LIGHT TRANSMISSION BY THE OCULAR MEDIA OF RAINBOW TROUT (SALMO GAIRDNERI) AND LAKE TROUT (SALVELINUS NAMAYCUSH)

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ABSTRACT

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Literature relating to the effects of light on eye tissues was reviewed at length.

A Beckman DB-G spectrophotometer was used to measure the transmission of light of all wavelengths between 200 and 800 mμ by the corneas, lenses and aqueous humor of lake and rainbow trout. The corneas of the two species differ significantly in transmission except at 350 mμ, with lake trout corneas transmitting more light than rainbow at longer wavelengths and less at shorter ones. Aqueous humor from the two species was found to transmit alike. The absolute transmission of lenses could not be measured, but the wavelengths where transmission was (a), greatest, (b), half its maximum value, and (c), zero were determined.

The absorption of light energy (not of the number of photons) from sunlight by corneas and aqueous humor at the water's surface and at a depth of 50 cm was calculated for each species, using the transmission data which had been

collected. It was assumed that all the light not transmitted was absorbed. Corneas, in each case, normally absorb enough light energy to produce photochemical changes, but probably not enough to heat them. If any abiotic wavelengths (shorter than 305 mμ) are present in sunlight, corneas protect the deeper eye structures by absorbing this radiation. Aqueous humor absorbs little light energy, but probably enough to produce photochemical reactions. Most of the incident solar radiation must be absorbed by the iris, lens and deeper eye tissues, where it may produce considerable heat and could initiate damaging photochemical changes. No photochemical reactions are now known in the cornea, lens or iris, and it therefore seems proper to suspect heating of the iris or deeper structures as the harmful agent, and to investigate its actions in future research.

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by

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INTRODUCTION

Corneal and lenticular lesions which develop during and after the second year of life in hatchery-raised lake trout can be prevented if the raceways containing the fish are shielded from the direct light of the sun. This suggests that sunlight has a critical direct or indirect role in the formation of these lesions. Since the lesions, which usually involve keratoconus, corneal opacity, and cataract, render the fish useless for stocking lakes, and since they resemble (in their early stages) little-understood optical lesions of higher animals, it has become important to investigate their causes. Sunlight could be involved in the onset of lesions in two ways: first, by direct abiotic action of light on the eye tissues, and second, by indirect action, e.g., by somehow upsetting eye metabolism. This is an investigation into each of these possibilities.

The Solar Spectrum

What is the composition of sunlight? Though the sun is thought to emit radiation across almost the entire electromagnetic spectrum, and though visible and infrared light pass easily through the earth's atmosphere, much ultraviolet light is absorbed. Parry Moon, whose 1940 article is a standard reference, brought his solar radiation curves to zero at 290 mμ, being unable to measure any light at shorter wavelengths on the ground.[24] The

"Revision of Rowland's Preliminary Table of Solar Spectrum Wavelengths with an Extension to the Present Limit of the Infra-red," another standard work, stops at 297.5 mμ,[1] and has been extended to 293.5.[15] Verhoeff and Bell, in 1916, claimed 305 mμ as a practical lower limit at sea level with the sun low, and 292 - 295 mμ at high altitudes with the sun high; hardly one-quarter of one per cent of the total solar irradiation, they say, is in the abiotic range (less than 305 mμ). Absorption by ozone below 300 mμ and by oxygen at 170 protect the earth from ultraviolet radiation. The most intense radiation is in the blue range of visible light (near 470 mμ) at high altitudes, and in the green (near 500 mμ) at low. At the equator, at sea level, direct solar flux will vary from about 480 to 540 calories per square centimeter per day on a clear day, depending on the season. At the poles, it varies from around 670 to zero during the long polar night.

With reference to fish, how much light is absorbed by water? Seven to ten per cent of all solar radiation is reflected from a water surface, and an equal amount is scattered back to the atmosphere by the water. The rest is absorbed by water, solutes, suspended material, and living things. Water absorbs most strongly in the infra-red, especially above 750 mμ, and transmits best at 470 mμ, with transmission falling off slightly in the near ultraviolet. Most solutes behave oppositely: They absorb strongly in the violet and blue, moderately at middle

visible wavelengths, and less in the infra-red. Thus, when solutes are present in small amounts, water transmits best in the blue or green; when in larger amounts, the wavlength of maximum transparency moves toward the red end of the spectrum.[17] The intensity of a beam of monochromatic light entering water at right angles is given by

$$I_d = I_o e^{-kd}$$

where d = depth

k = total extinction coefficient, the sum of extinction coefficients for water, particles and solutes.

Intensity therefore falls off logarithmically with depth.

The Effects of Light on Tissues

a. General Discussion

The molecular structure of a material determines which wavelengths of light it absorbs. Light will not harm tissues unless absorbed, of course, and absorption certainly does not guarantee damage. Light which is absorbed may produce excitation or ionization of the molecules. Ultraviolet (abiotic) light produces molecular excitations rather than ionizations, the most effective wavelengths for abiosis being 253 to 266 mμ.[19,9] It is easily absorbed by most tissues. Absorption of wide ranges of wavelengths characterizes heterogeneous materials, in which particles reflect or absorb without regard

to wavelength -- this is seen in the iris of the eye.

How might ultraviolet light affect cellular proteins? Proteins absorb, largely due to their aromatic acids (tryptophane, phenylalanine and tyrosine), between about 240 and 290 mμ, with a maximum around 275.[9] Burge, Schanz and others, around the turn of the century, performed experiments suggesting that intense ultraviolet light could make the proteins of egg white and lenses less soluble.[6,30] Henri and his wife believed the effect on egg albumin was most marked at very short wavelengths (below 220 mμ) and diminished to zero beyond 310 mμ.[2] It is possible, however, that they and the others mentioned were often observing heat effects. A discussion of the absorption of proteins may be found in Ellis and Wells, as revised by Heyroth.[9]

Nucleic acids are well-known as absorbers of ultraviolet; here the purine and pyrimidine bases absorb around 260 mμ. Small doses of ultraviolet are known to depress the rate of mitosis and cause bridge formation between chromosome strands.

True abiotic effects of light have a latent period, usually of several hours, while the effects of heat or chemicals usually are quicker to appear. Abiotic actions might theoretically seem to be possible to some extent throughout the spectrum, but in reality are confined to wavelengths below about 305 mμ for human cells and 295 mμ for bacteria, for above these levels, abiotic damage is either repaired as it occurs, or the cell is destroyed by

heat before abiotic effects develop.[2]

Ultraviolet light has been known to have bactericidal actions since at least 1877; Ellis and Wells have reviewed the early discoveries.[9] The first effect seems to be inhibition of reproduction, and the wavelengths most effective in bacterial abiosis are those which destroy nucleic acids, rather than proteins. Abiosis is prominent in the 210 - 296 mμ range, though. It seems impossible that ultraviolet light could kill bacteria living in other tissues, but this effect is probably very limited for the eye. Verhoeff showed that ultraviolet cannot under any conditions destroy bacteria in the cornea without severely injuring the tissue itself, and Hertel had to use a 20 - 30 minute exposure to a magnesium electrode spark to kill bacteria in tiny quartz boxes in the aqueous chamber of the eye.[29]

Everyone knows the effects of sunlight on the skin. Some of the best experiments in this area were done by Dr. de Laroquette, Surgeon Major of the French Army, in Algeria.[8,2] He concluded (and many more recent authors agree) that the first effect of sunburn is a heat erythema, with a critical temperature of about 30° C. This involves local capillary dilation, production of a histamine-like material, and leucocytic infiltration. After a latent period of one or two hours, a "photochemical erythema" develops. If this is severe, local hemorrhagic pigmentation, edema and desquamation occur, and the newly exposed

skin is darker than the old. It is interesting that the liminal exposure for solar erythema is the same as that for mild photophthalmia and corneal damage.[2] It has been shown in recent years that skin tumors can be produced by large doses of ultraviolet radiation; the paper by Freeman and Knox provides an example.[10]

But, specifically, how does light affect eye media? Again, we should look for abiotic effects and "other" effects, the most common other effects being those of heat; light generates heat when it is absorbed without respect to wavelength -- the amount of heat released depends purely on the energy content of the light absorbed. Severe heating of the eye certainly sets up painful danger signals, and probably almost never occurs during the lives of most animals. Gentle heating must be quite common in animals exposed to the sun.

Let us first look at the naturally-occurring effects of light. Erythopsia, the appearance of a red or pink tinge in everything seen, was once a source of confusion (see Walker[29] for a review of early thinking) but is now thought to be a result of color fatigue, with the red-sensing mechanism returning before the others.[2] (Erythopsia may also be caused by hemorrhage or by neurosis.)

Much more severe, usually, is vernal catarrh, a chronic conjunctivitis beginning in the spring, when days are getting longer, and disappearing as colder weather returns. It was originally associated with light because of

its summer occurence, and Birch-Hirschfeld produced similar symptoms in rabbits exposed to a "Uviol lampe" for ten minutes daily, at ten centimeters, for 180 days,[29] but his results have been discounted, and vernal catarrh has now long been regarded as not due to radiation of any kind.[2]

J. Hirschberg, in 1898, was the first to suggest sunlight as a possible cause of cataract; he used the high incidence in India as evidence.[3] Daland emphasized the commonness of cataract in the country, as oposed to the city.[3] W.E. Burge had this theory: cataractous human lenses from India contained unusually large amounts of calcium and silicon salts, and Indians are exposed to more sunlight than Europeans or North Americans, especially (he said) ultraviolet light. He could experimentally lower the solubility of proteins, and precipitate them, by exposing protein solutions or lenses to ultraviolet in the presence of calcium and silicon salts. Therefore, those classes of Indians whose diet included siliceous earths accumulated these salts in their lenses, where they were active with ultraviolet light from the sun in precipitating protein.[6] Two theories of the combined action of calcium salts and light were proposed by Weil, using as models theories about photographic emulsions.[30] Finally, in 1909, Handmann showed that senile cataract often begins in the part of the lens which he said was not exposed to the short-wavelength light of the sky, the lower half.[29] But Handmann's argument was neatly turned against him and

Burge by Verhoeff and Bell in the following way: the area of the lens which is really most exposed to sunlight is the pupillary area, not the lower half; why, then, does senile cataract often start in the lower half if it is light-induced? Furthermore, with respect to Burge's own argument, calcium, magnesium and other salts are deposited in the lens in other kinds of cataracts, (e.g., traumatic and inflammatory cataracts), and in fact this occurs in dead tissue anywhere, and is probably a result of the lesion, not the cause. And, Burge did his protein and lens experiments with light much more intense than that normally received from the sun by the lens. Finally, the wavelengths with which he could coagulate protein did not correspond to those of abiotic activity, as shown by Verhoeff and Bell.[2] So senile cataract seems not directly caused by sunlight.

Eclipse blindness and its relatives may be the oldest recorded natural forms of eye damage by light. Galileo injured his eye by looking at the sun through his telescope, and Galen reported symptoms in persons who had observed an eclipse. Very accurate symptomatic descriptions were made by Reed in 1761 and Soemmering in 1791; they are mentioned in Walker's thorough review of retinal injuries caused by light.[29] Eclipse blindness consists of an immediate scotoma after looking directly at the sun. This does not pass away, but leaves a more or less serious cloudiness or loss of vision for periods of from several weeks

to years. The retinal damage is central, usually small, and often corresponds to the image of the sun.

Eclipse blindness was originally casually ascribed to ultraviolet light, but Verhoeff and Bell point out that, (1), many cases have been received through eyeglasses, insufficiently darkened glass panes, or opera glasses, which cut out ultraviolet; (2), ultraviolet is so well absorbed by the lens that it can't reach the retina; (3), the maximum of solar energy lies in the blue region of visible light, with very little in the ultrviolet.[2] They believe that eclipse blindness is a thermal effect on the retina; they have caused similar symptoms in rabbits made to fix on less intense light sources for longer periods. Only the briefness of the exposure, the miosis usually prevailing, and the normal wandering of the eye prevent this condition from being more common.

Similar symptoms are found in people who have observed large electrical short circuits at close quarters (in such cases the pupils are often dilated when the short occurs) and in those who have onserved nearby lightning flashes -- a French policeman standing only yards from a lightning bolt on his post one night received a case of chorioretinitis with clouding of the vitreous which had not disappeared after three years.[2]

Snow blindness is somewhat related; snow is a good reflector of sunlight down to the extreme ultraviolet. Here a temporary photophthalmia occurs; it has been

analyzed by Verhoeff and Bell.[2]

Intentionally or not, man has managed to elicit a great many eye injuries with his own equipment. The electric arc was introduced in 1879 and 1880. Not only did it provide a strong source of ultraviolet light for various uses. but it also provided many of its users with cases of "ophthalmia electrica", photophthalmia very similar to snow blindness which, in severe cases, involved corneal injury.

Much more serious was the now-rare condition of glassblower's cataract. Though Snell in 1902 and Robinson in 1907 questioned statistics, it is commonly agreed that English glassblowers in the early 1900's frequently developed cataracts.[29] The cataract usually appeared first in the left eye, which was more exposed to the glass furnace than the right; when it began on the other side, the blower often said he had been turning his right side towards the furnace. Blowers developed a rusty brown spot on each cheek, more marked on the left side. The cataract often began before age 40 with a rosette-like or diffuse opacity in the lens cortex at the posterior pole. Striae sometimes appeared later, as in senile cataract. Glassblowers were usually thin and subject to asthma and pulmonary tuberculosis. Most had emphysema of the parotid gland. They perspired excessively during work, and so drank large amounts of all kinds of fluids.[2]

Causes originally suggested included venous stasis of

the vortex veins due to forced expiration, for cataracts could be produced by tying off these veins. Concentration of the aqueous humor by evaporation of tears and sweat was proposed.[2] But the most popular idea was that glassblower's cataract could be traced to light: its frequency and uniformity, and the fact that it began on the more exposed side, were regarded as suggesting this.[2] In 1910, Schanz and Stockhausen examined the radiation of the glass furnace and the glass shop conditions. The glassblower's head was exposed, they found, to a temperature of 110° C in taking the glass from the oven, and 45° C while blowing. But these were still not as great as the temperatures to which many iron and blast furnace workers were exposed without eye damage. So they believed that ultraviolet light of 350 - 400 mμ wavelengths, which was strong in the glass furnaces, was responsible, and that no <u>outer</u> eye trouble occurred because no wavelength less than 320 mμ (which they felt damaged the outer eye) were present.[29] But Aschkinass showed that, in the wavelengths strongest in the glass furnace light, 80 to 90 per cent of the radiation was absorbed by the cornea, and only 3 to 4 per cent by the lens, primarily the anterior cells.[2] Burge, still working on precipitation of protein in high-salt situations, claimed that ultraviolet from the furnace kept the lens protein in an easily-precipitated state until some accident of diet or metabolism raised the ion concentration in the eye, when precipitation occurred.[6] He

could not explain why the posterior pole was first affected, while the anterior pole was more strongly exposed. (It should be noted that the laminated structure of the vitreous might reduce heat transfer by convection and conduction at the rear of the lens, so that temperatures might rise more there than elsewhere.)

Yet the evidence <u>against</u> ultraviolet as a causative factor was strong: the spectrum of molten glass does not go below 320 mμ, and does not contain any abiotic radiation. Even if abiotic radiation were somehow present in small amounts, experiments (see below) showed that it would not penetrate the cornea. Experiments by Verhoeff and Bell showed that lenses exposed to strong light sources were damaged only on the anterior pole, and to depths no greater than 20 microns.[2]

Under the influence of the "Report of the Glass Worker's Cataract Committee," compiled by Bradford <u>et. al.</u>, it was eventually agreed by many that glassblower's cataract was only indireclty caused by light, which, perhaps by heating or causing water loss, damaged eye metabolism.[9] It was suggested that damage to the ciliary body was directly involved, since diseases of the fundus were known to produce cataractous changes at the posterior pole of the lens, attributed to impaired nutrition.[2]

Interest grew in trying to produce eye damage experimentally with light. Before discussing specific cases, we should note these general principles given by Verhoeff and Bell:[2]

1. the effects of heat appear immediately, but there is a latent period of one-half to twenty-four hours before abiotic effects are apparent;
2. abiotic damage follows the law of inverse squares and is proportional to the total amount of abiotic radiation received;
3. repeated exposures, if not too small or infrequent, will sum up to produce abiotic effects; and
4. only light of wavelengths less than 305 mμ produces distinct abiotic reactions.

b. The Conjunctiva: Experiments

Beginning with the front of the eye, Windmark reported in 1901 and 1902 that exposure to ultraviolet caused conjunctivitis in rabbit eyes which could be prevented by the interposition of thick glass or quinine sulfate solution, which absorbs ultraviolet.[29] This was duplicated many times by Verhoeff and Bell,[2] Burge,[6] (who claimed that the reaction was increased by the pressure of certain salts in the lid or corneal cells, and in frogs living in salt solutions), and Martin[22] (who obtained graded responses, depending on exposure). Verhoeff and Bell, at least, interposed a water cell to absorb the infrared, which is very potent in producing heat. Their elaborate studies led them to say that this type of conjunctivitis was very comparable to the skin inflammation seen after ultraviolet therapy. Conjunctival hyperemia becomes progressively more severe, and may involve edema and purulent exudation lasting for

three to nine days, even if the exposures used are insufficient to damage the cornea. More recently, Freeman and Knox say that pterygia and pinguecula of the conjunctivia can be related to sun exposure, and that carcinomas of the lids and perhaps conjunctivia can result from exposure to ultraviolet which produces skin tumors.[10] It may be that wavelengths near 296 or 297 mμ are most effective in producing conjunctivitis, with a range of 270 - 320 being less effective.[23]

c. The Cornea: Experiments

Reports of corneal damage from light are very numerous and extend over a long period. In 1889, Windmark reported swelling and necrosis of nuclei in the corneal epithelium, followed by small ulcerations which formed opaque areas.[29] Burge, in 1916, kept goldfish in solutions of 0.8 % calcium chloride and nitrate, 0.1 % sodium silicate, 1.0 % dextrose and tap water. On exposing one eye of each fish to ultraviolet, he found corneal opacities which cleared up within ten days in the tap water fish, but not in the others.[6] Martin reported corneal clouding and epithelial thickening in thirteen rabbits and three guinea pigs exposed repeatedly to moderate amounts of ultraviolet.[22] Verhoeff and Bell found, in rabbits, that exposure to wavelengths as short as 310 mμ caused only thermal clouding; 305 mμ slightly damaged the cornea in large doses; 295 mμ caused granulation of basal cells and central corneal haziness, thin epithelial stippling and loss,

opacity, and leucocytic infiltration. Wavelengths extending below 295 mμ, in large doses, caused all these changes plus semi-liquification of parts of the stroma down to Descemet's membrane. Pus cells and phagocytes were seen. The cornea usually reformed thinly within five days, and completely in five weeks, but in severe cases permanent opacities remained. Exposures of about two and one-half times those causing conjunctivitis were used to damage the cornea. They found that the cornea absorbed eighteen times as much abiotic radiation as it transmitted, for exposures of eighteen times that required for corneal damage were needed to damage deeper tissues.[2] Kinsey now claims that corneal sensitivity is greatest at 280 mμ.[18]

More recently, Freeman and Knox exposed animals to daily erythemal doses of ultraviolet. They describe the development of red vascular and white fibrous tumors identified as hemangioendotheliomas and fibrosarcomas, respectively, because of their rates of growth and cytology. No metastases were found. Rats, mice and hamsters, but not guinea pigs, developed tumors. Skin damage and the appearance of fibrin strands and leucocytes in the anterior chamber preceded tumor formation, and pigmented rats and mice (but not hamsters) developed tumors significantly faster than non-pigmented animals.[10] Lippincott and Blum found eye tumors of the same kinds in five per cent of those ultraviolet-exposed mice which developed skin tumors in their experiments. Hyperplasia of the corneal epithelium,

vesicle formation, focal vascularization of the substantia propria, leucocytic infiltration and pigmentation of the stroma were all seen. No definite carcinomas were found, which parallels the preponderance of sarcomas over carcinomas in mouse skin; in humans, on the other hand, carcinomas predominate both in skin and eye, and hemangioendotheliomas do not occur.[4] These authors report that Ash and Wilder, having examined many patients, feel (but apparently cannot prove) that human limbic tumors are more common in southern states, where light exposures are greater.

d. The Iris: Experiments

The iris is only damaged after exposures severe enough to damage the lens epithelium, say Verhoeff and Bell.[2] In rabbits exposed to this amount of light, they found pupillary constriction, hemorrhages near the pupil and congestion in the iris, and exudation of pus cells. They also noticed that lower exposures, causing conjunctivitis and keratitis but not lens damage, produced serum and fibrin in the anterior chamber, probably as a result of damage to the iris vessels. Blum and Lippincott found iridocyclitis and senchia in mice exposed to severe ultraviolet.[4]

e. The Lens: Experiments

There has been much experimentation suggesting that light can damage the lens. It is probably best to consider

it in the order in which it was published. Czerny and Deutschmann, in 1867 and 1882, respectively, exposed lenses (probably of rabbits) to sunlight and found turbidity developing in the cortex. The lens capsule was unchanged. Herzog, in 1903, found the same effects could be caused by light from a carbon arc focused through a plain glass lens, and, ultraviolet light being thereby excluded, it is likely that the turbidity was caused by heat.[29] During the period from 1889 to 1892, Windmark exposed rabbits to 1200 and 1400 cp zinc arcs through a heat-absorbing water bath with quartz sides. The cornea and iris were damaged, and the lens capsule in the pupillary area showed intense nuclear staining, mitosis with cell proliferation and destruction, and swelling of fiber bundles; transudate was found between the cortex and lens capsule. These effects were prevented by exposure through quinine sulfate, which absorbs ultraviolet.[29]

Hess in 1888 and Kiribuchi in 1900 showed that electrical sparks impinging on the supraorbital region caused central destruction of the lens epithelium, vacuolization of lens fibers, and peripheral epithelial proliferation leading to cataract. Burns, scars and vascular or nervous system damage were often seen in these cases, and might have caused some cataracts, but these writers believed most of the cataracts were caused by electrochemical changes.[29]

In 1904, Hess exposed rabbits, guinea pigs and frogs to a 3.5 - amp mercury vapor lamp at 10 to 30 cm. Forty-

eight hours after a six to twelve hour exposure, the lens changes observed earlier by Windmark occurred. In addition, a ring or "wall" of deeply staining cells developed in the lens epithelium under the iris -- probably cells heaped up by central epithelial proliferation. The interposition of glass plates which absorbed wavelengths less than 313 or even 280 mμ prevented these changes. Birch-Hirschfeld later showed that a simple quartz and water heat filter also prevented them.[29] In 1909, he produced the same damage by focusing a five amp arc light on rabbit lenses in situ for five minutes daily with a common glass lens. He claimed that the changes were produced by those wavelengths less than 400 mμ transmitted by the lens.[29] This seems a strange position, since Birch-Hirschfeld also held that the changes were prevented by using a heat filter which absorbed long wavelengths.

In 1912, Martin exposed rabbits to a mercury vapor lamp and obtained (in addition to corneal and iris damage) swelling of the lens capsular cells and, in 24 hours, extrusion of chromatin from nuclei in the pupillary area. Hess's wall of cells was seen under the iris. Regeneration was under way in two or three days. He repeated his experiments using similar exposures on three guinea pigs and thirteen rabbits; this time only one rabbit showed lens changes (proliferation of anterior capsular cells), and then only at the most severe exposure of the set. Martin thought this might have been the beginning of an anterior

capsular cataract, without the damage usually preceeding such a cataract under extreme exposures.[22]

Burge, in 1916, exposed goldfish living in 0.8 % $CaCl_2$ and $CaNO_3$, 0.1 % sodium silicate, 0.1 % dextrose, and tap water to a 2400 cp mercury vapor lamp for six hours. He said corneal opacity developed in fifteen hours and lens clouding, in two days in all fish; the conditions disappeared in tap-water fish but developed into cataracts in the others. He felt that during the fifteen hour latent period, the cornea was absorbing ions from the blood and protein was precipitating intracellularly.[9] Burge also made calcium chloride extracts of pig lenses and of egg white, and exposed them to ultraviolet light from a mercury vapor lamp, separated by a quartz spectroscope. In each case, he claimed coagulation at 254 mμ in fifteen minutes, at 265 mμ in sixty-five, at 280 - 302 mμ in one-hundred twenty minutes, and at other wavelengths later. Covering half the spectroscope slit with a rabbit cornea prevented coagulation at less than 297 mμ. He considered this evidence that the cornea passed some ultraviolet capable of precipitating proteins in the lens whenever enough salts were available.[6] It is pertinent that Schanz, in 1918 - 1920, exposed protein and acetone in quartz test tubes to ultraviolet, and said that exposure depressed protein solubility.[9]

Verhoeff and Bell published very comprehensive studies in 1916.[2] From experiments with filters, they said that

only wavelengths less than 305 mμ are abiotic (destroy the corneal epithelium or damage the lens capsule) in the rabbit eye. They focused sunlight on a rabbit's lens with a mirror. Though the iris was soon completely necrosed by heat, changes in the capsular epithelium did not appear until after twenty-four hours, and reached a peak only two to three days after exposure. These changes were of three types: irregular cellular swelling, cytoplasmic granulation (by large eosinophils and small basophils), and formation of a peripheral wall of cells as described by Hess. Very large exposures to a magnetite arc (rich in ultraviolet) caused these changes with vacuolization and eosinophilia of the lens below the epithelium for a depth of up to 20 microns. They never saw a visible opacity in a rabbit lens, but one lens put in normal saline after exposure developed pupillary opacity in 48 hours. Damage to the capsular epithelium was repaired by nuclear budding and repair of the original cells; no mitoses were seen, and they felt that earlier reports of mitosis in this tissue were wrong. They found that they could force a wall of epithelial cells to form under the iris and granulation to occur simply by injecting Lugol's solution into the anterior chamber, and they proposed that these were nonspecific reactions to irritation. Finally, Verhoeff and Bell reiterated the implication of Czerny, Deutschmann and Herzog's work; that even severe visible light can cause lens clouding, probably by heat.

In 1920, Hinrichs extrapolated on Burge's earlier work and said that frogs injected with 2 cc of 0.1 % NaCl or $CaCl_2$ in the dorsal lymph sac one-half hour before exposure to a mercury vapor lamp developed opacity in their exposed lenses, but uninjected frogs did not. He also said that exercised, ultraviolet-exposed frog lenses develop opacity faster in these same salt solutions than in tap water, and that exposed chick and hen lenses became opaque only in the salt solutions, and not in Ringer's solution.[16]

Vogt, also in 1920, claimed to have produced cataracts in rabbits with infrared light, and said that visible and ultraviolet light should have similar effects.[30] Rohrschneider produced lens opacities in guinea pigs with intense ultraviolet light exposures in 1928, but only after corneal damage which could have brought on the lens effect.[3] Blum and Lippincott said that cataracts in mice they exposed to ultraviolet were probably secondary to corneal adhesion to the lens. Lenses from these mice showed scattered epithelium-like cells, calcium deposits and destruction of fibrilation.[4]

Most recently it has been found that microwaves produce cataracts starting in the subcapsular region of the posterior cortex -- for a discussion, see Carpenter et. al..[7]

f. The Retina: Experiments

Finally we should consider retinal damage by light.

With a moderately dilated pupil, the intensity of the image on the macula could be enormously greater than on the cornea, and might damage the former before the later -- this, in fact, is eclipse blindness. The retina could be injured by light in three ways: by heat, by abiotic action on the retinal cells, or by overstimulation of the mechanism of perception, resulting in some physiological damage of it. Damage to rabbit retinas by heat was described by Verhoeff and Bell, and involved sharply defined redspots, in which the pigment epithelium was always the most severly damaged, followed by the rods and cones, the choriocapillaris, and the outer nuclear layer, in order. They discuss the histological details of the damage.[2]

Hallauer and others had reported that the lens absorbed all wavelengths less than 376 mμ, screening the retina. Verhoeff and Bell had shown that wavelengths as short as 310 mμ did no harm to the cornea or lens, and inferred that they would not damage the retina. Yet, Birch-Hirschfeld claimed to have produced retinal damage with ultraviolet light. To get a definite answer, Verhoeff and Bell first made calculations showing that no abiotic effects would be seen on the retina of a person who looked directly at a bare magnetite arc for two hours. Then they focused ultraviolet of wavelengths longer than 305 mμ on rabbit retinas, using a quartz lens and water cell. Not even after very severe exposures were they able to see any damage histologically. To rule out functional damage, they

exposed two monkeys given pupillary dilators to an arc lamp that was so intense that it caused an absolute scotoma for five minutes in one of the experimenters who looked at it for fifteen seconds. The monkeys were exposed for an hour and a half. After recovery, the monkeys' visual acuity, demonstrated by their ability to catch flies, did not seem to have lessened. From this work, Verhoeff and Bell said they felt it very unlikely that ultraviolet might damage the retina, and that Birch-Hirschfeld's observations could be attributed to his methods and interpretation. They thought instead that, after a certain amount of retinal fatigue, no more would occur no matter how much light was given, and that recovery might even start before the end of exposure. They exposed one eye of a woman with cancer of the orbit requiring removal to wavelengths greater than 305 mμ for a total of 55 minutes over an hour and a half. Her visual acuity in that eye was back to normal the next morning; later the eye was found histologically normal.

It could be argued that, if the lens protects the retina from ultraviolet, the aphakic eye would be susceptible to retinal damage. Verhoeff and Bell, however, calculated that to be harmed, such an eye would have to focus on their bare magnitite arc at a distance of three meters for almost half an hour continuously or for eight minutes daily for six days. It would not be damaged by focusing for four minutes daily for any number of days. Further, they did

not allow for pupillary constriction, the normal wandering of the eye, or for the usual thick cataract glasses, which would increase the required exposures. They present figures showing that fixation on any illuminant, including common arc lamps, for as long as a sensible person would fix on such a source plays much less energy on the retina than the minimum needed to cause damage; certainly one may fix on the sun for several seconds with no more than a temporary scotoma. The energy concentrated on the retina in such a fixation is about 113 x 10^6 erg/cm^2/sec, making the sun considerably more dangerous than any common artificial light source.[2]

Having considered light damage to the eye, we should say that light and other radiation has been used to produce beneficial eye changes. In 1910, Doelter found that radium, a strong beta and gamma emitter, would change colloids into crystalloids, and occasionally the opposite -- but more often the opposite could be done with ultraviolet light! Cohen and Levin, publishing in the 1919 JAMA, treated 24 cases of cataract with radium emissions. Some improvement was seen 87.5 % of these, and further development was arrested in every case. They made no hypotheses about the action of radium, but Weil suggested that the radiation disturbed a chemical equilibrium which had been or was being reached in every case, by causing ionization.[30]

Sulzer claimed that he had partially cleared an

opacity using ultraviolet therapy, and almost completely cleared it with nine subsequent applications of radiotherapy (presumably with radium).[30]

Concentrated light is now used to coagulate tissue in the retina in the treatment of retinal tumors. Melanomas in the rabbit eye can be destroyed completely in this way, with little danger to vision. Light beams are used to weld detached retinas back in place, as well.[12]

The Absorption of Light by the Eye Media

The first study of ultraviolet light and the eye was made by Brucke in 1845, in an attempt to explain the invisibility of the ultraviolet. Many related studies followed: Stokes, in 1852, found he could see wavelengths as short as 335 mμ; Helmholtz, though very myopic, claimed he could see a few emission lines in the 372 and 318 mμ regions. But in 1856, Eisenlohr threw doubt on all such observations by finding that the lens absorbs light in the 350 - 400 mμ range and fluoresces again in the blue, so that early investigators may have mistaken the fluorescence of their own lenses for true vision.[29]

There followed several experiments on lensless eyes. In 1883, deChardonnet coated a quartz plate with a silver film just thick enough to pass wavelengths between 301 and 343 mμ. Normal eyes could not see an arc light through the plate, but aphakic eyes could, and could follow movements of the light.[9] It was clear now that the retina could

respond to ultraviolet that would normally be absorbed by the lens.

How can lens fluorescence be distinguished from a true image? Walker suggests that fluorescence should appear as a blur impossible to focus, while wavelengths passing through the lens should be focused into a sharp image.[29] Of course, in the ultraviolet, some incident light might be focused into an image while some would be absorbed and fluoresced. Whether this occurs or no, there are people who claim to have seen the 317.5 mμ line in the tin spectrum and the mercury doublet at 313.2 and 312.6 mμ with normal eyes.[14,13]

These early experiments, though, say nothing about other eye media. How much light do they absorb? A certain amount of the incoming light is reflected at the boundaries between the cell layers of the cornea, in passing into the aqueous humor, in entering the lens, and again in entering the vitreous humor. Reflection losses are greater for short wavelengths than for long, and greater near the periphery of the cornea, aqueous humor or lens than centrally, as expressed for the rabbit cornea by Verhoeff and Bell in Figure 1.[2]

All the light not reflected is either scattered, transmitted, or absorbed. Transmitted light was the earliest to interest scientists: deChardonnet photographed the light passing the human eye in 1883, showing that absorption began at 397 mμ and became total, in his apparatus,

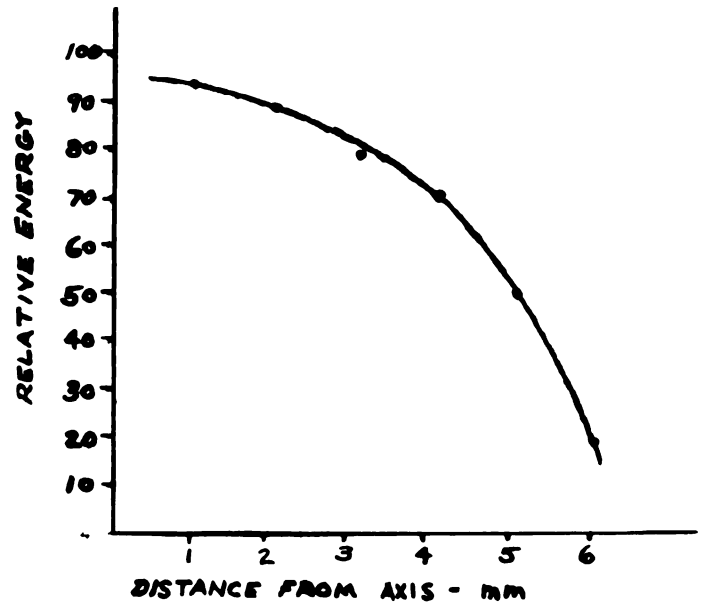

Figure 1: Energy Transmitted Through a Cornea as Function of its Curvature

st 372. He was the first to emphasize that it is the lens which limits the spectrum of normal vision: he, like others, showed aphakic eyes to be more sensitive to ultraviolet than others.[29] In 1909, Dhéré published studies on the absorption of proteins and albuminoids.[30] In the same year, Birch-Hirschfeld, Shanz and Stockhausen all used spectrographs to measure the absorption of various kinds of glass and the cornea, lens and vitreous humor of several animals, including humans.[29] By 1912, Martin had done the same.[22] This is by no means a complete review of the early investigations, and much more information may be

found in the works of Ellis and Wells, Verhoeff and Bell, and Walker.[9,2,29]

One very influential early study remains to be mentioned. In 1895, Aschkinass found that the eye media, except the cornea in some cases, absorbed visible and "near" infrared light much like certain thicknesses of water, that is, for cattle,[21]

Table 1: Aschkinass' approximations

part of the eye	corresponding thickness of H_2O
cornea	0.06 cm
aqueous humor	0.34 cm
lens	0.42 cm
vitreous humor	1.46 cm
whole eye	2.28 cm

Most of the absorption, he found, was in the infrared near 1000 mμ, though a second peak was located around 750 mμ. The pigment epithelium of the fundus and the iris absorbed light of all wavelengths, and the resemblance of the media to water did not extend to the ultraviolet.[2] This work had a great impact on related investigations elsewhere, and tables have been prepared of the energy absorption of the eye exposed to blackbody radiation of from 2000 to 5000° K; these may be found in Luckiesh, and, very unhappily, do not extend as far as the blackbody temperature of the sun.[21]

From the early studies above to the present, many experiments have been made on the absorption of light by the

eye media of different animals. Most of these were done with spectographs, so the absorption was measured only qualitatively, but a few of the later ones have used spectrophotometers. There is such a wealth of data that it is best expressed in pictorial form. In the bar graphs of Figure 2, the shaded areas represent light absorption, and the open areas, transmission.

How does light transmission or absorption change with time? In man, oxen and cattle, the old lens absorbs more strongly in the longer blue and infrared wavelengths than the young lens.[5,13,22,24] Injury or disease changes the absorption, usually in the same way as age. Short-term changes are seen after removal of the media: the 230 mμ transmission band of human aqueous humor moves towards longer wavelengths,[13] and the corneas of oxen, fish and perhaps other animals become cloudy, probably because of hydration.

We have already considered the harmful effects of light energy on the eye, and only a few points remain to be mentioned. First, some light energy certainly is converted to heat: Verhoeff and Bell say, for example, that wavelengths longer than 305 mμ absorbed by the cornea are converted almost entirely to heat.[9] Of the heat developed in the cornea, twenty to twenty-five per cent passes through the cornea and sclera; twenty to thirty per cent of this is then absorbed by the aqueous humor; thirty per cent of that passing the cornea and iris is then absorbed

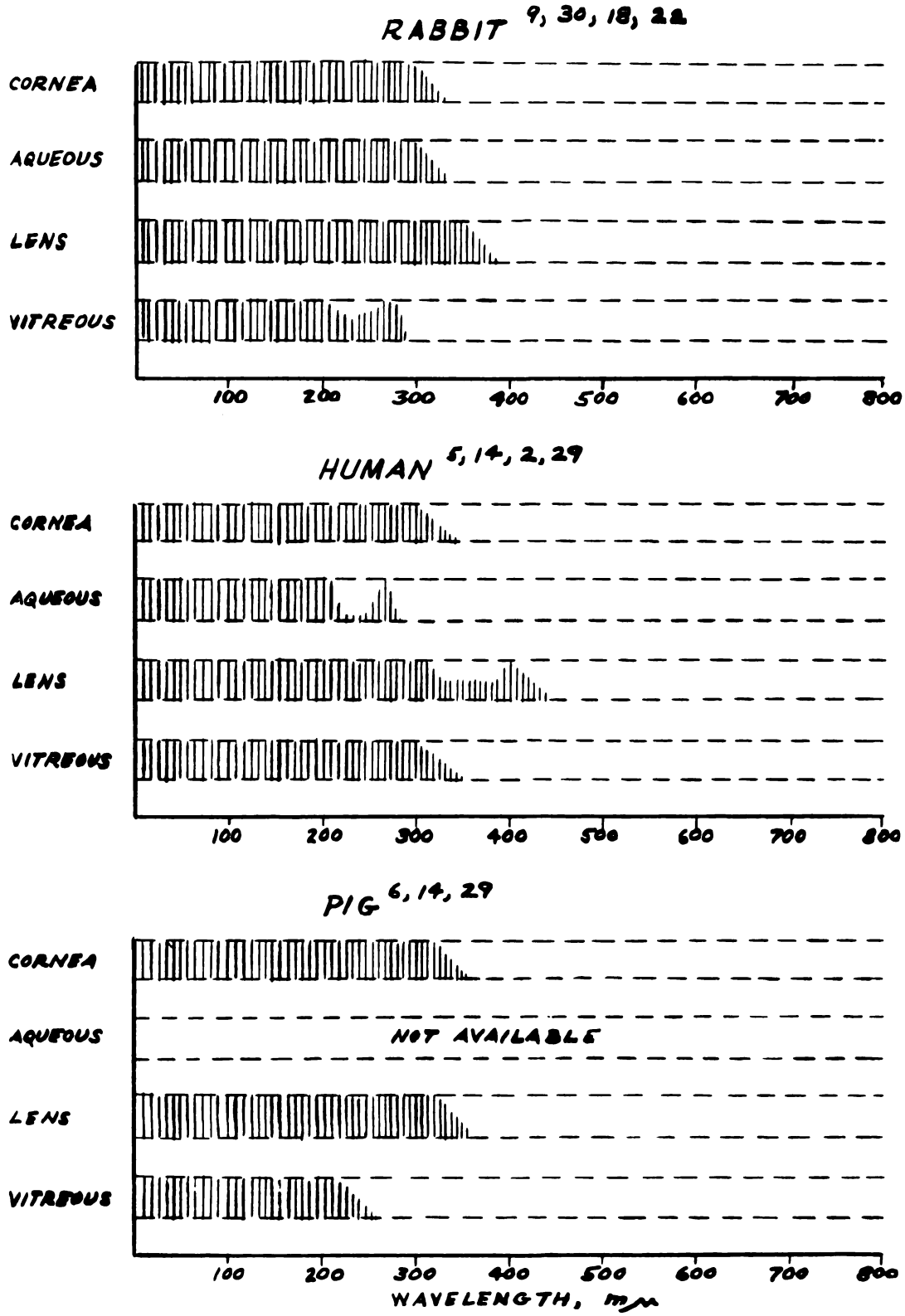

FIGURE 2: LIGHT ABSORPTION BY OCULAR MEDIA

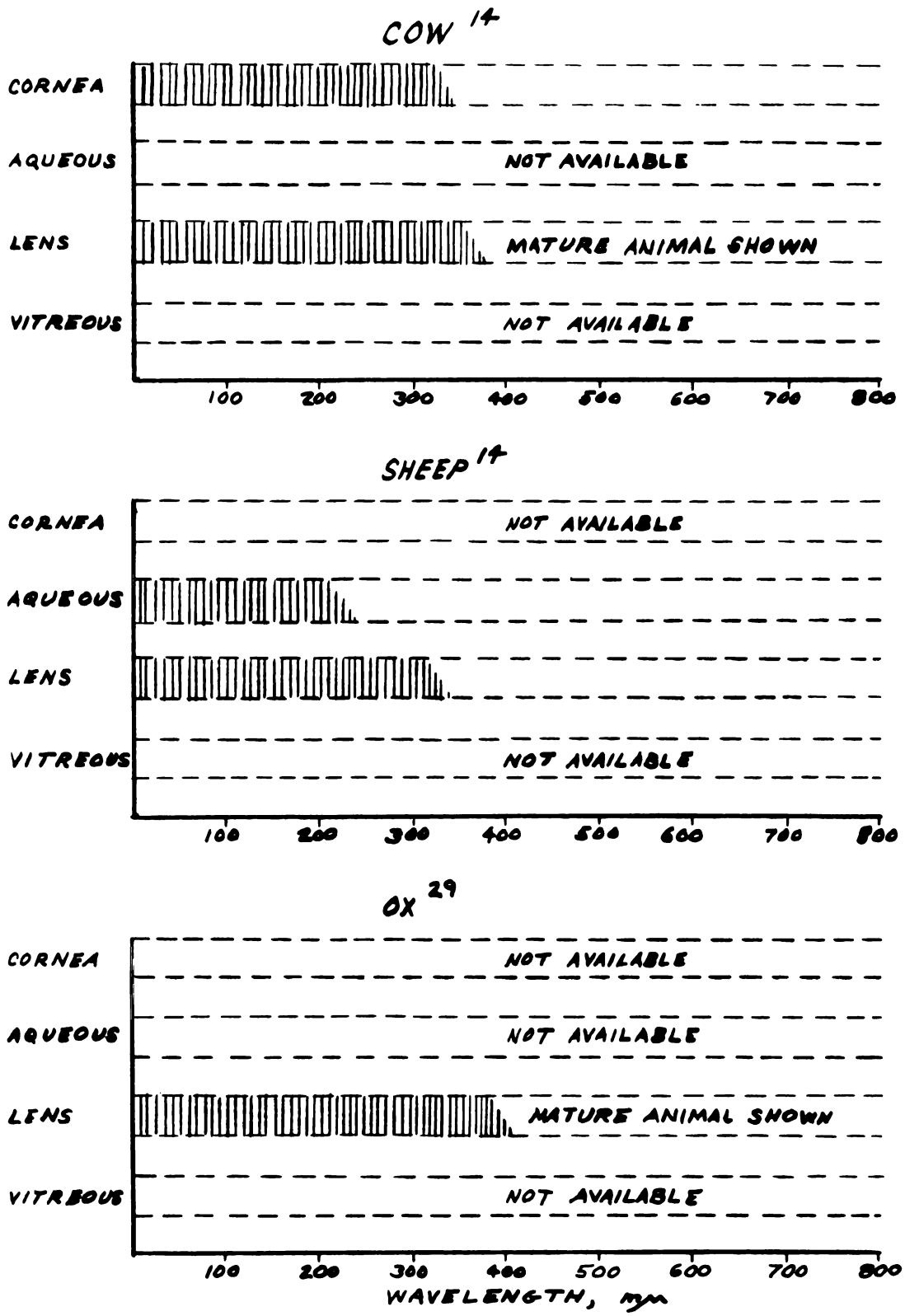

FIGURE 2, CONTINUED

by the lens; and sixty per cent of the heat reaching the vitreous is absorbed therein. In addition, the upper lid absorbs some heat. As a result of all this, only about three per cent of the heat applied to the cornea reaches the retina.[24] Heat is also generated by light absorbed by other eye structures.

Besides forming heat, some of the absorbed energy fluoresces in the lens, as already mentioned. Some has "abiotic" action by specifically deranging nucleoproteins, collagen, albumin or globulin, albuminoids or crystalbumin.[18] Finally, some may have specific photochemical actactions: ultraviolet below 360 mμ or sunlight have been shown to catalyze the oxidation of ascorbic acid by molecular oxygen in the aqueous humor, starting a chain of reactions which ultimately produces more $NADP^+$ (TPN^+), which is the limiting factor in the oxidation of glucose in the lens.[17]

This has been a review of light absorption by the ocular media, and its effects. It is clear that any investigation of light - induced damage must ask first, what light is absorbed by the tissues in question; second, how much energy is involved; and third, how might this cause damage? Light is clearly implicated in the ocular lesions seen in lake and, probably, rainbow trout, and the research reported here is an attempt to answer at least the first two of the questions above. It is hoped that this will pave the road to the answer to the third question.

MATERIALS AND METHODS

Equipment

A Beckman DB-G spectrophotometer with red-sensitive photomultiplier was used with a ten inch linear-log recorder to measure light transmission by the eye media. To hold corneas and lenses in its sample compartment, a special set of silica-window cells was made by Johnson Instrumentation Specialties of Ann Arbor, Michigan. One cell held the cornea or lens in trout Ringer's solution, while the other contained only the solution (see Table 2).

Table 2: Trout Ringer's Solution

component	gm/l	ionic strength, mM/l	
NaCl	7.37	Na^+	155.0
KCL	0.31	K^+	7.7
$CaCl_2$	0.10	Ca^{++}	0.9
$MgSO_4$	0.18	Mg^{++}	1.5
KH_2PO_4	0.46	Cl^-	132.0
Na_2HPO_4	2.02	PO_4	17.7
glucose	.50*	SO_4	1.5
		Total	316.3

* = added just before use

The sample cell held corneas between two round neoprene rubber washers (faucet or spigot washers), while lenses were forced into a hole drilled in a translucent, white piece of one-eigth inch acrylic plastic. A sketch of the cells follows.

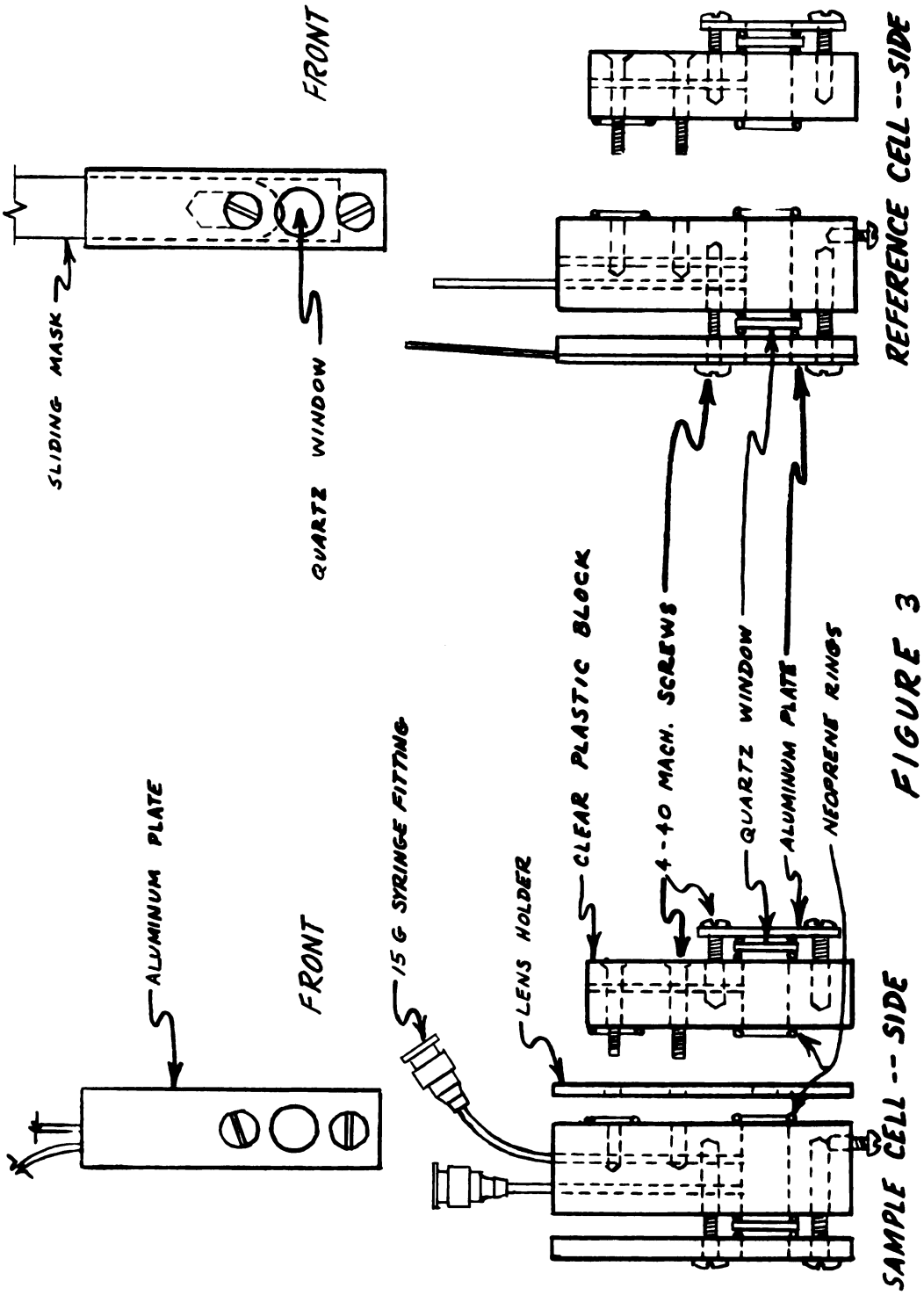
FRONT
SLIDING MASK
QUARTZ WINDOW
FRONT
ALUMINUM PLATE
REFERENCE CELL -- SIDE
15 G SYRINGE FITTING
LENS HOLDER
CLEAR PLASTIC BLOCK
4-40 MACH. SCREWS
QUARTZ WINDOW
ALUMINUM PLATE
NEOPRENE RINGS
SAMPLE CELL -- SIDE

FIGURE 3

Aqueous humor samples were placed in a standard Beckman microcell (no.97260, 10 mm path), with an adjustable Beckman beam attenuator used as reference cell. The spectrophotometer was fitted with temperature control tubing, so that cold tap water could be run around the cell compartment, keeping the temperature at 18 - 19° C while recording.

Animals

First, rainbow trout (Salmo gairdneri) eyes were used. These trout were kept at 12° C in aerated, flow-through, epoxy-painted wooden tanks inside a walk-in cold box until used. Fish weighing about 100 to 150 grams were chosen most often, but other sizes (usually larger) were tried occasionally. The eyes of any fish of about 75 grams or more would fit into the cells. Some rainbow trout showed symptoms of fin rot; a condition thought to be furunculosis was seen for a few weeks in the spring; and pathological eye conditions (keratoconus or cataracts, or both) were sometimes found. Data from experiments with damaged or cloudy eye media were treated separately. Our rainbow trout were obtained from the Michigan Department of Conservation hatchery at Grayling, where they were kept in uncovered, three feet deep outdoor raceways from a age of about six months.

Lake trout (Salvelinus namaycush) 29 months old and weighing about one hundred grams were obtained from the

Jordan River National Hatchery near Elmira, Michigan. The eye pathology previously described was seen in 10 to 20% of the fish from this hatchery. At the hatchery, the fish had been kept indoors until about two and one half inches long, then kept in uncovered outdoor raceways four to six feet deep. In our laboratory, lake trout were kept in the same facilities and under the same conditions as rainbow trout.

In addition, a dog aqueous humor sample was used. This was taken from a mongrel dog anesthetized with pentobarbital.

Techniques

Most fish to be used were killed with a sharp blow on the head; a few rainbow trout used for aqueous humor experiments were anesthetized with MS-222 (tricaine methanesulfonate, Sandoz Pharmaceuticals). One cornea was cut off around the limbus with scissors and placed in the sample cell; the other cornea and both lenses were removed and put in trout Ringer's solution until time for use. It was soon found that clouding of these stored tissues could be delayed for hours if the whole unused eye were kept intact until its cornea was needed, and if the Ringer's solution containing these tissues were refrigerated at 2-5 ^{o}C.

Prior to each experiment, the special sample and reference cells, set up for corneas or lenses, were both filled with Ringer's solution, and a spectral plot was run from 800 to 190 mμ; this was taken as representing 100% transmission (100 %T) at each wavelength. The sample cell

was removed and the proper tissue inserted. The chart paper was rewound so that a spectrum of the tissue was recorded below the 100 %T line and at the same paper speed. In the case of aqueous humor, the empty (air filled) microcell was used for a 100 %T measurement. Aqueous humor, removed with a syringe and 21 gauge needle inserted through the limbus, was centrifuged before reading to eliminate bubbles. Aqueous humor contaminated with blood was not used. Aqueous humor from two or more fish was sometimes pooled to provide enough (about 0.3 ml) to fill the cell.

The recorder and spectrophotometer were synchronized so that a recording could be divided into known intervals of wavelength. Many recordings were made of each type of tissue. At 50 mμ intervals (shorter intervals at critical areas), the heights of the transmission recordings for the tissues were measured as percentages of the height of the 100 %T recording at that wavelength. For each wavelength where these measurements were made, means and standard errors were calculated. Statistical tests, to be explained in the next section, were applied to detect differences between the transmission of lake and rainbow trout eye media.

Lenses interfered with the optics of the spectrophotometer, so that recordings of lenses always showed a steady 4-5 %T from 800 mμ down to a point between 300 and 400 mμ, where transmission dropped off quickly to zero. It was clear that these recordings did not accurately represent

the lens's transmission, but we had no equipment to hold the lens in a way which would eliminate its optical interference. Data for lenses was therefore limited to (1), the wavelength at which transmission was greatest, (2), the wavelength at which transmission was half its maximum value, and (3), the wavelength at which it reached zero. Means and standard errors were calculated for these values.

The light energy absorbed by fish corneas and aqueous humor in normal hatchery conditions was calculated, using assumptions and methods that will be explained in the next section.

RESULTS AND CALCULATIONS

Corneas

Figure 4 shows the light transmission between 200 and 800 mμ of 27 normal rainbow and 26 normal lake trout corneas. Means and standard errors are shown. At the wavelengths of 312, 325 and 350 mμ, and at 50 mμ intervals above 350, these curves have been compared using the two-tailed Student's *t*-test. Significant differences at the P = .01 level were found at 400 mμ and all longer wavelengths tested, but there was no significant difference at 350 mμ. The *t*-test could not be applied at 312 or 325 mμ because variance homogeneity was not obtained; the two-tailed nonparametric Mann-Whitney *U* test was applied in these cases, and significant differences were found at the P = .05 level. The *t* and *U* values for each wavelength appear in the appendix.

The energy absorbed by the corneas was calculated subject to the following conditions. It was assumed that the average cornea is 6 mm in diameter, flat, and perpendicular to the incident sunlight, and that all the light not transmitted by the cornea is absorbed. Light transmission by hatchery water was assumed to equal that of distilled water. The, using Gates' values for incident solar radiation in the middle latitudes[11] and Ruttner's figures for the depletion of sunlight by distilled water,[28] the energy absorbed by a cornea of each of these fish at the water's surface and at a depth of 50 cm was calculated

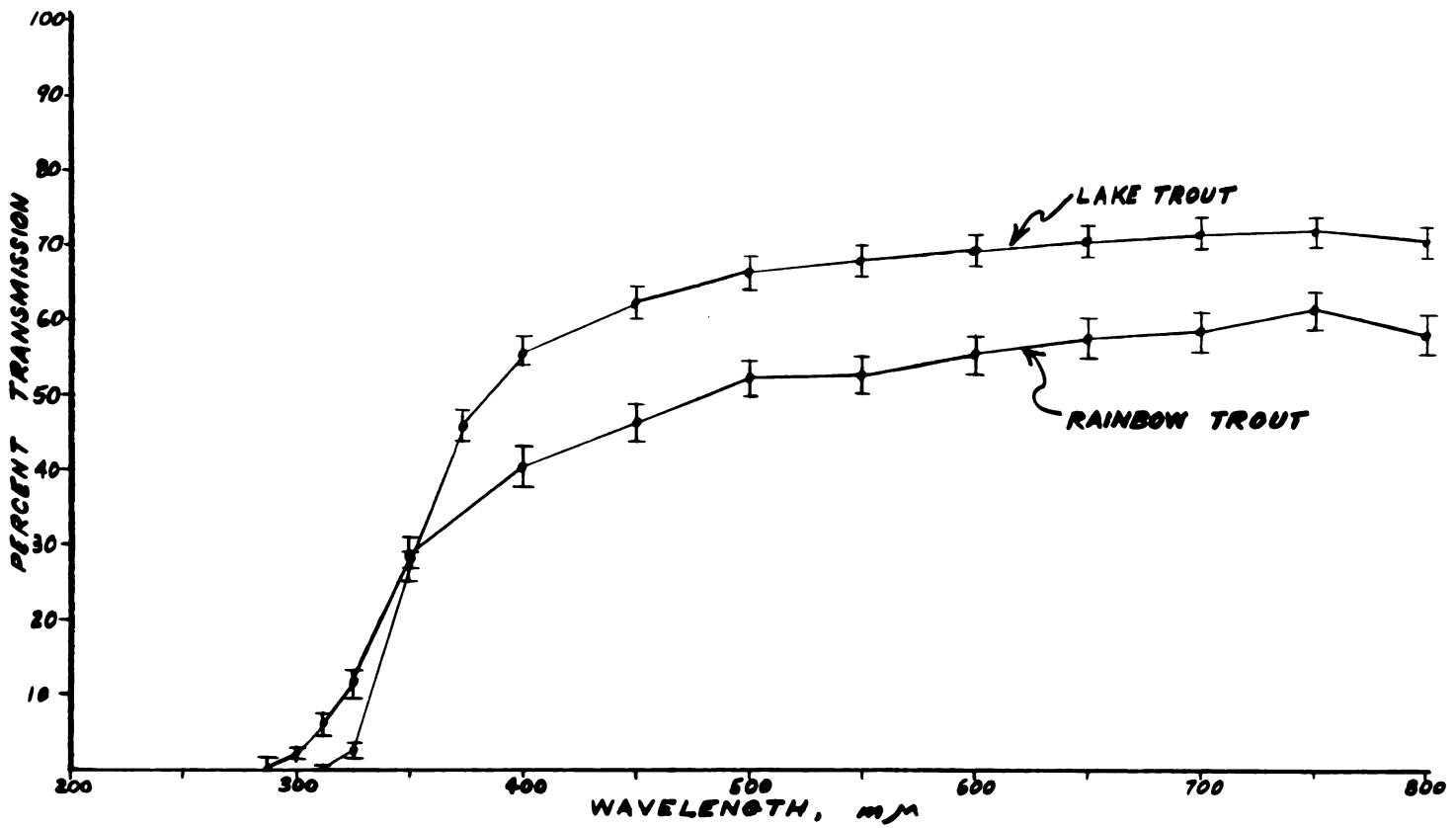

FIGURE 4: TRANSMISSION OF 26 LAKE AND 27 RAINBOW TROUT CORNEAS

(figure 5). Fifty centimeters is an average depth for fish in hatchery tanks, which are about a meter deep. Of course, corneas are neither flat nor perpendicular to the incident light; nor is all the light absorbed which is not transmitted--some is reflected and some is re-radiated. These assumptions were made to facilitate the calculations, and their effect is to make the estimates of energy absorption somewhat liberal. The fact that hatchery water and distilled water transmit alike is borne out by a comparison of water from three different hatcheries (Marquette and Grayling, operated by the Michigan Department of Conservation, and the Jordan River National Hatchery) and glass-distilled water. This comparison is shown in figure 6.

Aqueous Humor

The light transmission (means and standard errors) through aqueous humor in the 10 mm path microcell is shown shown for rainbow trout, lake trout and a mongrel dog on figures 7, 8, and 9, respectively. Nine trout of each species, and only one dog sample, were used. The lower lines on figures 7 and 8 show conspicuous dips at 415, 545 and 575 mμ. Dips at exactly these wavelengths were also seen when the transmission of a drop of whole rainbow trout blood or of packed cells from centrifuged blood, hemolyzed in distilled water, was measured against that of pure distilled water. The dips were not seen when the transmission of a drop of plasma in water was measured.

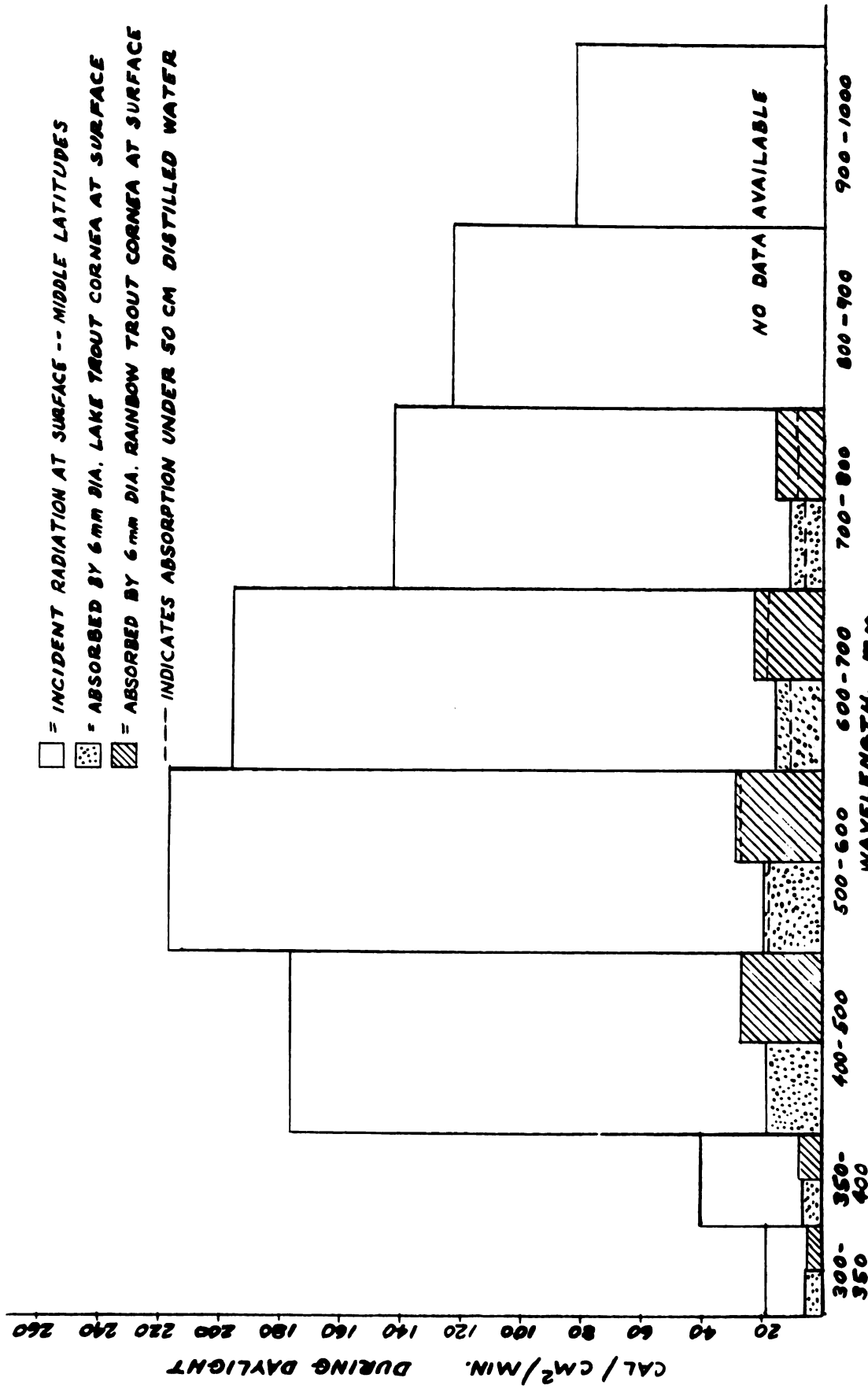

FIGURE 5: ENERGY ABSORPTION BY CORNEAS

FIGURE 6: LIGHT TRANSMISSION BY HATCHERY WATER

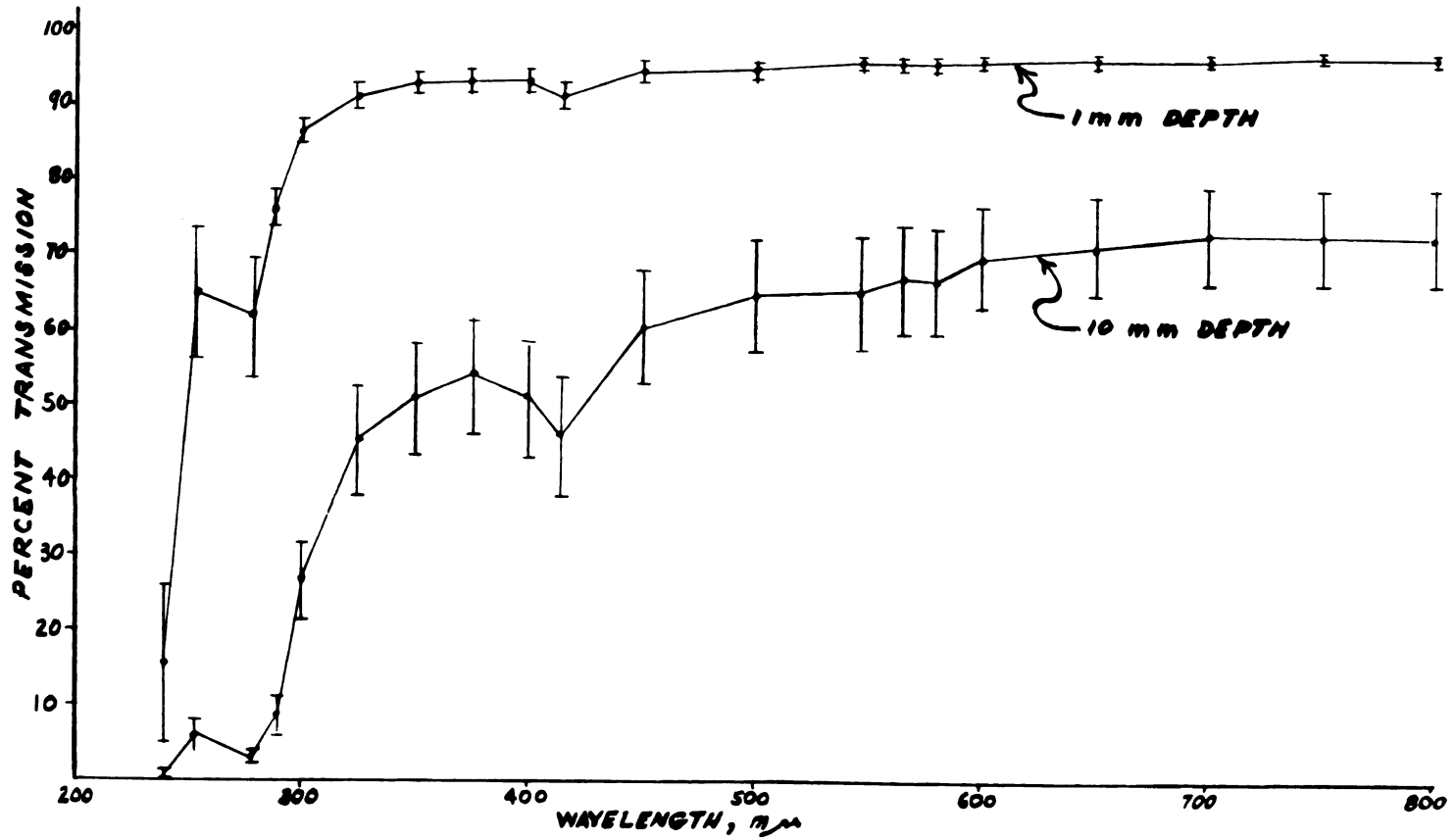

FIGURE 7: LIGHT TRANSMISSION BY RAINBOW TROUT AQUEOUS HUMOR

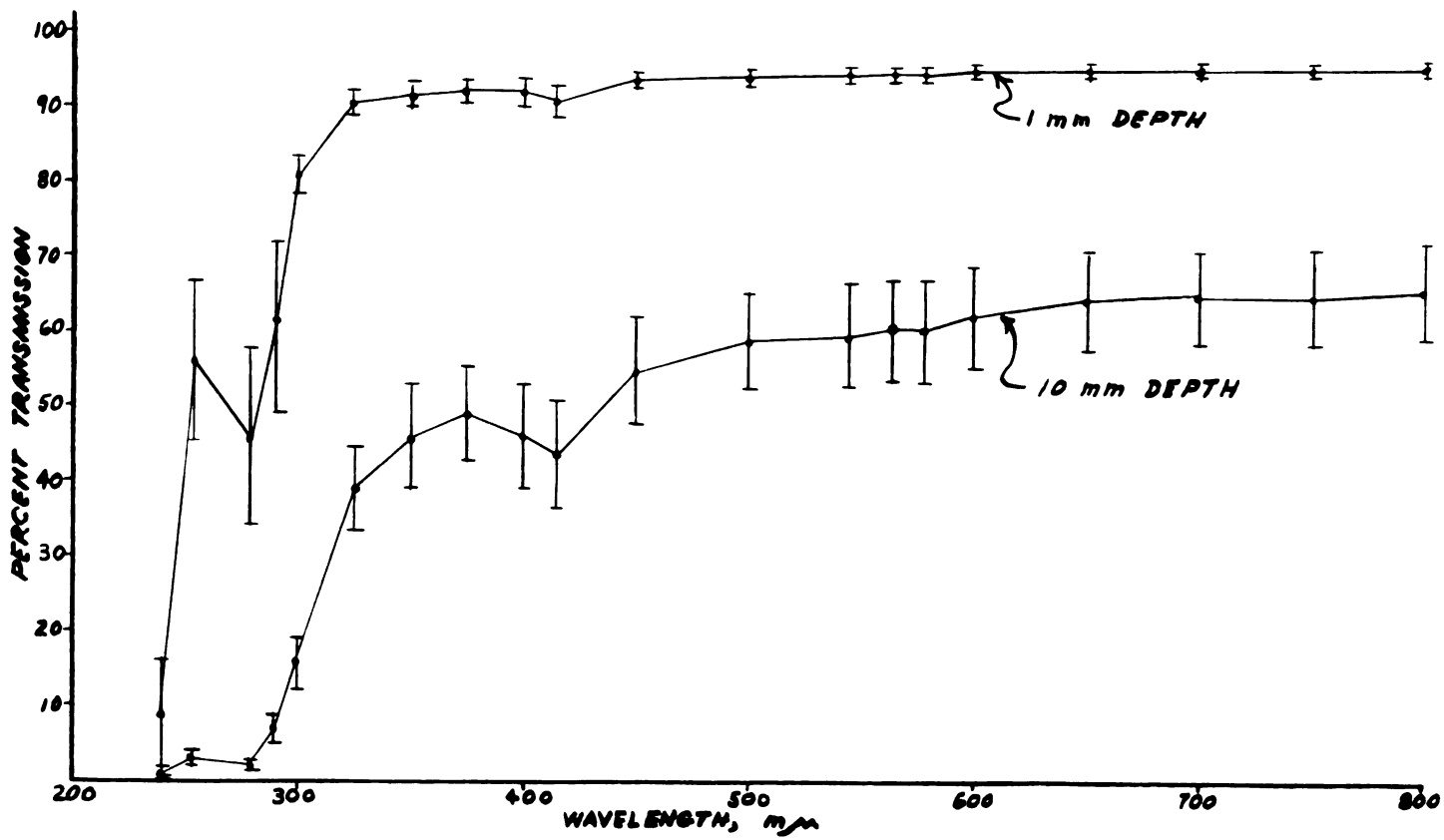

FIGURE 8: LIGHT TRANSMISSION BY LAKE TROUT AQUEOUS HUMOR

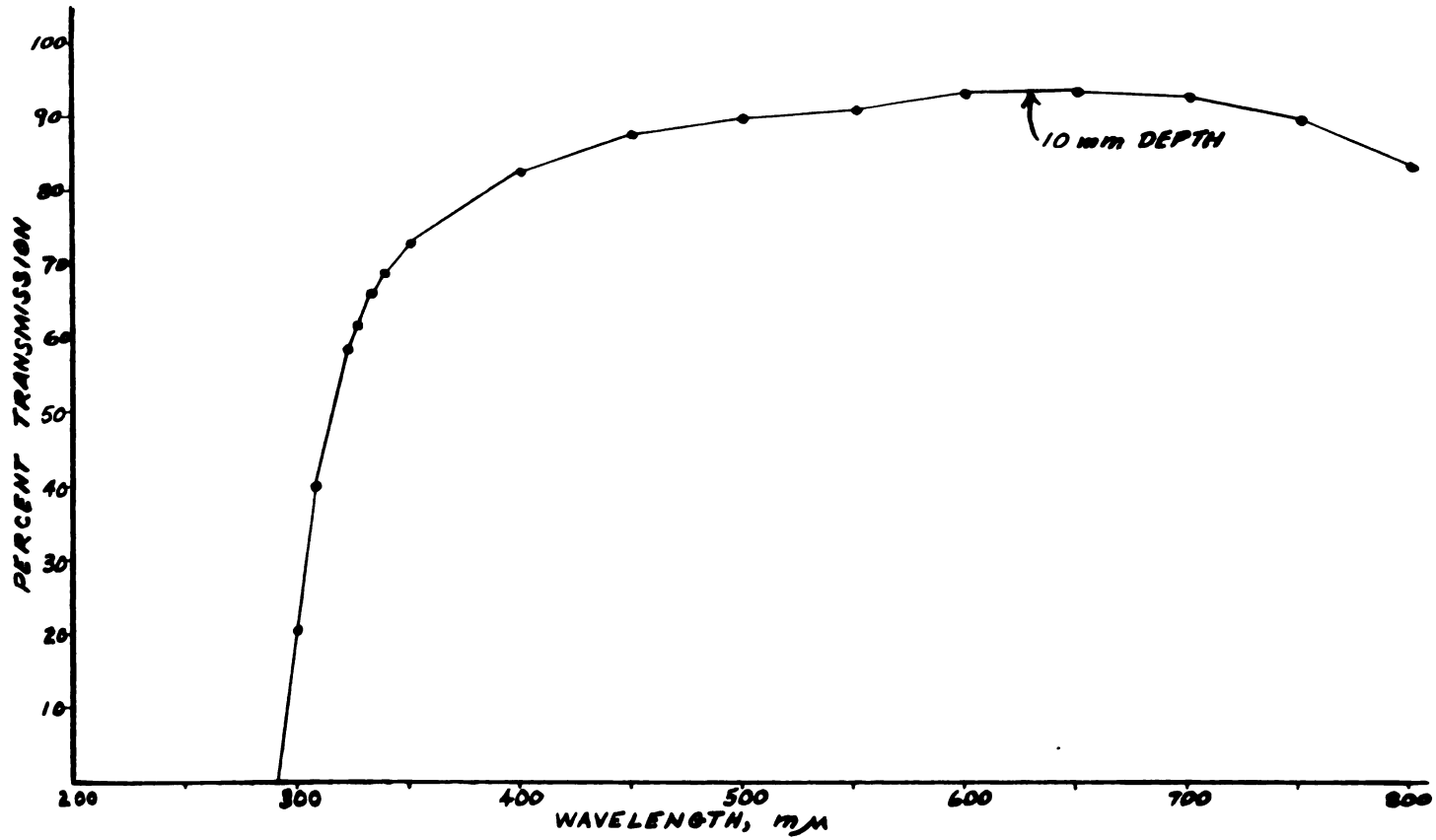

FIGURE 9: LIGHT TRANSMISSION BY DOG AQUEOUS HUMOR

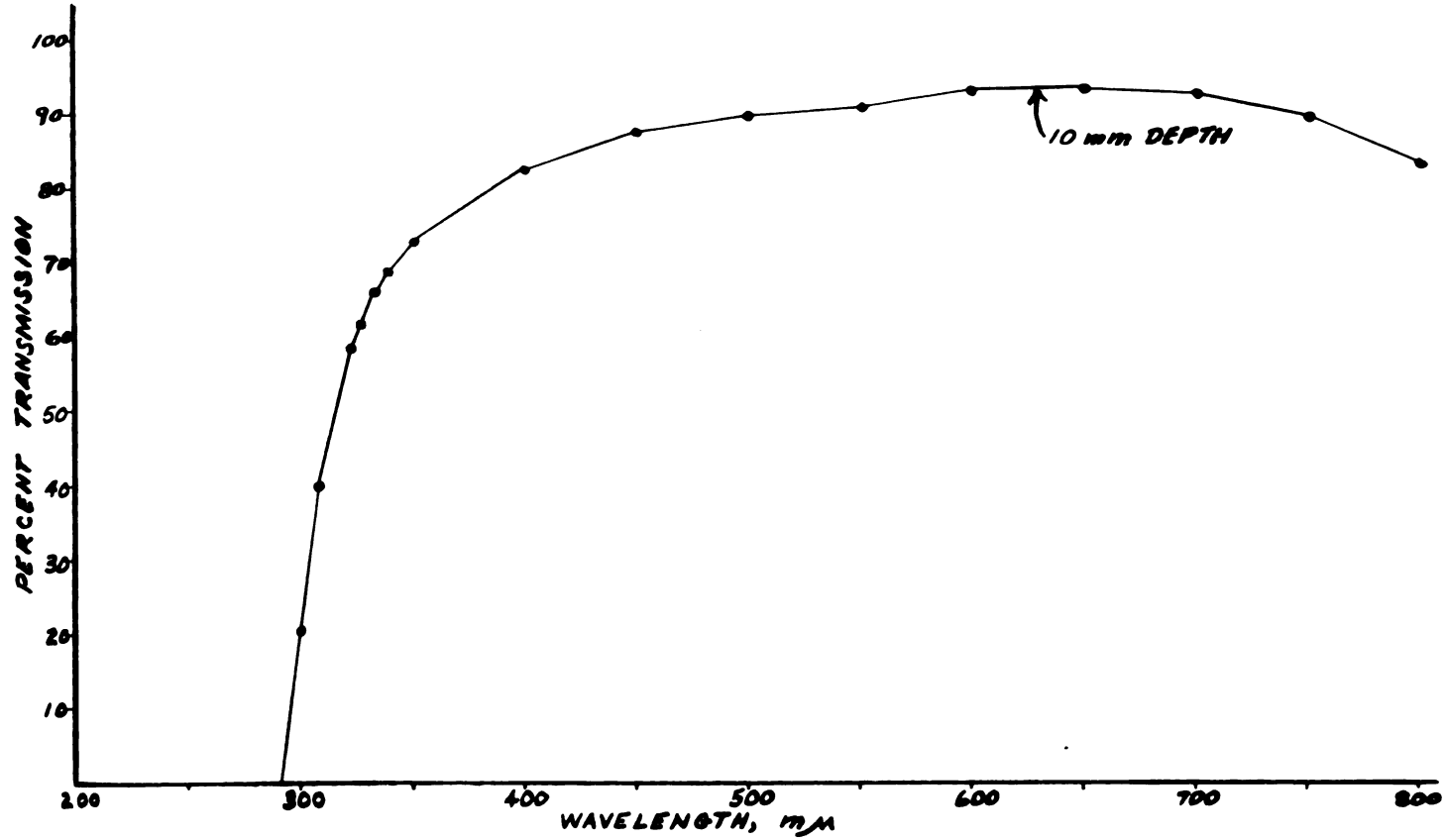

FIGURE 9: LIGHT TRANSMISSION BY DOG AQUEOUS HUMOR

It appears that these dips reflect light absorption by some water-soluble component of the red blood cells, which has come from the hemolyzed cells. This is probably hemoglobin from blood cells drawn into the syringe accidentally, perhaps from the iris vessels.

Aqueous humor in the anterior chamber normally averages about 1 mm thick behind the cornea of a 100-gram trout. To convert transmission through 10 mm of aqueous humor to that through 1 mm, the relation $I_d = I_o e^{-kd}$ was employed. Clearly,

$$\frac{I_d}{I_o} = \%T_d = e^{-kd}$$

and

$$\%T_{1mm} = (\%T_{10mm})^{0.1}$$

when I_d = intensity at depth d

I_o = intensity at surface (depth zero),

regardless of the value of the absorption coefficient, k. At each wavelength, the transmission through 10 mm of aqueous humor was raised to the one-tenth power, giving the values expressed by the upper lines on figures 7 and 8.

The transmission of light through 1 mm of aqueous humor was compared at each wavelength for which a mean is given using the two-tailed Student's *t*-test except at 290 mμ, where the lack of variance homogeneity dictated the use of a two-tailed Mann-Whitney *U* test. There is no significant difference between the rainbow and lake trout aqueous humor transmission means (or, at 290 mμ, medians). The *t*

and U values obtained are given in the appendix.

Assuming that all the light not transmitted was absorbed, the light energy absorbed by 1 mm of aqueous humor per square centimeter of surface area was calculated. The incident radiation was taken as solar flux at the surface minus the energy absorbed by the cornea for each species. Figure 10 displays the results of these calculations. The depletion of absorbed energy under 50 cm of distilled water is too small to be shown on this figure and is probably smaller than the limits of accuracy of the values of incident radiation used. The wavelength range in which the most energy is absorbed is now 400-500 mμ. Note that, to convert the y-axis values to the amount of energy absorbed by the aqueous humor in a real eye (which averages about 14 mm^3 in volume), they must be multiplied by 0.14. This volume was calculated from photomicrographs of frozen sections showing the dimensions of the anterior chamber, which is spherical towards the cornea, flat at the iris, and partly occupied by the spherical lens.

Lenses

Given in table 3 are the wavelengths between 200 and 800 mμ at which lens transmission was (a), greatest, (b), half its maximum value, and (c), zero. At the points of half-maximum transmission, the means of all observations for the two species were compared using the two-tailed Student's t-test, and a significant difference was found at the P = .05 level. Variance homogeneity could not be

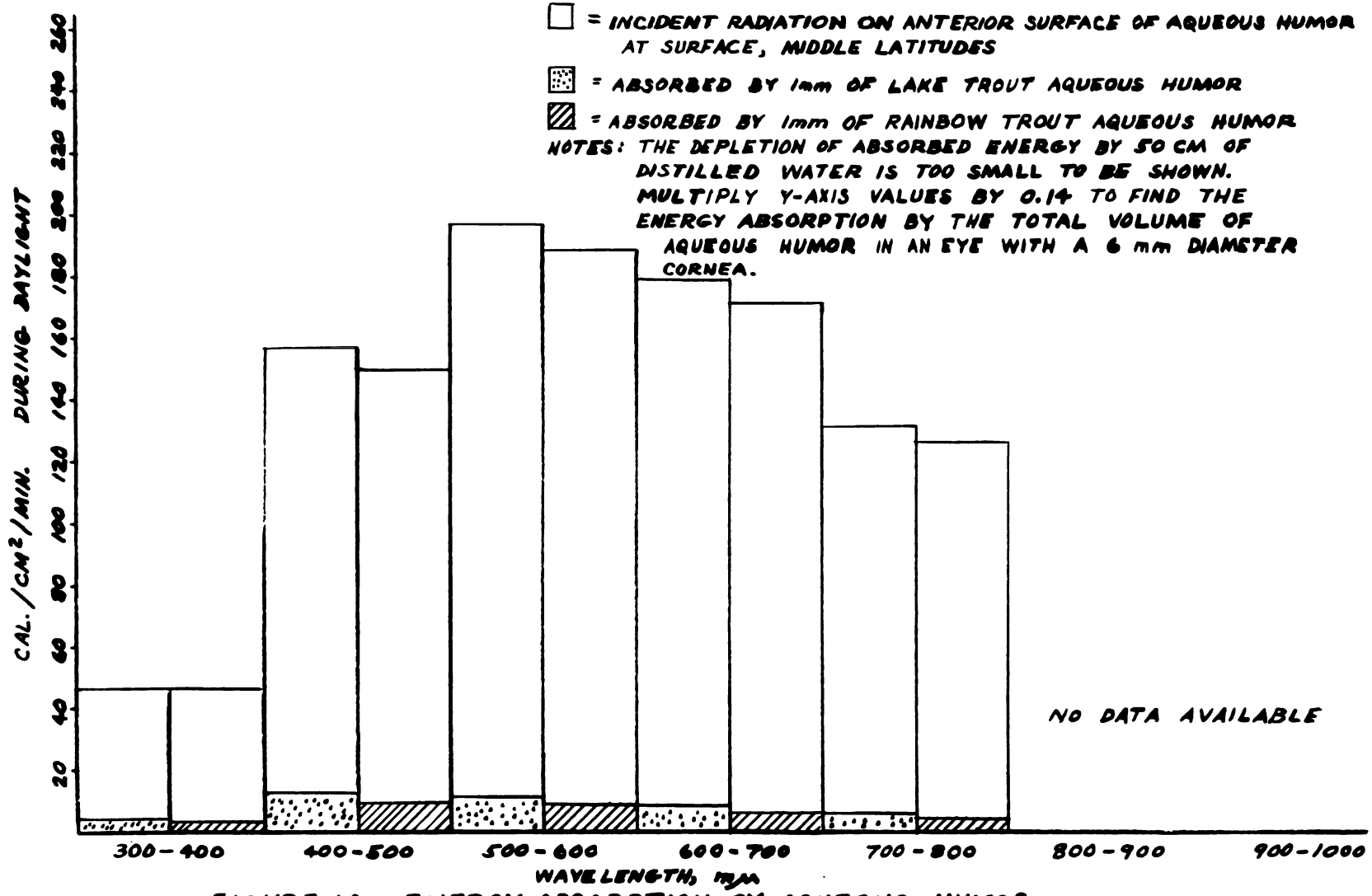

FIGURE 10: ENERGY ABSORPTION BY AQUEOUS HUMOR

Table 3: Light Transmission by Lenses

wavelengths of: (means ± S.E.)	zero %T	50 % of max.	maximum %T
rainbow trout	322.00 ± .61	356.27 ± 2.18	668.08 ± 28.03
lake trout	324.25 ± .81	365.25 ± 3.61	800.00 ± 00.00

obtained for the data at points of maximum and zero transmission, so the medians of the observations on the two species were compared using a two-tailed Mann-Whitney U test. Again, significant differences were found at the P = .05 level. Since one sample size (rainbow trout lenses) was larger than 20 and a large U value was encountered in one case (the wavelength where transmission reached zero), use was made of the fact that U becomes nearly normally distributed as n (the sample size) gets larger. The formula

$$z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{(n_1)(n_2)(n_1+n_2+1)}{12}}}$$

was applied in this case. The appropriate t, U, and z values are given in the appendix.

DISCUSSION

Corneas

The corneas of the lake trout used in this experiment transmitted more light than those of rainbow trout at wavelengths between 350 and 800 mμ. At 350 mμ, both transmitted light equally well; at shorter wavelengths, rainbow trout corneas were the better transmitters (figure 4). This means that lake trout corneas absorb more solar radiation (or light energy) at wavelengths below 350 mμ than rainbow trout corneas, and that the reverse is true above 350 mμ. Note that the solar flux is highest near the middle of the visible spectrum (500 - 600 mμ), and this is the region in which these corneas absorb the most light energy. If biological damage is being done *via* some nonspecific effect in which only the total amount of energy absorbed is important (e.g., heat), this visible region must certainly bear the blame for doing much of the harm. But it is possible that damage is done to the cornea by light of some *specific* wavelength(s) which powers unique photochemical reactions. If only a little light of the critical wavelength were absorbed, initiating subtle chemical changes leading to serious damage, the distribution of light energy shown in figure 5 might not indicate which wavelengths were responsible for the damage. Finally, one should realize that the cornea may not be damaged at all by sunlight. Perhaps the initial injury in these fish occurs deeper in the eye.

Corneas are not uniformly thick, of course, but thin centrally and thicker peripherally. The light beam in the spectrophotometer used here is rectangular (narrow and tall) and probably hit the corneas across their thinnest point. Light passing through them angularly or where they are thicker would be more fully absorbed. The corneal epithelium, constant in thickness, makes up a larger proportion of the cornea's thickness centrally than peripherally. If it absorbs differently than the corneal stroma -- and it probably does, because of its cellular structure -- its effect is different near the edges of the cornea than at the center. I was unable to determine the absorption of light by the epithelium alone.

Aqueous Humor

This substance, at a thickness of 1 mm, transmits light very well throughout the visible spectrum. The process of converting measurements made at 10 mm depth to 1 mm values brings all the observations closer together and lowers the standard errors. Of course, aqueous humor is not a uniform 1 mm thick all over the front of the eye, but 1 mm is a good working approximation.

Very little light energy is absorbed by aqueous humor in either species, but more is absorbed by lake trout than by rainbow aqueous humor in each wavelength range which was considered. Lake trout are believed to contract the eye pathologies which were described earlier more often than rainbow; if net energy absorption is nonspecifically

responsible, here may be a hint of the critical difference between the species. On the other hand, light transmission by aqueous humor is not statistically different between the species. The amount of energy absorbed by aqueous humor is so small that one must doubt whether the initial injury could be here, unless some specific photochemical reaction is involved (see Pirie[17]).

The open areas on figure 10 reveal the amount of light energy which penetrates both cornea and aqueous humor and is absorbed by deeper eye structures. This is, clearly, a very substantial amount of energy, comprising most of the incident solar radiation which reaches the water's surface. It seems very possible that this energy could injure the iris or other eye structures.

Lenses

Rainbow trout lenses reached their maximum light transmission, half maximum and zero transmission at shorter wavelengths than lake trout lenses in these experiments. It should be noted that the optical interference by the lenses which made the numerical transmission values useless also casts doubt on the values in table 3. If these are correct, though, and if it is assumed again that energy not transmitted is absorbed, lenses from 100-150 g lake trout must absorb more energy than those of rainbow trout of the same size. Unfortunately, there is no way to calculate the amount of energy absorbed by the lens from these data.

General Discussion

It is a principle of photochemistry (the Stark-Einstein law) that a single quantum of light, absorbed by a photosensitive molecule, can produce a photochemical reaction. Therefore, absorption of only a little light by a tissue can cause photochemical effects. One cannot help but admit that the cornea absorbs more than enough light to cause many photochemical reactions at the wavelengths studied. It is impossible, using these results, to tell whether such reactions are occurring, or, if so, at which wavelengths. The cornea also absorbs enough light energy to heat it somewhat. Its exposure to cold water on the outside, though, must keep its temperature fairly steady and low (0 - 15° C), and it is doubtful that enough heat is generated in the cornea itself to damage it.

Exactly the same considerations apply to the aqueous humor and lens, and the same conclusions can be drawn.

The bulk of the incident light energy reaches the iris and, after penetrating the lens, the retina. These tissues absorb most of the light energy which falls upon them, though some is reflected. That absorbed by the iris is certainly enough to cause many photochemical reactions, and to heat the iris considerably. Heat is not carried away from the iris well by convection because of the high viscosity of the aqueous humor surrounding it. Heat is carried away by the generous circulation of the iris, though. Any part of the iris, or of tissues near it in

the eye, might be damaged by heat or by photochemical changes caused by the absorption of light. The same arguments apply to the retina, of course, but any damage to it or the choroid layer should occur first at the focal point, where light intensity is greatest (these fish have no foveae).

Abiotic effects are only thought to occur at wavelengths less than 305 mμ. No light of abiotic wavelengths penetrates the normal lake trout cornea, and very little passes through the rainbow trout cornea. No eye structures except the cornea should be susceptible to abiotic effects, and the incident sunlight at the surface contains so little energy at these wavelengths that one must doubt that any abiotic effects occur at all in these fish eyes.

For wavelengths between 200 and 800 mμ, the amount of light and light energy absorbed by the corneas and aqueous humor of these trout is now known. This is a major step towards determining the nature of the process by which damage is done.

CONCLUSIONS

1. The transmission of light by normal rainbow and lake trout corneas between the wavelengths 200 and 800 mμ has been measured. Corneas of the two species differ significantly in transmission except at 350 mμ. Lake trout corneas transmit more light than rainbow at wavelengths longer than 350 mμ, but less light than rainbow at shorter wavelengths.

2. The amount of incident solar radiation absorbed by these corneas in 100 mμ intervals throughout the same wavelength range has been calculated. Enough light energy is absorbed in each species to initiate photochemical reactions at wavelengths in this range. It is unlikely that absorption of sunlight either heats the corneas significantly or produces abiotic effects. The cornea screens abiotic light from deeper eye structures in each species.

3. The transmission of light by aqueous humor of normal rainbow and lake trout was measured; there is no significant difference between the species. Absorption bands seen at 415, 545 and 575 mμ were probably caused by contamination of the samples with hemoglobin.

4. The light energy absorbed by aqueous humor in these species has been calculated. Absorption of this energy should be expected to produce very little heat and no abiotic effects in the anterior chamber of the eye.

5. The absolute transmission of the lenses of these fish was not measured, but the wavelengths between 200 and

800 mμ where transmission is (a), greatest, (b), half its maximum value, and (c), zero were tabulated.

6. Most of the incident solar radiation penetrates both cornea and aqueous humor in these fish, and must be absorbed by deeper eye structures. This absorption would be expected to produce considerable heat and might initiate photochemical reactions, especially in the iris, but also in the lens, vitreous body, retina or choroid layer.

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APPENDIX

A. Statistical Data for Comparison of Cornea Means (or Medians)

wavelength, mμ	statistic t	statistic U
312		128*
325		117*
350	0.2119	
400	4.3438**	
450	4.7939**	
500	4.9453**	
550	4.5941**	
600	4.5031**	
650	4.3774**	
700	4.2042**	
750	4.0700**	
800	4.0529**	

* = %T medians are significantly different at P = 0.05 level

** = %T means are significantly different at P = 0.01 level

B. Statistical Data for Comparison of Lens Means (or Medians)

-- for the wavelength of maximum transmission, U = 120*

-- for the wavelength of half-maximum transmission, z = 2.0053*

-- for the wavelength of zero transmission, t = 2.2284*

* = means (or medians) of wavelength measurements are significantly different at the P = 0.05 level

C. Statistical Data for Comparison of Aqueous Humor Means (or Medians) in 1 mm Depth

wavelength, mμ	statistic t	statistic U
240	0.5686	
255	0.6848	
280	0.1398	
290		35
300	1.8754	
325	0.4612	
350	0.5406	
375	0.4096	
400	0.4166	
415	0.1871	
450	0.4593	
500	0.6665	
545	0.8887	
565	0.7690	
580	0.7001	
600	0.7432	
650	0.6894	
700	0.6932	
750	0.6799	
800	0.8874	