.

recorded among the "No Cataract" totals on the necropsy sheet.)] Of the 2 "Questionables" ("?") at the time of examination (necropsy) 1 had "no comment or notation" on the data sheet and was automatically recorded as a likely "Questionable" when the data were pulled together for summary and tabulation. Hence, only 1 (2.7%) of the 37 recorded as having been examined for cataracts in this group is regarded as being a likely "Questionable". Therefore, for all practical purposes, the "Controls" or "Uncontaminateds" in this experiment were found to display no cataracts!

The "Contaminateds".

The results of our gross examinations of the eyes of whole animals and of the ocular tissues from these groups and individuals experimentally exposed to contaminated Elizabeth River sediments (i.e. the "Contaminateds") indicate that at the time of necropsy 2 [of the 4 reported as being uncertains ("?")] were not fully recorded in the data sheets, hence had to be recorded as "Uncertains" or "Possibles", (i.e."?"). The remaining 2 "Uncertains", which were recorded as such ("?"), represent 7.7 per cent of the total of 26 reported in this entire grouping. The other 24 (or 92.3%) showed No Cataracts at time of gross examination (necropsy). Results of NEI examinations were even more positive: Of the 2 slides listed as "Uncertains ("?") in the tally of NEI results both had lenses or significant parts of lenses missing. Removal of these 2 samples yields a diagnoses of 26 (100%) with "No Cataracts"! The VIMS results are somewhat different indicating that, after removal of the 2 samples with incomplete lenses, 7 with questionable cataracts (recorded as "?"), or 26.9%, remain. Further, our diagnostician recorded 2 of the 26 remaining (or 7.7%) as having cataracts and 17 (65.4%) with no detectable cataracts.

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The "Contaminateds".

The results of our gross examinations of the eyes of whole animals and of the ocular tissues from these groups and individuals experimentally exposed to contaminated Elizabeth River sediments (i.e. the "Contaminateds") indicate that at the time of necropsy 2 [of the 4 reported as being uncertains ("?")] were not fully recorded in the data sheets, hence had to be recorded as "Uncertains" or "Possibles", (i.e."?"). The remaining 2 "Uncertains", which were recorded as such ("?"), represent 7.7 per cent of the total of 26 reported in this entire grouping. The other 24 (or 92.3%) showed No Cataracts at time of gross examination (necropsy). Results of NEI examinations were even more positive: Of the 2 slides listed as "Uncertains ("?") in the tally of NEI results both had lenses or significant parts of lenses missing. Removal of these 2 samples yields a diagnoses of 26 (100%) with "No Cataracts"! The VIMS results are somewhat different indicating that, after removal of the 2 samples with incomplete lenses, 7 with questionable cataracts (recorded as "?"), or 26.9%, remain. Further, our diagnostician recorded 2 of the 26 remaining (or 7.7%) as having cataracts and 17 (65.4%) with no detectable cataracts.

Examination of the original and derived (summary) data sheets reveals that the VIMS examiner recorded at a much greater level of detail than did at NEI diagnostician, using more categories. It is possible that the fact that he was reading "in the blind" without foreknowledge of the identity of the specimen being diagnosed or its history (i.e. whether "Contaminated", "Uncontaminated" or "Control") or other technical or procedural factors described in the Materials and Methods section accounts for part of the divergences in diagnoses. Also, it is entirely possible the VIMS examiner is more uncertain than the NEI specialist of what actually constitutes a cataract in such preparations. Additional possibilities are; 1) he is "straining" in making the diagnoses, 2) he is taking greater care in diagnosis, or, 3) that the definitions or criteria in use at VIMS are different than those at NEI. [(Such differences in criteria have been noted in the diagnoses of conditions in other tissues made by hospital clinicians, who regularly work with human tissues and clinical concepts of disease, and VIMS researchers. In fact, confusion in terminology and definitions of lesions in fishes as well as higher vertebrates was one of the topics of a workshop at VIMS in 1984 (Hargis, 1984). Differences existed not only between fish pathologists and their veterinary and human (higher vertebrate) confreres but also between the fish pathologists, themselves.)] Re-examination of the sections and comparison of definitions and diagnostic methods and data between our two groups (NEI and VIMS) will be necessary to address some of the possibilities described immediately above and in the Materials and Methods section.

Considering the fact that the differences between the results of the two diagnosticians occur more strongly in the diagnoses dealing with those groups of animals exposed to contaminated Elizabeth River sediments (the

"Contaminated") than in the "Uncontaminated or Control" groups in Table III, these results are quite plausible. They may well indicate that cataracts actually were induced in the experiment (albeit very small and difficult to detect) by exposure to the contaminated Elizabeth River sediment and that they were detected in the sections by the VIMS observer but not by the NEI examiner or by the gross examiner. Induction of cataracts was an intention of the experimenters when the experimental design, including the dosage and conditions of dosing, was developed.

Results of Examinations of Wild Samples

Introduction

The eye samples taken from feral populations in the Elizabeth River at a series of 4 stations (ER 3, ER 7, ER 9 and ER 10) up the mainstem from high salinity stations downstream into low salinity areas upstream and passing through that reach of the river in which sediments are heavily contaminated by PAHs from petroleum and creosote plant spills and drainage [i.e. ER Station 7 (otherwise known as ER 217/218)] have been processed and read either at VIMS or NEI. The results of the comparison of analyses of the Elizabeth and Nansemond River (the reference estuary) collections are detailed in Table IV.

The Groupings of Samples -- "Uncontaminateds" and "Contaminateds".

The Nansemond is the reference river and Station 3 in the Elizabeth River is far downstream from ER Station 7 and, to our present knowledge, is relatively free of PAH-contamination in its sediments. For this reason the Nansemond and ER Sta. 3 are grouped together as the "Uncontaminateds" in Table IV as explained above in Materials and Methods.

Table IV

Results of Examination of Eye Tissues from Feral Sciaenids.
(Numbers in each category represent only those sections sufficiently complete to be read)

			Sex			Gross. Exam.			NEI/NIH				VIMS			
Group	Slides	Sects.	♂♂	♀♀	?	Cats	?	No Cats	Cats	Prob.	?	No Cats	Cats	Prob. Poss.	?	No Cats
Nansemond[1]	12	48	5	6	1	1[4]	3	8	0	0	0	12	0	0	4	7
ER Sta. 3	17	68	7	10	0	1	0	15[6]	0	0	0	16	2	1	7	7
"Uncontams."	29[10]	116	12	16	1	2	3	23	0	0	0	28[5]	2	1	11	14[5]
% Total			41.4	55.2	3.4	7.1	10.7	82.1	0	0	0	100.0	7.1	3.6	32.3	50.0
ER Sta. 7[3]	17	68	5	12	0	16[6]	0	0	4	1	2	8[6,7]	11	3[7]	1	0[8]
ER Sta. 10	14	56	10	4	0	13	0	1	5	0	0	5[9]	8	1	0	5
"Contams."	31[10]	124	15	16	0	29	0	1	9	1	2	13	19	4	1	5
% Total			48.4	51.6	0	96.7	0	3.3	36.0	4.0	3.0	52.0	65.5	13.8	3.4	17.2

1 Nansemond samples contained only 2 sciaenids, Atlantic Croaker and Spot. All others had 3, including Weakfish.
2 ER Station 3, the far downstream ER Station, was combined with Nansemond as "Controls" because PAHs are far lower in sediments there than at ER7, and likely exposures of fishes captured there seem much less.
3 ER7, the station or zone of heavy sediment-borne PAH's and ER10, just upstream, are grouped because damaged fish samples from ER7 and ER10 are considered as "Contaminateds" or "PAH exposed."
4 Cataract "left eye apparently".
5 Diagnosis technically impaired, 1 slide.
6 No comment on diagnosis, 1 slide.
7 1 lens missing. VIMS probables include 1 whose lens is mostly missing but whose epithelial and capsule remnants indicate probable cataract.
8 No comment on 2 by VIMS observer.
9 No comments on 4 by NEI/NIH observer.
10 No. of fish involved coincides with the no. of slides.

Elizabeth River Station 7 is that reach of the river where the sediments are very heavily PAH-contaminated and where the animals are exposed to the contaminant. ER Station 10, next but one (ER Sta. 9) to ER Station 7, seems to be a marshalling area for animals damaged at ER Station 7 or is itself a station where further environmental damage is inflicted or where "damage effects" resulting from exposure at ER Station 7 mature or are augmented. Extensive field sampling involving a total of over 45,000 animals of several species captured over the calendar years 1983 and 1984 confirms this relationship between damage effects in animals in ER Sta. 7 and ER Sta. 10. For purposes of this report ER7 and 10 are being called the "Contaminated and Contamination-influenced Zone" and the individuals are termed "Contaminateds" in Table IV. ER 9 samples, not all processed, are not included here.

As can be seen from Table IV, 29 individuals (represented by 29 slides) of 3 sciaenids (Atlantic Croaker, Weakfish and Spot) have been grouped for simplification. This grouping of 3 species precludes close comparisons with the experimental data represented in Table III which includes only one of these species, the Spot. (However, all animals were recorded separately elsewhere and data can be accumulated by species for future comparisons, if necessary.)

The "Uncontaminateds" or "Controls" versus the "Contaminateds".

As would be expected, Table IV shows that collections from the Nansemond (reference estuary) and (the far downriver) ER Station 3 the "Controls or Uncontaminated", clearly exhibit fewer recorded cataracts [as well as other eye (and general body lesions) not detailed here] than those from ER Stations 7 and 10 -- the "Contaminateds".

For example, under gross examination at necropsy the "Uncontaminateds" revealed that 23 (82.1%) of the 28 individuals recorded from the Nansemond and ER Station 3 showed "No Cataracts", while there were 3 (10.7%) questionables and 2 (7.1%) positive for cataracts. In strong contrast, the specimens from "Contaminated" stations (ER Stations 7 and 10) from the Elizabeth reveals that 29 of 30 (or 96.7%) animals had "grossly" detectable cataracts: Only 1 (3.3%) had "No Cataracts". Clearly, association of the fish with the contaminated sediments at ER Stations 7 and, perhaps also (cf. p 17 above) ER Station 10, produced cataracts (and other ocular disorders as described below) while those from ER Station 3 and the Nansemond had far fewer ocular problems.

Comparing the results of the gross examinations against the histopathological diagnoses and those produced by the two groups (NEI and VIMS) with each other shows some interesting differences. In making these comparisons it is worth noting that the numerical totals in each group do not agree with the grand totals or with each other because in some cases important parts of the eyes were lost during processing (technical impairment) making diagnosis of those eyes for cataracts impossible. In others the individuals making the diagnoses failed to make clear notations or neglected them entirely. Totals were adjusted to eliminate these unknowns: They and the percentages are accurate. The best points of comparison are the percentages! The same factors operated in producing the data from the controlled experiments detailed in Table III as discussed above.

As can be seen, the gross diagnoses made at time of necropsy seem most unequivocal with one exception (NEI in the "Uncontaminateds" grouping). The cause of this anomaly is unknown as yet. For example, gross examinations of the "Controls" or "Uncontaminateds" (Nansemond and ER 3) yielded 23, or 82.1%, "No Cats.", 3, or 10.7%, questionables ("?") and 2 (7.1%) "Cats" while the gross examination of the "Contaminated" (ER 7 and 10) yielded 29 of 30 (or 96.7%) with "Cats" and 1 (3.3%) with none.

As the results included in Table IV show, there were (again) differences between the results of the diagnosis of the two histopathology groups as well as between them and the "gross" results. The results of examination of the sections of the specimens from the "Uncontaminated" stations by VIMS' pathologist appear more closely to match those of the gross examinations in "positives" for cataracts (2 and 2) respectively and "uncertains" (11 and 3) respectively, while there are none in either category in the NEI diagnosis. Possible reasons for these differences are discussed above in reference to Table III and in Materials and Methods. As indicated there these results must be re-examined jointly to resolve these questions.

In comparing the specimens from the "Uncontaminated" composite samples with those from the "Contaminated" Elizabeth River stations (ER Stations 7 and 10) in Table IV it is clear that the numbers switched from the "?" and "No Cat" categories to the "Probable Cataract" and positive "Cataract" categories, respectively. This would be expected if there was a direct connection between exposure of the fish to the Contaminated-sediments and Sediment-exposed waters and fishes of ER Stations 7 and 10 of the Elizabeth versus the "Uncontaminated" (or "Less-contaminated") sediments and waters of the Nansemond and ER station 3. Along with the gross results reported by others (Huggett, Bender and Unger, In press and Hargis and Colvocoresses, In Press), our histopathology results indicate that there is a direct connection between PAH contaminated-sediments and sediment-influenced water found at ER Station 7 and the presence of cataracts, and it is a positive one.

On the basis of the data in Tables III and IV it seems possible to conclude that the gross examination may be as, perhaps even more, sensitive as the histopathological processing and diagnostic techniques employed. Care must be taken in interpretation of this "preliminary, possible" finding at this point, however, since the two histopathological examinations have produced disparate results between each other and also with those of the gross examination. As mentioned elsewhere one clear difference exists, the entire eye and most or all of the lens are being inspected carefully when the gross examinations are made at time of necropsy, while most of the histopathological diagnoses accomplished to date have been based only upon 4 sections, each but a few microns in thickness -- a very small portion of the eyeball and lens.

Examples of Other Lesions of the Eyes

This report has concentrated upon cataracts since they are the eye lesions most readily noted and enumerated under conditions of gross examination at time of necropsy and they have been the topic of special focus of our field and laboratory studies since the early days of this program. However, while making the histopathological examinations ocular lesions other than cataracts were observed early on. In recent analyses these lesions have been noted, classified and recorded. These include 1) thickening of the lens capsule, 2) hemorrhagic conditions of the vitreous (more common than in the aqueous) and aqueous chambers and their humours, 3) engorged choroid blood vessels (often distended all the way into the iris),

4) engorged choroid rete, 5) enlarged choroid spaces and, 6) distorted retinas. In some specimens one annulus seems to be enlarged more than its companion and the epithelium of some lenses is "tumorous". Examine and compare the various Figures F-I and their legends for these features.

It is not possible to carefully characterize and detail the appearance of retinas influenced by Elizabeth River contaminants at this time since several species (each with somewhat different retinal morphology) are involved and the normal morphology of each is not well-known as yet. In the Spot examined to date contaminant-influenced retinas often seem "thinner" overall and display more derangement of the various layers than those of individuals not exposed: In some, certain areas of the retina seem abnormally thickened or swollen (Figure F). The significance of the "bloodiness" (hemorraghes) of the vitreous and aqueous chambers and their fluids which also seem to accompany toxification is even more elusive. The appearance and significance of these "lesions" will have to be the subjects of later investigations and reports.

The enlarged choroid spaces, engorged choroid blood vessels and engorged rete commonly accompany severe cataracts in the same eyeball. There is a direct connection in at least two of the sciaenids (Atlantic croaker and weakfish) but its degree of coupling has not been carefully examined as yet. It would be surprising if the Spot (which may prove to be somewhat more refractory than the other two to contamination-induced eye lesions if preliminary indications are reinforced by later observations) does not exhibit similar responses relative to the choroid space, choroid blood vessels and the rete as its family relatives when sufficiently challenged.

In some instances engorgement of the choroid elements and enlargement of the spaces seems to appear even before cataracts are apparent in the sections. Perhaps they are precursors and can be related to developing or early cataracts (seen upon gross examination or by VIMS pathologists). Probably they are more likely to be visible in the sections than many cataracts which may be quite small and not readily apparent in the relatively sparse lens tissue found in the few sections on our slides. Some data on these points are available but their analysis must await processing of larger numbers and larger portions of the excised eyeballs. The significance of the occasionally observed lens capsule thickening and the tumorous condition of some of the lens epithelia also must await further sampling, study and analyses.

One further general result of these histopathological studies [which thus far involve some 10 marine/estuarine, estuarine and tidal freshwater species in all, though all have not yet been processed and only 8 are even mentioned herein (Table II)] has been the gathering of materials which will support special study and elucidation of the normal features of their eyeballs and associated ocular tissues. Doing so will provide valuable baseline information, assisting in future studies of pathological conditions of fish eyes, as well as for comparative work with ocular morphology and micromorphology of these species.

Larval and Early Juvenile Materials

Microscopic studies of the histological sections of whole or entire larval and juvenile Spot (L. xanthurus) have been primarily educational and preliminary in nature thus far. Of the several individuals, none have shown signs of disturbances in the optic cups or lens placodes of young larvae or in the developing eyeballs of older early juveniles. A large amount of material has yet to be examined. It remains to be seen (and cannot be predicted) what the slides included in that material will disclose in the way of histopathological conditions. But we can safely predict that study of them will add to our knowledge of the development of the elements of the eyeball of the Spot.

One especially interesting feature which may be clarified is the persistence and presence of the choroid-retinal-lenticular blood vessel in some of our sciaenids. Though it disappears early in the ontogenetic development of higher vertebrates it seems to persist in some individuals of into late stages of +1's and, perhaps, even older animals of some of the sciaenids under study here. Perhaps the juvenile specimens will clarify the development and history of this interesting feature.

Additional anatomical and physiological studies of eyeballs are needed to clarify the pathways of contaminants and toxic metabolites and other factors causing cataracts and other lesions of the eyes. Opthalmologists specializing in diseases of human eyes speculate, or state, that the major pathway of toxicants into the eyeball, or at least to the lens, is through the aqueous fluid of the aqueous or lateral chamber ("anterior" in higher vertebrates). We do not know the pathway(s) in fishes but given the obvious importance of the choroid blood vessels, including the rete (and perhaps the persistence of the chorid-retinal-lenticular blood vessel), in our sciaenids as well as the fact that the choroid and aqueous chambers seem less clearly separated morphologically, it would be surprising if a choroid-vitreous pathway of intoxication is not important in the teleosts under study.

CONCLUSIONS

Microscopic examinations have been made of slides made from sectioned eye lenses (fewest) or whole eyeballs (most). In all, 1156 sections of 319 slides representing almost as many individuals of 6 species were prepared and examined.

Cataracts (opacities of the lenses) were the principal focus of attention because they were noticed first in early laboratory and field observations, may serve as biological indicators of contamination in estuarine waters (especially of PAH-contamination) and are easily observed and recorded in the field on the shipboard sorting table or on the necropsy bench in the laboratory.

Histopathological results tend to confirm that the cataracts observed in our fish occur most frequently in populations from areas whose sediments are heavily contaminated by PAHs (among other organic as well as inorganic chemical species and compounds). Though certain shortcomings remain in our histological procedures and the resulting diagnoses of lenses and eyeballs (i.e. all too frequently the epithelium and its adherent capsule are separated from the lenses in our processed specimens, even those imbeded in plastic and in no cases did our slides contain more than a total of 20-25 microns thickness of tissue) they can be solved by more careful processing in the first case and by securing sections of more of the eyeball, as by the serial-sectioning or the "interrupted serial sectioning" techniques, in the second. For some special "cataractous" conditions or other ocular lesions, serial sections may be especially useful and, in fact, will be necessary to establish the requisite basic understanding of normal and abnormal ocular morphology of the species involved.

Regardless of their shortcomings, our sections have confirmed that many cataracts, ranging from very large to pinpoint opacities, observed by the naked eye at the time of necropsy of fresh whole fishes are indeed manifestations of morphological alterations, massive and minute, in the epithelium, cortexes (mostly) and/or nuclei (fewer) of the excised and sectioned lenses from the same fishes. (Obviously this confirmation can only occur when the few sections made coincide exactly with the locations of those lesions seen grossly at necropsy.) This finding is useful in establishing gross observations of cataracts as clear indications of micromorphological disturbances. Their occurrence in individual fish whose distribution coincides with sites heavily-contaminated by PAH's and their extreme rarity in samples in PAH-poor stations confirms the direct relationship between these morphological and micromorphological disturbances and PAH-contaminated sediments.

Differences between diagnoses made by cooperating opthalmological scientists at NEI/NIH and those made by the VIMS pathologist indicate that re-analysis of sectioned material, re-examination of data from both groups as well as concurrent diagnoses are in order. These reviews, if they prove possible to accomplish, may enable us to draw more from existing sections and to prepare better, more useful sections from as yet unprocessed (or uncollected) eyeballs. They may also result in better understanding of the criteria, definitions and diagnostic notations of the two groups and even increase the uniformity in these elements. Improvements in future diagnoses will result and enhanced scientific understanding of cataracts (and perhaps even humans -- using fish eyes as models) also will follow.

Several types of eye opacities ("cataracts") are seen in damaged fish eyes. They require separate, careful description. We hope to be able to do so. When proper descriptions are available, our understanding of damaged fish lenses will be increased. Again, this information may be useful in human eye pathology and its physiology. It seems certain that studies of fish eyes can aid veterinary and human opthalmology. Obviously, one can experiment with fishes and/or collect and obtain, excise, process, section and analyze fish eyes more readily and less expensively than with humans, and other primates, or with dogs and cats or even rats and mice (Couch and Hargis, 1984).

As indicated above, more than cataracts have been detected in our slides. Indeed, retinal tissues, vitreous and aqueous materials (lens capsules), and other ocular structures are affected by exposure to PAH-contaminated sediments and sedimented-exposed waters. Our sections show that in some specimens choroid blood vessels and choroid rete become engorged with blood as they have been toxified. Also, choroid spaces, i.e. the space which usually appears between the innermost and outermost layers of the choroid in processed eyeballs are enlarged. These easily observed features demonstrate the widespread effects that such toxicants have upon the eyeballs of fishes. (See Figures F, G, H, I, J and K which should be compared with Figures A, B, C, D, and E). These and other features of existing and future samples must be examined more carefully.

Our beginning studies of sectioned larval and juvenile spot (L. xanthurus) have not yielded much information about the development of sciaenid eyes in feral and laboratory conditions, but may be expected to as experience grows and more animals and other stages of development are studied. A large amount of already-processed material is at hand. It is, of course, too early to predict how much this material will tell us about the genesis and development of cataracts and other ocular lesions of older fishes. Surely, knowledge obtained from sections of larval and post-larval fishes will be useful in interpreting the results of experimental exposures of such animals. Spot is one of the few marine/estuarine species which can be spawned, reared, exposed and held under laboratory conditions. Since our larval and juvenile materials are of laboratory-reared and feral Spot, elucidation of them will be useful in later controlled studies, studies of ocular development and of development of induced-lesions. Such work is necessary in order to understand the physiological and morphological processes involved and the environmental significance of these phenomena.

Continuing work in these areas is clearly justified from several vantage points, studies and monitoring of environmental effects, bioassay and management of contaminants, improved understanding of ocular structure and physiology of fishes and higher vertebrates and the possible uses of fish eyes as models for studies of human eye pathology, natural and environmental.

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Comparative Opthalmology of the College of Veterinary Medicine of Cornell University reviewed our early slides and diagnoses.

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BIBLIOGRAPHY

Ali, M. A. (Ed.). 1974. Vision in Fishes. New Approaches in Research. NATO Advanced Study Institute Series Plenum Press, New York.

Anderson, B. G. and D. L. Mitchum. 1974. Atlas of Trout Histology. Bulletin No. 13 of the Wyoming Fish and Game Dept., Cheyenne, x-110.

Bieri, R. H., C. Hein, R. J. Huggett, P. Shou, H. Slone, C. Smith and C-W. Su. 1986. Polycyclic aromatic hydrocarbons in surface sediments from the Elizabeth River subestuary. Intern. J. Environ. Anal. Chem. 26:97-113.

Couch, J. A. and W. J. Hargis, Jr. 1984. Aquatic animals in toxicity testing. J. Amer. Coll. of Toxicol. 3(6):331-336

Fernald, R. D. 1985. Growth of the teleost eye: novel solutions to complex restraints. Env. Biol. of Fishes 13(2):113-123.

Grant, W. M. 1983. Toxicology of the Eye. xiii - 1083. Charles C. Thomas, Springfield, Ill.

Grizzle, J. M. and W. A. Rogers. 1976. Anatomy and Histology of the Channel Catfish. v-94. Agricultural Expt. Sta. Auburn Univ., Auburn, Ala., USA.

Hargis, W. J., Jr. (Ed.). 1986. Report of the Fish Histopathology Workshop. 414 pp. Edited Minutes of the Workshop held in April 1984 at VIMS/WM. Grant Report to the Grant College Program of the Virginia Graduate Marine Science Consortium and the Oxford Biological Laboratory of NMFS/NOAA/DOC.

Hargis, W. J., Jr., M. H. Roberts, Jr. and D. E. Zwerner. 1984. Effects of contaminated sediments and sediment-exposed effluent water on an estuarine fish: Acute Toxicity. Mar. Environ. Res. 14(1784):337-354.

Hargis, W. J., Jr. and J. A. Colvocoresses. In Press. EPA Workshop: Use of finfish as indicators of toxic contamination. Proc. 1986 EPA Airlie House Workshop.

Hnath, J. G. 1984. Investigate probable causes of eye problems in fish. Study Final Report, Study No. 602, Project No. F-35-R-9, Michigan.

Hogan, M. J. and L. E. Zimmerman. 1962. Opthalmic Pathology. An Atlas and Textbook. Second Edition. W. B. Saunders Company, Philadelphia.

Huggett, R. J., Jr., M. E. Bender and M. A. Unger. (In Press). Polynuclear Aromatic Hydrocarbons in the Elizabeth River, Virginia. IN: Fate & Effects of Sediment Bound Chemicals in Aquatic Systems. K. L. Dickson, A. W. Maki and W. Brungs (Eds.). SETAC Special Pub. No. 2, Pergammon Press.

Neilson, B. J. and S. C. Sturm. 1978. Elizabeth River water quality report. A report to the Hampton Roads Water Quality Agency. Special

Report No. 134 in Applied Science and Ocean Engineering of the Virginia Institute of Marine Science.

Poston, H. A., R. C. Riis, G. L. Rumsey and H. G. Ketola. 1978. Nutritionally induced cataracts in salmonids fed purified and practical diets. Marine Fisheries Review 40(10:45-46.

Riis, R. C. 1981. A report on cataracts. Prepared as an expert opinion relevant to the high incidence of fish cataracts in Washington State hatcheries, 53 pp. Spec. Report to Washington State.

Vazzoler, A. E. A. de M. and V. N. Phan. 1981. Ocorrencia de catarata em Micropogonias furnieri (Desmarest, 1822), na area entre Cabo Frio e Torres (23°S-29°S), Brasil! Investigacao de causas e estudo eletroforetico das proteinas totais dos cristalinos. Bolm. Inst. Oceanogr., Sao Paulo 30(10:65-76.

Walls, G. L. 1942. The Vertebrate Eye and Its Adaptive Radiation. Reprinted 1963. Hafner Publishing Co., New York.

Watanabe, Y. and J. Yamada. 1980. Histological characteristics of cataractous eye lenses in cultured masu salmon. Bull. Faculty of Fisheries Hokkaido University 31(4):290-296.

Weekers, R., Y. Delamarbelle, J. Luyckx-Bacus and J. Collingnon. 1974. Morphological changes of the lens with age and cataract. Pp. 25-43 in Pirie, A. (Ed.) The Human Lens in Relation to Cataract. Ciba Foundation Symposium 19 (new series). Elsevier, Eyestar Media, North Holland.

Yasutake, W. T. and J. H. Wales. 1983. Microscopic Anatomy of Salmonids: An Atlas. U. S. Department of the Interior, Fish and Wildlife Service, Resource Publication 150. 190 pp.

Zigman, S. 1980. Influence of aging and light on oxidation: reduction reactions in the lens. Pp. 181-184 in Srivastava, (Ed.), Red blood cell and lens metabolism. Elsevier North Holland, Inc.

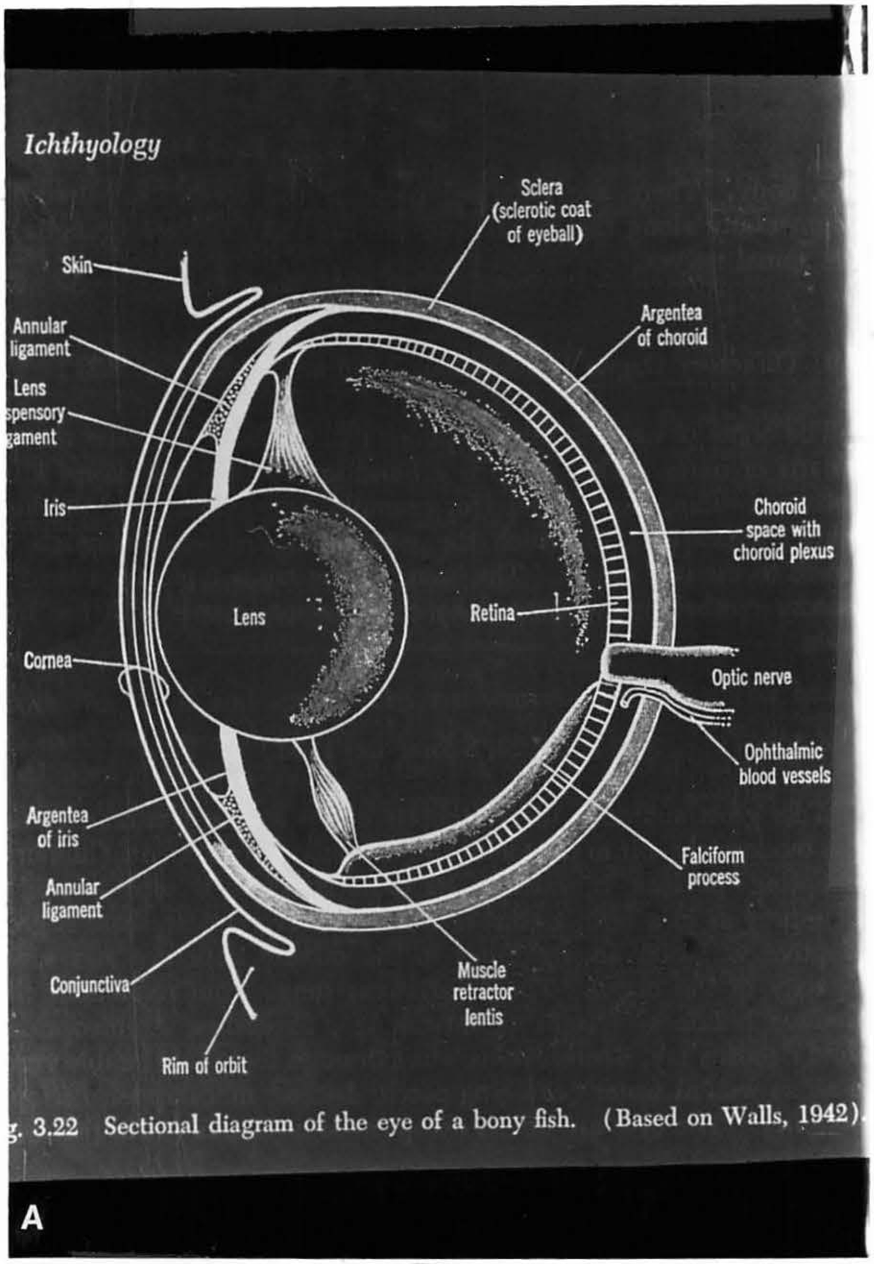
Zigman, S. 1985. Selected aspects of lens differentation. Biol. Bull. 168:189-213 (April 1985).

FIGURE A

DIAGRAMMATIC REPRESENTATION, SAGGITAL SECTION, TELEOST EYE

Showing Basic Morphological Features of the generalized teleost eye.

(From Lagler, Bardach and Miller, 1962, based upon Walls, 1942.)



g. 3.22 Sectional diagram of the eye of a bony fish. (Based on Walls, 1942).

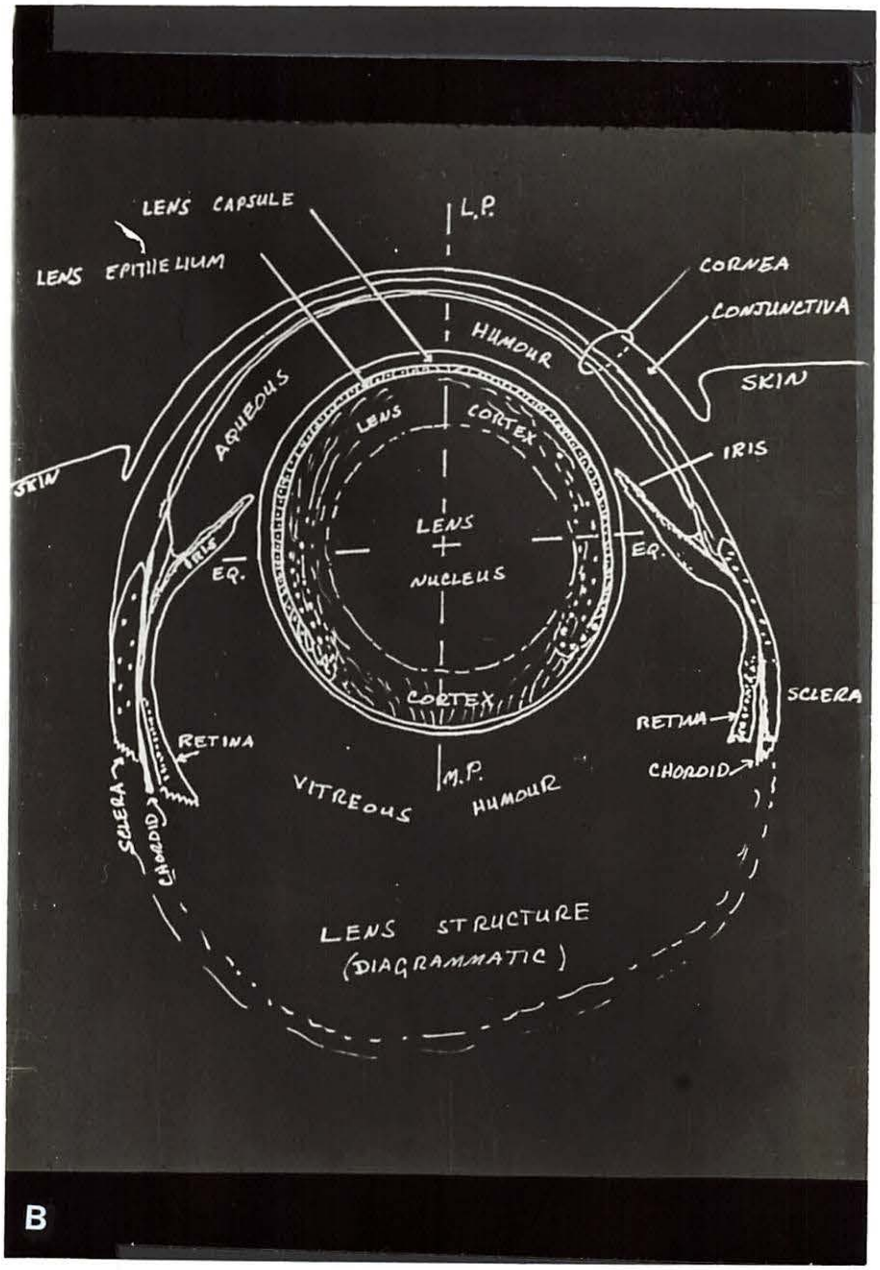
A

FIGURE B

DIAGRAMMATIC REPRESENTATION, SAGGITAL SECTION, TELEOST EYE

Showing lens structure and orientation.

(Original)



LENS CAPSULE
L.P.
LENS EPITHELIUM
CORNEA
CONJUNCTIVA
HUMOUR
SKIN
AQUEOUS
LENS
CORTEX
IRIS
SKIN
LENS
NUCLEUS
IRIS
EQ.
EQ.
CORTEX
SCLERA
RETINA
RETINA
CHOROID
M.P.
VITREOUS
HUMOUR
SCLERA
CHOROID
LENS STRUCTURE
(DIAGRAMMATIC)
B

B

FIGURE C

SAGITTAL SECTION, EYE OF SPOT (*L. xanthurus*)

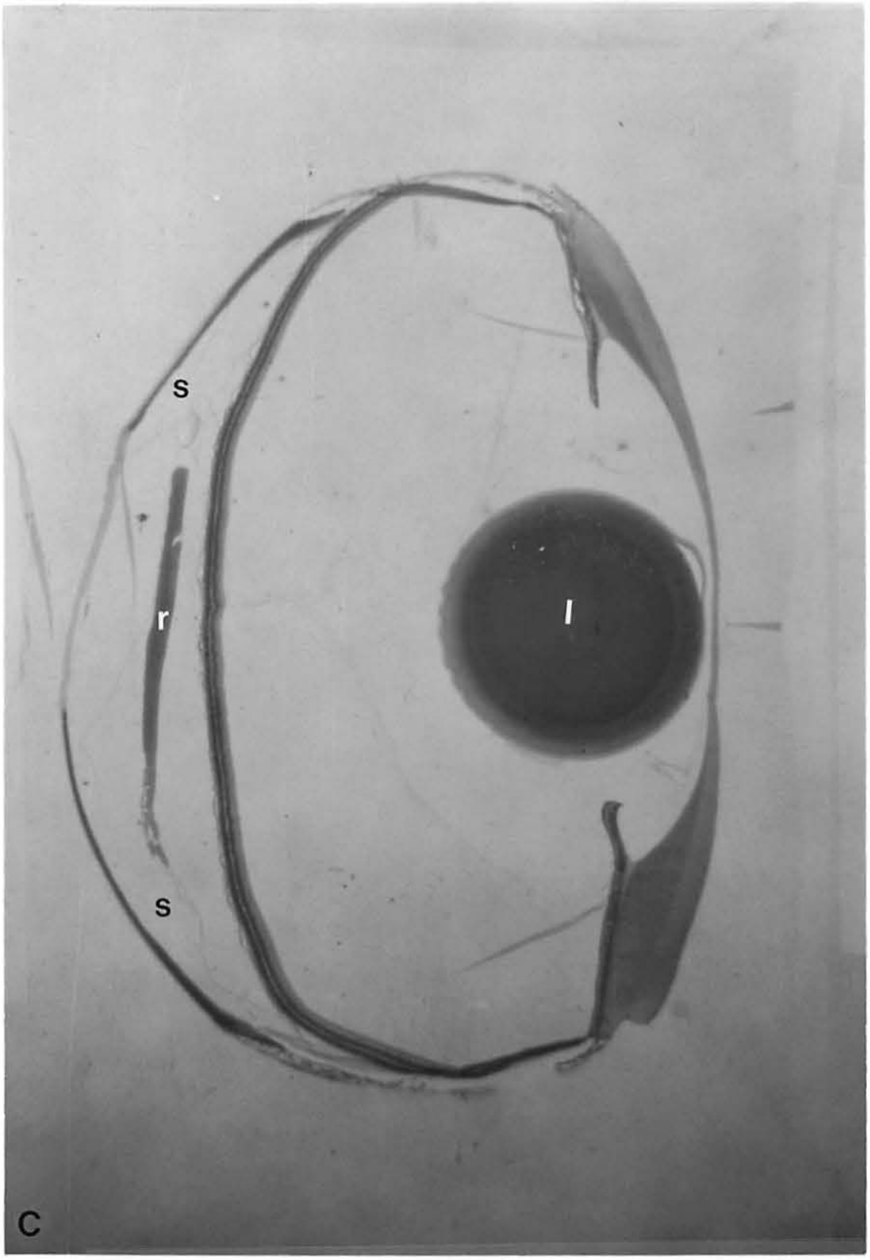
[From uncontaminated reference estuary (Nansemond River -- NR).]

Showing normal sizes of choroid rete (R) and normal appearance of lens (L).

Choroid space (S) somewhat large, probably a technical artifact.

Toluidine Blue (TB) Stain

7.5X



C

FIGURE D

SAGITTAL SECTION, EYE OF SPOT (*L. xanthurus*)

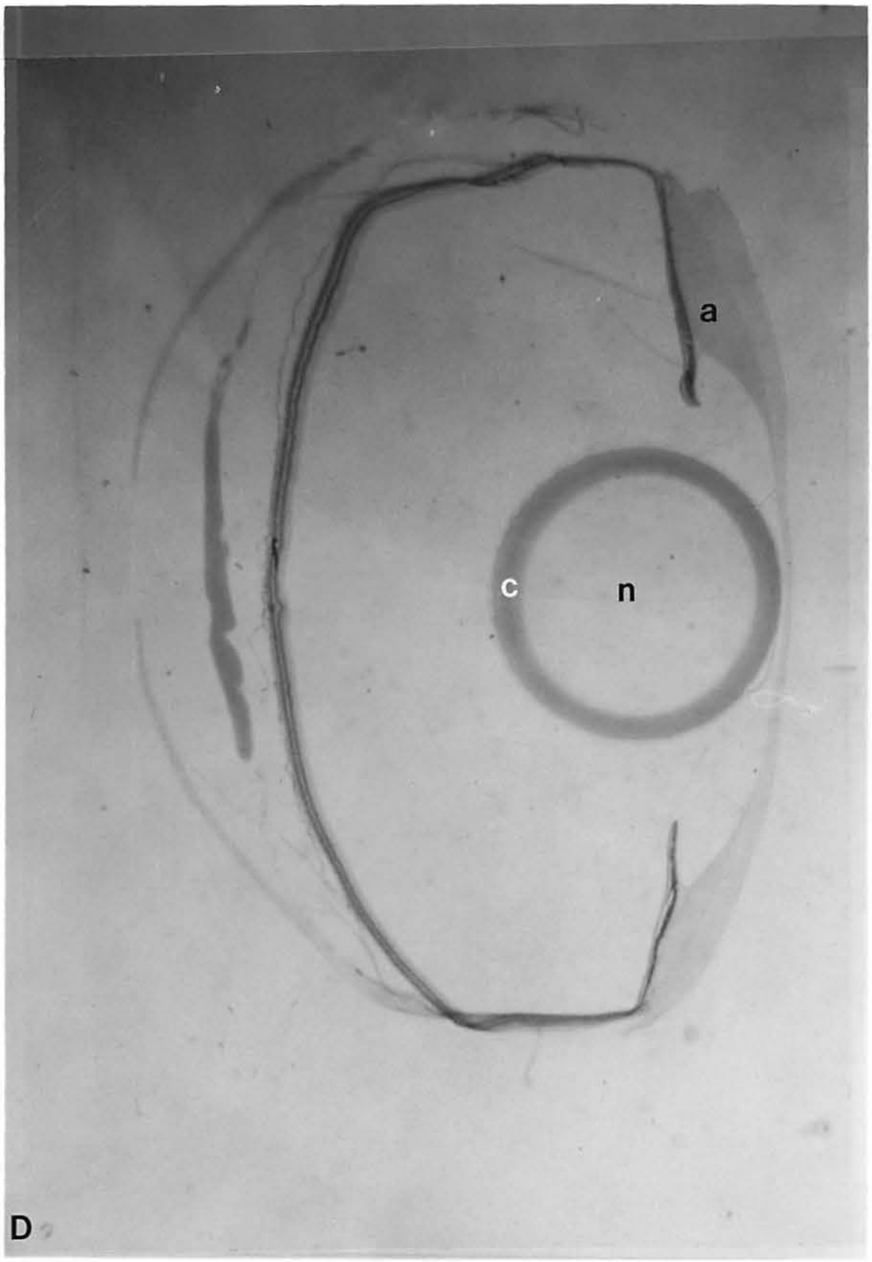
[From uncontaminated reference estuary (N.R.).]

Showing same features as Figure C (dorso-ventral position reversed) showing Nucleus (N) and Cortex (C) of the lens.

Note the single large annulus (A) commonly seen in spot. Its' mate below in the photo is much smaller.

Periodic-Acid-Schiff (PAS) Stain

7.5X



D

FIGURE E

SAGGITTAL SECTION, LENS OF SPOT (L. xanthurus)

[From "uncontaminated reference estuary, (N.R.).]

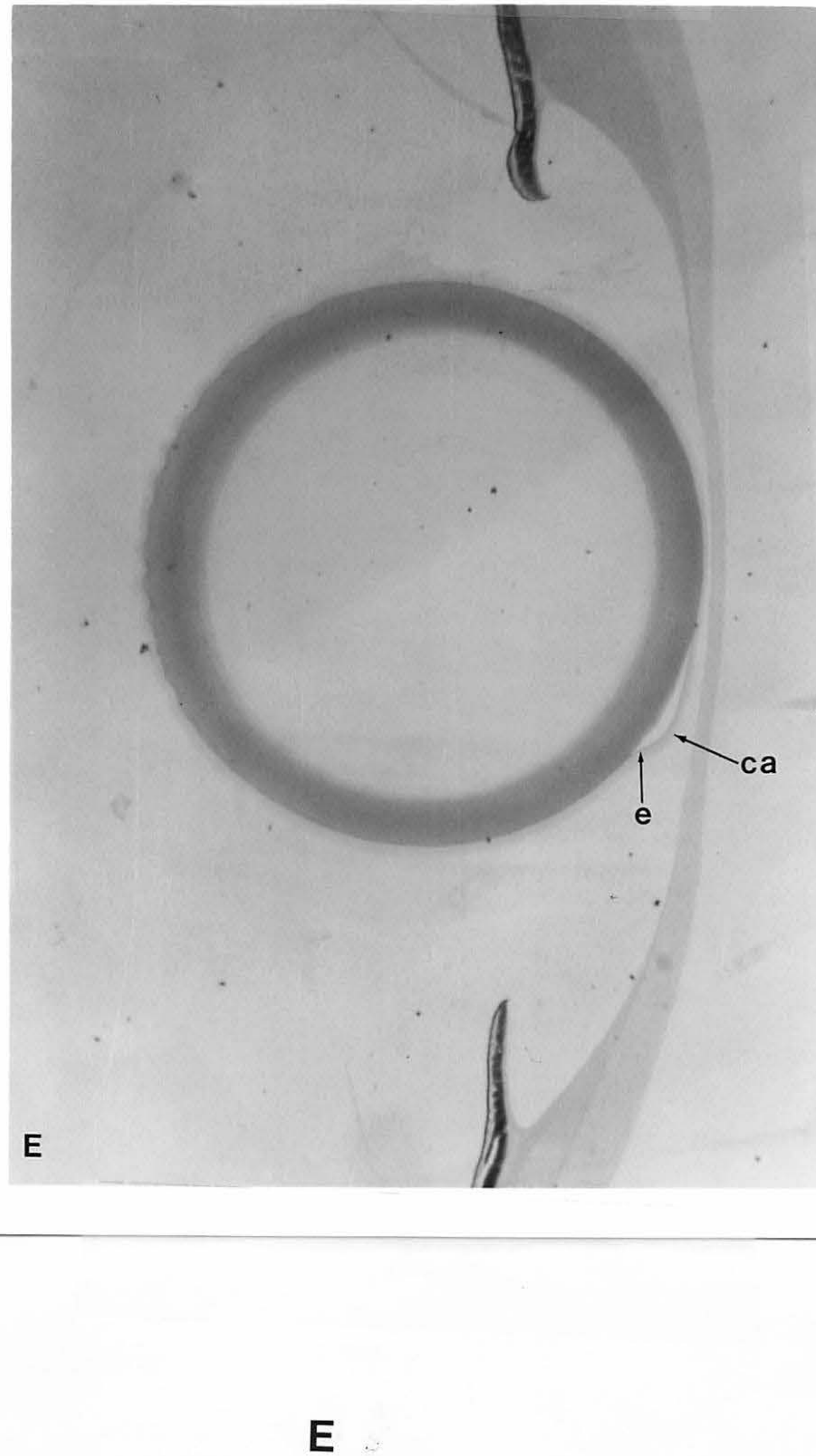
[Enlargement (15X) of lens of eye shown in Figure D).]

Showing the capsule (CA) and its underlying epithelium (E -- the living, dividing tissue responsible for production of the lens fibers inside and the capsule outside, shown here as a faintly darker line inside of the capsule).

The separation of the epithelium and capsule from the cortex (outer fibrous layer) of the lens in this location is an artifact of processing.

PAS Stain

15X



E

FIGURE F

SAGITTAL SECTION, EYE OF ATLANTIC CROAKER (_M. undulatus_)

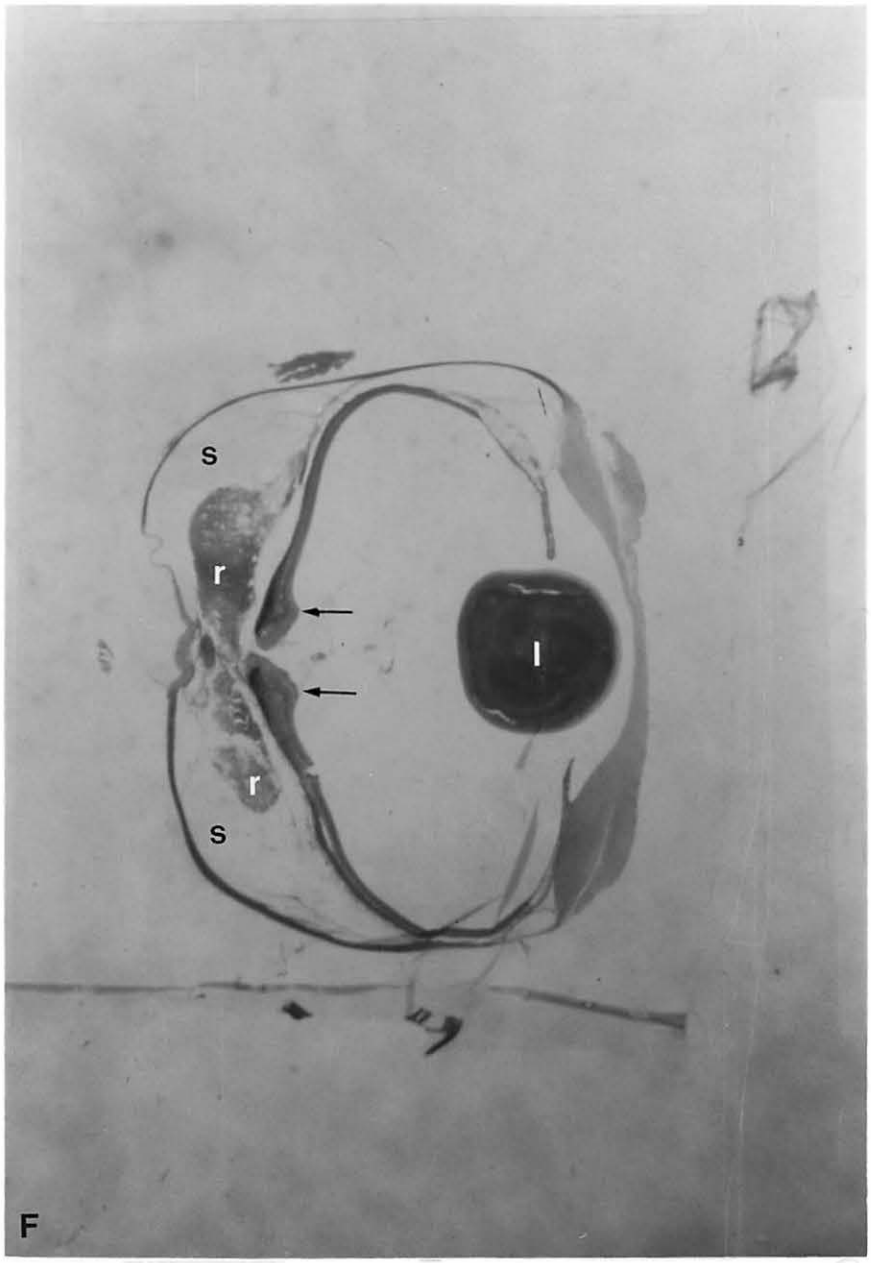
[From contaminated-sediment station (Elizabeth River -- E.R.7).]

Showing abnormally-shaped cataractous lens (L), enlarged choroid space (S) and engorged choroid rete (R). These features, i.e. large choroid space and engorged rete are common companions of cataractous lenses and are, undoubtedly, brought about by the toxic contaminant or its metabolites.

The retina seems abnormally swollen in the center (arrows).

T.B. Stain

7.5X



F

Figure G

SAGITTAL SECTION, LENS OF ATLANTIC CROAKER (*M. undulatus*)

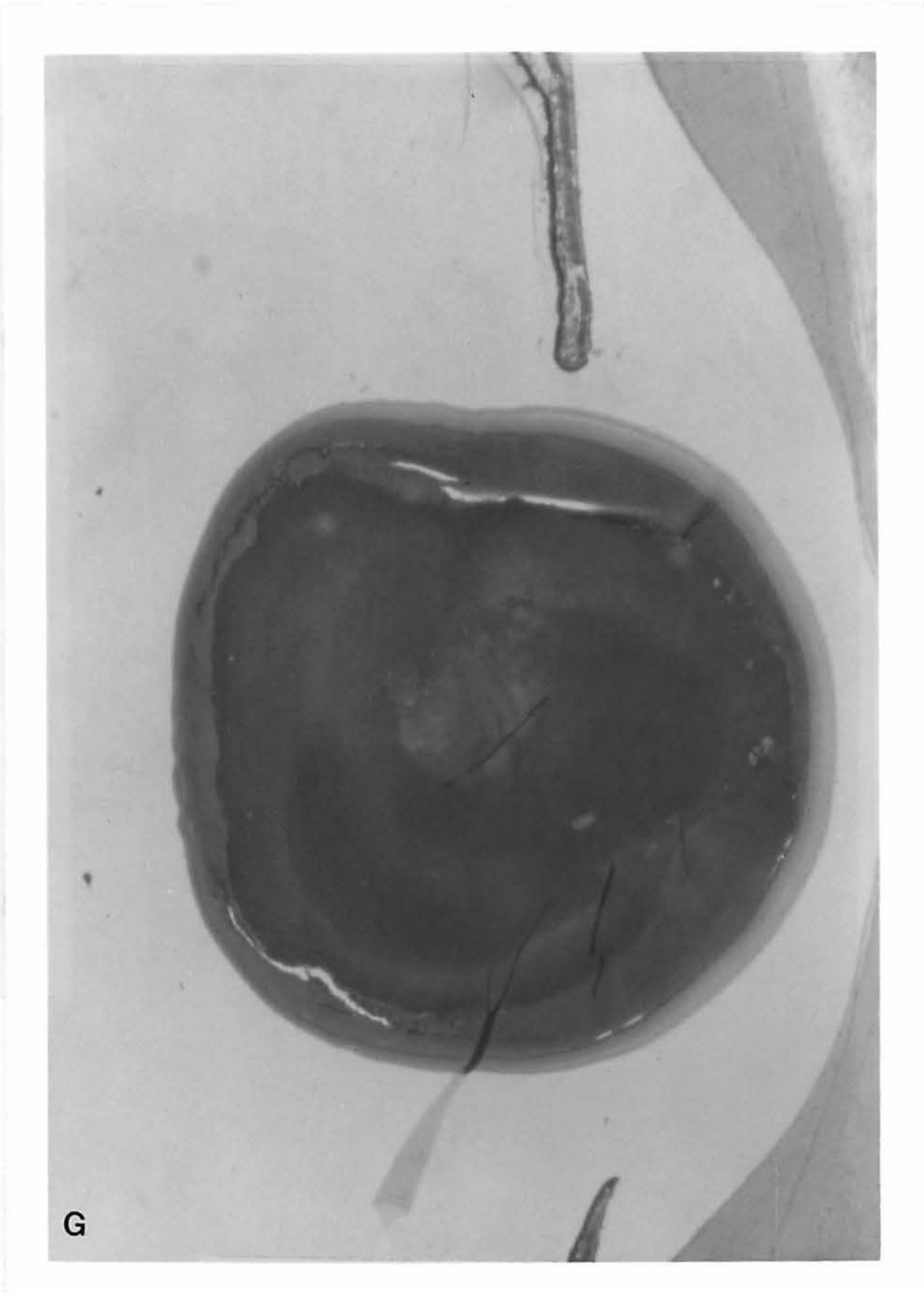
[From contaminated-sediment station (E.R. 7).]

Showing cataractous lens in greater detail.

Note involvement of nucleus (N) as well as the cortex (C) and the capsule (CA) which is abnormally enlarged. Abnormal vacuolation and epithelial growth also is involved.

T.B. Stain

31.25 X



G

FIGURE H

SAGITTAL SECTION, LENS OF SPOT (*L. xanthurus*)

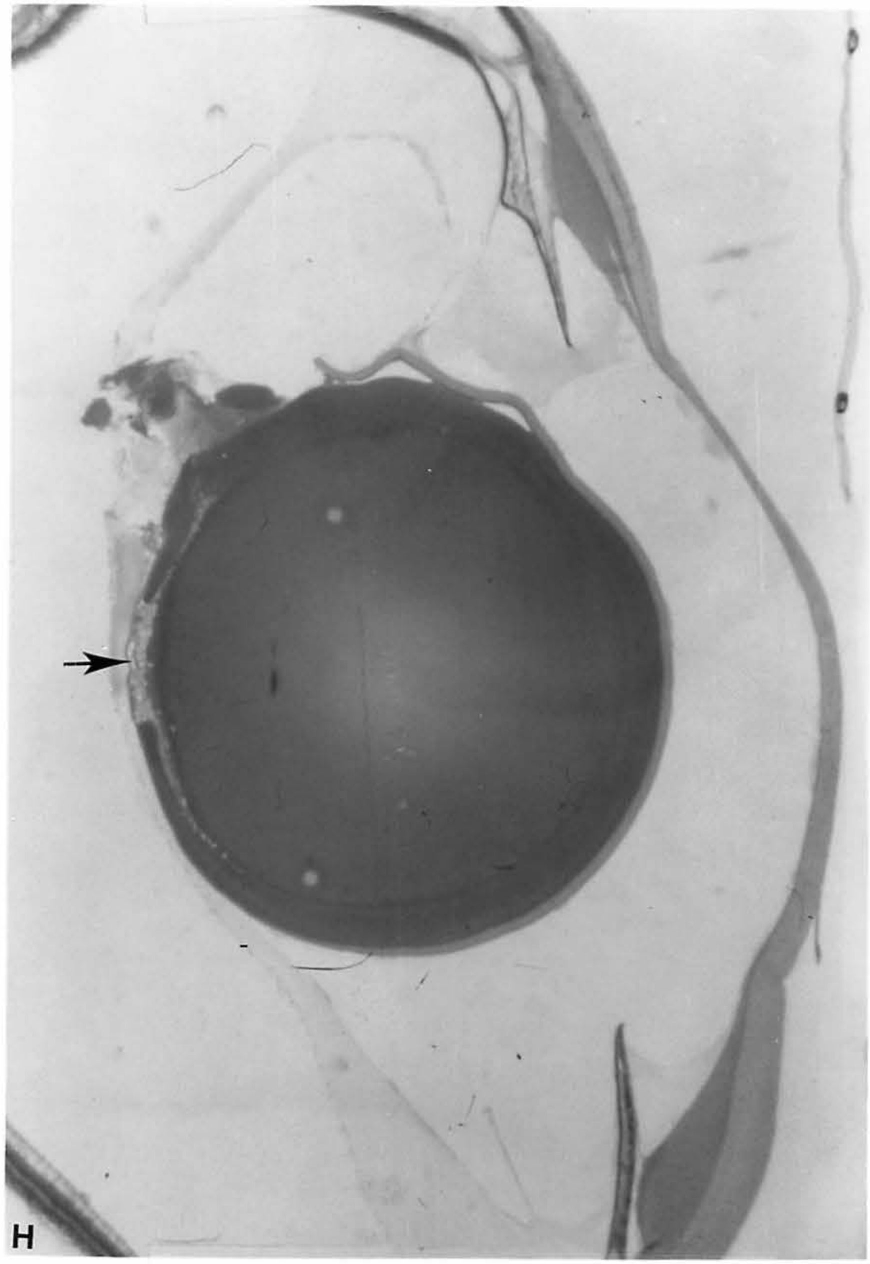
[From contaminated-sediment station (ER 7).]

Illustrating another manifestation of cataractous condition involving disruption of the epithelium and cortex of the medial pole (arrow) of the lens.

(Medial pole, to the left in the photographs, corresponds to the posterior pole in human lenses.)

T.B. Stain

15X



H

FIGURE I

SAGITTAL SECTION, EYE OF ATLANTIC CROAKER (*M. undulatus*)

[From contaminated-sediment station (E.R. 7).]

Eyeball was sectioned in paraffin in contrast to the others above which were embedded and sectioned in plastic.

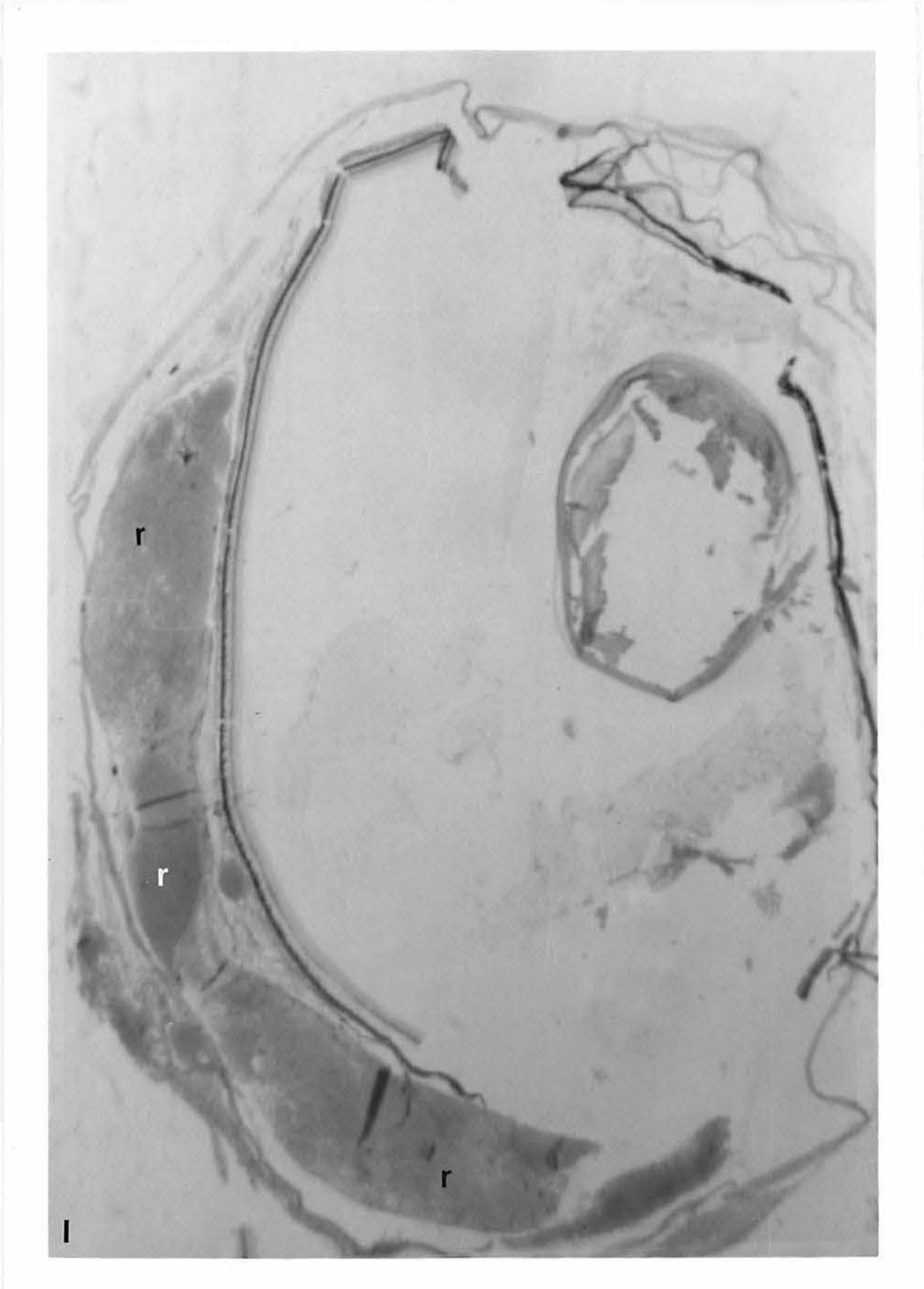
Comparison with Figures C, D, E, F, G and H show clearly the superiority of the preparations made by Ms. Groome of NEI/NIH as compared to ours.

Despite tissue disruption (especially the shattering of the lens), this slide displays the abnormal engorgement and enlargement of the choroid rete (R) graphically.

The remaining portions of the lens show it to have been cataractous as well.

Hematoxylin and Eosin (H&E) Stain

75.X



I

FIGURE J

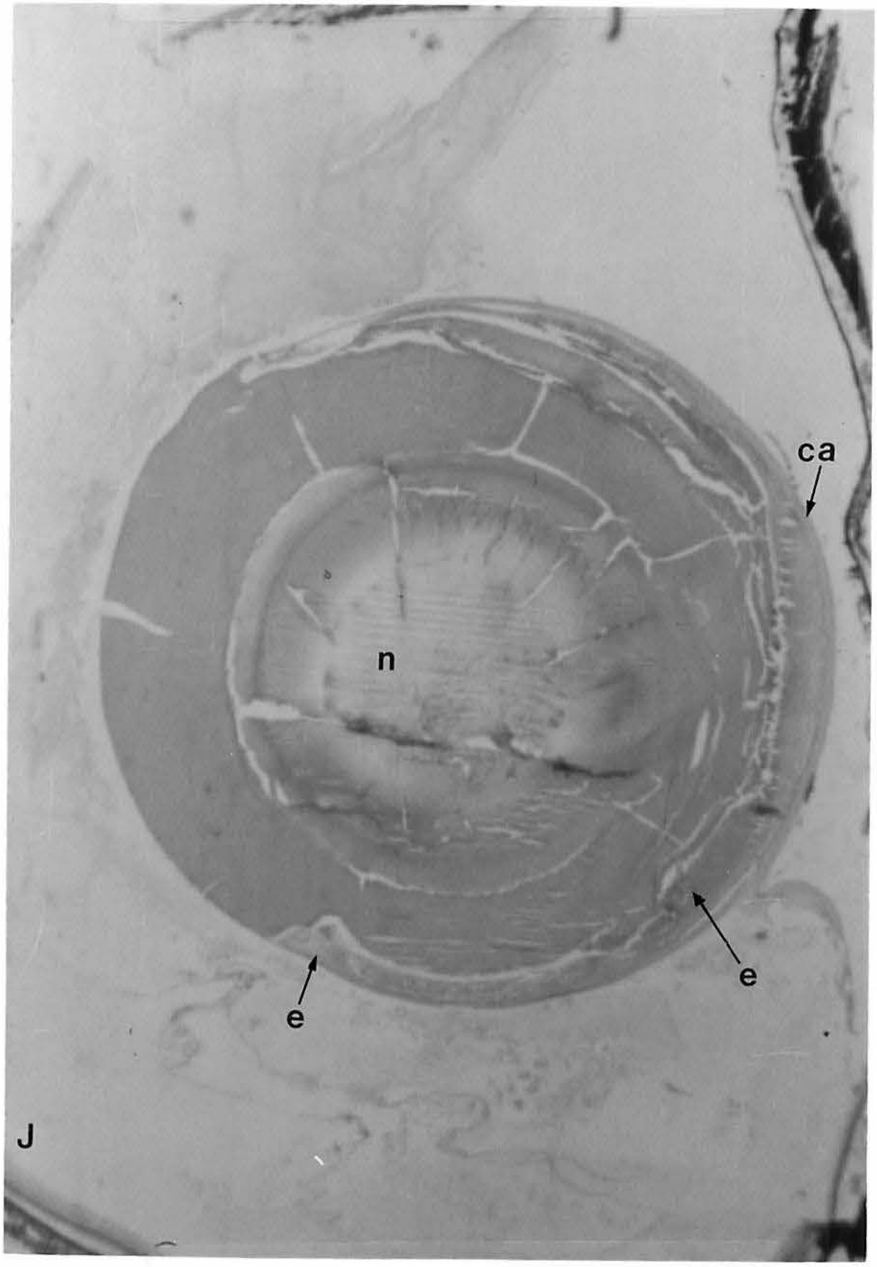
SAGITTAL SECTION, LENS OF WEAKFISH (C. regalis)

[From contaminated-sediment estuary (ER 7).]

Though shattered due to embedment in paraffin (not a good medium for the crystalline lens) the section clearly shows the extensive disruption of the epithelium (E), cortex (C) and nucleus (N) of the lens.

H&E Stain

15X



J

FIGURE K

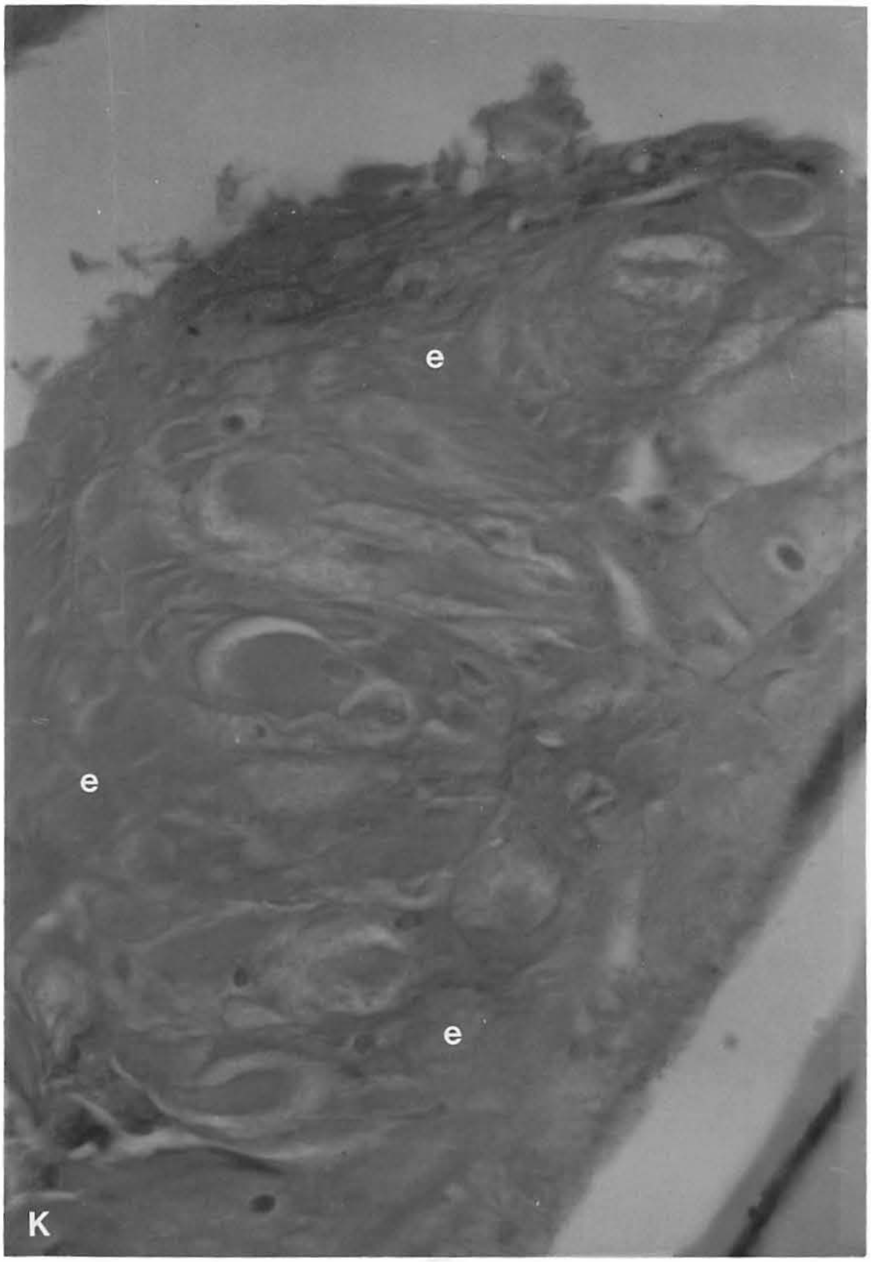
SAGITTAL SECTION, LENS OF WEAKFISH (C. regalis)

[From contaminated-sediment estuary (ER 7).]

Showing tumorous growth (hyperplasia?)
of the epithelium of this badly disturbed,
cataractous lens.

H&E Stain

ca. 400X



K