ray studies of other chloride solutions (*8*) (not HCl) have also shown a strong peak at 3.2 Å, as well as a tendency for the loss of the peak attributable to the O–H–O distance at 2.8 Å. Thus, chloride ions are often ascribed a "structure breaking" attribute (*9*).

We attribute the peaks at 2.56, 2.68, and 2.75 Å in the HCl solutions to strongly bonded oxygen pairs. Since such correlations do not appear in other chloride solutions (*8*), these O–O distances are probably related to the presence of excess protons in the water, and, moreover, appear to be shorter as the concentration of protons increases. Such distances are also suggested by the structures of crystalline di- and trihydrated HCl (*7*).

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Retinal Tapetum Lucidum: A Novel Reflecting System in the Eye of Teleosts

Abstract. *A new type of tapetum lucidum has been found in eyes of bony fishes (teleosts). It is retinal, lying in the processes of the pigment epithelial cells; it has a white appearance and reflects light diffusely. The cell processes are loaded with highly refractile lipid particles; as examined by electron microscopy these are about 400 nanometers in diameter, spherical, and homogeneous. This tapetum lucidum has been found in seven families of teleost fishes occurring in inshore waters and rivers of South Texas, all of which have high turbidity; the correlation indicates a role in dim-light vision.*

A reflecting layer, or tapetum lucidum, occurs in the eyes of many different kinds of animals and is generally regarded as an adaptation to dim-light vision. Several types of tapeta have been recognized in vertebrates (*1, 2*), and in fishes two types have been described. Both of these, an oriented reflecting layer in the chorioid of elasmobranchs and some primitive bony fishes (*3*) and a retinal reflector in pigment epithelial cells of teleosts (*1, 4*), contain guanine crystals. While investigating the eyes of fishes living in very turbid inshore waters of the Gulf of Mexico, we have encountered another type of tapetum lucidum having unusual properties.

The tapetum lucidum which we briefly describe is an integral part of the pigment epithelium and forms a diffusely reflecting white layer of a lipid nature. Fishes in which it occurs show eye-shine after dark-adaptation and, when the eye is opened and the retina has bleached, the fundus exhibits a whitish hue. The reflecting layer can be exposed by removal of the retina. Examination of this layer microscopically with light from above reveals a sheet of white tufts, each tuft representing a columnar epithelial cell (Fig. 1). Cells of this sort are shown in longitudinal section in Fig. 2.

Our first encounter with this tapetum was in the speckled sea trout *Cynoscion nebulosus*. Seeking to document its occurrence, we have examined many other species (63 species of 39 families) of the inshore waters and rivers of South Texas; among them a lipid tapetum occurs in seven families (Table 1). Further searching will probably reveal this kind of lipid tapetum in other families. Its appearance is generally the same except in the cusk eel *Otophidium* and the spadefish *Chaetodipterus*, where it has a bluish tint. Fishes of two families examined, namely Elopidae and Clupeidae, have a retinal tapetum lucidum of another kind, generally regarded as containing guanine particles (*1*), which is also a diffuse white reflector.

Table 1. Families of fishes containing retinal tapeta lucida.

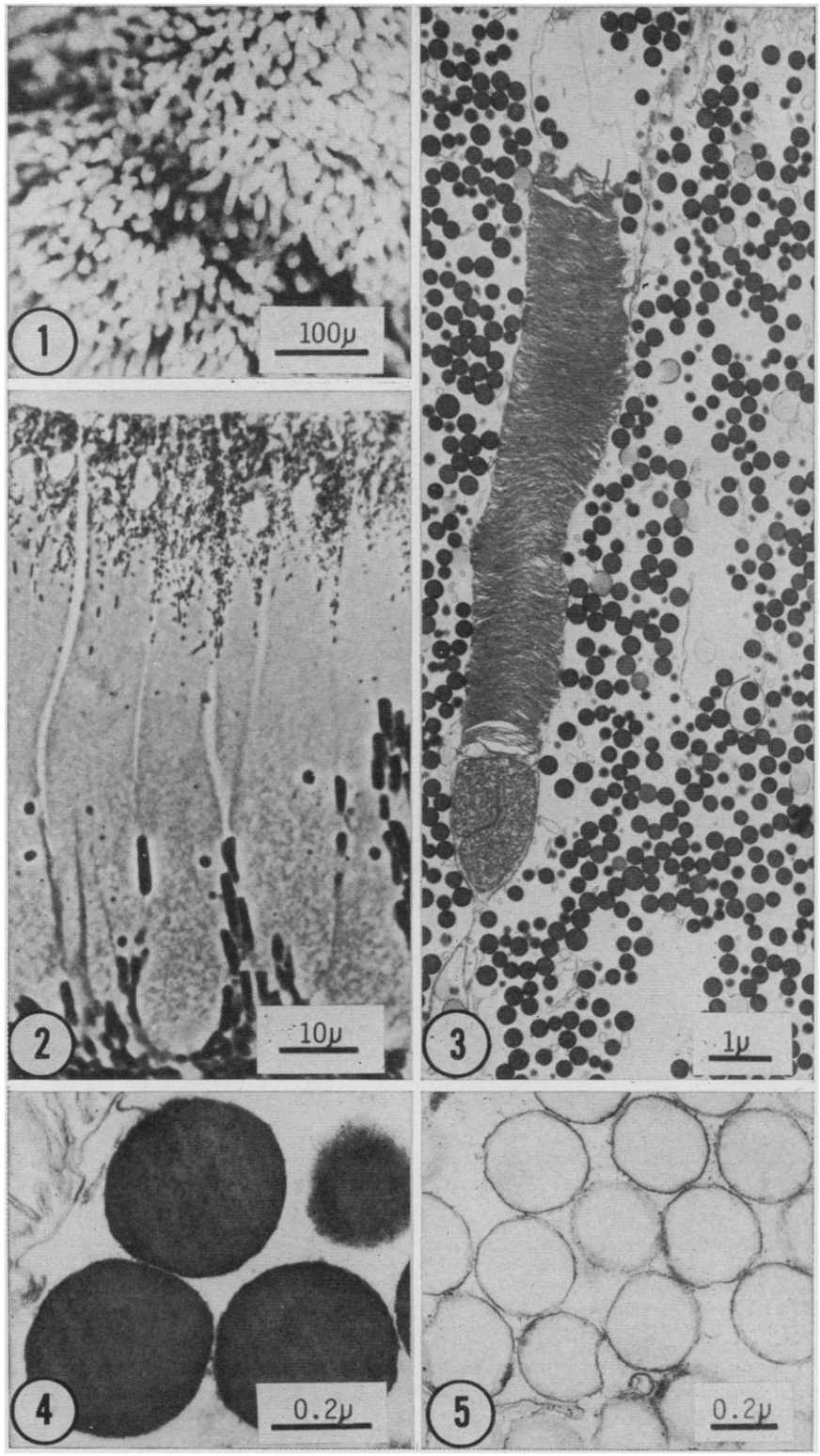
Family	Representatives
Ariidae	Marine catfishes
Ictaluridae	River catfish
Polynemidae	Threadfins
Sciaenidae	Croakers or weakfishes, *Menticirrhus, Cynoscion, Micropogon, Leiostomus, Bairdiella, Pogonias, Sciaenops*
Haemulidae	Grunts
Ophidiidae	Cusk eels
Ephippidae	Spadefishes

Attention has been focused on the sea trouts *Cynoscion nebulosus* and *C. arenarius*. The lipid material of the tapetum is contained in the processes of the pigment epithelial cells, which appear white in reflected light, brown in transmitted light. The pigment epithelial cells are easily ruptured, whereupon a dense cloud of minute whitish particles streams forth, gleaming brightly under superior illumination. The reflecting component is eliminated by some organic solvents (chloroform, methanol), exposing the dark retinal pigment beneath. Measurements of reflectance of fresh tapeta show that they reflect about equally well across the visible spectrum; reflectivity is about 45 percent.

The components of the eye execute normal retinomotor movements, the rods, cones, and pigment becoming radially displaced in light and darkness. In the light, the pigment granules migrate inward, partially obscuring the reflecting material, and the fundus becomes dull gray. In the dark-adapted state, the rod outer segments, lying inside the reflector, are in a position to receive diffuse light from the latter.

The lipid material is lost in the usual techniques employed in making histological preparations. It was possible to examine the lipid in the light microscope after Flemming fixation, or in frozen sections. The lipid nature of the reflecting material is suggested by solubility in fat solvents and by osmophilia. Although the material is sudanophilic, it is a negative to the acid hematin test and Nile blue sulfate test for phospholipids and to the Liebermann-Burchard test for cholesterol and its esters. The reflecting material has been extracted by chloroform and methanol (2 : 1 by volume). It is saponifiable; cholesterol and cholesteryl esters are counter-indicated by gas and thin-layer chromatography. A diffusely reflecting retinal tapetum containing cholesterol occurs in the opossum (*5*).

When viewed in the electron microscope, the tapetum granules are seen



Figs. 1–5. Pigment epithelial cells of the speckled sea trout *Cynoscion nebulosus*. Fig. 1. Pigment epithelium as viewed from the inside; each white process represents a single cell. Prepared by stripping the pigment epithelium from the remainder of the retina in the dark-adapted state. Fig. 2. Plastic-embedded, 1-μm section viewed in the phase microscope. Dark rods can be seen between some epithelial cells. The upper (outer) part of the cells contains melanosomes, nuclei (light areas), and mitochondria; lower (inner) part contains lipid granules. Fig. 3. Small portion of two pigment epithelial cells separated by a rod composed of an outer and inner segment. With the exception of a few membranes, this part of the epithelial cells is free of structures other than the lipid granules. Fig. 4. Lipid granules, apparently well fixed, in which the dense nature and lack of membrane boundary can be seen. Fig. 5. Lipid granules imperfectly fixed. Apparently only the periphery of the granules was fixed, the contents being extracted at some stage in dehydration. Entire preparation shows this kind of fixation.

to be dense spheres about 400 nm in diameter (Fig. 4). Such is the case when the tissue is first fixed in glutaraldehyde and acrolein in a combination of teleost Ringer and cacodylate buffer and then treated with osmium tetroxide (*6*). Other techniques of fixation produce less satisfactory fixation and often cause extraction of the lipid materials (Fig. 5). The granules are homogeneous in size and uniform in staining reaction. They do not appear to be surrounded by a membrane, although they are sometimes denser at the interface with the cytoplasm. The granules do not show any internal differentiation. High-resolution microscopy shows no evidence of a crystalline nature.

The tapetum granules are found throughout the pigment epithelial cell. In the light-adapted state (Fig. 2), the processes of these cells contain very little other than the granules, melanosomes being restricted to the outer part of the cell. In the latter region there are many granules, but they are associated with membraneous elements, nuclei, mitochondria, and melanosomes. In the inner portion of the cell lipid granules are almost exclusively the only component; occasionally membranes may be seen (Fig. 3). In the light-adapted state, melanosomes are found throughout the cells.

The lipid granules are so densely packed in places as to be almost contiguous (Fig. 5). Because of their size, density (that is, packing), and high refractile properties, we assume that the reflectivity of the system depends upon multiple back scattering. No other structural feature which could account for the reflectivity of the pigment epithelial cells has been observed in the cytoplasm of this region.

A retinal tapetum occurs in a surprisingly large number of inshore fishes of the Gulf, in about a quarter of the families examined. Its presence may be correlated with the high turbidity of rivers, bays, and coastal waters [the bays having attenuation coefficients k of up to 2.9 (*7*)]. Some of the families, however, are very widely distributed [for example, the sciaenids which live on sandy shores (*8*)]. It is absent from pelagic fishes, such as the jacks (Carangidae) and mackerels (Scombridae). Its role in dim-light vision remains to be determined.

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Lactate Dehydrogenase Isozymes: Further Kinetic Studies at High Enzyme Concentration

Abstract. *By competition with lactate dehydrogenase (LDH) for nicotinamide adenine dinucleotide (NAD), commonly occurring intracellular proteins, such as glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, and albumin, can protect LDH-1 and LDH-5 from inhibition and ternary complex formation with NAD and pyruvate. The existence of intracellular proteins that compete with LDH for NAD renders unphysiological a model for estimating the extent of intracellular LDH inhibition based on incubations of only LDH, NAD, and pyruvate.*

At low concentrations of enzyme, lactate dehydrogenase LDH-1, but not LDH-5, is inhibited by high concentrations of pyruvate. On the basis of this difference in kinetic behavior a theory was developed to explain tissue specific distributions of LDH-1 and LDH-5. It has been shown that LDH activity assayed under comparable conditions, except for substitution of physiological levels of enzyme, exhibits no substrate inhibition (*1*). Furthermore, Coulson and Rabin (*2*) and Griffin and Criddle (*3*) showed that LDH inhibition results from the enol form of pyruvate, which is present in most commercial preparations of pyruvate; the enol form of pyruvate enhances abortive ternary complex formation (*2, 3*). When enol pyruvate was chromatographically removed, even exceedingly high unphysiologic concentrations of the purified keto form failed to inhibit low concentrations of LDH. Our experiments were designed to determine whether certain commonly occurring intracellular compounds could alter pyruvate inhibition of LDH.

Our experiments reveal that certain intracellular compounds can alter the extent of LDH inhibition by pyruvate. Pyruvate inhibition of LDH has been attributed to the formation of an abortive ternary complex (*1–4*). As a reference, we incubated LDH, nicotinamide adenine dinucleotide (NAD), and pyruvate to form the abortive ternary complex and then investigated changes in LDH inhibition produced by addition of certain common intracellular proteins to the mixture of LDH, NAD, and pyruvate. We selected concentrations of these proteins similar to those of LDH. Such concentrations of these specific proteins may not be physiological, but comparable proteins that could compete with LDH for NAD probably exist intracellularly in at least these concentrations. The NAD concentration in our incubation mixtures (14 m*M*) is approximately 20- and 40-fold greater than physiological levels in rat liver and brain, respectively (*5*). As in our previous experiments (*1*), an Aminco-Bowman spectrophotofluorometer equipped with a stopped-flow apparatus (*6*) was used for measurement of LDH activity. Reactions were initiated in the 0.2-ml stopped-flow cuvette by mixing either NADH or pyruvate with the enzyme solution that had been incubated at room temperature (Tables 1 and 2). The initial linear decrease in reduced NAD (NADH) fluorescence was followed at 460 nm (340 nm excitation) for 0.1 to 5 seconds. Reactions were recorded on a Hewlett-Packard storage oscilloscope.

We recognize that incubation of the constituents of the abortive ternary complex prior to assay of LDH activity, though useful as a reference, is unphysiological for a variety of reasons and cannot therefore serve as a model of the situation in vivo. For example, concentrations of NAD and pyruvate are not stationary, but oscillate intracellularly over the time course of the incubations (*7*). Moreover, the cell contains many proteins that compete with LDH for NAD. Thus incubations solely of LDH, NAD, and pyruvate cannot be accepted as an indication of the extent of ternary complex formation in the cell. Rather they serve as a tool to explore the influence exerted by various commonly encountered intracellular compounds on the extent of formation of the abortive ternary complex. The extent to which a ternary complex forms in vivo with physiologic concentrations of LDH, NAD, pyruvate, and a variety of proteins that compete with LDH for NAD is unresolved and a major consideration of these experiments.

The results indicate that a variety of intracellular proteins compete with LDH for NAD and thereby reduce abortive ternary complex formation from that produced when LDH is incubated only with NAD and pyruvate. Even when the constituents of the

Table 1. Effect of several proteins on LDH inhibition and ternary complex formation. The incubating mixtures contained: $3.5 \times 10^{-6}M$ LDH-1 or LDH-5, 14.0 m*M* NAD, 2.0 or 10.0 m*M* pyruvate, $7 \times 10^{-6}M$ G-3-PD or $3.5 \times 10^{-6}M$ MDH or 2.5 mg of BSA per milliliter, all made up in 0.05*M* tris-HCl buffer, *p*H 7.4. Final concentrations in the cuvette were: $1.75 \times 10^{-6}M$ LDH-1 or LDH-5, 7.0 m*M* NAD, 1.0 or 5.0 m*M* pyruvate, $3.5 \times 10^{-6}M$ G-3-PD or $1.75 \times 10^{-6}M$ MDH or 1.25 mg of BSA per milliliter, and 0.7 m*M* NADH. Reactions were initiated by the addition of reduced coenzyme.

Final pyruvate in cuvette (m*M*)	Incubation (min)	Percent LDH activity* remaining after incubating LDH with:							
		NAD and pyruvate only		NAD, pyruvate, and G-3-PD		NAD, pyruvate, and MDH		NAD, pyruvate, and BSA	
		LDH-1	LDH-5	LDH-1	LDH-5	LDH-1	LDH-5	LDH-1	LDH-5
1.0	10	18	32	47	50	25	33	27	34
	30	6	10	15	17	15	20	10	13
5.0	10	7	13	52	53	20	16	15	17
	30	3	5	18	17	8	11	5	13

* Activity compared to that of control where NAD is deleted from the incubation medium.