Entrance of Ariguanabo River, Cuba, to its underground channel at San Antonio de los Baños.

Blind-fish rocks at base of Point Loma, San Diego, California.

# CAVE VERTEBRATES OF AMERICA

## *A STUDY IN DEGENERATIVE EVOLUTION*

BY

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1902

WASHINGTON, D. C.
PUBLISHED BY THE CARNEGIE INSTITUTION OF WASHINGTON
JUNE, 1909

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION NO. 104

Norwood Press
J. S. Cushing Co. — Berwick & Smith Co.
Norwood, Mass., U.S.A.

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# KEY TO DESCRIPTION OF PLATES AND TEXT FIGURES.

1. Pigment epithelium.
  *pi.* Densest pigmented section of the pigment epithelium, just below the nucleus.
2. Rods and cones.
3. Outer nuclear layer.
4. Outer reticular layer.
5. Horizontal cells.
6. Inner nuclear layer.
7. Spongioblastic layer.
8. Inner reticular layer.
9. Ganglionic layer.
10. Optic-fiber layer.

*a. o.* Ophthalmic artery.
*am.* Ameloid bodies of the pigment epithelium.
*b.* Brille.
*bac.* Rod.
*ci. p.* Ciliary process.
*cj.* Conjunctiva.
*cj. s.* Conjunctival sac.
*chr.* or *chd.* Choroid.
*chr. l.* Choroidal lymph.
*chr. f.* Choroidal fissure.
*cn.* Cones.
*cn. nl.* Cone nuclei.
*cor.* or *crn.* Cornea.
*cps.* or *cpl. sng.* Blood-corpuscles in normal vessels.
*cps. s.* Stagnant blood-corpuscles.
*d.* Dorsal aspect of eye.
*dr.* Dermis.
*e. m.* End member of cone.
*F. cj.* Fornix conjunctiva.
*fr. ol.* Olfactory pit.
*hd.* or *hyl.* Hyaloid membrane.
*H. gl.* Harder's gland.
*i.* Iris.
*i.* 1. Outer layer of iris.
*i.* 2. Inner layer of iris.
*i. c.* Interpolated cells.
*l.* Left side of eye.
*l.*$^1$, *l.*$^2$, *l.*$^3$ First, second, and third labial scale.
*lns.* or *l.* Lens.
*l. c.* Lens capsule.
*M.* Müllerian nuclei.
*m. m.* Middle member of cone.
*msc.* or *mu.* Eye muscle.
*nl.* Nucleus.
*nl. con.* Cone nuclei.
*nl. f.* Nuclear fragments.
*nl. g.* Nuclei of the ganglionic cells.
*nl. l.* or *nl.*$^1$ Elongate nuclei of the pars ciliaris.
*nl. Muel.* Müllerian nuclei.
*n. op.* Optic nerve.
*n. s.* Nasal scale.
*oc.* Eye.
*o. c.* Ocular scale.
*o. f.* Orbital fat.
*o. s.* Ocular scale.
*ot.* Otolith.
*p.* Pupil.
*p. i.* Palpabra inferior.
*p. s.* Palpabra superior.
*pi. s.* Pigment appearing in optic cavity with senescence.
*pi. sph.* Pigment spheres.
*p. l.* Pigment layer.
*po. s.* Preocular scale.
*pr. nl.* Processes of the cone nuclei.
*pupl.* Pupil.
*r.* Right side or retina.
*r.* or *rt.* Retina.
*ro.* Rostral.
*scl.* Sclera.
*scl. c.* Scleral cartilage.
*subo.* or *sb. orb.* Suborbital.
*v.* Ventral aspect.
*vit.* Vitreous body.
*x.* Flattened cells beneath pigmented layer, of doubtful significance.
*y.* Flattened cells beneath inner nuclear layer, of doubtful significance.

# PREFACE.

## INTRODUCTORY.

A cave is a unit of environment so well circumscribed and of such simplicity that we may know its contents, its elements, and its conditions nearly as well as the experimental zoologist knows the contents and conditions of his aquarium. These contents and conditions are of rare uniformity, changing but little from day to night, from season to season, or from decade to decade. The point of chief interest in the cave environment is the total absence of light in all parts except about its mouth. Probably no animals have a more intimate environmental adaptation than those inhabiting caves. This adaptation is largely of color and structure of eye, which modifications are surpassed only by the functional adaptation of the tactile apparatus of the blind forms.

While no one has followed, and although we may not be able to follow in detail, the steps through which the cave animal has acquired this environmental adaptation, a knowledge of the present condition of their unchanging environment gives us a knowledge of what it has been during their entire period of development.

We know, or can know, what the present stage of their adaptation is. Not infrequently we know what the condition of the animal was at the start of its cave experiences and enough of the steps along its line of evolution (indicated by the degrees of adaptation reached by different members of the group) to enable us to form so clear a picture of its entire route of evolution that we may conjecture what elements of the environment caused the modifications, and by what process they were brought about. We have, in other words, a long experiment conducted by nature unrolled before us.

I propose in this work to give an account of the cave as an environment; to bring together in a revised form the papers on blind and cave vertebrate animals so far published by myself and my students, together with further observations on the species previously considered, to consider the habitat, mode of life, and the origin of the Cuban blind fishes, and to give an account of their eyes.

My first experience with blind vertebrates was in 1886, when Superintendent Funk sent to Indiana University a living blind fish which had been taken from a well at Corydon, Indiana, and which proved to be a new species, *Typhlichthys wyandotte*, the only representative of the genus so far taken north of the Ohio River. Later, when a stay in southern California came in prospect, a study of the blind fish, *Typhlogobius*, living under rocks along the base of Point Loma, was one of the first definite plans formed.

When, in 1890, I returned to Indiana and was once more within reach of the caves, the problem again came up. My laboratory is excellently located for the study of cave faunas, the series of caves to which Wyandotte, Marengo, Mammoth, Colossal, and Nickajack belong, beginning in or about the campus of Indiana University. But while seemingly ideally located, and in spite of the fact that numerous trips were made to Indiana caves, especially those from which blind fishes had been reported, no blind fishes were found till 1896.

In May, 1896, I was again looking for blind fishes east of Mitchell, Indiana, this region being drained by underground streams. East of Mitchell several of these find their exit in caves of romantic beauty in the escarpment flanking the valley of White River (plate A). The roof over one of the streams has fallen in at two places, Dalton's Spring and Twin Caves. At Dalton's Spring the cave-stream runs above ground for about 100 yards when it again enters its subterranean course. Within sight of the lower opening of the "spring" I saw two blind fishes swimming in a quiet pool. I secured about 20 specimens and had found the stream which in its varying reaches has furnished me with an unlimited supply of specimens which have enabled me to give the complete history of the eye of this species, *Amblyopsis spelæus* De Kay. More material has been obtained from this cave than from all others put together. In 1903 the State legislature of Indiana placed the land, about 182 acres, on which are the entrances and exits to this stream in the keeping of the trustees of Indiana University. While some litigation has arisen as to the ownership of the farm, it will probably be permanently preserved as a State park.

## ACKNOWLEDGMENTS.

Through grants from the Elizabeth Thompson Science Fund and from the American Association for the Advancement of Science I have been able to visit the cave regions of southwestern Missouri, about San Marcos, Texas, Corydon, Indiana, and Mammoth Cave, Kentucky. In 1902, through a grant from the American Association for the Advancement of Science and assistance from various other sources, I was able to visit the blind-fish caves of Cuba. Subsequently the Carnegie Institution of Washington aided me in making additional investigations in Cuba. The part of the present volume dealing with Stygicola and Lucifuga is my final report on the work carried on with this aid, and in it a detailed account of the Cuban work is given.

Prof. S. A. Forbes kindly lent the drawing for figure A, plate 1. The drawings of sections of eyes were made under my direction by Mrs. E. R. Bieling in the laboratory of Prof. R. Wiedersheim, in the University of Freiburg, Germany, and I am indebted to Professor Wiedersheim for placing his laboratory at my disposal.

I am under many obligations to various friends, both at home and in Cuba. Mr. Oscar Riddle, Dr. John Beede, Mr. John Haseman, Mr. Norman McIndoo, and Mr. T. L. Hankinson acted as volunteer assistants on various Cuban trips, always working without remuneration and in part paying their own expenses. The late Prof. Jose T. Torralbas, Prof. Carlos de la Torre, Mr. Pascual Ferreiro, Dr. Felix Garcia, and the Director of the Cuban Agricultural Station, Prof. F. S. Earl, assisted me materially in various ways.

The assistance of my friend, Mr. Francesco Martinez, has been invaluable. His finca, the "Isabella," is at the margin of the cave region of Cuba, and in the interval between our trips he ferreted out unsuspected caves, determined their richness in blind fishes, and put himself at our disposal in guiding us to his various finds.

Prof. D. W. Dennis of Earlham College, Richmond, Indiana, made the microphotographs in a manner to leave nothing desired (plates 9, 10, 16–23).

Mr. Lewis H. Wild, under the direction of Prof. J. Reighard, made a series of photographs of entire eggs and embryos (plate 7).

Mr. Samuel Garman sent me my first specimens of the blind fish, *Troglichthys.*

Dr. B. W. Evermann of the Bureau of Fisheries and the late Prof. W. Norman secured me specimens of *Typhlomolge.*

Prof. Wm. Roux, Dr. F. R. Lillie, and others kindly consented to the republication of articles issued in the journals under their editorship.

I desire also to express my high appreciation of the interest taken by the authorities of Indiana University, especially by President William Lowe Bryan, in the various trips and plans necessary to bring this work to a successful conclusion.

The present work forms No. 97 of the Contributions from the Zoological Laboratory of the Indiana University.

Finally, I wish to express my indebtedness to her who as Rosa Smith guided me to the blind-fish rocks at the base of Point Loma, and who as Mrs. R. S. Eigenmann collected for me at the same place, has acted as editor of the various papers that have appeared, and through the twelve years during which my leisure has largely gone to the blind vertebrates has ever been ready with advice, encouragement, and assistance.

## CONCLUSIONS OF GENERAL IMPORT.

(1) The bleached condition of animals living in the dark, an individual environmental adaptation, is transmissible and finally becomes hereditarily fixed. (See page 80.)

(2) Ornamental secondary sexual characters not being found in blind fishes are, when present, probably due to visual selection. (See page 94.)

(3) Individual degeneration of the eye may begin in even earlier stages of development until nearly the entire development becomes affected, that is, functional adaptations are transmissible. (See pages 172 and 235.)

# A GENERAL CONSIDERATION OF CAVES AND THE CAVE FAUNA

# CAVES AND THE CAVE FAUNA.

## CAVES IN THEIR RELATIONS TO THE REST OF THE UNIVERSE.

The environment favorable to animal life is limited to a thin layer of water, earth, and air. From its deepest to its most elevated point this layer does not much exceed 10 miles [1] in thickness. At no particular point does it exceed much more than half this thickness; and usually the layer is but a few feet thick. About half the total thickness is below sea-level and the other half above it. The places where the ocean has a depth of 5 miles are few, but in these places the greatest depth of possible environment is found. The favorableness of the environment diminishes rapidly with the depth. The depth of the possible environment at any point on land above the surface is very limited, and beneath the surface it depends on conditions; solid rocks may limit it to the surface and soil may permit mammals, and especially insects, to burrow several feet beneath the surface. Underground watercourses, which are caves in the formation, may enable animals to live several hundred feet beneath the surface of the ground. The animals thrown out by artesian wells attest this. *Typhlomolge* is occasionally thrown out of the artesian well 190 feet deep at San Marcos, Texas. The plant environment stops at the surface of the ground; [2] animal life diminishes rapidly within a few feet of the surface unless trees cover the ground. Animal environment definitely stops at the tops of trees, though the air above them may be temporarily visited.

While the depth of the environment at any point is only a few feet on land, because the surface of the land itself rises to a few miles above sea-level, the total depth of the environment above sea-level is considerable. The fauna rapidly diminishes in either direction from sea-level, and were it not that the extreme limits of the environment, above and below, furnish rare, sometimes peculiarly adapted forms, sometimes relicts, the numbers of individuals and types found would not repay the exploration of the ocean depths and mountain heights.

Since the environment varies within the limits of the possible existence of living matter, from the extreme of wetness and dryness, of heat and cold, of depth and height, of light and dark, etc., we may divide the environment into many distinct units within which the conditions are similar or alike. It is profitable at present to call attention only to *discontinuous* and *continuous* units of environment. Similar or identical conditions may stretch uninterruptedly in one or more directions indefinitely, permitting the free movement of its inhabitants from one part to another. The *continuous* unit of environment of greatest extent is furnished by the ocean at considerable depths. Light and temperature conditions and seasonal fluctuations are reduced to the minimum and are nearly uniform under the whole surface of the ocean, furnishing an ideal of the type of the continuous environment. This particular environment is continuous not only as to space, but also as to time.

The surface of the ocean forms an equally continuous area, but because temperature and light conditions differ greatly in different parts of the globe we must here deal not with a single but with several distinct units of environment, each large in extent. If we assume the conditions in the north polar sea to be identical with

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[1] Highest mountain, deepest ocean.

[2] Some fungi are found in caves.

those of the south polar sea, these form a *discontinuous* unit of environment, a unit whose parts do not form a portion of a continuous area and whose inhabitants can not migrate from one part to the other.

If we assume the conditions in the equatorial Atlantic to be the same as those of the equatorial Pacific, we are again dealing with a discontinuous unit—discontinuous because the inhabitants of one part can not migrate to the other. If we examine these two units more closely, it becomes evident that the Arctic and Antarctic oceans have always formed a discontinuous unit. Arctic conditions have never prevailed between the two. On the other hand, the equatorial Atlantic and the equatorial Pacific were formerly connected in Colombia and formed one continuous environment. The land area and the fresh waters near the equator from Para to the Andes form a continuous unit of environment, and the Galapagos Islands to the west of it form a discontinuous unit, each separate island forming a continuous unit of a smaller order. It is evident that there are degrees of discontinuity, depending in part on the length of time the discontinuity has existed, and in part on the space separating the nearest parts of the unit.

Caves are discontinuous units of environment whose elements have always been separate. It is possible that in some areas a large complex of different underground channels exists. An east to west fault has lowered the southern part of Texas, or has raised the northern part, many feet. The dividing line is an abrupt escarpment across the State. This fault has favored the formation of underground watercourses, and inasmuch as river valleys do not cut down to the underground channels, it is possible that they form a network of channels or a continuous unit which permits the ready migration of its inhabitants from one part to another.

The lower area on the southern slope of Cuba, between Cañas on the west and an undetermined point east of Union, is drained by underground rivers. No valleys cut down to these rivers, and since this part of Cuba has sunk in recent times, the land being only a few feet above sea-level, it is possible that we again have a complex of underground channels permitting the migration of its inhabitants. However, it is also possible that the streams run in separate courses. The absence of *Lucifuga* from the eastern caves favors this hypothesis. At best we have here several degrees of continuity.

The large streams cut the cave region of Kentucky, Indiana, and Missouri into sections, their beds lying deeper than the caves. These caves are, therefore, part of a discontinuous environment. These facts must be constantly borne in mind in considering the origin and dispersal of cave faunas.

It is quite out of the question in this connection to give even a partial list of North American caves, or an account of the North American cave regions. The region to which Mammoth Cave belongs reaches from near Bloomington, Indiana, through Kentucky into Tennessee and embraces many thousand square miles of territory. Only the larger streams whose rapidly deepening channels have made the caves possible flow on the surface. "One may travel on horseback all day, through certain parts of Kentucky, without crossing a single running stream; all the rain water that falls being carried down through the sink holes into caverns below where are the gathering beds that feed the few large open streams of the region, of which Green River is an example. It is reported that there are 4,000 sink holes and 500 known caverns in Edmondson County (Kentucky) alone."[1]

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[1] For an account of the principal caves of North America see Hovey, Celebrated American Caverns, Cincinnati, 1882 and 1896; and Packard, The Cave Fauna of North America, Memoirs of the Nat. Acad. Sci. vol. 4, 1888.

## THE NATURE OF THE CAVE ENVIRONMENT.

Each cave is a distinct unit of environment and needs special consideration. In the present work we can deal only with the general features of this environment. The chief element for consideration is the absence or reduction of the amount of light and the relative constancy of other physical conditions. On this basis a cave may be divided into three regions: (1) the twilight region just within the cave, bounded by the distance to which light penetrates from without — this part shades generally from epigean conditions to the real cave conditions; (2) the region of fluctuating temperatures; (3) the inner cave region.

These different sections occupy greatly variable parts of different caves. In Mammoth Cave the twilight region is large enough to contain a tennis court and reaches some distance beyond the "iron door." Some Cuban caves are entirely of the twilight character, usually containing an abundant fauna, consisting largely of occasional, regular, or accidental visitors from the outside. The second region in Mammoth Cave reaches to the Mammoth Dome. On a cold winter day I found ice stalagmites on the floor of the entrance gallery just before it enters the dome. In certain of the ice caves the entire portion beyond the twilight area may belong to this section. In caves of the tropics, on the other hand, it may not exist at all. The third part is the cave *par excellence* — the inner section, but little influenced by external conditions. Here there is absolute darkness at all times, both day and night, summer and winter following each other without very decided change in temperature.

The temperature differs in the various parts of the same cave and also changes slightly with the seasons. In the center of the Shawnee Cave at Mitchell the fluctuations in temperature during a week do not equal the error of the recording thermograph arising from unequal trimming of the paper, the absorption of water, etc. The total fluctuation during a year is 2.2° C. It is remarkable that this record of cave temperature is taken in a cave open at both ends with a current of air flowing through it at times. The instrument is placed where it would be least affected by these currents, that is, in a large room near the center of the cave about 15 feet above water-level.

Glacières, or ice caves, are found in various places. They exist wherever the prevailing direction of the winds and nature of the cave causes a strong inflow of air during the winter, reducing the temperature to below the freezing point. The summer winds do not blow in the same direction, and convection currents are prevented by the nature of the cave.[1]

Between June, 1906, and February, 1908, the fluctuations in the temperature in the water where it leaves Shawnee Cave ranged from a maximum of 17.3° C. to 7.4°, or through about 10° C.[2]

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[1] A very extensive list and excellent account of glacières is given by Balch in his Glacières or Freezing Caverns, 1900. Concerning the cause of glacières, he says, on page 148: "The cold air of winter sinks into and permeates the cave, and in course of time freezes up all the water which, in the shape of melting snow or cold winter rain or spring water, finds its way in; and once ice is formed it remains long after ice in the surrounding open country has melted away, because heat penetrates with difficulty into the cave."

[2] This range becomes interesting when compared with the range of temperatures in a lake. Professor Birge gives the ranges of the water at the surface and at the depth of 18 m. for Lake Mendota:

| | |
|---|---|
| Surface, 1895 . . . 0° to 24° | Bottom, 1895 . . . 1.5° to 17.1° |
| Surface, 1896 . . . 0° to 26° | Bottom, 1896 . . . 2° to 16° |

Conditions of moisture, while practically uniform in some parts of caves, fluctuate in others more than any other element of environment. The maximum degree of moisture is naturally found in the pools and streams. On the other hand, in the upper parts of Mammoth and Wyandotte Caves the dust lies undisturbed for years. In Mammoth Cave the tracks of oxen made in 1860 are now shown to visitors, and I am told that in Wyandotte the still older tracks of the moccasined Indians are perceptible to-day. There are, however, parts of caves where the moisture dripping through from above is considerably increased after a rain, and the River Styx in Mammoth Cave rises 60 feet above low-water mark. The creek in Shawnee Cave sometimes fills parts of the cave to the ceiling.

The conditions of the water also change very greatly. At ordinary times it may be very clear; after rain it may carry a large amount of sediment. In its low condition it may flow very quietly, in its high condition be a torrent. The water, then, fluctuates in amount, clearness, and swiftness, with meteoric conditions.

Charts of simultaneous records on two self-registering barometers show the close agreement in changing barometric pressures inside a cave and outside it. One of the instruments was placed about 90 feet above the exit of the cave, the other near the middle of Shawnee Cave. Records chosen on account of peculiarities in the rise and fall of the pressure at certain times leave no room for doubt that barometric changes similar to those of the outside take place in the caves.

The following table shows the temperatures for air and water in Donaldson and Shawnee Caves in 1906 and 1907:

*Temperatures for air and water in Donaldson and Shawnee Caves.*

| Time. | Temperature of air in center of Donaldson Cave. | Maximum temperature of water at its exit from Shawnee Cave. | Time. | Temperature of air in center of Donaldson Cave. | Maximum temperature of water at its exit from Shawnee Cave. | Minimum temperature at same place. | Time. | Temperature of air in center of Donaldson Cave. | Maximum temperature of water at its exit from Shawnee Cave. | Minimum temperature at same place. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1906. | | | 1907. | | | | 1907. | | | |
| July ..... | 12.7 | 12.4 | Jan. .... | 11.5 | 11.6 [2] | 9.5 | July ..... | 11.9 | ....[1] | .... |
| August ... | 12.9 | 12.5 | Feb. .... | 11.5 | 11.3 | 8.9 | August ... | 12.7 | 16.1 | 13.1 |
| September | 13.2 | 12.6 | March .. | 11.5 | 12.6 | 9.9 | September | 12.7 | 17.3 [1] | 13 |
| October .. | 12.7 | 11.8 | April .... | 11.5 | 12.1 | 10.2 | October .. | 12.2 | 13.4 | 12.3 |
| November | 11 | 10.3 | May .... | 11.5 | 12.8 | 11.6 | November | 11.9 | 12.5 | 11.5 |
| December | 12.2 | 10. | June .... | 11.7 | 15.1 | 12.5 | December | 11.7 | 12.1 | 7.4 |

[1] The higher temperatures are caused by rains and last only a few hours after a heavy rain. During the first 10 days in September, 1907, the temperature of the water was 14.5, 15.6, 17.3, 16, 14.9, 14.6, 13.9, and 15.3 on successive days. During the last 10 days of the month it ranged from 15° to 15.5°.

[2] From the 1st to the 15th the temperature was between 10.6 and 11.6.

Currents in water and air differ materially in different caves and at times in the same cave. In the Cuban blind-fish caves there is neither appreciable air-current nor water-current, so that the evaporation from the quiet surface of the water forms a covering crust of carbonate of lime and magnesium. In the blind-fish caves at Mitchell, Indiana, a small current of water flows during normal conditions. The stream becomes a raging torrent in high water.

Currents in the air may be caused, (1) by the flow of water; (2) by the epigean air-currents; (3) by changes in the atmospheric pressure; and (4) by differences in temperature.[1]

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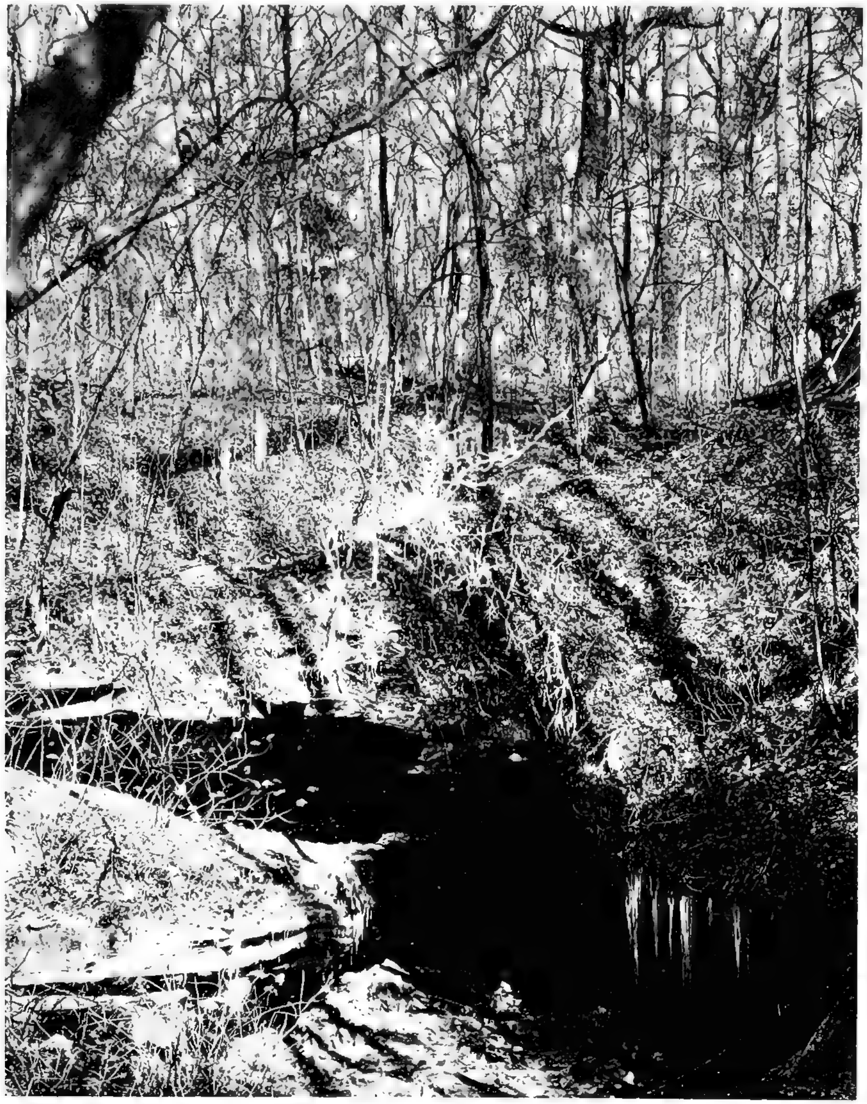
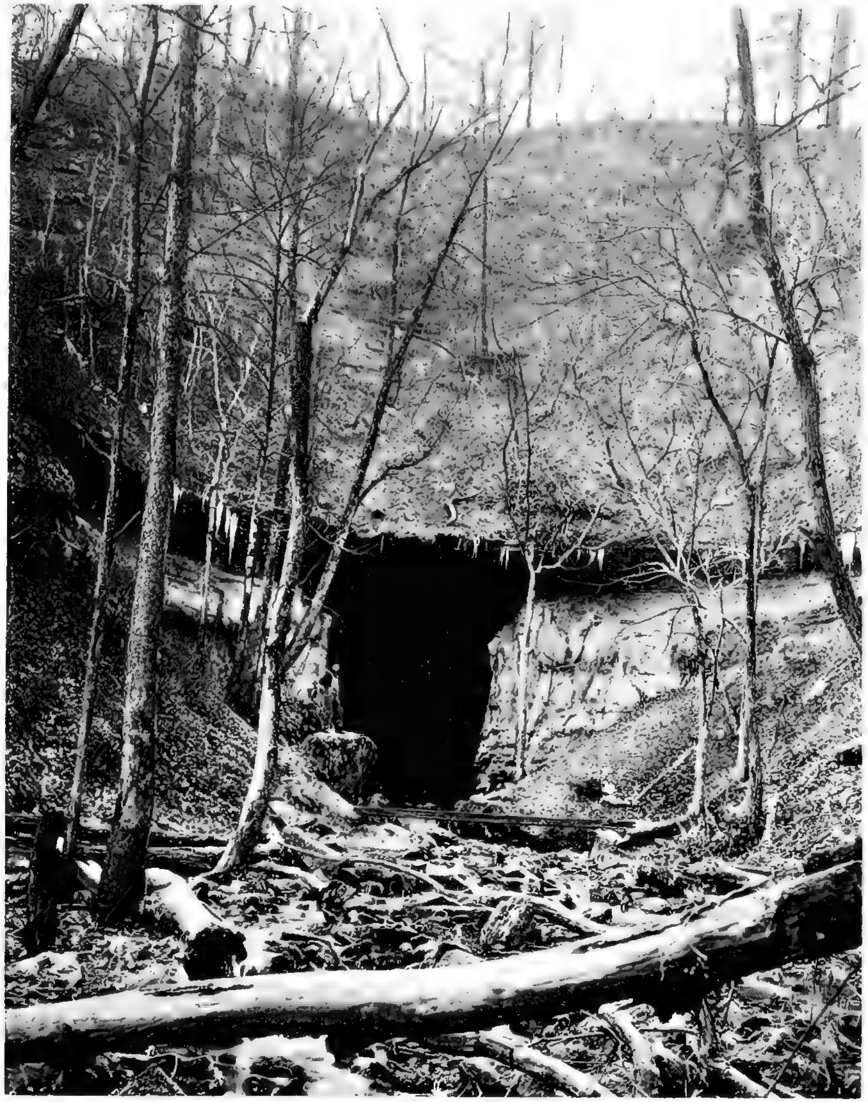
[1] A detailed study of the currents of air and temperature of the water in the Mitchell Caves will be published within a year.



Twin Cave.



Shawnee Cave.

Entrance to (Lower Twin Cave) and exit (Shawnee Cave) of underground river on the Indiana University Cave Farm. The two openings are at opposite ends of an underground tube about three-quarters of a mile long. During winter the warmer air of the cave flows out of the upper opening. The moisture in the outflowing air congeals, forming the heavy frost seen on the shrubs above the opening.

In Mammoth Cave a very perceptible air-current flows into the top of the dome from Little Bat Avenue. It probably descends to the bottom of the dome and then ascends at the side to flow out at Sparks Avenue. This current was flowing at the rate of 8,640 feet per hour on November 30, 1902. It is probably caused by a thin fall of water which descends from the roof of the dome to the bottom.

By far the most violent air-current may be caused by a change in the atmospheric pressure in the air without. These currents are perceptible only in caves of considerable extent, and become violent when the opening is insignificant compared with the size of the cave.

When the weight of superincumbent air is lightened, the compressed air in the cave expands and there is an outrush of air through the opening. If, on the other hand, the barometric pressure increases when the superincumbent air column gains in weight, there is an inrush of air. I have been at the entrance of Mammoth Cave when the internal and external pressures were so equalized that the anemometer would show ingoing and outgoing currents alternating irregularly every few minutes. In 1902 I was also at the entrance [1] when the anemometer showed the following rates per hour for air going in: November 29, 9 a. m., 46,350 feet; 6 p. m., 39,840 feet; November 30, 7 a. m., 50,290 feet; $9^{h}$ $40^{m}$ a. m., 55,830 feet; and $12^{h}$ $30^{m}$ p. m., 7,800 feet.

Mr. A. M. Banta reports from Mammoth Cave that on January 31, 1903, "At the gate the air-currents were surprisingly fitful. The current was running in 40 seconds, stopped 15 seconds, flowed out 8 seconds, stopped 10 seconds, and then ran in for 2 minutes, when we left." His records give the following rates per hour of air going in during February, 1903: February 18, 12 m., 76,464 feet; $5^{h}$ $30^{m}$ p. m., 77,396 feet; $6^{h}$ $20^{m}$ p. m., 79,896 feet; February 19, 10 a. m., 76,692 feet; 12 m., 68,904 feet; and February 21, 9 a. m., 56,556 feet.

I know of no direct record of currents due to changing temperature on the outside. Until direct observation with an anemometer had been made the general impression among the guides at Mammoth Cave was that air rushed in during one part of the year and out during the other. On cold winter days at Mitchell frost on the bushes showed that a gentle current of the damp cave air was flowing out from the upper part of the cave. The strength of the convection currents is undoubtedly dependent in large measure upon the shape of the cave and the nature of the opening. But the influence of water-currents or winds might at any time be sufficient to change the direction of the convection currents.

Nothing very definite can be said about the size of the environment afforded by a cave.[2] While it is known that some caves are much larger than others, it is never certain how large the unexplored or unexplorable part of a cave may be, how far the smaller cracks lead, and in how far they may establish intercommunication between neighboring caves.

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[1] A wall partially closes the entrance avenue so that the air passes in and out through a narrow gate where the currents were measured.

[2] Hovey (The Mammoth Cave of Kentucky 1897, p. 64) makes the longest course in Mammoth Cave from the entrance to Grogham Hall about 4.5 miles; the total length of all the known channels is several times that. The width and height may vary greatly from the many cracks where one has to crawl to Chief City between 450 feet (Hovey) to 541 feet (Call) long, and an average width of 175 feet (Hovey) to 190 (Call), with a maximum width of 287 feet.

Blatchley says of Marengo (p. 157), "Marengo Cave has been advertised far and near as containing 7 miles of underground passages. Our measurements showed its total length to be 3,850 feet, or 0.7 of one mile. The main channels of Wyandotte Cave we determined to be 4.21 miles long." Very many of the caves are but a few inches in diameter and too small to be entered.

The Mitchell Caves can be traced for over 2 miles. Given that they are 3 kilometers long, their average width is perhaps 8.3 meters. This would give an area of 25,000 square meters. As a stream flows their entire length a direct comparison can be made with epigean conditions by taking a stream of similar size and length above ground, with territory equaling the width of the cave. The fauna of the epigean area of equal size is incomparably richer than the subterranean one.[1]

The biological environment of cave animals is comparatively simple. While much has been written on them, the only account of the interrelation of the animals of any cave has recently appeared in a publication by one of my students, Mr. A. M. Banta (publication No. 67 of the Carnegie Institution of Washington).

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[1] For a discussion of the age of caves see page 17.

## THE BLIND VERTEBRATES AND CAVE VERTEBRATES OF NORTH AMERICA.

The blind vertebrates do not belong to one class nor do those within one class belong to one family. The blind fauna is very diverse in character and origin, but not all families of vertebrates are represented. A certain predisposition in habit and structure must be present to enable a species to dispense with light and to live in caves. A large blind epigean animal might secure its food and meet its mate, but it could not escape its enemies. Large blind forms are therefore impossible. While the size of a sun-fish (*Lepomis*) might not preclude it from entering caves, the fact that it detects its prey by sight excludes it entirely from the possibly blind. There is, on the other hand, no reason why members of the nocturnal Siluridæ, for instance, should not become blind.

No large mammals are blind, nor have large mammals permanently taken up their abode in caves. Bears visit caves, and raccoons, minks, and ground hogs also enter them. The latter two confine their underground wanderings mostly to small caves or to caves of their own making. None of these animals permanently live in caves; they are all twilight animals and depend on light for their continued existence; they have normal eyes and are not otherwise modified for life in caves.

Blatchley reports that a number of cats have established themselves in Wyandotte Cave, where they bring forth and rear their young. Nothing is known about their adaptations. They have exterminated the cave rats and are said to place themselves in a narrow passage of the cave and capture bats passing through.

*Neotoma pennsylvanica*, a wood rat widely distributed in eastern America, has entered caves. It was formerly found in Wyandotte Cave, but has been exterminated there. In various caves white-footed mice are found, but they are not blind.

The common mole (*Scalops aquaticus*), the long-tailed mole (*Parascalops breweri*), and the star-nosed mole (*Condylura cristata*) burrow in the ground and are partly or entirely blind. They are not found in caves.

Bats, which are twilight animals, but have minute eyes, do not depend on their eyes to secure food; they fly at night because their food is then abroad. There are in North America and the West Indies a large number of bats partly or totally blind. Many, if not all of those of the temperate region, winter in caves; a smaller number spend only the day there. They do not secure much, if any, of their food in caves and simply use them as shelters in a more systematic manner than bears do.

There are no blind birds, and no birds, as far as I know, permanently live in caves. The phœbe utilizes the entrances as it uses all other similarly sheltered places to nest. In Cuba a small owl is sometimes found in caves, but I know of none that makes it a permanent home. Many owls are adjusted to existence in twilight, but that they are dependent on their eyes is shown by the increase in size of their eyes. Other animals, depending on their eyes but living in the dusk, have similarly enlarged eyes. This is especially well shown by marine fishes living at twilight depth.

There are no cave reptiles, nor do reptiles temporarily enter caves for shelter, as do mammals. One turtle found a little distance inside of one cave was evidently accidental. I have never seen a snake in a cave, but once secured a copperhead at the entrance to one. But there are numerous blind lizards and snakes that burrow in the ground. *Aniella*, a small, legless, burrowing lizard of California, probably indicates their origin. This lizard has well-developed eyes. It burrows in sand and gravel. I have frequently seen it cautiously thrust its head out of the ground for an instant as if to take a survey of the field. It evidently still uses its eyes.

Amphisbænians,[1] which are widely distributed over the warm parts of the globe, burrow in the ground or live in ant hills, and are partially or totally blind. The blind snakes, members of the Typhlopidæ, have similar habits.[2]

Many salamanders live in damp earth under logs or rocks. It is but natural, therefore, that they should be found in or about the entrances to caves, where sheltering rocks are not infrequent. Others are true cave animals. Two of the salamanders in North America that habitually live in caves have apparently quite normal eyes. They are *Spelerpes maculicauda* found from Indiana and Kentucky to Missouri, and *Spelerpes stejnegeri* from southwestern Missouri. Two others living in caves have quite degenerate eyes, *Typhlotriton spelæus* from caves in southwestern Missouri, and *Typhlomolge rathbuni* from the caves of Texas. *Proteus*, the nearest relative of the latter, lives in the caves of Carniola. There are no blind epigean salamanders. Of *Anura* there are no permanent residents in caves, nor are there any blind forms. A jumping animal would be sure to meet with disaster in a cave if it practiced its usual mode of progression.

The classes of vertebrates furnishing the largest number of blind forms are the fish and fish-like vertebrates. Excluding the Branchiostoma, the Cyclostomes have for the most part degenerated eyes. *Polistotrema stouti* is quite blind.

*Benthabatis moresbyi* Alcock is a blind Torpedinid Selachian from Travancore, from a depth of 430 fathoms.

Of the lowest teleosts the Siluridæ are represented by *Gronias nigrilabris* Cope, which occurs in a cave near Philadelphia.[3] The eyes of many other cat-fishes are not highly organized and but little used in detecting food.[4] Other cat-fishes are occasionally met in caves, but no others are permanent residents.

The cave fishes of North America, *par excellence*, are the Amblyopsidæ. All the members of this family, 8 in number, have degenerate eyes; 5 have mere vestiges; 6 permanently live in caves; 1 is known only from a spring and another from open streams. These will be considered in detail later.

In Cuba 2 fishes belonging to a marine family, the Brotulidæ, have become adapted to a cave life in fresh water. Both are blind. Many of their marine relatives are also blind.

Along the coast from San Pedro, California, to Encenada, Lower California, but more particularly at the foot of Point Loma, a blind goby lives under rocks embedded in sand between high and low tide.

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[1] "All the members of the family are burrowers, and many live in ant nests. They bore narrow galleries in the earth, in which they are able to progress backwards as well as forwards. On the ground they progress on a straight line, by slight vertical undulations, not by lateral movements, as in other limbless reptiles; the tail of many species appears to be more or less prehensile. The food of these lizards consists of small insects and worms. * * * As many as 65 species are characterized in this account; 39 are American, of which only 2 (*Chirotes* and *Rhineura*) occur north of the Tropic of Cancer, and 4 (*Amphisbæna*) in the West Indies." — Boulenger, Catalogue of Lizards, vol. II, p. 430, 1885.

[2] There are altogether about 100 species reaching, in the Americas, as far north as Cuba: *Typhlops lumbricalis*, Yucatan; *Typhlops microstomus*, Mexico; and *Typhlops tenuis*, Guatemala and Mexico.

[3] Two blind cat-fishes have recently been described from Brazil.

[4] Herrick found that the cat-fishes detect their food, not by means of their eyes or olfactory organs, but by the touch and taste organs over the body and in the barbels.

The fishes, blind or partly blind, living in the depth of the ocean bordering the American continents are *Ipnops murrayi* Günther and *Ipnops agassizii* Garman. The former lives at depths varying from 955 to 2,158 fathoms and is very widely distributed. The second one is known from the type specimens from Lat. 2° 34′ N., 92° 6′ W., at a depth of 1,360 fathoms. *Ipnops* stands alone in a family and is the only vertebrate in which no eyes have been found.

The Brotulidæ have several members blind, or with very minute eyes, in various parts of the globe. With the exception of the fresh-water species of Cuba, the only ones found in the neighborhood of America are *Aphyonus mollis* Goode and Bean, 955 fathoms, and *Alexeterion parfaiti* Vaillant, 2,736 meters. Other deep-sea blind fishes are *Aphyonus gelatinosus* Günther between Australia and New Guinea, 1,400 fathoms; *Mancalias shufeldtii* Goode and Bean, 372 fathoms; *Paroneirodes glomerosus* Goode and Bean, 1,260 fathoms; *Tauredophidium hextii* Goode and Bean, Bay of Bengal, 1,310 fathoms; *Typhlonus nasus* Günther, north of Australia and Celebes, 2,150 and 2,440 fathoms.

## THE ORIGIN AND DISPERSAL OF CAVE ANIMALS.

It has been shown that many cave animals have good eyes. Epigean animals with degenerate or no eyes are not rare, hence the origin of the cave fauna and of the blind fauna are two distinct questions. This was first recognized by H. Garman and indorsed by Eigenmann and by Hamann. Other writers have usually confused the two questions, and indeed they may become one when they concern an animal that has become blind concomitantly with its cave colonization. A consideration of the forms that are not found in caves, and the reasons why they are not found there, is in this connection possibly more illuminating than the direct consideration of the cave forms.

Caves may have become populated by one of the four following processes:

(1) Animals may by accident have been carried into caves.
(2) Animals may, step by step, have colonized caves, becoming adapted to the environment as successive generations gradually entered deeper and deeper recesses of the caves.
(3) Animals which had elsewhere become adjusted to do without light may have gathered voluntarily in caves.
(4) Animals may have developed along with the development of the caves.

*First process:* This process was imagined by Lankester to operate as follows:

> Supposing a number of some species of arthropod or fish to be swept into a cavern or to be carried from less to greater depths in the sea, those individuals with perfect eyes would follow the glimmer of light and eventually escape to the outer air or the shallower depths, leaving behind those with imperfect eyes to breed in the dark place. A natural selection would thus be effected. In every succeeding generation this would be the case, and even those with weak but still seeing eyes would in the course of time escape, until only a pure race of eyeless or blind animals would be left in the cavern or deep sea.

While this is a possible mode of origin of cave animals, and even of blind ones, it is highly improbable that many or even any animals depending, as he supposes, on their eyes have thus come to first colonize the cave. Fishes are annually swept into caves, but these are not able to permanently establish themselves in them. To do this the fish must have peculiar habits, special methods of feeding and mating, before an accidental colonization can become successful, and if they are so adapted for a cave existence, they would probably voluntarily colonize the caves, without waiting for an accident.[1] The Amblyopsidæ are a small family of fishes, 8 species being known. They form a very small part of the large fish fauna about the North American caves. But since 6, possibly 7, of the species of this family are cave dwellers, and only one of the numerous other fishes is permanently at home in the caves, we must suppose, if the theory under consideration is the correct one, that the accident of being carried into caves happened to 6 or 7 out of 8 of the Amblyopsidæ, and to only 1 of all the other fishes about the caves. The absurdity of this supposition is self-evident. A comparison of the abysmal fauna with the pelagic and shore faunas would probably give us similar results.

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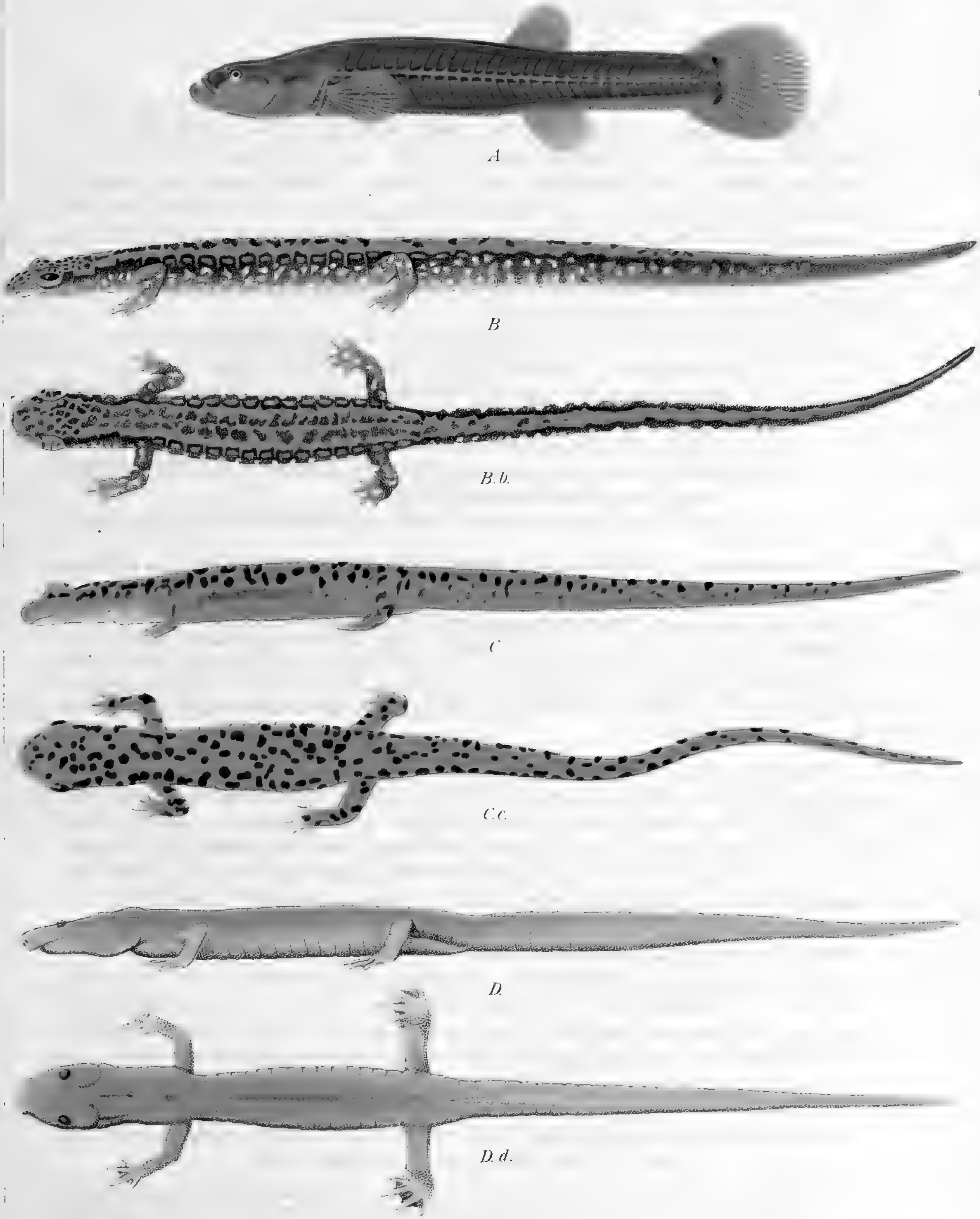
[1] A distinction ought possibly to be made between the aquatic cave animals that will be discussed under the "fourth process," and non-aquatic forms. Non-aquatic cave animals are later immigrants of caves. These must either be voluntary recruits from the twilight fauna about the entrance of the cave or they must have become otherwise adjusted to live in the dark. There is no difficulty in accounting for the presence of Myriopoda on this score nor for the other forms habitually found under bark and under rocks. Myriopods are everywhere abundant in the caves of North America and they (if any animals) may have accidentally been carried into caves with sticks of wood or trunks of trees.



Mrs E R Biehng del.

A. Chologaster papilliferus.
B, Bb. Spelerpes stejnegeri. 112 mm. Wilson's Cave, Sarcoxie, Missouri.
C, Cc. Spelerpes maculicauda. 130.5 mm. Wilson's Cave, Sarcoxie, Missouri.
D, Dd. Typhlotriton spelæus. 134 mm. Marble Cave, Missouri.

*Second process:* The second theory is that of Herbert Spencer:

> The existence of these blind cave animals can be accounted for only by supposing that their remote ancestors began making excursions into the cave, and, finding it profitable, extended them, generation after generation, farther in, undergoing the required adaptations little by little. — Popular Science Monthly, XLIII, 487 and 488.

I can offer no objection to this theory. It presupposes the existence of caves, and it is perfectly possible that many cave animals have arisen in this way. The abundant twilight fauna in the entrance of caves argues in favor of it. *Spelerpes maculicauda* and other salamanders, which are so frequently found a short distance within caves and even in remote recesses, seem to be present colonizers that bear out Spencer's view, though it is possible that these should be grouped with the animals next to be considered.

*Spelerpes maculicauda* has not yet been affected, as far as its structure is concerned, by its habits. It is a nascent cave form that may result in the future in a single blind species of wide distribution, or a number of species in the groups of caves that are geographically separated from each other. There can be no question whatever, in its case, about an accidental carrying into caves, for if it enter caves by accident it must be continually meeting accidents through a very wide region.

*Third process:* This view was first expressed by Garman (Science, Oct. 28, 1892, p. 240):

> The originals of the cave species [non-aquatic, especially] of Kentucky were probably already adjusted to a life in the earth before the caves were formed * *.

The writer [1] independently came to the same conclusion.

This theory makes the cave simply the collecting ground of animals adapted to a cave existence, and leaves the origin of this adaptation an open question. Garman imagined that the animals become adjusted to cave existence in crevices of rocks. Since these crevices are but caves on a small scale, his suggestion simply tends to account for the aggregation of the animals found in the caves of Kentucky, not for their becoming cave animals in the first instance.

If at this point we might call mutation to our aid, we would have a satisfactory explanation. If mutants arose among any species of animals adapted to cave existence, they would find their way into caves or crevices if such existed. What would happen if there were none need not concern us. But while mutation might account for the positive adaptive modifications in cave animals, it does not account for the negative or degenerative changes, and the more venerable theory of special creation is of equal potency.

*Fourth process:* It is certain that in some cases cave animals have developed concomitantly with the caves. It seems quite possible that in more cases than we have thought the adaptation of an animal to a very complex environment can only be explained as the result of concomitant development of environment and

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[1] In answer to the statement made by Eigenmann, Krause [Promethius, No. 457, p. 652, 1898] said: "Nicht weil sie in dunklen Höhlen leben, seien ihre Gesichtsorgane verkümmert, nicht die Entziehung des Lichtes habe diese Organe zurückgehen lassen, sondern umgekehrt, weil sie sich schon in der Oberwelt dem Leben ohne Licht angepasst hatten, wären sie wohl vorbereitet gewesen, in den Höhlen, von völliger Dunkelheit umgeben, so — glänzend, könnte man beinahe sagen — zu reüssiren. * * * Nun, wer's glaubt, mag ja auch bei dem Glauben selig werden können, dass die Höhlen gleichsam zum Tummelplatz und Elysium der Blinden aller Thierklassen erschaffen seien. Wir haben diese Sirenenklänge aus dem mystischen Dunkel der Gegner des Lichtes und der Entwickelungslehre schon öfters gehört; sie stehen in Harmonie mit den immer starker hevortretenden Bestrebungen, dem Lamarckismus, Darwinismus und selbst dem Weismannismus ein Bein zu stellen." This quotation is possibly sufficient to indicate the general tenor of the rest of his article.

animal. Certain parasitic insects are in the habit of boring through the hard mud walls of the nests of mud wasps to deposit their eggs. It seems difficult to explain the origin of so complex a habit and of the organ sufficient to pierce the hard wall. A mutation to account for it seems inconceivable. It is, however, quite possible that the hard wall is a partial adaptation against these very enemies, and that the habit of building heavier and heavier walls, and the development of more and more efficient organs for piercing them were developed as armor plate and armor-piercing shells are interrelated developments.

From the hills about Horse Cave, Kentucky, one sees valleys about 250 feet deep stretching out in four directions. Of the river that is responsible for them nothing is to be seen. It is 185 feet beneath the bottom of the valley at the town of Horse Cave. The hills are capped with over 70 feet of sandstone. The river has had a continuous existence from the time it formed the valley in the sandstone capping, through its later history when it continued the process of valley formation in the limestone underlying it, and later still when it hollowed out its underground channel in the limestone. There is nowhere any indication that there has been a cataclysm in the history of the river. It lies south of the glacial area. What is true of the river may be true of the inhabitants still within it. There is no reason to think that the ancestors of the blind fishes may not have lived in the stream when it flowed over the sandstone capping the hills.[1] Some fishes of any stream stay in the light, others in the shade, others under rocks. The ancestors of the blind fishes probably lived in the shade under rocks and became adjusted to the dark or dusk, existing there long before the caves were formed. When placed in open pools *Amblyopsis* still has that habit. What more natural than that this fish should descend farther and farther with the river after it began its subterranean course — not suddenly, but gradually? At first only part of its water found its way underground; but when all its water could flow beneath the surface under normal conditions, a part flowed above ground after every freshet, just as the water of Lost River of Indiana does at present. It could not sink beneath the ground at all until Greene River, into which it empties, had cut a considerable distance beneath the surface of the limestone, and thus gave the water in the limestone rifts a chance to flow out and be replaced with fresh water from the river above. As the stream sank beneath the surface, naturally those fishes depending on light for food and courtship left it, and only those either negatively heliotropic or positively stereotropic remained.

The blind aquatic fauna looked at from this standpoint is not a new acquisition of the present cave stream, but a relict of the fauna of the river when it still flowed above ground. The cave and its fauna have developed hand in hand. The presence of the cave fishes and other aquatic cave dwellers do not so much need explanation (they were present long ago) as does the absence of all of the other forms that must have been present when the stream flowed in its epigean valley. The prime requisite for a candidate for underground existence is a negative reaction to light, or positive stereotropism, or both.

It must also be evident that a fish depending on its sight to procure its food can never become a cave form. Sun-fishes, which are annually carried into the present fully developed caves, belong to this class of fishes. They are always

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[1] Shaler, 1875, considers that during the glacial epoch the conditions in the caves of Kentucky were such that the present fauna could not have existed there.

poor when found in the caves, and will never be able to establish themselves in them. On the other hand, there are no reasons why fishes detecting their prey either by smell or touch should not be capable of colonizing caves. The cat-fishes and Amblyopsidæ belong to the latter class. It is surprising that more cat-fishes have not established themselves in caves. Among the Amblyopsidæ, even those with functional eyes depend on touch and vibrations for their food. *Chologaster* has well-developed tactile organs and poor eyes. It is found chiefly at the mouths of underground streams, but also in the underground streams themselves. The tactile organs are not different in kind from those of other fishes, and their high development is not more marked than their development in the barbels of the cat-fishes. The characters which distinguish *Chologaster* as a fish capable of securing its food in the dark are emphasized in *Typhlichthys*, and the tactile organs are still more highly developed in *Amblyopsis*. The eyes of the last two genera are so degenerate that it is needless in this connection to speak of degrees of degeneration. On account of the structure of their eyes and their loss of protective pigment, they are incapable of existence in open waters. With the partial and total adaptation to underground existence in the Amblyopsidæ and their negative reaction to light, it is scarcely possible that for this family the idea of accidental colonization can be entertained for a moment. Their structure is not as much due to their habitat as their habitat is due to their structure and habit.

*Typhlogobius* lives in the holes of shrimps, under rocks, on the coast of southern California. It is a living example of the origin of blind forms in dark places remote from caves. Here again the "accidental" idea is preposterous, since no fish could by accident be carried into the devious windings of the burrows they inhabit. Moreover, a number of related species of gobies occur in the neighborhood. They live ordinarily in the open, but always retreat into the burrows of crustaceans when disturbed. The origin of the blind species by the gradual change from an occasional burrow seeker to a permanent dweller in the dark, and the consequent degeneration of the eye, is evident here at once. Among insects the same process and the same results are noted. We have everywhere the connection of diurnal species with nocturnal, dark-loving, and blind forms, a transition, the result of habit entered into with intent, but no evidence of such a connection as the result of accident; also numerous instances of daylight species being swept into caves, but no instance of one establishing itself there.

Attention has been called to the difference in the time of origin of the aquatic and non-aquatic cave-dwellers. The latter are later immigrants. They necessarily arrived after some channels had been cleared of water through the stream burrowing into still lower channels. The non-aquatic forms are derived, in part at least, by migrations from the twilight forms that may have developed with the twilight region, and in part they are active immigrants of stereotropic or negatively phototropic forms like the *Spelerpes*. Some of them, like the myriopods, may even be accidentally brought in with their food and habitat,[1] but even here the active voluntary immigration is, at least, as probable as the accidental one.

Species widely distributed over a continuous environment may have become distributed from one center of development. The same may be said of the species found in distant, discontinuous environments where it can be shown that the discontinuity is of recent origin. The same can not be said of species distributed in

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[1] Decaying logs have been carried into and are found in various parts of the Mitchell caves.

isolated elements of a discontinuous environment that can not, in the nature of the case, at any time have formed parts of a continuous environment.

*Amblyopsis* is found on both sides of the Ohio River. The caves of the two sides have certainly never formed part of the same complex. It is possible, though scarcely probable, that the caves south of the Ohio, inhabited by *Typhlichthys* at one time, formed a continuous environment. It seems evident that *Amblyopsis* could not have migrated from the caves south of the Ohio to those north of the Ohio. The different colonies probably had similar but independent histories. The cave salamander, *Spelerpes maculicauda,* is widely distributed in the Mississippi Valley. It enters caves wherever they are found within its area of distribution. It is becoming adapted to a cave existence in widely isolated places. What is at present taking place with *Spelerpes* may have taken place with *Amblyopsis*, except that *Spelerpes* found its caves ready made, while *Amblyopsis* was present during their making.

The ancestry of the Amblyopsidæ we may assume to have had a tendency to seek dark places, wherever found, and incipient blind forms would thus arise over their entire distribution. Certainly the fearless, conspicuous blind fishes, as at present developed, would have no chance of surviving in the open water. Their wide dispersal after their present characters had been assumed would be out of the question entirely, except through subterranean waters. The same would not be true of the incipient cave forms when they had reached the stage at present found in *Chologaster.* This genus has the habit of hiding underneath objects in the darker sides of an aquarium. These dark-seeking creatures would be especially well fitted to become distributed in caves throughout their habitat. S. Garman's able argument for the single origin and dispersal of the blind fishes through epigean waters was based on the supposition that the cis-Mississippi and trans-Mississippi forms were identical. The differences between these species are such as to warrant the inference, not only that they have been independently segregated, but that they are descended from different genera. The external differences between these species are insignificant, but this is to be expected in an environment where all the elements that make for external color markings are lacking. The similarity between *Typhlichthys* and *Amblyopsis* is so great that the former has been considered to be the young of the latter. For reasons that will be fully set forth there is every probability that the Cuban blind fishes developed with the caves which they inhabit.

In conclusion it may be said:

(1) That the cave fauna is in large part the result of the formation of the caves themselves, that environment and habitat developed *pari passu.*

(2) That to this original fauna have been added and are being added species (such as *Spelerpes maculicauda*) which, because they are negatively heliotropic or positively stereotropic, are gradually becoming adapted to the deeper and deeper recesses of caves.

(3) That to the fauna of the larger caves may also have been added animals which had become adjusted to cave existence in crevices, under banks or rocks, etc., that is, in small caves.

(4) That accident has played little or no part in developing the cave fauna.

## THE ORIGIN OF THE FOOD SUPPLY OF CAVES.

Cave existence, reduced to its simplest terms, is the securing of food and the meeting of mates in absolute darkness. Food is so scarce that no large predaceous animals have taken up their abode in caves, hence the largest cave animals, such as the cave fishes, have no enemies aside from parasites and disease germs. Of the cave fishes *Chologaster* reaches a length of but 62 mm.; *Typhlichthys*, 55 mm.; *Troglichthys*, 55 mm.; *Amblyopsis*, 135 mm.; *Lucifuga*, 104 mm., and *Stygicola*, 152 mm. All are insignificant in size.

The density of the population of any cave, other things equal, is inversely proportional to the size of the cave. No food is generated in caves by the growth of plants. Directly or indirectly all food consumed in a cave must be imported. It may come in through various openings; usually there are only one or two openings of any consequence: (*a*) the "entrance" in a dry cave, (*b*) the entrance and point of inflow of the stream in a wet cave. That cave is best supplied with food per square yard which has the smallest area over which the limited supply must be distributed. There is, of course, a great difference in the amount of food carried in through different openings. An entrance sloping upward naturally will not admit as much decaying vegetation as one sloping downward. A narrow crack through which water may enter a cave will not admit as much as a large opening, through which in times of flood the water may carry tree trunks. These matters equalized, I may repeat that that cave is best supplied with food per square yard which has the smallest area over which the limited supply entering a given opening must be distributed. The density of the fauna varies as the amount of food, and hence, other things equal, inversely as the size of the cave.

## AGE OF CAVES IN RELATION TO THE VARIETY OF CAVE FAUNA.

Desired lines of research are the relation of the abundance of the cave fauna to the age of the particular cave and the comparative degree of adjustment of the animals to caves of different ages. We have in North America a series of caves reaching from Howe's and other northern caves in the glaciated region to the Ohio Valley caves near the edge of glaciation, and the caves of Texas and Cuba never affected by glaciation.[1]

Howe's Cave in central New York is exceedingly poor in animals, the Texas caves are as correspondingly rich, but no detailed comparison has been made. It is also known that the Ohio Valley cave salamander, *Spelerpes maculicauda*, has well-developed eyes, that the Missouri salamander, *Typhlotriton*, has degenerate eyes, and that the Texas salamander, *Typhlomolge*, has very much more degenerate eyes. The degree of degeneration seems here coördinate with the age of the cave. Also that the Missouri blind fish has more degenerate eyes than those of the Ohio Valley. In a general way the older caves appear to have more intimately adapted or more profoundly modified forms than the newer. But here again we lack entirely a detailed study.

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[1] Shaler, 1875, estimates the age of the Kentucky caves at between 750,000 and 2,000,000 years. He further maintains that, during the glacial epoch Kentucky was populated by an Arctic fauna and that the cave fauna was not derived from this, but from the present fauna of Kentucky, "since the glacial period." I agree with him that the present cave fauna of Indiana and Kentucky was derived from or developed concomitantly with the present epigean fauna, but am in doubt about the nature of the fauna during the glacial period.

## DIVERGENCE IN EPIGEAN AND CONVERGENCE IN SUBTERRANEAN FISHES.

The struggle for existence with the biological environment as the result of the geometric rate of increase tends to divergence in habit and form. It does this by preserving variants whenever such possess a character diverging sufficiently in amount to give the variant a personal advantage over his fellows — always provided the divergent character is transmissible.

Whether we call the diverging individuals variants in the old sense, or mutants in the new, it is to the selection of those among them best adapted to utilize the foods of various sorts, to occupy localities of various kinds, to escape the enemies of various sorts, and to leave others similar to them in their place when they die, that we owe the specific divergence in structure, shape, color, food habits and breeding habits of a given family — say the American Characins. The entire process tends to the divergence and multiplicity of species.

The Characins are a family of fresh-water fishes that, in America, range from the border of the United States to some distance south of Buenos Aires. They form about one-third of the entire South American fresh-water fauna, and have diverged in adaptation to diverse food, diverse habitat, and diverse enemies to fill nearly every niche open to fishes. The ends of three of the lines of adaptation to different food give us mud-eating forms, with long intestinal tract and no teeth; flesh eaters, with shear-like teeth, that make bathing dangerous to life and that cut their way out of nets; and conical-toothed forms, with sharp, needle-like teeth and comparatively huge fangs. Greater diversity could scarcely be imagined, and one is led to suspect that some of the forms are over-adapted. In their divergence in form they have reached almost every conceivable shape as we shall see in a moment.

The struggle for existence with any unit of physical environment, whether there be geometric rate of increase or not, tends to convergence in habit and form. There is no more striking instance of this than the acceptance of the annual or deciduous habit of most of the plants inhabiting the temperate zones with their seasonal changes, nor is there a more striking illustration of the struggle with other individuals than the diversity of form and habit of various forest plants for ground and light space. Records of the simultaneous and similar changes in the form in the mass of species of any area during changing physical conditions are not wanting. For instance, Scott says:

> The steps of modernization which may be observed in following out the history of many different groups of mammals are seen to keep curiously parallel, as may be noticed, for example, in the series of skulls figured by Kowalevsky, where we find similar changes occurring in such families as the pigs, deer, antelopes, horses, elephants, etc. Indeed, one may speak with propriety of a Puerco, or Wasatch, or White River type of skull, which will be found exemplified in widely separate orders.

On some riffles of the San Juan River of Cuba I found a small fish that is very strikingly like other fishes inhabiting similar localities in the eastern United States. The former is a goby, a marine form, *Philypnus dormitator*, which has become adjusted to conditions found about the riffles of streams; the others are darters, *Hadropterus*, belonging to an entirely different family of fresh-water fishes. The similarity of various "darters" which live on the bottom of our streams to various gobies and blennies that occupy a similar position along the marine shores has repeatedly been noticed.

In the tropics live many burrowing lizards and snakes. *Rhineura*, one of the lizards, lives and acts like an earthworm, and so like an earthworm has it become that only a close inspection reveals its true nature. Even the chickens following the plows in Florida and Cuba are said to be taken in by the similarity of some of the burrowing lizards to earthworms.

The Characins again furnish striking illustrations. Diverging among themselves, as has been noted above, they have approached, or paralleled, many members of the diverse families of North American fresh-water fishes. Our shads and fresh-water herrings have their counterparts in *Elopomorphus*, *Potamorhina*, and *Psectrogaster;* our salmon are paralleled by *Salminus* and *Catabasis;* our minnows are paralleled by *Tetragonopterus* and its relatives. It will take but a slight flight of the imagination to detect the striking similarity of some of the Hydrocyninæ to our garpikes; our mullets are duplicated by *Prochilodus;* our topminnows are mimicked by *Nannostomus;* and even our festive darters are duplicated by the species of *Characidium*, members of this most remarkable family.

In a dark cave, all those differences between related species which would strike the eye, such as protective coloration, recognition marks, decorations of any sort, etc., are absent, and related species tend to look alike. It was not until after a detailed examination of many specimens that I could invariably distinguish *Lucifuga* and *Stygicola*, the Cuban blind fishes, from each other.

On the surface the specimens of *Troglichthys rosæ* very closely resemble *Typhlichthys subterraneus* from Mammoth Cave, differing slightly in the proportion and in the pectoral and caudal fins. These fins are longer in *rosæ*. It is, however, quite evident from a study of their eyes that we have to deal here with a case of convergence of two very distinct forms. They have converged because of the similarity of their environment and especially owing to the absence of those elements in their environment that lead to external protective adaptations. It would be difficult to distinguish specimens of similar size of *Amblyopsis* from either *subterraneus* or *rosæ* were it not that it possesses ventrals.

The eye of *T. subterraneus* is surrounded by a very thin layer of tissue representing the sclera and choroid. The two layers are not separable. In this respect it approaches the condition in the epigean, eyed member of the family, *Chologaster*. For other reasons, that need not be given here, it is quite certain that *Typhlichthys* is the descendant of a *Chologaster*. The intensity of coloration and the structure of the eye are the chief points of difference. The eye of *rosæ* is but about one-third the diameter of that of *subterraneus*, measuring 0.06 mm. or thereabout. It is the most degenerate, as distinguished from undeveloped, vertebrate eye. The point of importance in the present instance is the presence of comparatively enormous scleral cartilages.[1] These have not degenerated in proportion to the degeneration of the eye and in some cases are several times as long as the eye, projecting far beyond it, or are puckered to make their disproportionate size fit the vanishing eye. This species is unquestionably descended from a species with well-developed scleral cartilages, for it is not conceivable that the sclera as found in *Chologaster* could, by any freak or chance, give rise during degeneration

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[1] Kohl mistook the nature of these structures, as he did of every other connected with these eyes, except the lens and ganglionic cells.

to scleral cartilages, and if it did they would not develop several sizes too large for the eye. At present no known epigean species of the Amblyopsidæ possesses scleral cartilages. The ancestry of *rosæ* is hence unknown. *Amblyopsis* has the scleral cartilages, and the eye of *rosæ* passed through a condition similar to that possessed by *Amblyopsis*, but the latter species has ventral fins and is hence ruled out as a possible ancestor of *rosæ*. The epigean ancestry of *Amblyopsis* is also unknown. The ancestry of *Typhlichthys* being quite distinct from that of *rosæ*, the latter species is referred to a separate genus, *Troglichthys*.

Judging from the degree of degeneration of the eye, *Troglichthys* has lived in caves and has done without the use of its eyes longer than any other known vertebrate. (*Ipnops*, being a deep-sea form, is not considered.)

The species of *Typhlichthys* differ from each other in only a few inconspicuous respects. (See page 53.)

## CONCLUSIONS.

(1) The possible physical environment of animals is composed of units, each of which is distinguished by a combination of conditions peculiar to it.

(2) A unit may embrace one continuous area.

(3) A unit may have extended in the past over a continuous area, but may now be broken up into separate, though similar, parts between which the migration of animals is not possible.

(4) A unit may always have existed of separate and distinct parts (units of a smaller order) which together form a discontinuous unit.

(5) An animal distributed over a continuous, or parts of a formerly continuous, unit may have arisen at a single center of dispersal.

(6) An animal distributed over a discontinuous unit must have had separate places of origin or have originated at a time when the parts of the unit were continuous.

(7) Each cave consists of a twilight section, a fluctuating temperature section, and the cave *par excellence.*

(8) The environment in the third section is chiefly characterized by (*a*) the absence of light; (*b*) the constancy of meteorological conditions between seasons; (*c*) the absence of food except such as is imported.

(9) All classes of vertebrates, except birds, have blind members.

(10) Some cave animals (aquatic) have developed *pari passu* with the development of an underground stream and are among the few inhabitants remaining to the stream of its inhabitants during its epigean period.

(11) Some cave animals (non-aquatic) have gradually colonized caves after their formation.

(12) Some cave animals became elsewhere adjusted to live in the dark and later migrated into caves.

(13) Accident had little or nothing to do with the colonization of caves.

(14) Some widely distributed cave species have independently arisen in different places from a widely distributed epigean species.

(15) Directly or indirectly all of the food supply of a cave must be imported.

(16) Smaller caves have a relatively richer fauna, because the food supply is more abundant.

(17) Older caves have a more varied and richer fauna.

(18) Cave animals tend to converge in their evolution; epigean animals, to diverge.

# BLIND AND CAVE VERTEBRATES AND THEIR EYES.

# MAMMALS.

## EYES OF THE COMMON MOLE.

Dr. J. R. Slonaker has found that the eye of the mole (*Scalops aquaticus machrinus*) lies embedded in the muscle beneath the skin, where it appears as an inconspicuous dark spot. It is situated well forward on the side of the snout. The eye is degenerate and is no longer capable of functioning in distinct vision. The most noticeable changes which have occurred are:

1. The great reduction in the size of the eye.
2. The much crowded condition of the retina as a result of the decrease in size of the eye as a whole.
3. The noticeable reduction in the size, or the complete absence, of the aqueous and vitreous chambers.
4. The varied modification of the shape and size of the lens, also the peculiar cell structure of the lens.

All the structures of the normal mammalian eye are present in some form or other. (1) The conditions found in the adult and at birth have been studied. Very little difference is seen in these two stages excepting an increase in size.

The eye muscles and the optic nerve are easily traced back to the skull. At birth the nerve presents in its course from the eye to the skull a peculiar arrangement. The course is marked by numerous cells and few or no fibers. At the eye there is a rapid change from this cell condition to the fiber condition of the nerve tract. The fibers have not apparently grown much beyond the limits of the eye. In the adult the fibers can be traced to the skull.

The eye cleft is very small and of practically the same diameter in both horizontal and vertical sections through it. It meets the eye at such an angle that it is impossible for rays of light, should any enter, to pass through the eye along the axis of vision.

All the elements of the normal retina are present, but, owing to the much crowded condition, the ganglion-cell layer is much increased in thickness.

The lens, which is found in a great variety of shapes and sizes, is composed of peculiar cartilage-like cells with well-defined nuclei. It is therefore incapable of functioning as a normal lens.

It is very doubtful, therefore, whether the eye of the mole functions in any sense. At best it can do no more than distinguish between light and darkness.

## THE CAVE RAT AND ITS EYES.[1]

The cave rat, *Neotoma magister*, ranges eastward to southern New York and south to Alabama, and is not confined to caves. It lives in "cliffs, caves, and rock ledges of the mountains, descending into the lowlands, where limestone caves afford it security."

In White's Cave, near Mammoth Cave, Kentucky, it has its nests near the entrance, in the twilight region. In Mammoth Cave I found it in Mammoth Dome, and it occurs also farther in, far removed from the twilight area.

Rhoads (Jour. Cin. Soc. Nat. Hist., XIX, No. 2, 55, 1897) says of it:

> Any suspicion of blindness or deficient eyesight, such as is exemplified in some of the lower orders of animal life in the cave, can not attach to this mammal. As in all the more strictly nocturnal rodents, the eyes of this species are greatly developed; nevertheless, they are able to make most intelligent use of them in broad daylight, if need be.

In his "Origin of Species," sixth edition, vol. I, page 171, Darwin says that the eyes of *Neotoma* of Mammoth Cave are "lustrous and of large size; and these animals, as I am informed by Professor Silliman, after having been exposed for about a month to a graduated light, acquired a dim perception of objects." The cave rat, *Neotoma*, is still abundant in Mammoth Cave. Its tracks are numerous, and in places little paths have been made by the rats where they run backward and forward along ledges of rock. Since, however, a track once made in a cave remains unchanged by wind or weather, the abundance of rats, as judged by their tracks, may be misleading. A number of traps were set in the rotunda. During three days one trap was sprung and one had the bait removed. No rats were caught in the traps and none were caught alive. The author discovered one rat rolling a mouse trap about which was too small for it to enter. When approached with a light, the rat turned about and stared at the light. It then ran to a pile of rocks, but did not attempt to hide; instead, the rat ran to one end of the pile, then along the top back to where it had stood, then stopped and again stared at the light.

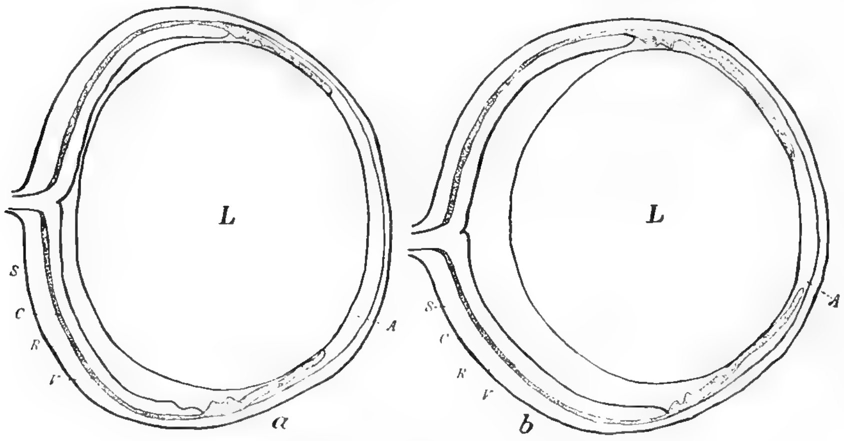

FIG. 1. (*a*) Eye of Mammoth Cave Rat. (*b*) Eye of Common Gray Rat.

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[1] The histology of the eye is condensed from Dr. J. B. Slonaker's account, from which figures 1 and 2 are taken. See Proc. Ind. Acad. Sci. for 1898, p. 255, 1899.

An attempt to catch the rat sent it running back and forth along the ledges of rock at the side of the cave. Finally the rat appeared at the ground again, and despairing of catching it alive, it was killed. Its eyes seemed to be large and protruding very much as in the common rat. Without question the rat noticed the light. It had no hesitation in running from place to place. Later four of these rats were sent by express. Only one arrived alive; one had been partly eaten by the others. The living one was quite gentle. It permitted itself to be stroked. Occasionally it pushed an object away with a sideward motion of the forefoot. If provoked it snapped at the object. During daylight it sat quietly in a nest it formed for itself of cotton batting, which it pulled into a fluffy mass. At night it frequently moved about in its cage. Turning on an electric light near its face always produced a twitching of the eyelids, so there can be no doubt that the light was perceived. An object held some distance from the cage on one side or another was always perceived, but just how precise its vision was has not been determined. Its hearing was acute.

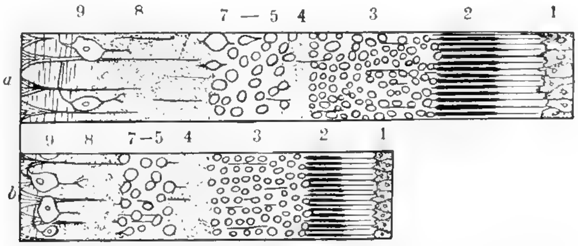

FIG. 2. Retinas of Neotoma and Common Gray Rat Compared.
(*a*) Mammoth Cave Rat. (*b*) Common Gray Rat.

Its eyes were as prominent as those of the gray rat. If there was any difference, its eyes were larger in proportion to the size of the body weight than those of the gray rat. The lens in both cases was enormously large in proportion to the eye. The pupil was capable of very wide dilation. A microscopic comparison of the retinas also showed little difference. Bits of retina from corresponding parts of the eye of a cave rat and a gray rat were hardened by the same process, sectioned the same thickness, and stained alike. The results are given in figures 1 and 2.

There is little difference except in the thickness of the retina, that of the cave rat being thicker. However, the difference may be due to the differences in the ages of the animals, the cave rat being fully grown, the gray rat only half grown. The thickness of the retinas are proportionate to the size of the eye. The increased thickness is largely due to the larger size of the cells of corresponding layers of the retina. For instance, the rods and cones are decidedly longer and larger in the cave rat. But with the exceptions given the two retinas are nearly alike.

## THE CAVE SALAMANDERS.

The salamanders, of which there are many species in the United States, habitually live under rocks, logs, and the bark of decaying trees. These all shun the light except during the breeding season. Others habitually live in the water and are principally nocturnal in their habits, hiding under the banks, logs, or rocks in the water during daylight. The eyes of the cave salamanders of North America, of which there are four species, range in their structure from the perfectly normal to the most degenerate known among the Batrachia.

*Spelerpes maculicauda* (Cope) (plate 1, fig. C) is common in the caves of the Mississippi Valley. As far as I have been able to determine, its eyes have not undergone any degeneration. It is abundant and so nearly allied to *Spelerpes longicauda* Green, an epigean species of very wide distribution, that formerly the two were considered identical (plate 2, fig. A).

*Spelerpes stejnegeri* Eigenmann (plate 1, fig. B) is found in the twilight regions of the caves of southwestern Missouri. Its eyes are also normal. Other species of *Spelerpes* [1] are sometimes found in caves.

*Typhlotriton spelæus* Stejneger (plate 1, fig. D) is restricted to the western caves of the Mississippi Valley. It has so far been found in Marble Cave and Rockhouse Cave, and smaller caves in the same neighborhood in southwestern Missouri. It is found under rocks in and out of the water. This is the most interesting form, inasmuch as it is a much more typical cave animal than *Spelerpes*, but has not yet reached the degenerate condition of *Typhlomolge*. Its eyes are apparently normal in the larva, but in the adult have undergone marked degeneration. The eyelids are disappearing and the rods and cones are no longer present in the adult. The eyes of this species will be dealt with below.

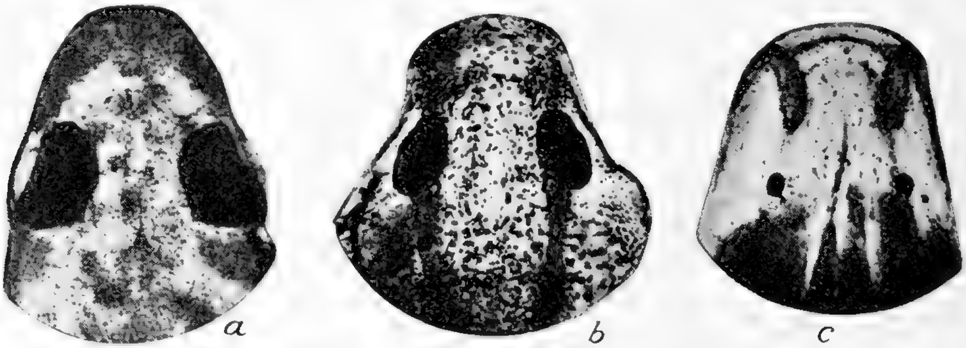

FIG. 3. (*a*) Head of *Spelerpes maculicauda*, 54 mm. long. (*b*) Head of *Typhlotriton spelæus*, 54 mm. long. (*c*) Head of *Typhlomolge rathbuni*, 47.5 mm. long.

*Typhlomolge rathbuni* Stejneger (plate 2, fig. B) is found in the underground streams near San Marcos, Texas. It has been taken from the artesian well at San Marcos and a surface well. It has also been noticed in one of the caves near that place, Ezel's, in which the underground water can be reached. It is said to have come out of some artesian wells south of San Antonio. It is a perennibranch and spends all of its time in the water. Its remarkably long and slender legs are not able to support its body when out of the water. Figure 3 shows

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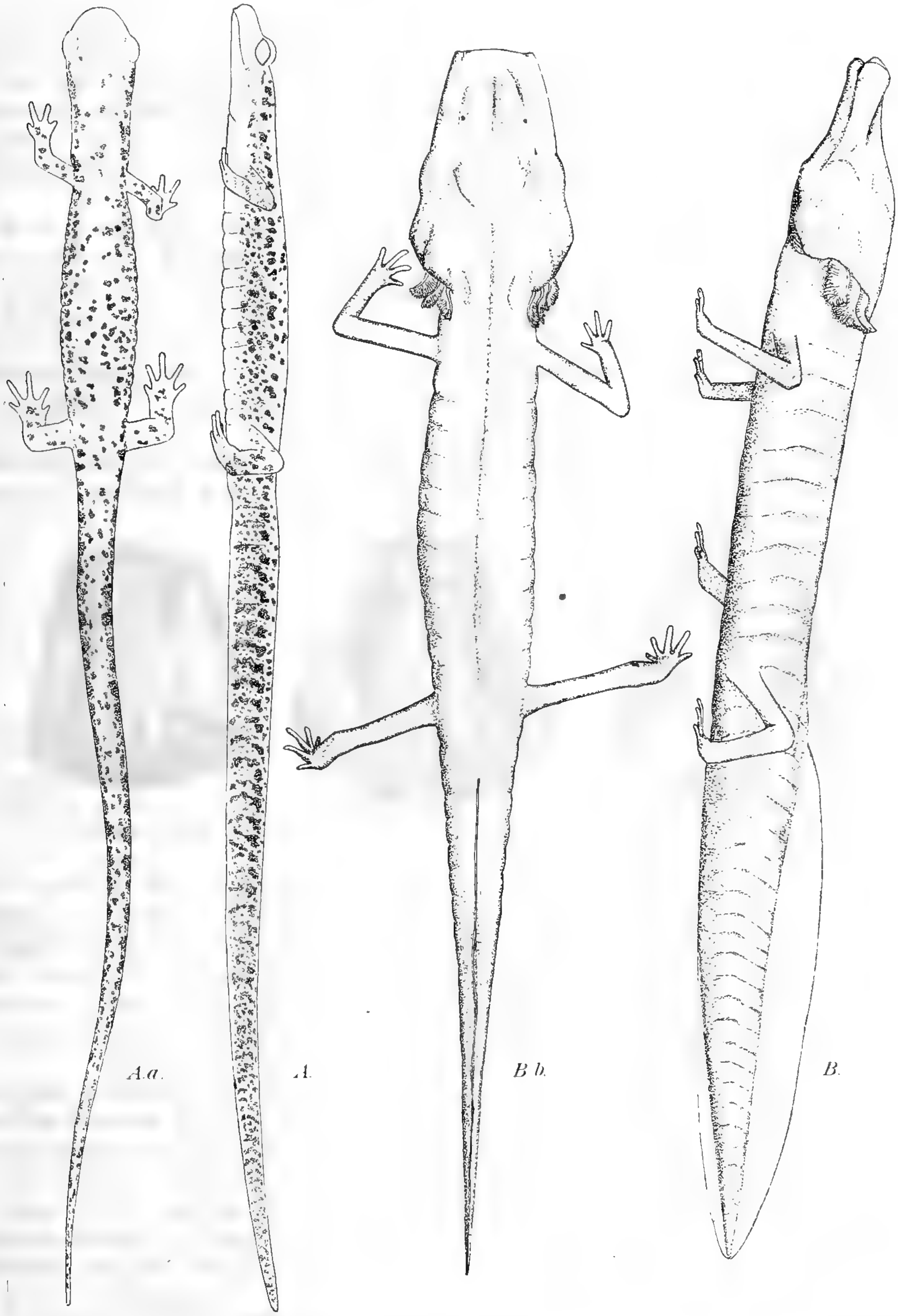
[1] *Bilineatus* is frequently found about the caves of Bloomington, Indiana.



A, Aa. Spelerpes longicauda. 147.5 mm. Carlisle, Pennsylvania.
B, Bb. Typhlomolge rathbuni. 88 mm. San Marcos, Texas.

the heads of three cave salamanders of North America. The heads were subjected to the same treatment to prepare them for photography, and photographs were taken under approximately the same magnification.

In February, 1896, the first recorded specimens of this species were cast up from an artesian well about 190 feet deep, bored by the U. S. Fish Commission. Other specimens have since been thrown up at the rate of 30 to 50 a year.

The following notes on the habit of this cave salamander are by the late Professor Norman of the University of Texas.

Unless disturbed, the salamanders appear at all times either resting, or very slowly and cautiously walking along. They move a few steps at a time, wait awhile, and go again. They have no particular pose when quiet except that they always rest on their 4 feet, holding themselves up from the bottom of the vessel and frequently retaining the exact position of the legs at the moment the motion is arrested. If the vessel contain, for example, watercress, they crawl in among the branches and stop as when walking on firm bottom, with the legs in such a position as fits easiest for gliding in among the twigs.

They are never seen to move faster than a slow, easy walk, except when disturbed by external stimuli. Then one of three methods of locomotion may follow: (1) the walking speed may pass into a grotesque run by long strides and corresponding winds of the body; or, (2) this passes into a combined movement of legs and tail, the last acting as fin; (3) at its greatest speed the legs are laid lengthwise against the body, and the tail only is used for locomotion.

The legs are exceedingly slender and weak. If the animal is placed on a table out of water, the body falls to the table, and at best the animal may wriggle a few inches; but in water the weight of the salamander is so little that the legs are amply strong for its locomotion. Dr. Stejneger lost sight of this point when he guessed that the animal used its tail for locomotion and its legs as feelers. He stated as follows:

> Viewed in connection with the well-developed, finned swimming-tail, it can be safely assumed that these extraordinarily slender and elongated legs are not used for locomotion, and the conviction is irresistible that in the inky darkness of the subterranean waters they serve the animal as feelers.

The motion in water is, for the most part, slow and cautious, the movement of the long legs being apparently calculated to produce the least commotion in the water. The motion suggests that of a cat creeping upon its prey, or the elephantine progression of the snapping turtle. The feet are lifted high in walking, and the body is kept from the bottom by the full length of the fore arm and leg. In ordinary progression the body slopes from nose to tail, which drags (plate 2, fig. B). The method of moving the limbs is as follows: Left hand and when this is nearly ready to place, or usually when placed, the right foot. When the right foot is placed, then the right hand and then the left foot. As the hand of one side is not raised till the foot of the same side is placed, the enormous strides of the long-legged creature cause it to step on its hand or even beyond. Its natural gait is a deliberate progression by means of its feet with three feet usually on the ground. Any attempt at great rapidity by this means of locomotion results in a most undignified and futile wriggle. When going slowly, the head is held sloping upward. When walking rapidly, it is held sloping down, so that the snout is near the ground.

No definite information has been obtained as to their habits in nature. They show no reaction to light, either as a response by motion to the direction of the rays or to the quantity of light. If kept in a vessel, half of which is dark and the other half light, the animal is found about as often in one as the other, and on emerging into light from the dark it indicates in no way an awareness of the difference. If in a tangle of plants, as watercress, they are found about the same as in any other part of the vessel.

If they are headed against a current, the flowing water acts as stimulus, urging them on. If the current strikes them from behind, they move more rapidly in the direction of flow.

The sense of touch is highly developed. There is, however, no experimental evidence that this is confined to any particular region. If the surface of the body is touched anywhere except at the blunt truncated snout, the animal responds at once by moving away. If the stimulus causes it to swim away, it may go (say 12 or 16 inches) till it strikes the side of the vessel, after which it soon comes to a standstill. If, however, it is struck, say with the flat side of a scalpel handle, sufficiently hard to move the entire animal even an inch backward, it may not react, and this may often be repeated before it reacts by moving away. A possible explanation of this fact is that in normal life it is every day striking itself against obstacles, especially the sides of the vessel (when in confinement).

The animal is exceedingly sensitive to any motion of the water. Where one is kept in water about an inch deep, with its head near the surface, waves of water set going by a gentle puff of the breath act as a sure stimulus.

But little evidence thus far shows in favor of a sense of smell. All attempts at feeding (except one) have been in vain. No attention was given to meat or other articles placed near it. Examination of a dead specimen showed chitinous remains of such crustacea as *Cyclops*.

If a glass rod or other object is held a little to one side and in front of the animal, it will cautiously turn its head in the direction of the rod. If the latter is then made to describe an arc about the side of the salamander, the head will follow it with a continuous motion, expressive of the greatest caution, as far as it can be followed without moving any of the limbs. A sudden jar, produced by tapping the rod on the bottom of the aquarium at such a time, causes the salamander to jerk its head back and rear back on its limbs as far as it can. The same effect is produced if the rod is introduced too rapidly.

If a piece of crayfish tail is held by pincers in the fingers a short distance in front or on one side of the head of the salamander, there is the same cautious motion forward till the snout comes in contact with it. There is then a momentary hesitation, followed by a sudden snap and seizure.

The salamander may be pulled from side to side by the meat, after it has once secured a hold, without causing it to let go. All of its caution is apparently directed in approaching the food without disturbance. After it has secured a hold it will struggle to maintain it.

## THE EYES OF TYPHLOMOLGE RATHBUNI.[1]

The U. S. Fish Commission, through Dr. B. W. Evermann, sent me four specimens of this salamander and a number of its eggs. Of these, one adult had been received in Washington, April 8, 1896, and three young, of different sizes, March 1, 1896. A few eggs were laid about March 15, 1896. The late Professor Norman, of the University of Texas, and Professor Bray, of the same place, secured me an additional number. Later, I visited the caves and the artesian well at San Marcos, and have been able to observe the living specimens. The specimens sent by Professor Evermann were preserved in alcohol; those sent by Professor Norman had been killed in Perenyi's fluid. The sections were stained chiefly in Biondi-Ehrlich's tricolor mixture.

The following gives the dimension of the eyes in a number of individuals. Professor Norman sent only the heads, so the length of his specimens sent can be given only approximately. The sizes (in millimeters) were obtained by comparing the distance between the eyes, with the same distance in entire specimens.

*Dimensions of the Eyes of Typhlomolge in Millimeters.*

| LENGTH OF SPECIMEN. | DISTANCE BETWEEN EYES. | DIAMETER OF LEFT EYE. | | DIAMETER OF RIGHT EYE. | |
|---|---|---|---|---|---|
| | | Longitudinal. | Transverse. | Longitudinal. | Transverse. |
| mm. | mm. | | | | |
| 30 | 1.44 | 0.336 | 0.232 | 0.368 | 0.240 |
| 47 | 1.92 | .432 | .320 | .432 | .304 |
| 70 | 3.10 | .544 | .384 | .608 | .368 |
| .. | .... | .496 | .432 | .544 | .384 |
| 90 | 4.00 | .592 | .400 | .592 | .448 |

The eye of *Typhlomolge* is, in many respects, much more degenerate than that of its European caverniculous relative, *Proteus*. In *Proteus* the six muscles are all present; in *Typhlomolge* they have entirely disappeared. In the former all the layers normal to the retina are present; in the latter the conditions are much simpler. In *Proteus* the lens is still present and blood-vessels still enter the eye; in *Typhlomolge* no trace of the lens could be found, except in one individual, and blood-vessels no longer enter the eye. While some of the asymmetry may have been caused by reagents, it is evident that there is a great deal of fluctuation in the shape of the eye. The eye is irregular-oval in outline as seen from above, but the optic nerve enters it at the posterior half of its inner face. The eye increases materially in size from the smallest to the largest of specimens examined. This increase is not directly proportional to the increase in the length of the animal, so the young have relatively larger eyes (fig. 4).

The eye lies immediately beneath the skin, to which it is attached by a connective tissue mass which is horizontally elongate. The axis of the eye makes an acute angle with the surface of the skin, the eye being directed outward and forward. The dermis over the eye does not differ from that in the neighboring tissues. The epidermis, in the largest individual, is perceptibly thinner over the eye, *i.e.* from the continuation of the axis of the eye to the surface of the epidermis. The measurement, in the largest individual, of the epidermis at a point over the eye and 320 $\mu$ above and below this point gives the following: thickness over the eye 73 $\mu$, 320 $\mu$ above the middle of the eye 96 $\mu$, 320 $\mu$ down from the eye 80 $\mu$.

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[1] See Trans. Am. Microsc. Soc. xxi. p. 49, 1900.

The same elements are found over the eye that are evident in other regions. There is no indication of a past free orbital rim; the dermis and epidermis are directly continuous over the eye. There are no eye muscles and no glandular structures connected with the eye. It is surrounded on all sides, except where it becomes associated with the skin, by loose connective tissue meshes filled with fatty tissue, and is bound to the dermis by many fibers running in various directions, and among these a few pigment cells are found.

### SCLERA AND CHOROID.

(*a*) *Largest specimens:* Cartilaginous elements are found in the sclera of but two eyes. In one individual, 90 mm. long, the left eye possesses a cartilage, while there is none in the right eye. It is in this case placed just above the entrance of the optic nerve and measures 96 μ in thickness, 160 μ vertically, and 204 μ antero-posteriorly. In all other cases the sclera is a thin, flocculent layer not distinctly separable from the layers beneath it. It is thickest about the entrance of the optic nerve. Over the front of the eye there are a few denser strands, which may represent the remains of the cornea. Over the sides of the eye of the largest individual the sclera measures from 4 μ to nothing. About the entrance of the optic nerve it attains a thickness of 14 μ, and contains many flat nuclei with a length up to 17 μ.

FIG. 4. Outline Sketch of Part of Section of Head of Specimen of *Typhlomolge rathbuni*, 90 mm. long, showing Position of Eye.

The choroid reaches a thickness of 20 μ near the entrance of the optic nerve, and dwindles regularly from this point to the distal face of the eye. Blood-vessels are found in it next to the pigmented epithelium of the eye. Otherwise it is a mass of pigment interlarded with streaks of colorless tissue containing nuclei. Over the front of the eye, next to the epithelium, there are a number of colorless cells with large, granular nuclei.

(*b*) Essentially the same conditions exist in younger specimens, but the parts are relatively thinner. The ophthalmic artery, extending approximately parallel with the optic nerve during its distal course, is sometimes surrounded by pigment.

## THE PIGMENT LAYER, EXCLUSIVE OF THE IRIDEAL PARTS.

The pigment layer is a thin, compact layer, densely pigmented. In an individual 30 mm. long it is about 8 μ in thickness. As there are no rods and cones, the inner surface of this layer is similar to the outer, that is, the cells form a pavement epithelium. In places, however, processes of the cells extend in among the cells of the nuclear layers, for a distance of 40 μ in some cases (fig. 5*a*), to the inner reticular layer. In the individuals 70 to 90 mm. long, the pigment epithelium reaches 16 μ in thickness. The only indication of a lens was found in the eye of a specimen 72 mm. long. In this a small lenticular group of cells lay in the opening of the pupil. It measured 24 × 40 μ (fig. 6).

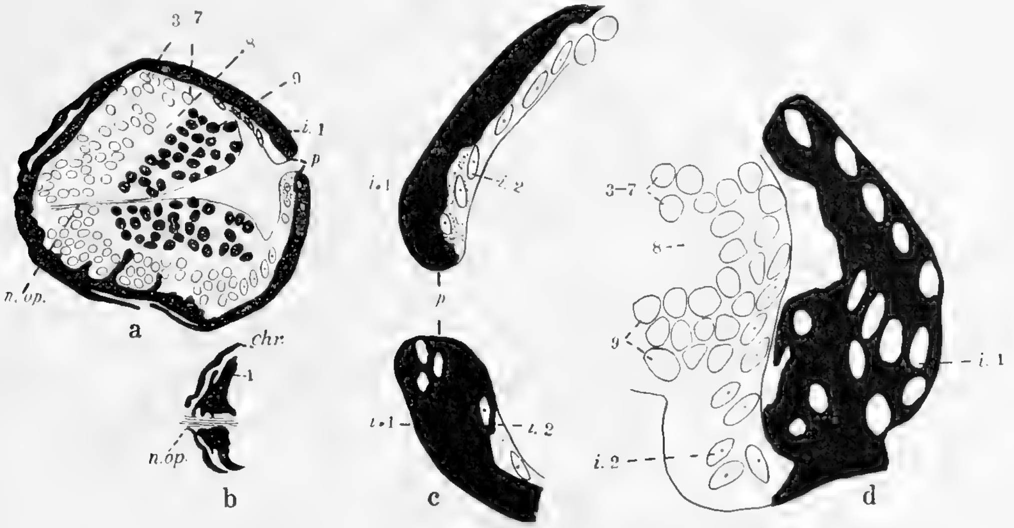

FIG. 5. (*a*) Right Eye of Specimen of *Typhlomolge* 30 mm. long. (*b*) Exit of Optic Nerve of Same. (*c*) Iris of Left Eye of Same Specimen. (*d*) Upper Half of Iris of Right Eye of Specimen of *Typhlomolge* 70 mm. long.

FIG. 6. Lens of Specimen 72 mm. long.

## THE IRIS AND ORA SERRATA.

Marked changes take place in the iris from the smallest to the largest individuals examined, so that these must be dealt with seriatim.

The smallest individual is 30 mm. long (fig. 5 *a* and *c*). On the left side the pupil measures 22 μ in diameter; the distance from the margin of the pupil to the ora serrata measures approximately 100 μ. The epithelial part of this iris consists of an outer layer of dense pigment considerably (14 μ) thicker than the pigment epithelium of the rest of the eye. At the pupil this pigment appears rolled into the inner surface of the iris, where it is continuous with the inner layer of cells, which consists of a layer of ordinary pigmentless epithelium 6 μ thick, with the nuclei elongate and placed obliquely, and 24 μ in length. A few of these ordinarily pigmentless cells show pigment. There is a distinct thickening of the iris at the margin of the pupil. The pigment cells lying on the inner face of this region are much less densely pigmented than those of the outer layer, and their nuclei are quite evident. The pupil is closed with colorless cells belonging to the choroid (fig. 7 *a*).

Very marked changes have been brought about in the specimen 70 mm. long. The pupil is now an oblique channel and the lower margin of the iris overlaps the upper margin. On the left it is more nearly as in the younger stages, but wider (48 $\mu$). The free margin of the iris now reaches the enormous thickness of

Fig. 7. (a) Right Eye of Specimen 70 mm. long. (b) Right Eye of Specimen 90 mm. long.

56 $\mu$ to 80 $\mu$. The pigmented epithelium has rolled in more, so that the elongated nuclei, free from pigment, are crowded together in the region of the ora serrata. The pupil is filled in part with pigment, evidently of choroidal origin (fig. 7 *a*).

In the right eye of the specimen 90 mm. long the choroidal pigment has forced its way into the interior of the eye and forms a conical-shaped mass like a plug in the iris and extends into the depth of the vitreous cavity. Apparently on the external half of the iris the pigmented layer has become rolled in and folded upon itself in the interior of the eye, giving rise to a pigment mass over 100 $\mu$ thick. No such mass is present in the left eye. The pigment on the inner or upper half of the iris is as in the younger stages. The choroidal pigment entering the eye is in solid, vermiform strands (fig. 7 *b*).

## THE RETINA.

The retina of *Typhlomolge* is much simpler than that of *Proteus*. In the latter all the layers typical of the perfect retina are still distinguishable. In the former the outer reticular layer has entirely disappeared, and the layers between the rods and cones and the inner reticular layer form a mass of cells that are homogeneous as far as ordinary histological methods permit one to determine. There are nowhere the slightest evidences of any rods or cones, either in the largest or smallest individual. The nuclei of the outer nuclear, the horizontal, and inner nuclear layers are alike. Müllerian fiber-nuclei have not been distinguished as such. This layer consists of about five series of nuclei and measures 44 $\mu$ in thickness in the smallest (30 mm.), and 48 $\mu$ in the largest (90 mm.), specimen; it is between 32 and 48 $\mu$ in the specimen 70 mm. long.

The inner reticular layer is thin, but well defined. It is 6 $\mu$ thick in the smallest specimen and 16 $\mu$ in the specimen 70 mm. long. In section the ganglionic layer forms a U-shaped mass of cells. In the larger specimens it is about 60 $\mu$ thick and made up of from five to seven series of cells. The vitreous cavity is a widely flaring, trumpet-shaped structure, with its pointed end reaching to near the center of the eye (fig. 7 *a*). In the older specimens it is filled by fibers and cellular tissue, apparently continuous with the choroid ingrowth from the pupil (fig. 7 *b*).

The optic nerve is 17 $\mu$ in diameter in the 30 mm. specimen. In the largest specimen it is 24 $\mu$ thick without its sheaths. At its passage through the pigmented layer of the retina it is contracted to a width of but 14 $\mu$. Within this layer it expands to 28 $\mu$. After passing directly through the ganglionic layer it is distributed to the cells of this layer, some of the fibers being bent at an acute angle to reach the cells near the entrance of the nerve into this layer. A large number of isolated pigment granules are found associated with the nuclei of the optic nerve within the eye from its entrance to the ganglionic layer. There is no sheath of pigment such as that found in *Typhlogobius*. Pigment cells are also occasionally present in the very center of the eye (fig. 7 *a* 2), and are presumably associated with the optic nerve. The sheath of the optic nerve consists of a direct continuation of the choroid layer, which is for a shorter distance pigmented, and of a continuation of the sclera (fig. 8).

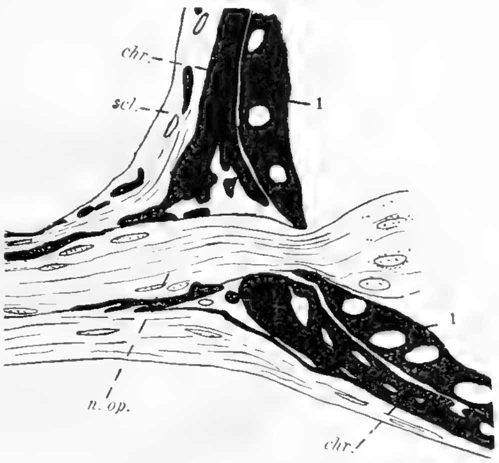

FIG. 8. Exit of Optic Nerve of Eye shown in fig. 7 *b*.

Blood-vessels do not enter the eye with the nerve, and none were with certainty detected except in the largest individual, where they are closely associated with the choroidal mass of tissue that has grown into the eye through the pupil.

## THE EYES OF TYPHLOTRITON SPELÆUS STEJNEGER.[1]

A single specimen of a salamander was discovered in Rockhouse Cave, Barrie County, Missouri, by Mr. F. A. Sampson in July, 1891. The specimen was described by Dr. Stejneger (Proc. U. S. Nat. Mus., vol. xv, p. 115), as *Typhlotriton spelæus*. His diagnosis reads as follows:

> Vertebræ opistocœlous; parasphenoid teeth; vomerine teeth; eyes concealed under the continuous skin of the head; tongue attached in front and along the median line, free laterally and posteriorly; maxillar and mandibular teeth small and numerous; vomerine teeth in 2 strongly curved series; parasphenoid patches separate; nostrils very small; toes 5; 16 costal grooves, or 18 if counting the axillary and groin grooves; tail slightly compressed, not finned; toes nearly half-webbed; vomerine teeth in two V-shaped series with the curvatures directed forward; gular fold strong, very concave anteriorly; color uniformly pale.

He further wrote, before he discovered *Typhlomolge* in the underground streams of Texas:

> Although many of our salamanders are known to inhabit caves, this seems to be the only one, so far discovered, which, like some of the other animals exclusively living in caves, has become blind or nearly so.

A preliminary note by Eigenmann and Denny (Proc. Ind. Acad. Sci. for 1898, p. 252, 1899) completes the list of papers dealing with this species.

In the spring of 1897, I visited Rockhouse Cave and secured a number of larvæ, which Dr. Stejneger pronounced the larvæ of *Typhlotriton*. Later Mr. E. A. Schultze informed me that he had seen this salamander in the underground passage leading to Blondi's Throne Room in Marble Cave, Stone County, Missouri. In September of 1898, I visited this cave and secured 4 adults and 3 larvæ of *Typhlotriton*. A large number of the larvæ were obtained from Rockhouse Cave a few days later. Those from the latter cave were found under loose stones and gravel in the rivulet at the mouth of the cave. They had been exposed to the light. It is scarcely supposable that those from Marble Cave had ever been subjected to light. In the caves both larvæ and adults are found under stones, the old ones in and out of the water. Occasionally one is seen lying on the bottom of a pool.

In the aquarium the larvæ creep into or under anything available; a glass tube serves as a "hiding" place. The rubber tube admitting water to the aquarium is sometimes occupied by several during a temporary cessation of the flow of water. A wire screen sloping from the bottom of the aquarium formed the most popular collecting place for the larvæ. They collected beneath this, though it offered no protection from the light. From these observations it seems probable that stereotropism rather than negative heliotropism accounts for the presence of this species in the caves, and that this reaction has been retained after the long stay of the species in caves necessary to account for the changes in its eyes.

The eyes of the larvæ when examined from the surface appear perfectly normal, but they are little used in distinguishing objects. When hungry they will strike at a stick held in the hand as they would at food. A stick lying undisturbed at the bottom of the aquarium is not molested. They strike at a worm when touched by it, or when it approaches close enough for its motion to be perceived.

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[1] By Carl H. Eigenmann and Winfield Augustus Denny. See Biol. Bull. II. p. 33, 1900.

In the larvæ up to 90 mm. long the skin passes over the eye without forming a free orbital rim and the eye does not protrude beyond the general contour of the head. In the adult from 97 mm. on, the eye forms a beadlike projection. There are in the adult distinct lids. These are closed over the eye, covering it entirely, the slit being much too small for the eye. The lower lid is free from pigment, but the upper lid, which closes over the lower, is as thickly pigmented as any other part of the body.

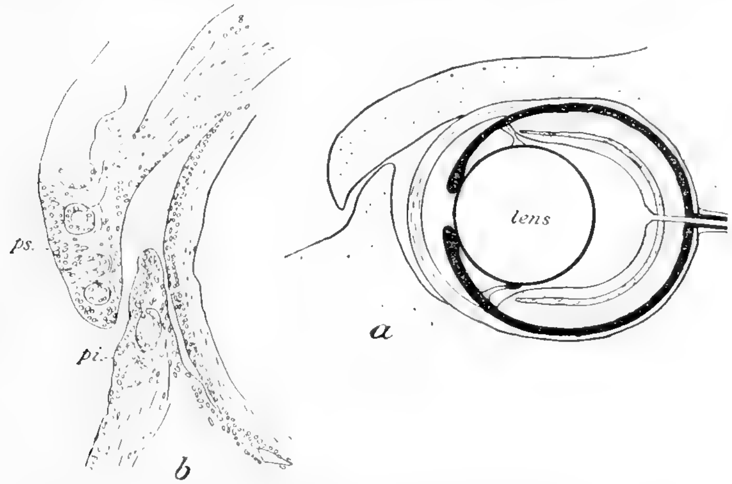

Fig. 9. (*a*) Diagrammatic Representation of Eye of *Typhlotriton* drawn to scale.
(*b*) Vertical Section through Cornea and Lids of Adult.

Stejneger says of the eyes that they are "small, only slightly raised, and covered by the continuous skin of the head, with only a shallow groove to indicate the opening between the lids, the underlying eyes visible as two ill-defined dusky spots."

In sections the lids are seen to overlap one another some distance, forming an obscure, free orbital rim. Figure 9 *b* is a median section of the lids and corneal epithelium of an eye 0.954 mm. in diameter, taken from an adult specimen 106 mm. in length. In this section the upper lid overlaps the lower lid 0.216 mm., or more than one-fifth the diameter of the eye. Passing from the median section toward the corners of the eye, the lower lid unites with the underlying tissue first. When observed from the top, the upper lid covers the eye entirely. The orbital slit is 0.17 mm. in length. The conjunctival pocket extends some distance forward and backward beyond the slit. The eye increases in size but little from the larval to the adult stage and its growth is not proportional to the growth in length of the animal. (See comparative measurements of the eyes at the close of the chapter.)

The following is a series of measurements (in millimeters) on the larvæ of *Typhlotriton*:

| Locality. | Length of specimen. | Size of pupil. | Length of eye. | Length from optic nerve to front of lens. | Vertical diameter. |
|---|---|---|---|---|---|
| Rockhouse Cave | 54 | 0.432 | 1.30 | 0.80 | |
| Rockhouse Cave | 78 | 0.640 | 1.50 | 1.20 | 1.248 |
| Marble Cave | 88 | ............ | 1.60 | ............ | 1.28 |

Sections of the adult and larva from Marble Cave were made in the usual manner. The six normal eye muscles are present in *Typhlotriton*. The m. recti form a sheath about the optic nerve in its distal part and spread out from it near the eye. In the adult the sclera is a layer of uniform thickness except in the region of the entrance of the optic nerve. It is not usually separated from the adjoining parts of the eye, but in places is retracted a short distance from the choroid coat by the action of reagents. It is for the most part fibrous, with few compressed nuclei, and varies from 18 $\mu$ to 40 $\mu$ in thickness. In the larva a narrow cartilaginous band surrounds all but the ventral wall of the eye. In a specimen 35 mm. long the width of the band is about 30 $\mu$, its thickness 16 $\mu$. In three adult specimens the sclera of only one had any traces of cartilage. In the right eye of the adult specimen 103 mm. long a cartilage about 36 $\mu$ thick, 60 $\mu$ wide, and not more than 40 $\mu$ long is found on the upper face of the eye. The absence of this cartilage in the adult has probably no connection with the degeneration of the eye. Its presence is probably a larval characteristic which disappears as the gills disappear during the metamorphosis.

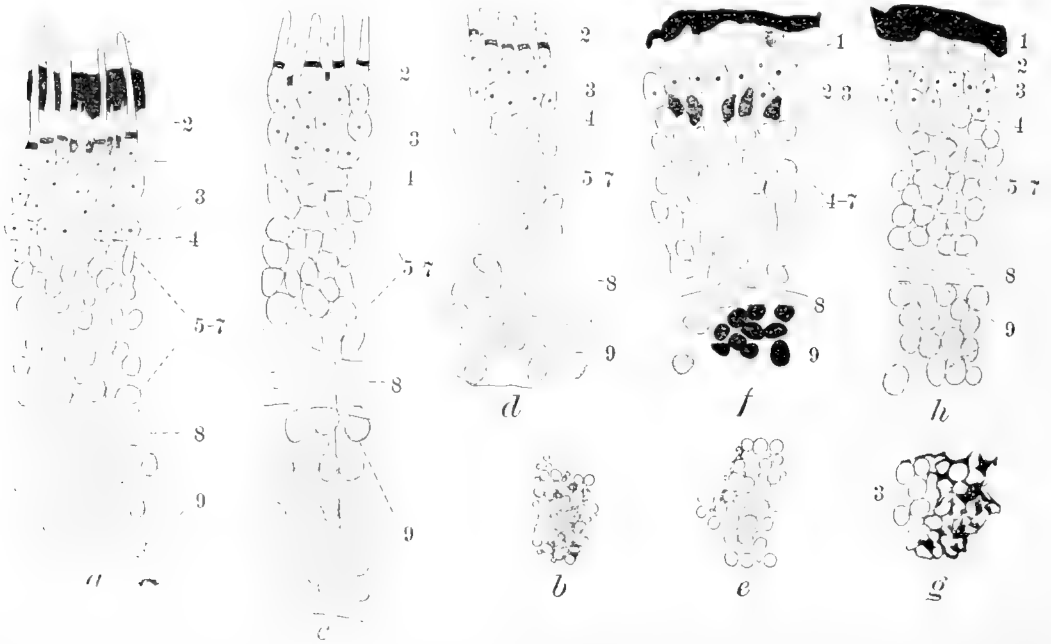

FIG. 10. (*a*) Section of Retina, exclusive of Pigment Cells, of Larva 35 mm. long. (*b*) Tangential Section through Rods and Cones about on Level with Innermost Extent of Pigment (seen on Right) showing Relative Sizes and Abundance of Rods and Cones. (*c*) Section of Retina of Larva 48 mm. long. (*d*) Section of Retina of Larva 90 mm. long. (*e*) Tangential Section showing Rods and Cones at about Inner Limit of Pigment (seen on Left). (*f*) Section of Retina of Adult 106 mm. long. (*g*) Tangential Section at about Inner Limit of Pigment. (*h*) Section of Retina of Adult 97 mm. long.

The average thickness of the cornea is 40 $\mu$. In the adult it is covered by a layer of stratified epithelium, 25 $\mu$ in thickness, consisting of three rows of cells. The cells of the inner row are columnar in shape, those of the middle row rounded, and those of the outer row very much flattened and elongated (fig. 9 *b*).

In the adult the choroid coat is usually separated from the pigment layer, but adheres closely to the sclera. In general it is thicker at the back part of the eye, and quite decidedly so at the entrance of the optic nerve. The lens is normal. Its size is given in the table on page 40.

The layers of the retina are well developed in the larva. The retina of the larva differs from that of an *Amblystoma* larva in the greater thickness of its ganglionic layer. This layer is, in the young larva of *Typhlotriton*, composed of 5 or 6 layers of cells. This thickness may in part be an artifact, since the retinæ examined are shrunken away from the pigment epithelium and the ganglionic layer is in contact with the lens. In the larva 90 mm. long this layer has been reduced to not more than 3 series of cells. Aside from the differences noted above, the eye of the larval *Typhlotriton* is apparently normal in all of its histological details. The relative thickness in the different sizes of the larvæ may be gathered from figures 10 *a* to *d* and from the comparative table at the end of this chapter.

Figures 10 *a* to *f* are drawn with the same magnification and show the relative thickness of the different layers in the retinæ of the larvæ of different sizes and of the adult. The adult retina is reduced in thickness by the absence of the rods and cones and the (partial?) atrophy of the outer reticular layer and by the thinning of the ganglionic layer. The ganglionic layer in the adult contains from two to five rows of cells. In this respect, the adult approaches the condition found in the *Amblystoma* more than the young does. The inner reticular layer is comparatively thick, that of the young being thicker than that of the adult.

In the adult the inner nuclear layer is continuous with the outer nuclear layer. (See fig. 10 *f*.)

The inner nuclear layer consists of about 7 series of cells in the smallest larva and of 4 to 7 in the largest. The cells in the preparations available can not be separated into bipolar and spongioblastic layers, nor are the horizontal cell layers distinguishable. The outer reticular layer is well differentiated, but quite thin in the larvæ, and is irregular in outline, adapting itself to the overlying nuclei which encroach on its outlines. In the adult this layer is indistinguishable by the same methods that make it conspicuous in the larva. In places there appeared an open space where the outer reticular layer should be (fig. 10 *h* 4), but none of its structure remains. It is fair to suppose that the fibers forming this layer are resorbed during the metamorphosis. This layer seems to be the very first obliterated by the processes of degeneration both ontogenetic and phylogenetic in this as in other vertebrates with a degenerating eye.

The greatest change during and shortly after metamorphosis takes place in the layer of the rods and cones. In the larva 35 mm. long, from the mouth of Rockhouse Cave, the rods reach an extreme length of 50 $\mu$. The relative sizes and number of these as compared with the much smaller cones may be gathered from figure 12.

In the larva 90 mm. long the outer segments of the rods are much shorter and stain less conspicuously than in the younger. The nuclei of the outer nuclear layer are distinctly in 2 layers, whereas in the younger specimen they are in 3 less regular layers. The cones are correspondingly fainter than in the young. It is surprising that whereas in the larva 90 mm. long we find the rods and cones well developed, they have greatly degenerated or practically disappeared in the adult only a few mm. longer. In an adult specimen 97 mm. long the rods have retained their normal shape and position, but no differentiation into inner and outer segments was detected. In longer ones most of the nuclei of the outer series have become rounded at both ends. But one cone was found in eyes of the adult over 100 mm. long. It is shown in figure 11 *a*. In an adult specimen 103 mm. long filmy rods are still evident. They appear as conical spaces above the nuclei free from pigment rather than as possessing any

FIG. 11. (*a*) Only Cone found in Eyes of Adults. (*b*, *c*) Difference in Shape of Outermost Series of Cells in Outer Nuclear Layer.

demonstrable structure. Just at the margin of the place where the pigment has been torn from the retina one of these is drawn out to a great length. The pigment in this individual extends in places down between the nuclei of the cones. This latter condition appears in a very exaggerated form in the eye of *Typhlomolge*. In tangential section this condition and the filmy rods give rise to the appearance represented in figure 10 *g*.

Distinct signs of ontogenetic degeneration are also seen in other parts of the retina. For instance, many nuclei of the inner series of the outer nuclear layer are shriveled. In some eyes the ganglionic nuclei have for the greater part lost their granular structure and show a homogeneous pasty condition, only a few cells with granular nuclei being present (fig. 10 *f*). The same is true in large part of the inner nuclei of the inner nuclear layer. This condition of the ganglionic nuclei is not entirely confined to the adult but is also found in the larva.

Some of the modifications in the shapes of the outer nuclei in the adult are shown in the figures. In figure 11 *b* the upper part of the nucleus is very much elongated. This form is of frequent occurrence. In figure 11 *c* is shown the common form where the nuclei are simple elliptical bodies, which give no evidence whatever of any processes uniting them with the other elements of the retina. The Müllerian fibers are profusely present and of very large size in both larva and adult.

In both adult and young the optic nerve enters as a single strand and passes entirely through the layers. A heavy mass of pigment is found following the optic nerve to within a short distance of the brain.

*Average Measurements of the Eyes of Typhlotriton.*

| | Length of Specimen. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 35 mm. | 48 mm. | 62 mm. | 90 mm. | 97 mm. | 103 mm. | 106 mm. |
| Vertical diameter of eye | 810 | 800 | .. | 960 | .. | 800 | 1170 |
| From front of lens to back of eye | 600 | 672 | .. | 720 | 720 | 720 | 1134 |
| Outer nuclear layer with the rods | 76 | 42 | 112 | 36 | 28 | 28 | .. |
| Outer reticular layer | 1 | 2 | .. | .. | .. | .. | .. |
| Inner nuclear layer | 76 | 72 | 80 | 50 | 48 | 72 | 72 |
| Inner reticular layer | 16 | 20 | 16 | 24 | 8 | 8 | 13 |
| Ganglionic layer | 68 | 56 | 64 | 32 | 24 | 26 | 26 |
| Pigment layer | 4 | 16 | .. | .. | 8 | 20 | 22 |
| Optic nerve | 20 | 25 | .. | .. | .. | 23 | 29 |
| Lens | 342 | 300 | .. | 500 | 432 | 430 | 504 |

### CONCLUSIONS AS TO THE EYE OF TYPHLOTRITON SPELÆUS.

(1) The eye lies just beneath the skin. The skin is but little thinner over the eye than elsewhere and shows no structural characters different from those of neighboring regions.

(2) The eye muscles have vanished.

(3) The lens has vanished and its place has in part become filled by an ingrowth of choroidal tissue containing pigment.

(4) The vitreal body is very small, if present at all. The vitreal cavity is a funnel or trumpet-shaped space.

(5) The pigmented layer of the retina is a pavement epithelium with indistinct cell boundaries and with occasional pigmented processes extending into or through the nuclear layers.

(6) Rods and cones are not found.

(7) The outer reticular layer has disappeared.

(8) The inner and outer nuclear layers form one layer of cells indistinguishable from each other.

(9) The inner reticular layer, as usual in degenerate eyes, is relatively well developed.

(10) The ganglionic layer is well represented and connected with the brain by the well-developed optic nerve.

(11) The epithelial part of the iris is at first simple, with an outer pigmented and an inner colorless layer. With age the margins of the iris become folded inward in such a way that the pigmented layer may be thrown into folds in the interior of the eye, while the colorless layer is but little affected.

(12) Pigment granules, and rarely pigmented cells, are associated in the eye with the optic nerve.

(13) The eye is more degenerate than that of the European *Proteus*. It is less degenerate than that of the North American blind fishes, *Amblyopsis*, *Typhlichthys*, and *Troglichthys*, but much more so than that of the species of *Chologaster*.

### SUMMARY IN REGARD TO TYPHLOTRITON.

(1) *Typhlotriton* is an incipient blind salamander living in the caves of southwestern Missouri.

(2) It detects its food by the sense of touch without the use of its eyes.

(3) It is stereotropic.

(4) Its eyes show the early stages in the steps of degeneration from those of salamanders living in the open to those of the degenerate *Typhlomolge* from the caves of Texas. The lids are in process of obliteration, the upper overlapping the lower so that the eye is always covered in the adult. The sclera possesses a cartilaginous band in the larval stages but not in the adult. The disappearance of the cartilage is probably an incident of the metamorphosis, not of the degeneration the eye is undergoing. The lens is normal. The retina is normal in the larva with a proportionally thicker ganglionic layer than in the related epigean forms.

(5) Marked ontogenetic degenerations take place during and shortly after the metamorphosis. (*a*) The outer reticular layer disappears. (*b*) The rods and cones lose their complexity of structure, such as differentiation into inner and outer segments, and finally are lost altogether.

# THE BLIND REPTILES.

## AMPHISBÆNA PUNCTATA.[1]

*Amphisbæna punctata* (Bell) is a blind, legless lizard which burrows in the ground. It is common in Cuba, to which place it is restricted. How deep it burrows can not be stated, but it is often turned up by the plow. The specimens obtained ranged from 103 to 245 mm. in length. The head is short, hard and pointed, and the tip of the upper jaw projects slightly beyond the tip of the lower. In shape, arrangement of the dermal plates, and color of the ventral surface of the body it closely resembles an earthworm. The dorsal surface is flesh-color with small brown spots. The tail is short and flattened dorso-ventrally. In a specimen 245 mm. in length, there were 225 annuli on the dorsal side, 202 on the ventral, and 15 on the tail. In this specimen the tail was one-thirteenth and the head one-thirty-fifth the length of the body.

### METHODS.

The lizards were put alive into formalin. They were afterwards put into alcohol. For decalcification, the heads were placed in 5 per cent nitric acid from 20 to 30 days. A shorter period did not give satisfactory results. Some heads were embedded in paraffin and others in paraffin and celloidin. In using the latter method the head was embedded in celloidin in the usual manner and hardened in chloroform. From chloroform the block was transferred to soft paraffin for 24 hours and thence to hard paraffin for 24 hours, after which it was embedded in paraffin. The best results were obtained from those embedded in paraffin and celloidin. Several methods of staining were used; iron hæmatoxylin with eosin as a counter stain gave the best results. The more modern methods of treating the retina with silver could not be applied for lack of fresh specimens. On account of the extreme toughness of the cuticle it was impossible to get complete series of sections. For comparison the eye of *Anolis carolinensis* has been examined.

### GENERAL ACCOUNT OF THE EYE.

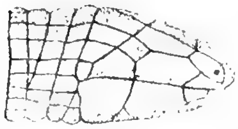

FIG. 12. Head of *Amphisbæna punctata* (Bell) showing Location and Relative Size of Eye.

The eye of *Amphisbæna* appears indistinctly as a small black spot beneath the ocular plate (fig. 12). In a specimen 225 mm. in length, the eye is 352 μ beneath the surface, 420 μ in width, and 360 μ in depth. The conjunctival sac is 116 μ in diameter. The conjunctiva is very thin over the cornea, but measures 4 μ in thickness over the anterior part of the sac.

The dermis and epidermis have the same structure over the eye as over the regions near by. This corresponds with what Eigenmann ("The Eyes of *Rhineura floridana*," 1902) found in *Rhineura*, although the eye of *Rhineura* is a much more degenerate organ than the eye of *Amphisbæna*, but to what extent the eye is degenerated from a more elaborate structure can not be stated. Few organs are stationary, and this one is probably still in process of reduction. The writer has been unable to obtain the young, and there is no means of finding out from the adult whether the eye is degenerat-

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[1] By Fernandus Payne. See Biol. Bull. XI. 60, 1906.

ing at present or not. In each specimen examined the eyes appeared in about the same state of degeneration.

The eye measures 1,224 $\mu$ in circumference and the pupil 104 $\mu$ in diameter. The uveal part of the iris on each side of the pupil measures 250 $\mu$. The pupil and iris occupy 49.3 per cent, or very nearly half, of the entire circumference.

Harder's gland is very much larger than the eye. In a cross-section through the central part of the eye, the antero-posterior diameter of the gland is approximately three times and the medio-lateral diameter four times the medio-lateral diameter of the eye. It is divided into two distinct lobes, the anterior being much smaller than the posterior. The gland completely surrounds the eye except over the anterior face. Its secretion is poured into the conjunctival sac and from thence into the mouth cavity. The large size of the gland in *Typhlops* led Duvernoy to the conclusion that its function was not connected with the eye. As its secretion, in *Amphisbæna*, is poured into the conjunctival sac and thence into the mouth cavity, its function must have been, primarily at least, connected with the eye. No eye muscles are present in *Amphisbæna*. The eye is directed outward and forward and makes an angle of about 60° with a line drawn tangent to the dermal plate which covers it.

Whether the eye is still used as a sense-organ is not certain, but since the parts are so well developed and the eye is not buried very deeply beneath the surface, it is probable that it is at least susceptible to light.

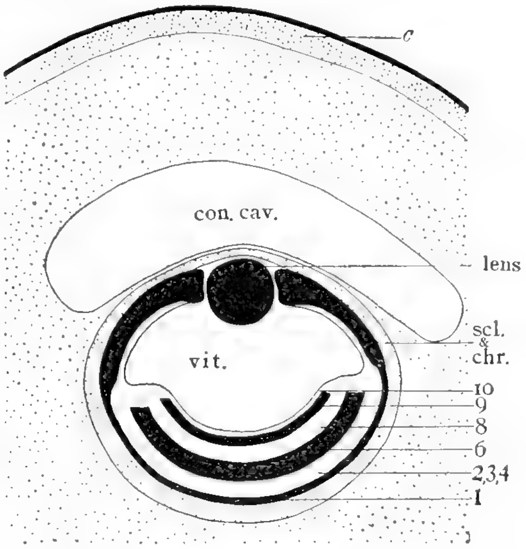

Fig. 13. Diagram of Eye, showing Parts in their Relation and Distance of Eye beneath Surface.
1, pigment layer; 2, cones; 3, outer nuclear layer; 4, outer reticular layer; 6, inner nuclear layer; 8. inner reticular layer; 9, ganglion-cell layer; 10, fiber layer; *lens*, lens; *scl.*, sclerotic; *chr.*, choroid; *cor.*, cornea; *scl. c.*, scleral cartilage; *n. op.*, optic nerve; *vit.* vitreous cavity; *con. cav.*, conjunctival cavity; *C.*, outer covering of eye; *M.*, Müllerian fiber; *L.*, membrana limitans externa.

*The Sclera.* — The sclera (*scl.*, fig. 14) has apparently undergone no degeneration whatever. It compares favorably with that of *Anolis*. In fact, there is but little difference in its structure in the two eyes. At the proximal part of the eye, the sclera measures 12 $\mu$ in thickness, while at the same place in *Anolis* it measures 15 $\mu$. It is continuous over the front of the lens as the cornea, which together with the thin wall of the conjunctival sac at this place measures 7 $\mu$. Scleral cartilages extend from about the middle of the eye back almost to the optic nerve. On each side of the sclera, and forming a part of it, are thin irregular layers of pigment in patches.

## MINUTE ANATOMY OF THE EYE

*The Choroid.* — If the blood-vessels in the choroid still persist, the preparations do not show them. All that can be seen is a number of densely pigmented cells, around and between which are filaments of connective tissue (*chr.*, fig. 14). At the entrance of the optic nerve, this layer measures 8 $\mu$ in thickness, but gradually becomes less forward and vanishes entirely a short distance back of the enlarged end of the pigment layer. The pecten, present in *Anolis*, is not seen in *Amphisbæna*.

*The Lens.* — The lens has retained its natural shape and position (*lens*, fig. 14). It is almost spherical and measures 80 $\mu$ in diameter. In most of the sections an outer layer of cells extends around the anterior surface of the lens. The interior in nearly every case stained as a structureless mass, but in a few sections it appeared to be made up of large irregularly shaped cells with small nuclei. If any fibrous cells still persisted, they did not show. No capsule is present.

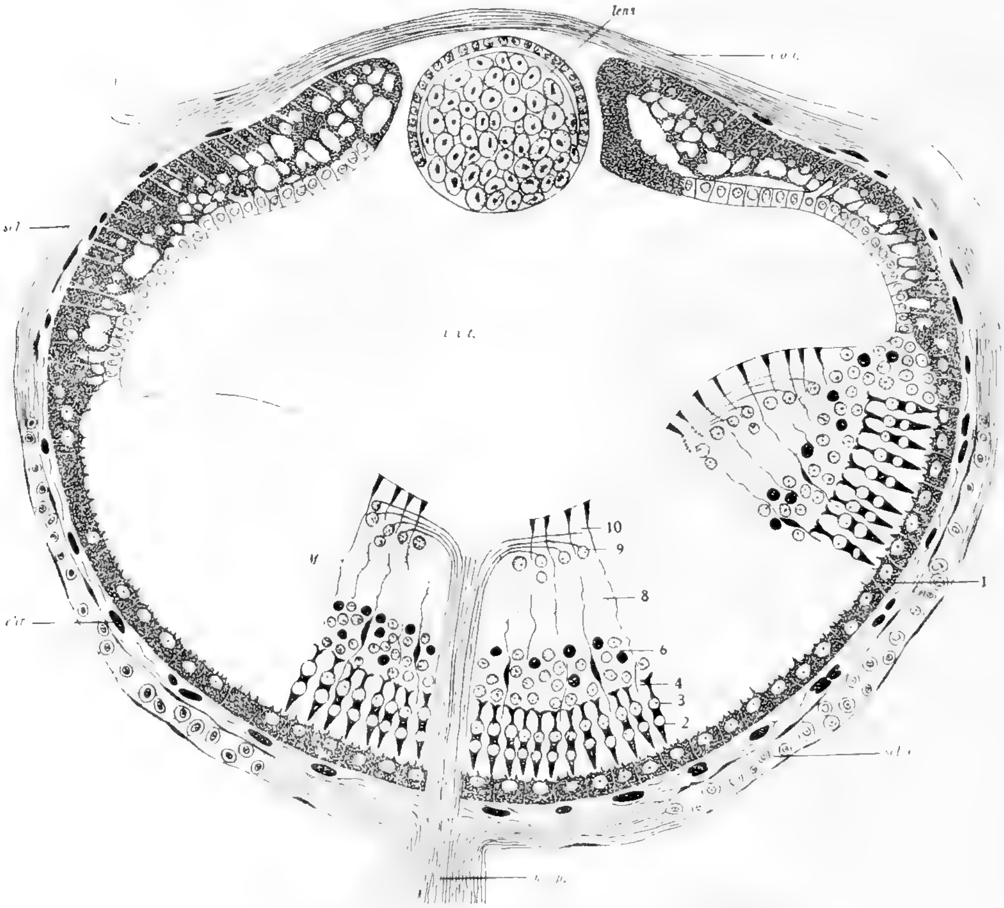

Fig. 14. Horizontal Section of Eye showing Different Parts. Retina diagrammatic. For explanation of letters see fig. 13.

*The Vitreous Body.* — The vitreous body (*vit.*, fig. 14) occupies the greater part of the eyeball and has certainly undergone but little change. The aqueous cavity has entirely disappeared.

*The Iris.* — Only the uveal part of the iris remains. It is continuous with the pigment epithelium of the retina and has the same structure. In the thickest part it measures 68 $\mu$. The cells are similar to those of the pigment layer, except that their radial diameter is much greater. The ciliary processes are no longer present.

*The Optic Nerve.* — The optic nerve can be traced from the eye, through and along the side of Harder's gland. While the nerve could be traced no farther on account of an incomplete series of sections, there is no doubt that the connection with the brain still exists. The nerve fibers enter the eye in a compact mass, pass through the layers of the retina until they reach the nerve fiber layer, where they spread out and connect with the nerve cells of the ganglionic layer in the usual manner.

*The Retina.* — While the retina has undergone considerable change, all of the layers are still present (fig. 15 *a*). It measures 78 $\mu$ in thickness. In *Anolis* about half-way between the anterior and posterior parts of the eye it is 179 $\mu$ in thickness. If the macula lutea is still present, the preparations do not show it.

*The Pigment Layer.* — The pigment layer (1, fig. 14), which bounds the retina externally, consists of a single stratum of rectangular cells separated by a small amount of clear intercellular substance. These cells have large oval nuclei free from pigment, almost transparent and with small nucleoli. At the back part of the eye, where the pigment layer measures 8 $\mu$, the transverse diameter of the cells is greater than the radial diameter, but toward the anterior part, where the layer becomes thicker, the radial diameter becomes much the greater. The greatest thickness of this layer is near the lens, where it measures 68 $\mu$. The outer surface of the pigment cells — that which lies next to the choroid — is smooth and slightly convex. The inner surface, on the other hand, is very irregular. The cells at this place are very densely laden with pigment and prolonged into filamentous processes which extend between and amongst the cones. In fact, the cones may be said to be embedded in the pigment cells. This layer differs but little from that of *Anolis*, except at the anterior part of the eye, where it becomes much thicker.

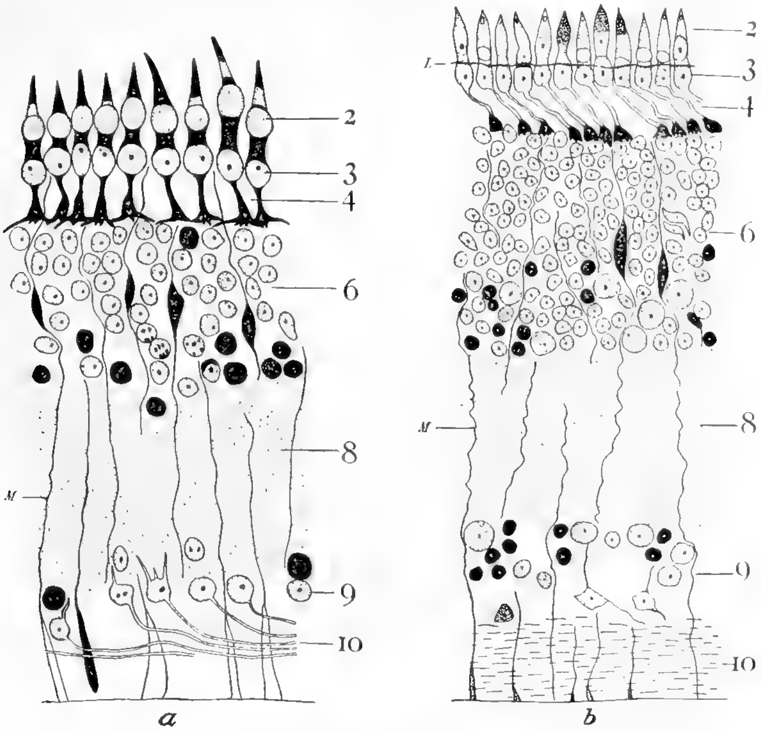

FIG. 15. (*a*) Horizontal Section of Retina of *Amphisbœna punctata*, showing Different Layers. (*b*) Horizontal Section of Retina of *Anolis*.

*The Cones.* — No rods are present. The cones (2, fig. 15 *a*) consist of an upper and a basal part. The basal part is elliptical in shape and stains uniformly throughout, while the outer portion is longer and somewhat triangular in shape, with the smaller side of the triangle resting on the inner elliptical part. This layer measures 10 $\mu$ in depth, while the same layer in *Anolis* measures 13 $\mu$.

*The Outer Nuclear Layer.* — This layer is made up of a single stratum of nuclei with small dark nucleoli (3, fig. 15 *a*). Some of these nuclei are almost spherical, while others are oval in shape. They are connected with the cones by broad processes which stain darkly. These processes may be very short, in which case the cone comes in close proximity to the nucleus; or they may be drawn out into filaments as long as or longer than the nuclei themselves. From the inner part of the nuclei extend processes which broaden toward the base and send numerous ramifications into the inner stratum of the outer reticular layer. There is a striking difference here between this eye and the normal one. The processes from the base of the nuclei pass straight through the outer reticular layer, while in certain sections of the normal eye they pass through at an angle of about 45° (3, fig. 15 *b*).

*The Outer Reticular Layer.*—The outer reticular layer (4, fig. 15 *a*) is penetrated by the processes from the nuclei of the outer nuclear layer and by a few Müllerian fibers. If processes from horizontal cells are present, they were not brought out by the method of staining which was used. Again, there is but little difference in the thickness of this layer in the two eyes, as it measures 6 $\mu$ in *Amphisbæna* and 7 $\mu$ in *Anolis.*

*The Inner Nuclear Layer.* — The inner nuclear layer is a compact mass of somewhat irregular spherical nuclei and is 24 $\mu$ in thickness (6, fig. 15 *a*). The corresponding layer in *Anolis* is 59 $\mu$. Spongioblast and bipolar cells can not be differentiated from each other. All of the nuclei appear to be very much alike, except the nucleated enlargements of the fibers of Müller, which have no definite shape and which stain very densely. However, some nuclei, more especially those of the inner stratum, stain a very deep black color, and show no structure whatever. Parts of certain other nuclei stain densely, while the rest retains its original identity. Some of the nuclei have 4 to 6 nucleoli. In *Anolis* two other kinds of nuclei appear. A few flattened horizontal nuclei can be seen near the middle of the layer and in the inner stratum are a number of large spherical nuclei. Penetrating this layer are many fibers of Müller. Each fiber as it passes through is characterized by a nucleated enlargement.

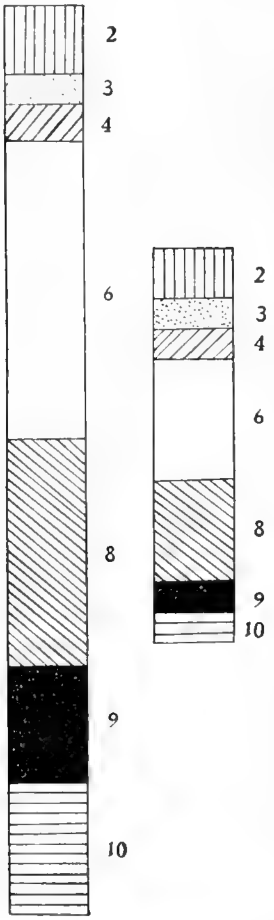

Fig. 16. Diagram showing Comparative Measurements of Retina in Eyes of *Anolis* and *Amphisbæna*.

*The Inner Reticular Layer.* — The inner reticular layer measures 20 $\mu$ in thickness as against 45 $\mu$ in *Anolis* (8, figs. 15 *a* and 15 *b*). The method of staining brought out no definite structures. The fibers of Müller pass through it as fine vertical filaments. Occasionally there is a nucleus from the nuclear layer or from the ganglionic layer which lies embedded in the edge of this layer.

*The Ganglionic Layer.* — The ganglionic layer (9, fig. 15 *a*) consists of a single layer of nuclei 6 μ in diameter, with now and then another nucleus above or below the single layer. From the outer side of these nuclei, fibers which run out and penetrate the inner reticular layer can be traced for a short distance. On the opposite side are also fibers which continue as fibers of the nerve fiber layer. In *Anolis* this layer measures 23 μ and is made up of loosely connected nuclei, some of which are large and spherical, others are smaller and irregular, while still others stain very densely.

*The Nerve-fiber Layer.* — The nerve-fiber layer is 6 μ in depth, while in *Anolis* it is 26 μ.

*The Fibers of Müller.* — The Müllerian fibers can be traced from the membrana limitans interna to the outer nuclear layer. They commence at the inner surface of the retina by a broad conical foot which extends into the ganglionic layer. Through the inner reticular layer the fibers pass as fine filaments, but in the inner nuclear layer each fiber is characterized by an irregularly shaped nucleus, which stains densely and shows no structure. The membrana limitans externa is not visible. These fibers differ but little from those in *Anolis*, except that those in *Anolis* can be traced to the membrana limitans externa, which is plainly visible.

## RHINEURA FLORIDANA.[1]

### HABITS OF RHINEURA.

*Rhineura floridana* Baird is a legless, burrowing, blind Amphisbænian lizard. It is abundant in some parts of Florida. The largest individual secured by the author measured 340 mm. The tail is very short, flattened dorso-ventrally, and the upper surface of its distal half is strongly rugose. Each of the transverse rings is here, with numerous tubercles. The mouth is small; the tip of the lower jaw is some distance behind the tip of the upper jaw. In shape, color, and arrangement of its dermal plates it strikingly resembles an earthworm. This resemblance is heightened by its vermiform progression through the rhythmic movements of its annular plates. Its forward and backward locomotion in its burrows is entirely due to this vermiform movement. It burrows rapidly, and for this its small, hard, conical head is well adapted. The point of the snout is turned down and the head then thrust upward in a rooting fashion. An individual will readily disappear in from half a minute to two minutes. By placing it in a glass vessel partly filled with earth its burrowing can readily be seen from below. If placed on a bare surface, it for a time will wriggle actively from side to side, snake fashion, but without much effect as far as locomotion is concerned. The tail, under such circumstances, is dragged behind, as if it had no vital connection with the head. Rarely there is a suggestion of a bracing with the tip of the tail against the floor. In one minute an individual moved 250 mm. In an attempt at rooting, after the snout had become wedged under the edge of an immovable object, the whole body to the tip of the tail was repeatedly lifted off the floor.

*Rhineura* is, as far as known, one of the two blind vertebrates that have been found in the fossil state. Baur described a species of *Rhineura* (*R. hatcherii*) and another Amphisbænian (*Hypsorhina antigua*) from the Miocene beds of South Dakota. Baur says nothing concerning the dermal plates, so that nothing is definitely known about the eyes of this fossil *Rhineura*. Since all the genera of the family Amphisbænidæ have rudimentary eyes, the eyes were very probably degenerate before the genera became separated. It seems quite certain that any fossil members of an existing genus all of whose living species have degenerate eyes, must have had eyes that were to a greater or less extent degenerate. The time suggested by this find of Baur during which the eyes of *Rhineura* have been degenerating is surprisingly long, extending as it does through about 5 to 10 per cent of the formation of sedimentary rocks.

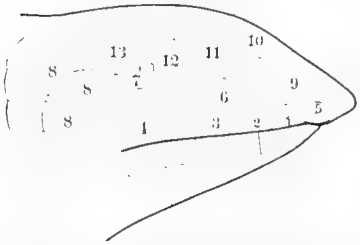

FIG. 17. Side View of Head of *Rhineura* showing Surface Plates and Position of Eye in Relation to them.

*Rhineura* is a burrowing animal, and blind animals which burrow in the ground are not found in naturally made caves. The latter are largely populated by species that tend to hide in crevices or natural cavities under rocks. It would seem from this that the cave fauna was incipient before the existence of caves, and that the latter were colonized as soon as they were large enough to admit their present inhabitants.

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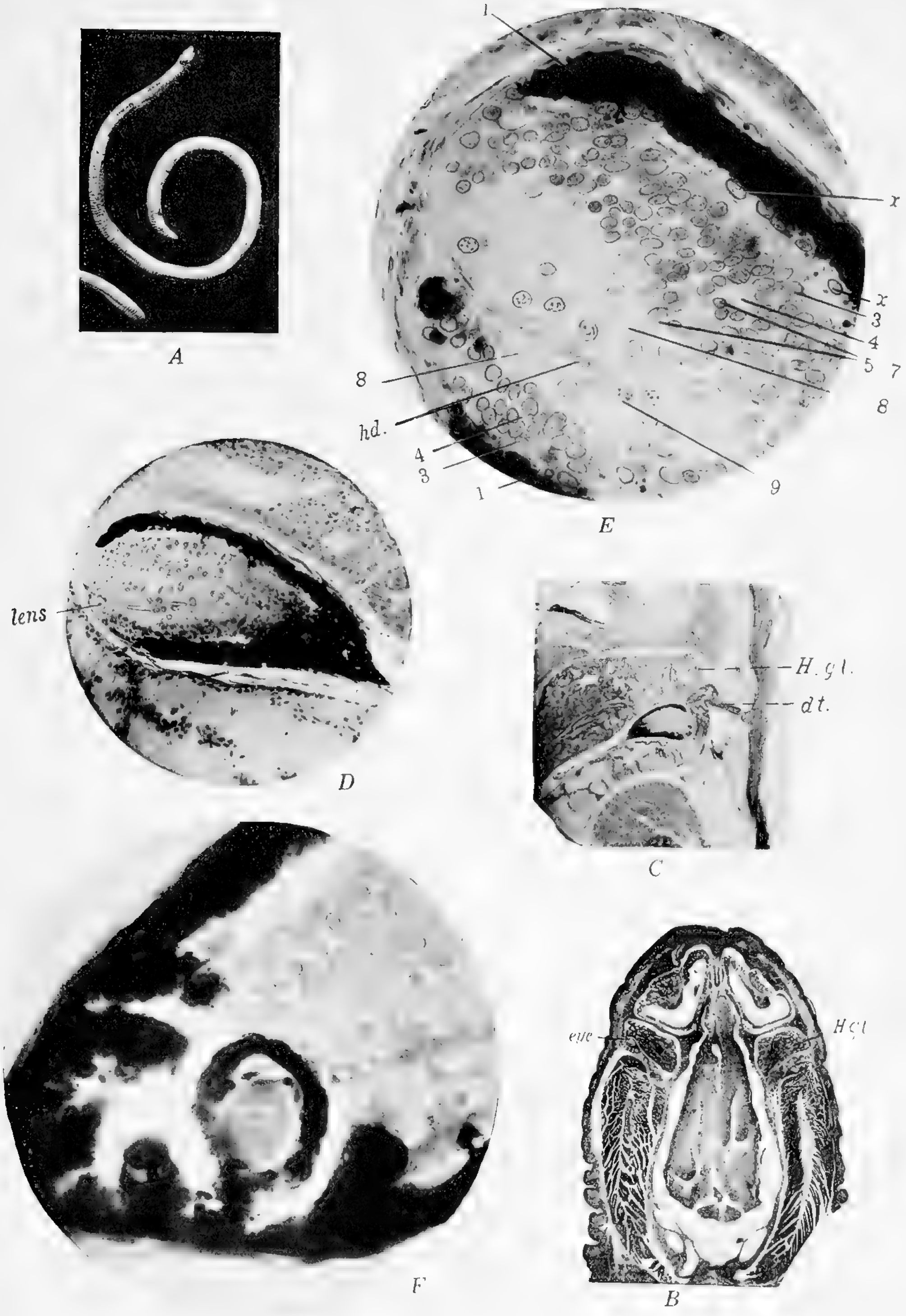
[1] See Proc. Wash. Acad. Sci., IV. p. 533, 1902.



Rhineura floridana.

A. Side and dorsal views of tail.

B. Horizontal section of head, showing Harder's gland and position of eye.

C. Horizontal section through right eye, showing solid strand of cells, extending from Harder's gland to near epidermis.

D. Horizontal section of left eye, showing extent of pigmentation and lens.

E. Distal part of another section of same eye, showing different layers of retina at their highest development. 2 mm. objective.

F. Proximal part of another eye, showing cyst represented diagrammatically in text-fig. 19c. 2 mm. objective.

## GENERAL ACCOUNT OF THE EYE OF RHINEURA.

The eye of *Rhineura floridana* is not visible externally, nor is there any indication where it formerly came to the surface. The side of the head is continuously covered with plates. There are 4 labials (1, 2, 3, and 4, of fig. 17), the posterior of which is comparatively large. Above the labials from in front backward lie a single nasal (5), a single loreal (6), a single preorbital (7), and a group of temporals (8). Above this series of plates lie a supranasal (9), joined to its fellow of the other side, a prefrontal (11), and 2 supraciliaries (12, 13). In heads cleared with xylol the black eye can be seen to lie underneath the angle between the 2 supraciliaries and the preorbital.

The dermis and epidermis over the eye are not different from these structures over neighboring regions except that in one instance (plate 3 C, *dt*) a solid column of cells 32 μ thick extends from Harder's gland to near the epidermis, without, however, fusing with the latter. Fisher found that in *Trogonophis* the epidermis is reduced to half its thickness and free from pigment over the eye. In *Amphisbæna strauchi* and *A. darwini* the skin is not thinner and the pigment is little or not at all less over the eye. A conjunctival sac has been described for various Amphisbænians. No such structure is present in *Rhineura*.

Harder's gland (plate 3, figs. B, C, *H.gl.*) is out of all proportion to the size of the eye. In a horizontal section it measures about 4 times as long as the eye (medio-laterally) and 3 times as wide (antero-posteriorly). Duvernoy found that in *Typhlops* Harder's gland is 10 times as great as the eye. It is divided into 2 distinct lobes, that over the anterior face of the eye is histologically quite different from that over the posterior face. In vertical section the gland is seen to entirely surround the eye except sometimes at its lower posterior quarter. The large size of Harder's gland has given rise (Duvernoy) to the conclusion that its function is not connected with the eye. Its secretion is poured directly into the tear duct and through it into the nasal cavity.

The distance of the eye beneath the outer surface of the epidermis measures between 320 and 560 μ in specimens between 280 and 310 mm. long. It is surrounded by 2 layers of connective tissue. These are thin over the distal half of the eye. Over the proximal narrow end of the eye they become thick; and since they are prolonged beyond the eye, stain a different tint, and readily become separated, they are easily distinguishable. They probably represent the sclera and choroid. If so, the choroid is practically free from pigment except possibly in rare instances where a few pigment granules were detected in cells closely applied to the eye. There is no indication of any differentiation into a cornea or capsule of any sort. The fibrous sheaths are at the proximal end drawn out into a cone. A supposed scleral cartilage has been found in one individual. Here a bar about 20 μ thick extends from over the center of the distal face of the eye for 160 μ around its posterior face. It stains and has the structure of bone rather than of cartilage. No traces of any muscles have been found connected with the eye.

The eye is directed outward and forward. Its axis is horizontal and makes an angle of about 60° with the sagittal plane of the body. It does not occupy a definitely fixed position on its axis, for in the eye of one side the choroid fissure was found directed caudad, in the other eye ventrad. It is irregularly pear-shaped, with its anterior face convex, its posterior face flat or even concave. The eyes in 3 specimens give the following measurements in microns:

*Measurements (in microns) of eyes of Rhineura.*

| LENGTH OF SPECIMEN. | MEDIO-LATERAL DIAMETER. |  | ANTERO-POSTERIOR DIAMETER. |  | DISTANCE FROM SURFACE. |  |
|---|---|---|---|---|---|---|
|  | Left eye. | Right eye. | Left eye. | Right eye. | Left eye. | Right eye. |
| mm. |  |  |  |  |  |  |
| 275 | 320 | 320 | 128 | 176 | 480 | 336 |
| 280 | 312 | 298 | 160 | 181 | 320 | 368 |
| 310 | 320 | 320 | 216 | 176 | 560 | 560 |

## MINUTE ANATOMY OF THE EYE OF RHINEURA.

All the structures vary greatly in different eyes so that the terms "sometimes," "usually," "frequently," etc., have to be used much more than is desirable. This can not be avoided unless each eye is given a distinct description.

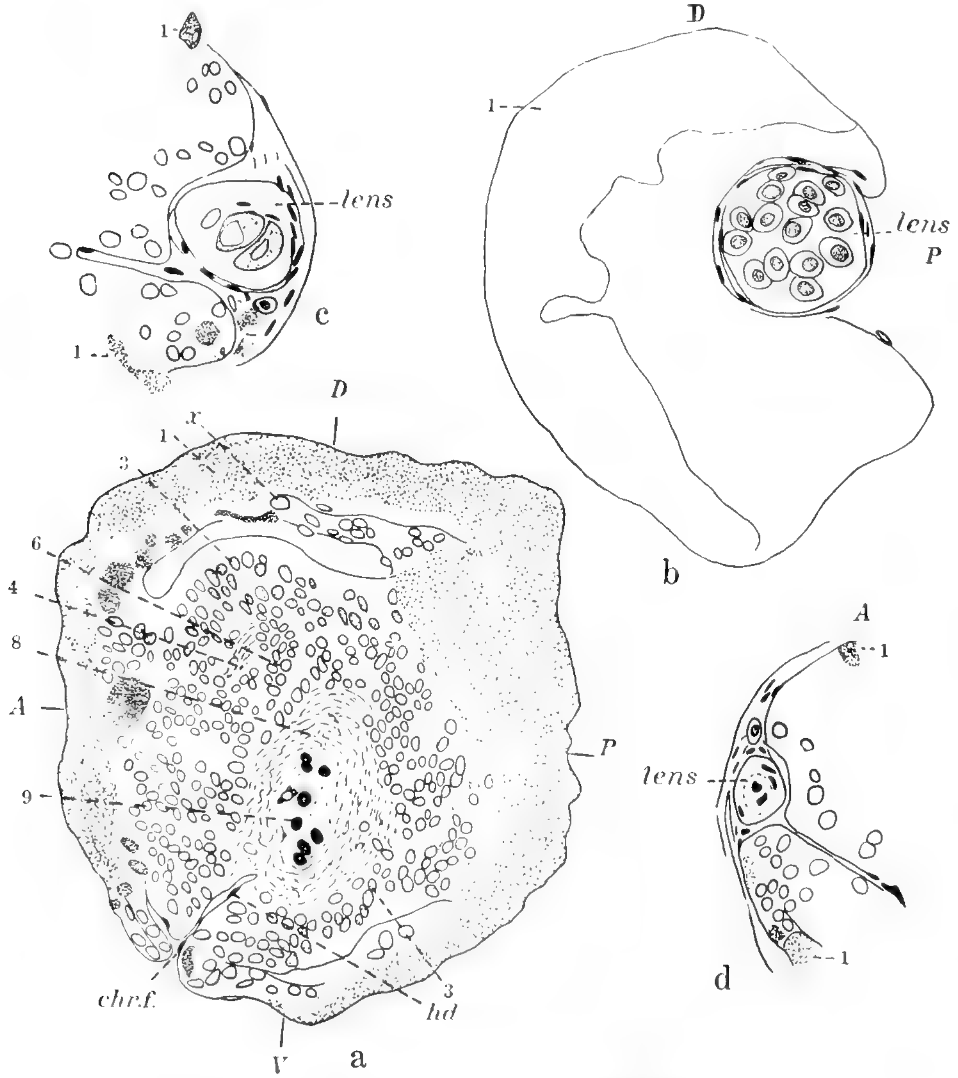


FIG. 18. (*a*) Sagittal Section through Middle of Left Eye of *Rhineura*, about 300 mm. long.
(*b*) Vertical Section through Distal Part of Eye of *Rhineura*, showing Lens with Capsule.
(*c*) Lens of Right Eye of Individual, 275 mm. long. Horizontal Section.
(*d*) Left Lens of Same Individual.

(*a*) *The Iris.* — In the structure of the irideal region the eye of this species is unique among the degenerate vertebrate eyes so far described. In all other eyes, with the possible exception of *Troglichthys*, elements of an iris are distinctly recognizable. In *Rhineura* the fold of double epithelium between the pigmented and unpigmented part of the retina whose margin is the margin of the pupil has been obliterated and the pupillary edge forms the extreme outer edge of the blunt end of the pear (*p*, fig. 19 *a*). The pigmented layer of the retina in other words merges directly into the unpigmented layers of the retina. The entire thickness of the retina is thus exposed at the distal face of the eye.

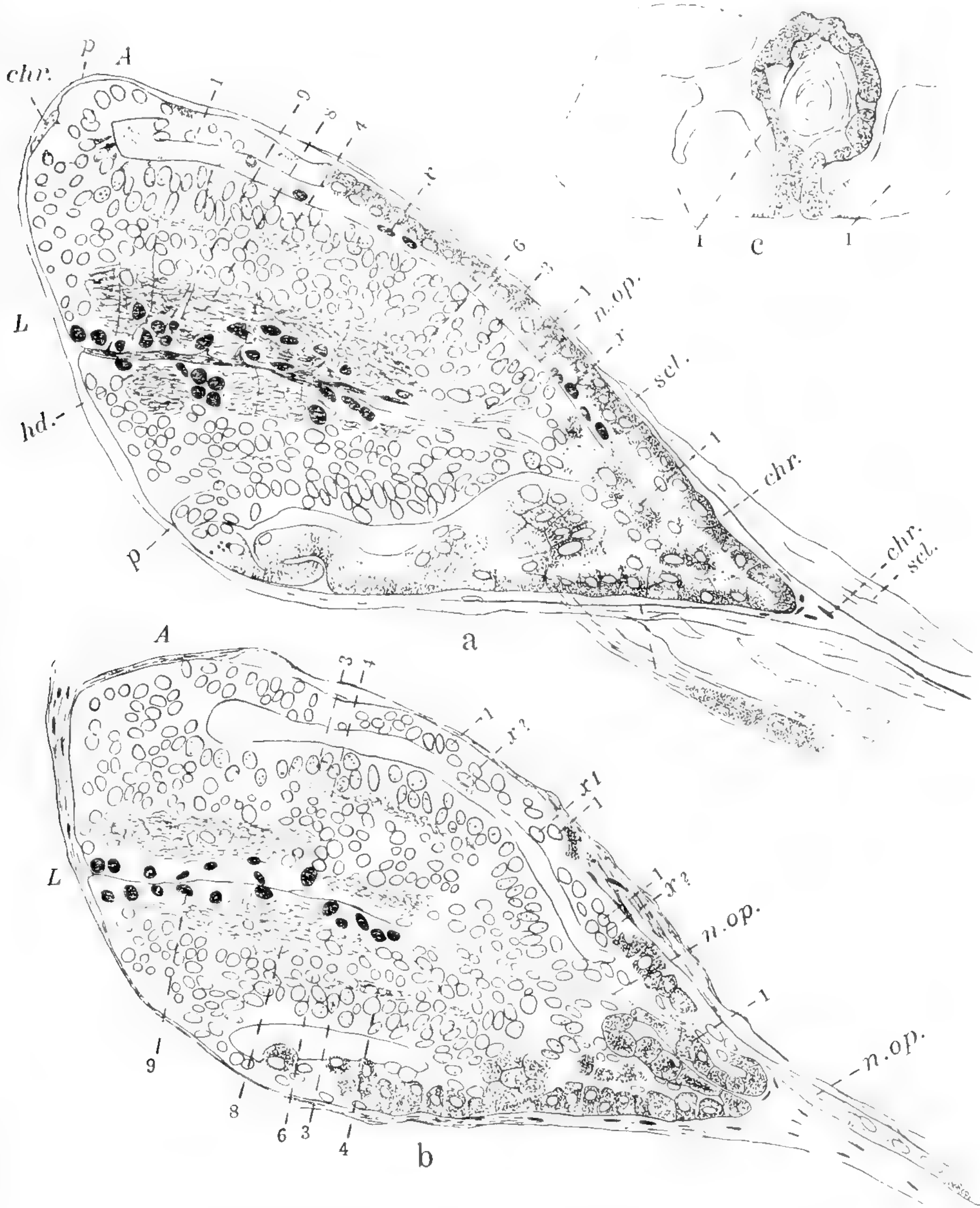


FIG. 19. (*a*) Horizontal Section of Left Eye of Specimen, 280 mm. long.
(*b*) Another Section through Same Eye, showing Exit of Optic Nerve, the Pigmentless Condition over Anterior Face of Eye, and Invaginated Pigment at End of Pear.
(*c*) Outline of Pigment in Proximal End of Right Eye of same Individual, showing Invagination of Pigment to form a Cyst.

(*b*) *The Vitreous Body.* — The vitreous cavity is represented by a vertical slit extending from the axis of the eye downward to the edge. The choroid fissure (fig. 18, *chr.f.*) thus remains permanently open in so far as the edges of the opposite sides of the fissure are not united. A space a few microns wide was found in one eye. In other cases there is no real cavity and no vitreous body. The hyaloid membrane (fig. 18 and fig. 19, *hd*) is represented by a few cells with elongated nuclei. Blood-vessels were not found in it.[1]

(*c*) *The Lens.* — In two specimens no traces of a lens were found, but in two other specimens a lens was present. There being no pupil and no vitreous cavity, the lens is situated in a little depression in the distal face of the retina (figs. 18 *b*, *c*, *d*). The lenses differ greatly from each other. In the better developed instances (fig. 18 *b*) it is composed of a spherical mass of cells. The nuclei are granular and are surrounded by a hyaline cell body. These little capsules are closely packed in a slightly darker matrix. The whole lens is surrounded by a fibrous capsule containing elongated nuclei. Both eyes of one individual are provided with lenses as described. In another individual the 2 lenses differ materially not only from those described, but from each other both in structure and size. The left lens consists of a lenticular nodule containing about 6 dense nuclei (fig. 18 *d*). On the right side (fig. 18 *c*) the lens is much larger. It consists of 2 large nucleated capsules surrounded by a matrix containing a few dense elongated nuclei similar to those of the capsule surrounding it (figs. 18 *b*, *c*, and *d*, are drawn to the same scale). The difference exclusive of size between the 2 lenses *c* and *d* and the lens represented in figure 18 *b*, may be due to differences in the method of preparation.

(*d*) *The Retina.* — The numbers in the following paragraphs are not consecutive, but are those used to designate the corresponding layers in the figures.

(1) *The pigment epithelium* forms a complete outer layer of the eye exclusive of its distal face and a narrow strip along the choroid fissure. The extent to which this epithelium is pigmented differs greatly in different eyes. A region along either side of the choroid fissure is free from pigment, occasionally parts of the anterior face of the eye are free from pigment (fig. 19 *b*), and very frequently the cells of this layer around the distal margin of the eye are free from pigment. Over the anterior face of the eye this layer is usually composed of a regular layer of cells whether these are free from pigment or not (figs. 19 *a*, *b*). On the posterior face the series of cells is not nearly so regular. The pigmented epithelium is here invaginated and folded upon itself in various ways. The infoldings are sometimes solid masses of pigment cells, but sometimes they form hollow spheres which contain a mass of concentrically arranged unpigmented material, probably of choroidal origin (plate 3, F, and text-fig. 19 *c*.) What the significance of these cysts may be I can not conjecture. Indications of similar structures were seen in the eyes of *Amblyopsis*.

The narrow stalk of the pear-shaped eye is usually filled with an irregular jumble of pigment cells. In favorable sections it is seen that these are also the result of an invagination of the pigment epithelium from the pointed end of the eye (fig. 19 *b*). The pigment epithelium has not been reduced at the same rate as the rest of the retina; as a consequence it is infolded in various ways. Small

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[1] The figures were drawn with camera lucida from sections mounted in balsam; 2 mm. objective and 4 eyepiece. The horizontal sections were made from above down and are so drawn that the anterior face of the figure is toward the top of the page.

pigment cells are sometimes found in the inner layers of the retina among the ganglionic cells and along the optic nerve within the eye. Pigment cells were also found in the eyes of *Typhlomolge* (figs. 5 *a* and 7 *a*, *z*). There are rarely any pigment cells over the distal face of the eye.

(1 *a*) *X*, *nuclei.* — In the eyes of *Rhineura*, *Typhlichthys*, and *Troglichthys* a few cells with elongated, tangentially placed nuclei are present between the pigmented epithelium and the outer nuclear layer. They are distinctly outside of the outer limiting membrane (figs. 19 *a*, *b*; plate 3, fig. E, *x*). The origin of these nuclei is difficult to explain. Possibly they are derived from the pigment epithelium which in some of the unpigmented regions (fig. 19 *b*, *x*) is more than one layer deep. If the outer layer should become pigmented, the inner nuclei, if they remained unpigmented, might give rise to these longitudinal cells.

(2) *Rods and cones* are not present. There is in some cases a distinct space between the pigment epithelium and the outer nuclear layer. This space when present is partially filled with filmy, hazy structures, but nothing suggesting definitely either a rod or cone was detected (fig. 19 *a* and plate 3, fig. E).

(3) *The outer nuclear layer* consists of about 2 series of elliptical nuclei. They form a compact and distinct layer a few microns from the outer limiting membrane (figs. 18 *a*, 19 *a*, *b*, and plate 3, fig. E).

(4) *The outer reticular* layer is represented by a series of distinct but irregular gaps between the outer nuclei and the inner nuclei. Horizontal cells are not present (figs. 19 *a*, *b*, *c*, and plate 3, fig. E).

(6) *The inner nuclei* are smaller, rounded, and less granular than the outer nuclei. They do not form as compact a layer as the outer nuclei. It is impossible to distinguish between bipolar and spongioblastic cells (6 in figs. 18, 19, and plate 3).

(8) *The inner reticular layers*, as is usual in degenerate eyes, are well developed in the eyes of *Rhineura*. They are frequently crossed by Müllerian fibers.

(9) *The ganglionic layer* is represented by a number of nuclei loosely grouped about the vitreous slit. The individual nuclei are distinctly larger than those of the inner nuclear layer and less oval than those of the outer nuclear layer (9 in the figures).

(10) A distinct *optic fiber layer* is not present and the *optic nerve* is nowhere within the eye a compact strand of fibers. A loose flocculent strand of fibers passes through the proximal part of the retina. Its path through the pigmented layer is difficult to trace. Beyond the eye the optic nerve can be followed by means of the fibrous sheaths and pigment cells associated with it rather than by the presence of any fibers with a distinctly nervous structure. The optic nerve leaves the eye, not at the proximal end or the narrow end of the pear, but anterior to the pigment mass in the narrow part of the pear (fig. 19 *b*, *n.op.*).

## TYPHLOPS LUMBRICALIS.[1]

*Typhlops lumbricalis* (Linnæus), a blind snake, is generally distributed in the West Indies and Guiana. The specimens examined were obtained in the neighborhood of Cañas, Province Pinar del Rio, Cuba. It is a burrowing form that lives just beneath the surface, being thrown out even by the plow.

The snakes were first placed in formalin and after a few days were transferred to alcohol. Only one young specimen was obtained, and it was preserved in Zenker's fluid. For decalcification, the heads of some were placed for at least 3 days in 10 per cent nitric acid and others in Perenyi's fluid from 1 to 2 weeks. One series was stained by the iron hæmatoxylin process, the others with hæmalum and eosin. It was very difficult to obtain satisfactory sections and especially complete series from the specimens, since no method was found to decalcify properly and to get the integument in condition for sectioning.

The lengths of the individuals examined were 10, 20, 21, and 21.5 cm. The color is brown above, on the ventral side it is yellowish white. The body is covered with scales of uniform size, while those of the head are somewhat larger. The surface of the entire body is very smooth and shining and rather hard. The tail, which is about one-twentieth of the body's length, ends in a short, sharp spine. The mouth is small and lies on the ventral side some distance back from the tip of the snout.

### GENERAL ACCOUNT OF EYES IN SNAKE.

Snakes differ from other animals in having the edges of the two eyelids entirely grown together. A disk-shaped, conjunctival sac is thus formed and the layers over the eye between this sac and the exterior form the "brille." Six weakly developed muscles are present. The 4 straight ones arise in the neighborhood of the foramen opticus, while the 2 oblique ones arise from the surface of the prefrontal which is turned toward the eye socket.

Closely connected with the eye is Harder's gland, whose function is doubtful. Leading from this gland is a single duct, which either empties into the duct from Jacobson's gland or directly into the mouth cavity. The secretions of the gland are thus not functional in connection with the eye.

The sclera consists of closely woven fibers. Ciliary muscles are not found, but next to the iris is a great bundle of equatorial muscle fibers running obliquely, which seem to be a continuation of the iris musculature. The ciliary processes are weakly developed.

The retina consists of the usual layers. The nerve-fiber layer is very thin (0.003 to 0.004 mm.).

The ganglion-cell layer consists of a single, rarely two, layer of small cells, each with a very large nucleus (0.012 to 0.013 mm.). The inner reticular layer contains, at apparently regular intervals, elongated, oval nuclei (0.042 to 0.045 mm.). The inner nuclear layer consists of two kinds of cells (0.052 to 0.054 mm.). The outer reticular layer is very thin (0.004 to 0.005 mm.).

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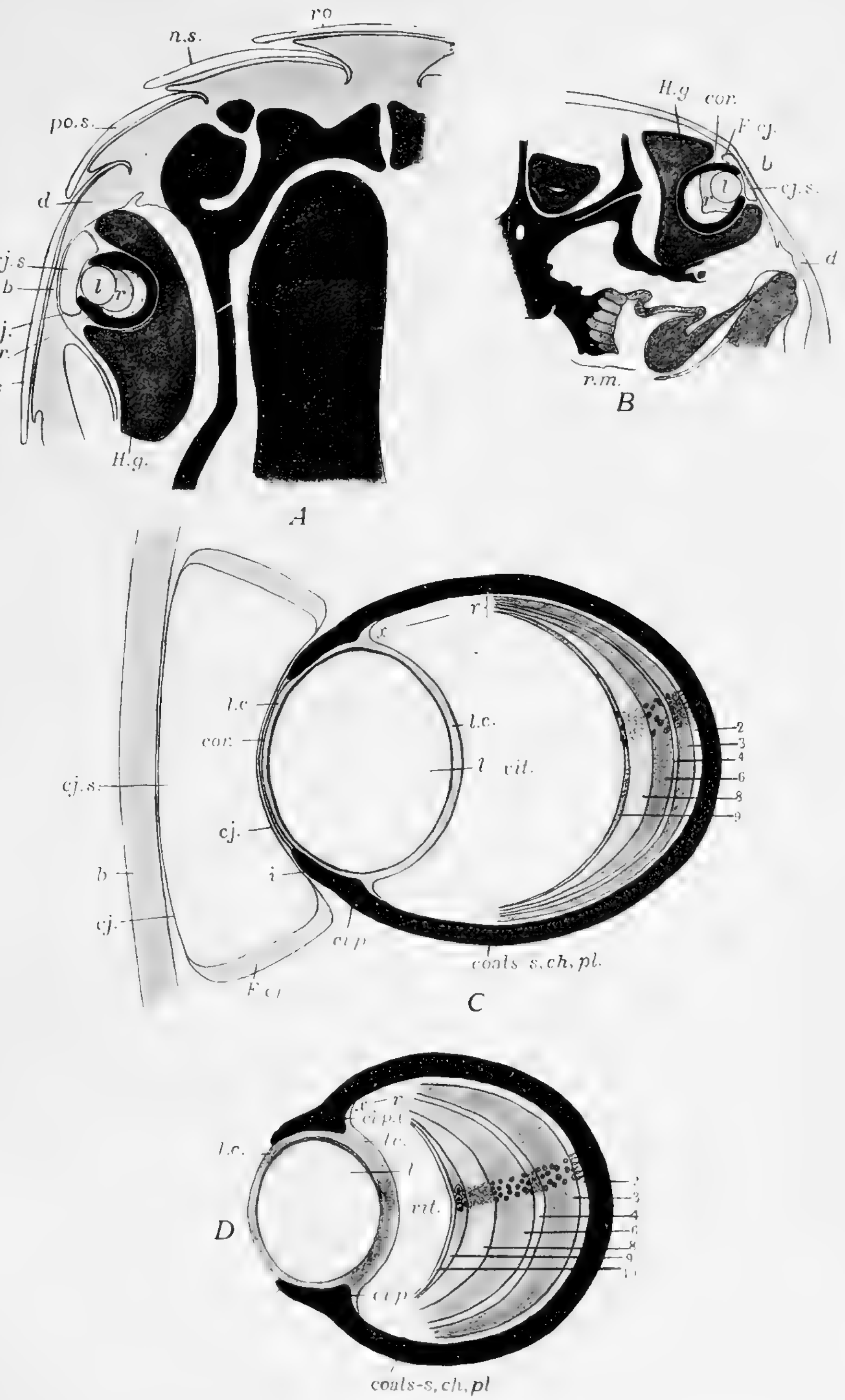
[1] By Effa Funk Muhse. See Biol. Bull. VI. p. 261, 1903.



Eye of Typhlops lumbricalis.

A. Horizontal section, from specimen 20 cm. long. A and B two-thirds objective, 2 inch eyepiece.

B. Transverse section, from specimen 21 cm. long. (Scales not shown.)

C. Diagram of eye of adult.

D. Diagram of eye of young.

The sensory epithelium consists of the outer nuclear layer and the cone layer which is made up of single and twin cones. There are no rods. A single cone consists of two sections, an outer extremely small section, 5 to 6 $\mu$ in length and an inner much larger section, almost completely filled with a larger, pear-shaped, strongly refractive body, the ellipsoid, 14 to 16 $\mu$ in length and 8 to 9 $\mu$ across its widest part, which is turned toward the limiting membrane. The twin cone consists of two parts, one similar to a simple cone, the other cylindrical and very slender, its structure being otherwise like that of a simple cone. It is probable that the two parts of the twin cone are connected with but one nucleus. The nuclei of the cones vary greatly in form, and leading from these into the inner layers of the retina are relatively very large fibers or processes. Passing between the limiting membranes are the radial supporting Müllerian fibers.

### THE EYES OF TYPHLOPS VERMICULARIS.

The work thus far on blind snakes has been done by Kohl on *Typhlops vermicularis*, a species found in Greece and the southwestern part of Asia, and on *Typhlops braminus*, a species found in the islands of the Indian Ocean and in Africa south of the equator, accounts of which are given in his "Rudimentäre Wirbelthieraugen."[1] He found that in depth the eye of *Typhlops vermicularis* is equal to about one-sixth that of *Tropidonotus*. The brille is thicker in *Typhlops* than in *Tropidonotus* and compared with the axial diameter of the respective eyes it is seven times thicker. In *Typhlops* the brille is equal in thickness to about half that of the ordinary skin of the head. In *Tropidonotus* it is equal to one-fourth.

The cornea of *Typhlops* measures 0.0052 mm., and compared with the relative sizes of the eyes is equal to about half that of *Tropidonotus*, which measures 0.064 mm. The conjunctiva is thickened at the edge of the disk-shaped sac and consists here of gland cells, the fornix conjunctiva. The supporting membranes of the eyeball, choroid, and sclera are relatively equal to about half those of *Tropidonotus*.

Harder's gland in *Typhlops* is many times larger than the eyeball. The six muscles are present. The lens is elliptical, while that of *Tropidonotus* is almost globular. The ratio of the lens volume of *Typhlops* to the eye volume is 1 to 14.04, while in *Tropidonotus* it is 1 to 3.6. The lens epithelium of the former is relatively 6 times greater than that of *Tropidonotus*.

The retina at the back of the eye of *Typhlops*, and the retina of *Tropidonotus* bear the actual ratio of 8 to 13, while compared with the eye axis in each case the *Typhlops* retina is 4 times greater. The fovea centralis and area are absent.

The fiber layer has its greatest thickness near the exit of the nerve and gradually becomes thinner until, near the iris, scarcely a fiber is found. The globular ganglion cells are arranged in a single layer except occasionally for short distances, when they lie in a double row. The inner nuclear layer seems to be subdivided into four layers.

There are no twin cones. Each cone consists of a cone cell, stalk, middle and end members. The cone nuclei lie in two series, but the stalks vary in length so that the distal ends of the cone members reach nearly the same level.

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[1] Kohl, Dr. C., Rudimentäre Wirbelthieraugen, Erster Theil, Heft 13, Bibliotheca Zoologica. Verlag von Theodor Fischer, 1892, Cassel.

### THE EYES OF TYPHLOPS LUMBRICALIS.

The eye shows through the large ocular scale, which entirely covers it. It appears as a black spot surrounded by an unpigmented circle. The preocular, also a large scale, overlaps the ocular and reaches just to the edge of the eye (figs. 20 *a*, *b*).

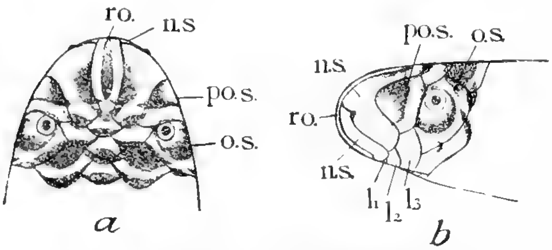

FIG. 20. (*a*) Dorsal View of Head of *Typhlops*, 21 cm. long
(*b*) Lateral View of Head of same Specimen.

Compared with one of the garter snakes and in proportion to the size of the head, the eye of *Typhlops lumbricalis* is situated farther from the surface and occupies far less space, while Harder's gland, associated with the eye in both, is relatively much larger in *Typhlops*. In a specimen of *Typhlops lumbricalis* 21 cm. in length, the eye measured 0.306 mm. in width, and 0.387 mm. in depth. The greatest width of the gland of the same was 0.711 mm. and the length was 1.067 mm. The gland completely surrounds the eye up to the edges of the conjunctival sac (plate 4, figs. A, B). In proportion to the size of the eyes, the gland of the garter snake is much smaller than that of *Typhlops lumbricalis*, but compared with *Rhineura floridana* the gland of *Typhlops lumbricalis* is but little more than half as large.

The eye is covered by layers of epidermis and dermis that differ from these same layers on neighboring parts by being thinner, more compact, and free from pigment and glands. The ocular scale, however, which covers the eye region, does not differ in thickness from the other scales of the head (plate 4, fig. A).

A conjunctival sac is present with a diameter at least as great as the greatest width of the eye bulb. The conjunctiva, which forms this sac, is very thin over the cornea and next to the brille, where it measures 0.003 mm. At the edge of the sac it is differentiated into glands, the fornix conjunctiva, and measures 0.016 mm. (plate 4, figs. B and C, *F. cj.*).

In horizontal section, the eye axis is seen to be turned forward about 30° away from a line at right angle to the horizontal axis of the body.

Eye muscles are present, but from the sections used, the exact number could not be determined.

*Choroid and Sclera.* — The dense pigmentation makes it impossible to distinguish between the different coats at every point. Beyond the retina with its pigment layer is an open vascular space, and this is followed by another dark layer, the two together representing the choroid. The choroidal pigmentary layer seems to consist of long fibers circularly arranged. The sclera can be followed by starting with the outer covering of the optic nerve and tracing its continuation about the eye.

*Iris and Ciliary Processes.* — Here again the pigmentation makes it difficult to determine the structure. Both iris and ciliary processes are present, for the black layer extends over the anterior surface of the lens, leaving a pupil equal in diameter to about one-fourth of the circumference of the lens. At points near the equator of the lens this dark layer is enlarged into the ciliary processes and in connection with the capsule helps to hold the lens in place.

*Cornea.* — This structure is present and can be traced to the region of the ciliary processes.

*Lens.* — A large lens is present, its depth being equal to about two-fifths of the eye depth. From the sections little could be determined about its structure. A well-developed capsule surrounds it (plate 4, fig. c).

*Retina.* — The same layers are present that are found in snakes in general, but the comparative thickness of the various layers is different. In the garter snakes, for instance, the retina is of a uniformly even thickness even to the ciliary process, a single layer of cells continues on over the surface of the processes and iris, but in *Typhlops lumbricalis* the retina at the back of the eye is very thick and gradually becomes thinner till it ends a short distance from the ciliary processes (plate 4, fig. c). At this point the arrangement could not be definitely determined in the sections. At the back the retina, exclusive of the pigment layer, measures 0.0725 mm.

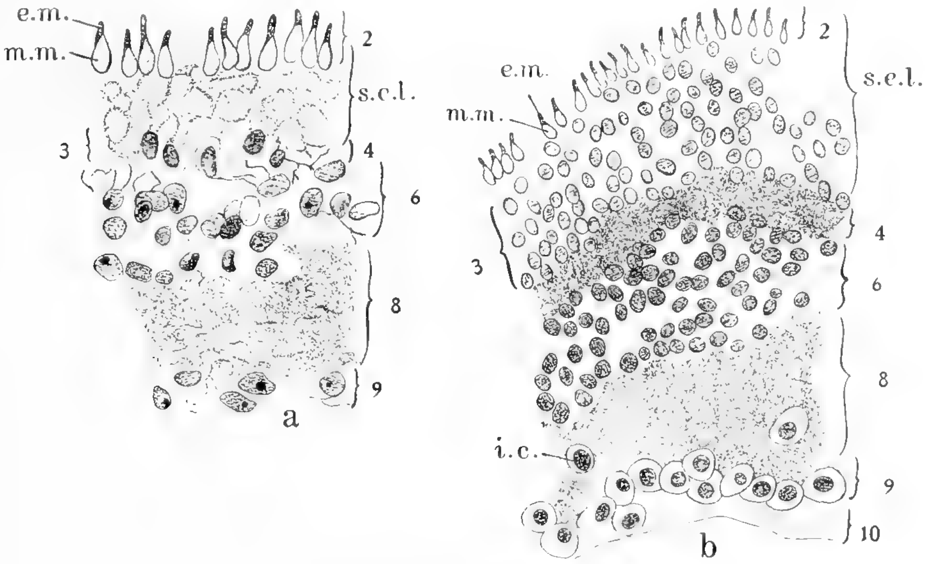

FIG. 21. (*a*) Section of the Retina of an Adult Specimen, 21 cm. long.
(*b*) Section of the Retina of a Specimen, 10 cm. long.

Ends of fibers were seen projecting inward from the ganglion-cell layer, but no definite fiber layer could be distinguished (10 in fig. 21 *b*).

The *ganglion-cell layer* (9 in the figures) consists of a single row of large nucleated cells, somewhat irregularly arranged (0.008 mm.). The inner reticular layer (8) consists of a mass of fibers interwoven in a close network. This layer measures, at the back of the eye, 0.015 mm.

The *inner nuclear layer* (6) consists of at least 3 layers of cells, loosely arranged. The course of some of the fibers can be followed among these cells. This layer measures 0.016 mm.

The *outer reticular layer* (4) is very thin and consists of a few fibers so arranged as to leave a great number of spaces between the two nuclear layers. The distance between the nuclear layers is about 0.005 mm.

*Cones.* — The sensory epithelium shows two distinct parts, an inner layer of nuclei (3) and an outer row of cones (2). In the sections these two were so separated that a loose tissue was visible, consisting probably of the limiting membrane and ends of the Müllerian fibers. The outer nuclear layer in the adult consists of a single row of nuclei, with a mass of quite homogeneous material about them. This part of the sensory epithelium measures 0.018 mm. The cones are pear-shaped bodies with the smaller end pointing outward, and at intervals of every four or five a shorter one occurs. Each element is differentiated into two parts. By the iron hæmatoxylin process of staining, the outer small end is densely stained, while the body of the element is a light granular mass (fig. 21 *a*).

The *pigment layer* (1) is a continuous layer of even thickness, similar in every respect to that of the garter snake.

One young specimen, 10 cm. in length, was examined. The eye as a whole, as well as the lens, is nearly spherical. The eye measures in width 0.290 mm. and 0.322 mm. in depth. All parts are so developed that the vitreous cavity is relatively much smaller than that of the adult. The coats are thicker, the ciliary processes better developed, the lens capsule thicker, and the retina at the back actually measures one and two-thirds the depth of the adult retina. The elements of each layer are much more numerous than in the adult, and they are packed much more closely together (fig. 21 *b*). The ganglion nuclei are apparently arranged one against the other. In the inner reticular layer occur the "interpolated cells." These were not found in the sections of the adult eye that were examined. The cells of the inner nuclear layer are smaller and arranged in five or six rows. There is a well-developed outer reticular layer similar in its make-up to the inner reticular. Instead of a single row of cone nuclei with its surrounding homogeneous mass, as in the adult, this layer in the young consists of five or six rows of small closely arranged cells. The cones likewise are smaller and more numerous (fig. 21 *b*).

*Comparative Measurement of Retinal Layers in millimeters.*

| | Fiber layer. | Ganglion-cell layer. | Inner reticular layer. | Inner nuclear layer. | Outer reticular layer. | Sensory epithelium. | Total depth. |
|---|---|---|---|---|---|---|---|
| Tropidonotus natrix.............. | 0.003 | 0.012 | 0.042 | 0.052 | 0.004 | 0.0196 | 0.1331 |
| Typhlops vermicularis ........... | .0018 | .0081 | .0155 | .0221 | .0022 | .0324 | .0821 |
| Typhlops lumbricalis (adult) ...... | | .008 | .015 | .016 | .005 | .030 | .0725 |
| Typhlops lumbricalis (young, 10 cm.) | .005 | .010 | .024 | .032 | .008 | .040 | .1206 |

*Relative Proportions of Eye Parts.*

| | Tropidonotus natrix. | Typhlops vermicularis. | Typhlops lumbricalis (adult). |
|---|---|---|---|
| Eye depth. | 2.5541 mm. | 0.4399 mm. | 0.4032 mm. |
| Brille: | Eye axis:: 1 : 77.4 | 1 : 10.77 | 1 : 12.5 |
| Cornea: | Eye axis:: 1 : 39.9 | 1 : 84.6 | 1 : 85 |
| Lens depth: | Eye axis:: 1 : 1.56 | 1 : 3.03 | 1 : 2.5 |
| Coats: | Eye axis:: 1 : 21.63 | 1 : 38.58 | 1 : 25.4 |
| Retina at back: | Eye axis:: 1 : 19.19 | 1 : 5.36 | 1 : 5.5 |

## CONCLUSIONS AS TO THE EYES OF BLIND REPTILES.

### AMPHISBÆNA.

(1) The eye muscles have entirely disappeared.

(2) Only the uveal parts of the iris remain.

(3) The lens has retained its shape and position, but its structure has been greatly changed. No capsule is present.

(4) Harder's gland is many times larger than the eye and pours its secretion into the conjunctival cavity and thence into the mouth.

(5) The sclera, scleral cartilages, cornea, vitreous body, and pigment epithelium have undergone but little change unless it be in the reduction in size.

(6) The cuticle passes over the eye unchanged.

(7) The aqueous cavity is no longer present.

(8) All the layers of the retina are still present. As shown in figure 6, the great reductions in the depth of the layers, in comparison with those of *Anolis*, have taken place in the nerve fiber, ganglion cell, inner reticular and inner nuclear layers.

(9) If the eye has been reduced from an eye of the average size, all parts have certainly undergone considerable change, and this change has been approximately equal among the several parts.

(10) The retina does not show such a profound change as either the iris, muscles, or lens. However, it has been greatly changed, as it extends only 50.7 per cent of the distance around the eye.

(11) The eye of *Amphisbæna* shows that the more active parts of the eye are the ones to degenerate first. They are the parts which have been most affected.

### RHINEURA.

(1) The eye of *Rhineura* has reached its present stage as the result of a process of degeneration that probably began in the early Miocene.

(2) The dermis and epidermis pass over the eye without any modifications. The conjunctival pocket has vanished.

(3) Harder's gland is many times as large as the eye and pours its secretion into the tear duct and thus into the nasal cavity.

(4) The eye muscles have disappeared.

(5) A cornea is not differentiated.

(6) The lens is absent in half the eyes examined and varies greatly in those in which it is present.

(7) The vitreous body has practically disappeared.

(8) The pigment epithelium is variously pigmented. It is of greater extent than is sufficient to cover the retina and has been variously invaginated or puckered over the proximal and posterior faces of the eye.

(9) An uveal part of the iris is not present.

(10) The eye of *Rhineura* does not represent a phylogenetically primitive stage; it is an end product of evolution as truly as the most highly developed eye.

(11) The adult eye shows few indications that there has been a cessation of development at any definite ontogenetic stage. It does not resemble as a whole any ontogenetic stage.

(12) An arrest in the ontogenetic development has taken place in so far as the number of cell multiplications concerned in forming the anlage of the various parts of the eye have decreased in number, and in the lack of union of the lips of the choroid fissure.

(13) It is possible that the absence of cones or rods is due to an arrest in the histogenesis of the retina, but since these structures are normally formed in the young of *Typhlotriton* and disappear with age, it is possible that their absence in the adult eye of *Rhineura* is also due to ontogenetic degeneration.

(14) The irregularity in the structure and existence of the lens and the great reduction of the vitreous body offer evidence in favor of the idea of the ontogenetically and the phylogenetically earlier disappearance of the ontogenetically and phylogenetically newer structures.

(15) Horizontal nuclei found between the pigment epithelium and the outer limiting membrane are probably derived from the proximal layer of the optic cup.

(16) The different layers of the retina have reached a degree of differentiation out of proportion to the great reduction of the dioptric apparatus and general structure of the eye.

### TYPHLOPS.

(1) The dermis and epidermis over the eye differ from the same over neighboring parts, by being thinner, more compact, and free from pigment and glands.

(2) The conjunctival sac is present and has a width at least as great as the eye.

(3) Harder's gland surrounds all but the distal part covered by the conjunctival sac.

(4) Eye muscles present, but their number and structure could not be made out.

(5) A large lens with capsule is present.

(6) The various layers of the normal snake retina are present but the comparative thickness is different.

# EYES OF THE CYCLOSTOME POLISTOTREMA STOUTI.

The eyes of this myxinoid of the Pacific coast were examined by Allen and by Stockard. Allen found that they show a very primitive structure, which is in reality the result of a complex process of degeneration. The eyeball is found embedded in a mass of fat about three times its size. In one case, the eye was found to lie some distance beneath the outer surface of the mass of fat. Normally, however, the corneal surface lies on a level with the surface of the fat and is often flattened to form a rather extensive free surface. No eye muscles nor traces of such were discovered. No oculomotor nerves were found. No traces of them are discoverable in embryonic life (Kupffer). The choroid and sclerotic coats are represented by a very thin layer of unpigmented, non-vascular connective tissue without any appreciable distinction between corneal and sclerotic portions. The retina remains in the early condition of an optic cup, the outer layer (pigment layer) not being fused with the remaining layers. All specimens showed the layer in question to be widely separated from the bulk of the retina. This pigment layer is composed of a single layer of cubical cells devoid of pigment as far as could be ascertained. A layer corresponding to that of the rods and cones in higher vertebrates is clearly present. The nuclei of these structures (outer nuclear layer) are strikingly well developed and regularly arranged. Certain characteristic cells of the inner nuclear layer could be readily made out. The ganglionic layer is represented by cells scattered irregularly throughout the inner reticular layer. Fibers from these last-named cells can be traced in a more or less direct course to the optic nerve. The outer rim of the optic cup is in many cases differentiated in such a manner as to suggest a rudimentary iris. A structure unmistakably like an iris was found in one specimen examined. The cellular structure of this rudimentary iris is almost identical with that of the pigment layer. No indications of muscle fibers or pigment are to be seen. Certain deeply staining coagula within the optic cup give evidence of a vitreous body. Some large, clearly-marked cells, probably those of the vitreous body, are found attached to the surface of the retina. Evidences of a choroid fissure are to be seen in the fact that the ventral part of the retina is thinner than the dorsal in almost all specimens. In one case the choroid fissure was found to persist. The most striking feature, however, is the extreme variation. The optic nerve enters the eye at various angles. Variation occurs in all parts of the eye and is especially notable in the measurements of the thickness of the retina and the dimensions of the eye as a whole.

Stockard found that the lens-bud results from a contact of only a portion of the optic cup with the ectoderm. This structure continues to develop for a time until, in an embryo considerably more advanced and measuring 15 mm. in length, one sees the lens-bud with a slight indication of a constriction about the periphery of its area of union with the ectoderm, as if it were preparing to pinch off. Here the progressive development of the lens ceases and degeneration begins. It soon disappears entirely. He considers the cessation of development in the lens due to the absence of a durable contact with the optic cup upon which lens formation is directly dependent.

# THE FISHES.

## GENERAL REMARKS ON THE EYES OF FISHES.

It is not the intention to review the literature on the normal eyes of fishes. A list of papers dealing with their macroscopic aspect has been furnished by Ziegenhagen in 1895, while those dealing with minute structure have been enumerated by Krause in 1886 and Cajal in 1894. The current literature is discussed periodically by Virchow in "Die Ergebnisse der Anatomie und Entwickelungsgeschichte."

The topographical relationship of the cells of the retina obtained an entirely new light by the application of the methylene-blue method chiefly on the part of Dogiel, and the Golgi method principally through Ramón y Cajal. The layers of the retina of fishes as made out by Ramón y Cajal are as follows, beginning at the periphery and going toward the center of the eye:

1. Epithelial-pigment layer.
2. Rods and cones.
3. Outer nuclear layer.
4. Outer molecular layer.
5. Horizontal cells.
6. Bipolar cells.
7. Spongioblasts.
8. Inner molecular layer.
9. Ganglionic layer.
10. Optic fiber layer.

Throughout this work the layers are designated on the figures and frequently in the text by these numbers. The literature bearing on the eyes of the blind species will be given under the different species.

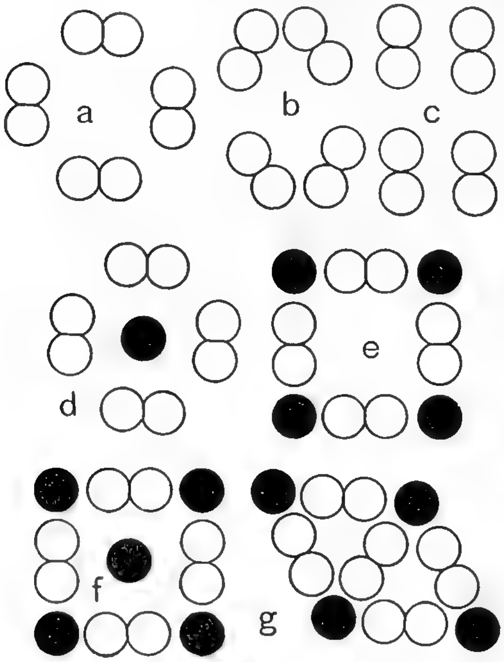

Fig. 22. Types of Single and Double Cones in Retinas of Various Fishes.

The horizontal relations, especially the mosaic of the single and twin cones in the retinas of fishes, has been dealt with by Hanover, Müller, Krause, Friis, Ryder, Beer, Eigenmann, and Shafer.

It was found that in many fishes the single and twin cones form a regular mosaic. The number of parts entering into each unit of the retinal mosaic is remarkably constant for any species, but differs considerably in different species of fishes. The "shape" of the unit differs in different parts of the retina. The pattern may be made up of twin cones only.[1] The axes or lines joining the centers of the components of each twin if continued may be at right angles to each other and form a square (fig. 22 *a*), or they may be approximately parallel (*Sebastodes*, *c*), or they may be variously inclined to each other and form rhombs (*Scorpæna*, *b*).

In other genera (*Perca*, *Micropterus*, *Etheostoma*, and *Pimephales*) a single cone is placed in the center of each of the units of 4 twin cones (*d*). In still others (*Blennius*, *e*) a single cone is added at each angle of the unit, and in still others

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[1] Krause found only single cones in the eel.

(*Salmo, Coregonus, f*), a single cone is found both at each angle and in the center of the unit. The most complicated unit (*Esox, g*) is composed of 5 twins, 4 forming the sides and 1 a diagonal, and of 4 single cones, 1 in each corner. These patterns are all regular, but not mathematically so.

In some families (Siluridæ and Catostomidæ) no regularity could be made out. In general the number of rods is inversely proportional to the number of single cones.

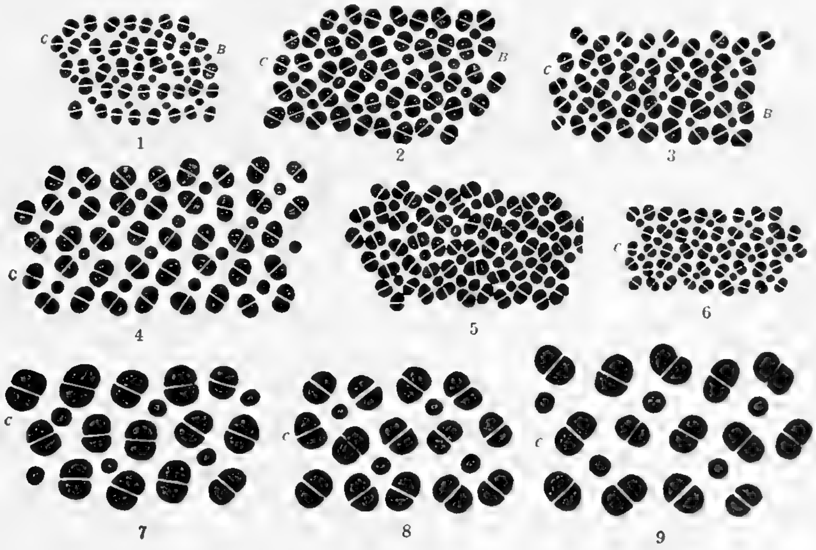

FIG. 23. 1 to 6 show Section of Eye of Bass 6 cm. long. The Eye measured 3.8 mm. in Diameter from Cornea to Back, and 4.7 mm. from Anterior to Posterior Edges. 7, 8, and 9 show Sections of Eye of Bass 33.5 cm. long. The Eye was 10 mm. in Diameter from Cornea to Back, and 13 mm. from Anterior to Posterior Edges. All figures drawn to the same magnification. *C*, part turned toward cornea; *B*, part pointing from cornea.

In the black bass, the only species in which the pattern was examined over the entire eye, the number of components in each unit of the mosaic is the same, but the shape of the pattern varies regularly from a rectus at the anterior and posterior faces of the eye, to a rhomb above and below. The elements of the unit and the entire unit increase in size with the growth of the eye. New elements are not added after the pattern has been established.

## THE EYES OF ZYGONECTES NOTATUS.

Of the eyes of a number of species of normal fishes, namely those of *Cymatogaster aggregatus*, *Carassius auratus*, *Ameiurus* sp., *Coregonus* sp., and *Zygonectes notatus* examined, I shall briefly describe the eyes of but one.

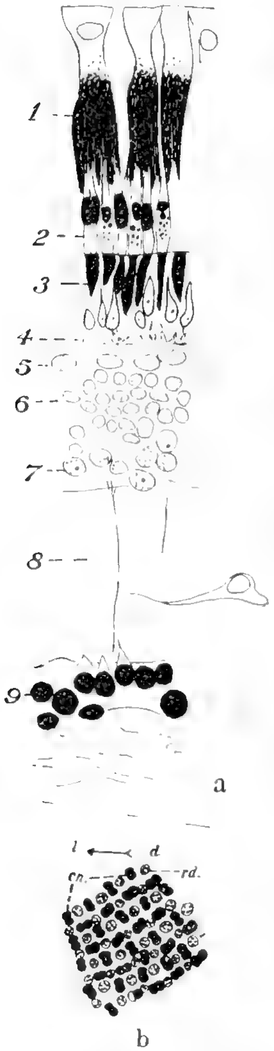

Fig. 24. (*a*) Section through Retina of *Zygonectes notatus*. (*b*) Cone Pattern of Same Fish.

*Zygonectes notatus* (Rafinesque) was selected for comparison, since it is a member of the Cyprinodontidæ, a family closely related to the Amblyopsidæ. I am not aware that this species has any advantage over other species of the family. It has large, well-developed eyes, that we may assume to be fully and normally developed. The material examined was alcoholic. It had been preserved by simply placing in alcohol without any intention of future histological examination, but the structures were all well preserved for making out the horizontal relations of the single and twin cones. The protoplasmic and nervous processes of the cells were of course not brought out as with Golgi's method.

A specimen 38 mm. long had the eye 2.24 mm. in length, 2 mm. in vertical diameter, 1.12 mm. from axis of optic nerve to front of iris, 1.6 mm. from axis of optic nerve to front of cornea; lens 0.96 mm. in diameter; pigment layer measures 56 $\mu$; outer nuclear layer, 36 $\mu$; outer reticular, 4 $\mu$; tangential cell layer, 9 $\mu$; inner nuclear, 40 $\mu$; inner reticular, 52 $\mu$; ganglionic layer, 12 $\mu$; optic-fiber layer, 28 $\mu$; total thickness of retina, 237 $\mu$.

The regularity of arrangement of single and twin cones is very striking. The basal part of the single cones contains refractive granules increasing in size outward where the series ends in a lenticular vacuolated body separating the granular from the distal part of the rod. The twin cones are all without granulation. This marked difference between the two enables one to distinguish between them at a glance in tangential sections. The twin cones are arranged in series in such a manner that the axes joining the cones in any neighboring series are at right angles to each other, while in every alternate series they extend in approximately the same or parallel directions. The single cones alternate in all directions with twin cones (fig. 24 *b*).

The outer nuclei are irregular, compressed, and elongate, forming two distinct layers. The outer molecular layer has an irregular outer boundary produced by the process extending toward the outer cells. The inner nuclear layer is divided into an outer layer of small bipolar cells and an inner layer of larger, more coarsely granulated spongioblastic cells. When any breaks occur in the retina, owing to mechanical or chemical causes, they usually occur between these outer bi-polar and inner spongiose cells of the inner nuclear layer.

## TYPHLOGOBIUS: THE POINT LOMA BLIND FISH AND ITS RELATIVES.

San Diego Bay is in part surrounded by mud flats which are covered by water at high tide. Sand beaches take the place of the mud flats where the channel approaches the shores. On the ocean shores a sandy beach stretches several miles to the southeast from the mouth of the bay, while on the west rises the point of land called Point Loma. The entire ocean beach at the base of this promontory is rocky. In many places all the earth has been removed by the action of the waves, leaving the bare rock; in other places, and more especially between the outer point and Ballast Point, large bowlders lie embedded in the sand (frontispiece). These are all covered at high tide, while but a few small pools remain about the rocks at low tide. Many of these rocks are covered with seaweeds, actineans, and especially large chitons. All these localities are inhabited by relatives of the Point Loma blind fish. The sloughs traversing the mud flats of the bay are inhabited by *Gillichthys mirabilis* Cooper, the young of which is represented in figure 25 *a*.

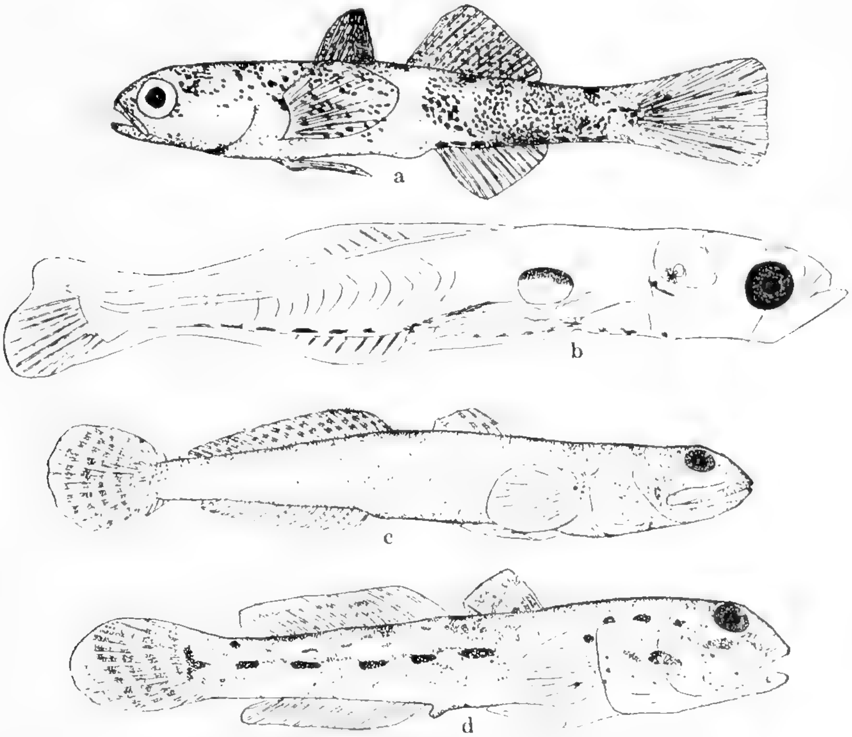

FIG. 25. (*a*) Young *Gillichthys mirabilis* Girard. From mud flats of San Diego Bay.
(*b*) Larva of *Clevelandia* or *Lepidogobius*. From surface of San Diego Bay.
(*c*) *Clevelandia ios* Jordan and Gilbert. From San Diego Bay.
(*d*) *Quietula y-cauda* Jenkins and Evermann. From San Diego Bay.

In the mud flats every tide pool as large as a man's hand contains *Clevelandia ios* (fig. 25 *c*); nearer low-water mark in similar localities *Quietula y-cauda* are found, but less abundant than *Clevelandia ios*. On digging in the sandy beaches of the bay specimens of another species of this group, *Ilypnus gilberti*, are sometimes found buried in the sand. In the crab holes under the rocks about Point Loma occurs the most remarkable of this family, the Point Loma blind fish, *Typhlogobius californiensis* (fig. 26 *a*). In deep water off Point Loma lives still another goby, *Gobius nicholsoni*.

It is thus seen that almost every nook available has been taken possession of by these diminutive fishes. All of them have the two ventrals united along the median line and a thin membrane stretched across their bases to form a pouch. By appressing the ventrals and then raising them, a partial vacuum is formed in this pouch and the fish is enabled to cling to any substance with which its ventral happens to be in contact. In confinement the blind fish frequently utilizes the surface of the water of an aquarium for a surface of attachment.

All the species in the bay have the habit, if disturbed, of hiding in crab or clam holes. *Clevelandia* will sit on its tail and pectorals until the hand is near it; then with a quickness which would do honor to a Johnnie Darter, with a flirt of the tail and a stroke of the pectoral, it disappears into its hole, from which, however, it at once thrusts its head to await developments. Several of them frequently take refuge in the same hole.

*Gillichthys* is the largest of these gobies. About San Diego the young are abundant throughout the year. The adult can be caught with hook and line in quantities, especially just at the return of tide during summer. Toward their spawning season they retire to their respective crab holes, and no morsel, however tempting, will lure them forth. At San Diego they begin to spawn about the end of March. The young, when first observed, have but few color cells. They are very active, jumping several times their own length if left dry in a watch crystal. The young of this species but little resemble the adult. The maxillary does not reach beyond the eye, the color is in more or less well-defined crossbars, and the scales, which in the adult are cycloid, have several large teeth.

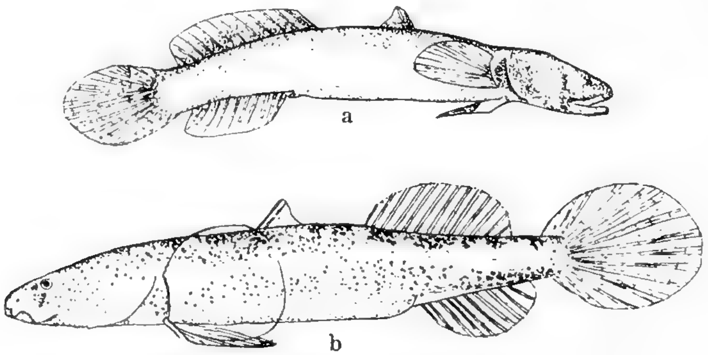

FIG. 26. (*a*) *Typhlogobius californiensis* Steind. From base of Point Loma.
(*b*) *Typhlogobius* about 25 mm. long.

*Clevelandia* is by far the most abundant of the gobies, and in fact the most abundant of any fish in the bay of San Diego. They are found everywhere between high and low water mark, and doubtless form an important item of the food of the larger fishes. They spawn in the early part of May. The young rise to the surface at night, and are then sometimes taken in the surface dredge. They can, however, be procured more abundantly in the latter part of May in the pools left at low tide about the piles of wharves.

The most remarkable of the gobies is undoubtedly the blind one inhabiting the crab holes under rocks at Point Loma. In its pink color and general appearance it much resembles the blind fishes inhabiting the caves of southern Indiana. Its peculiarities are doubtless due to its habits. The entire bay region is inhabited by a carideoid crustacean which burrows in the mud, which, like the blind fish, is pink in color. Its holes in the bay are frequented by *Clevelandia*, etc., while at the base of Point Loma, where the waves sometimes dash with great force, the blind fish is its associate.

On rough days few fishes are seen, though ever so many stones are overturned. On mild days, on the contrary, at very low tides quantities are found almost invariably in company with one of the crustaceans mentioned above. Sometimes the fishes live quite out of water on the damp gravel and sand under a rock, but more frequently small pools of water fill all the depressions under the rocks, and the fishes swim rapidly away to hide in the crab holes, several of which always branch from the cavity in which the rock has lain. Very rarely are the fishes found swimming in rocky tide pools.

In the bay the gobies habitually live outside of the holes, descending into them only when frightened; but at Point Loma they rarely leave their subterranean abodes, and to this fact we must attribute their present condition. How long these fishes have lived after their present fashion it would be hard to conjecture. The period which would produce such decided structural changes can not be a brief one. The scales have entirely disappeared, the color has been reduced, the spinous dorsal has been greatly reduced, the eyes have become stunted, and the whole frontal region of the skull and the optic nerves have been profoundly changed.

The skin, especially that of the head, has become highly sensitized. The skin of the snout is variously folded and puckered. The nares are situated at the end of a fleshy protuberance which projects well forward, just over the mouth. At the chin are various short tentacles, and a row of papillæ (which probably bear sensory hairs) extends along each ramus of the lower jaw and along the margin of the lower limb of the preopercle. The eye is, however, the part most seriously affected. It is quite evident and apparently functional in the young (fig. 26 *b*). Objects thrust in front of the fish are always perceived, but the field of vision is quite limited. With age the skin over the eyes thickens and they are scarcely evident externally. As far as I could determine they do not see at this time, and certainly detect their food chiefly, if not altogether, by the sense of touch. A hungry individual will swim over meats, a fish, or a mussel, etc., intended for its food without perceiving it by sight or smell, but as it comes in contact with any part of the skin, especially that of the head region, the sluggish movements are instantaneously transformed, and a stroke of the fins brings the mouth immediately in position for operations.

Ritter's experiments showed that it would not choose between light and dark, but, "On the whole, both from these observations on the living fish, and from the structural conditions, . . . I am of the opinion that the power of perceiving light is not wholly lost even in the adult."

The optic nerve is very slender and the lens proportionately very large.

In the youngest individual caught (fig. 26 *b*), the membranes of the fins were thin, the color cells well formed and arranged not unlike those of the young *Gillichthys*. The movements were similar to those of the other gobies, and not at all sluggish like those of the adult. Their favorite position is standing or sitting with the broad pectorals extending out at right angles to the body. In this position the fish can, with a sudden stroke of its pectorals, move quickly and rapidly. In the old fish the fins are thick and smaller in proportion, and all the vivacity seems to have disappeared. The color has degenerated, or at least not developed in proportion to the growth of the fish.

All these gobies are tenacious of life, especially the blind ones. Several of the latter have been kept in a half-gallon jar of water for several weeks without change of water, and others have been kept several months in confinement in my laboratory. When the water becomes somewhat stale, they frequently rise to the surface and use the water as a plane to which they attach themselves by means of their ventrals. The earliest date at which I procured young was October 25. The smallest caught at that time is represented in figure 26 *b*.

The covering of the ovarian egg consists first of a finely striate membrane, the zona radiata of all teleostean eggs. Exterior to this is a network of threads with the meshes coarsest at the entodermic pole and forming almost a continuous membrane at the ectodermic pole. When the eggs are deposited, the meshwork of threads is stripped off the egg and remains attached to the zona radiata around the micropyle. In the eggs deposited naturally by the females in confinement the threads were wound together to form a cord at the micropylar end of the egg. The cords of many of these eggs were attached to each other, and the eggs thus came to be laid in bunches like those of grapes. In their natural habitat the eggs are fastened by the threads to the lower surfaces of the rocks under which they live, and the membranes are expanded into long club-shaped bags. The yellow of the blind-fish egg is entirely confined to the yolk, which contains many oil globules. The granular protoplasm is opaque. In females with ripe eggs they are frequently to be seen forming a yellow band along the flanks.

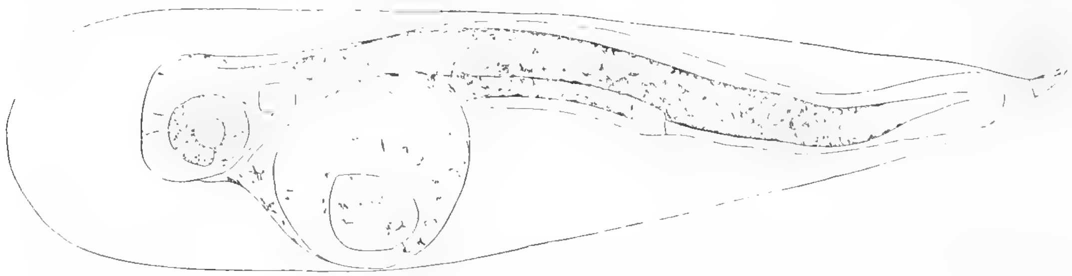

FIG. 27. Larval Typhlogobius in its membrane.

The eye in the larvæ just about to be hatched (fig. 27) is apparently normal.

The histology of the adult eye was studied by Ritter, who comes to the following conclusion:

> 1. In the smallest examples studied the eyes, though very small, are distinctly visible even in preserved specimens — so distinctly that the lens is plainly seen. In the largest examples, on the other hand, they are so deeply buried in the tissue as to appear even in the living animals as mere black specks, while in preserved ones they are in many cases wholly invisible.
>
> 2. Neither in small nor in large specimens does the epidermis over the eye differ in thickness or structure from that of adjacent regions. In the large individuals the much greater thickness of the tissue here is brought about by an increase in the sub-epidermal connective tissue, the growth of which can be seen taking place in the embryonal connective-tissue cells that are found here.
>
> 3. As is the case with rudimentary organs generally, the eye is subject to great individual variation in size, form, and degree of differentiation.
>
> 4. The only parts of the normal teleostean eye, no traces of which have been found, are the argentea, the lamina suprachoroidea, the processus falciformis, the cones of the retina, the vitreous body proper, the lens capsule, and in one specimen the lens itself.
>
> 5. In the parts present the rudimentary condition of the organ is seen in the very slight development of the choroid, no cellular elements being present in this excepting in the chorio-capillaris, and here to a quite limited extent, the rest of that layer being composed exclusively of pigment;

in the fact that the choroid gland is composed entirely of pigment; in the fact that the iris, though of fully the normal thickness, is almost entirely of pigment, there being on its outer surface in some specimens a small amount of cellular material, which probably represents the ligamentum annulare; in the great proportional thickness of the pigment layer of the retina and the entire absence in it of anything excepting pigment; in the incomplete differentiation of the layers of the retina, there being in some individuals scarcely more than a trace of the external reticular layer separating the two nuclear layers, and there being in no specimen studied a retina sufficiently developed to enable one to homologize with certainty the layers marked out; in the minute size of the optic nerve, and the fact that it is ensheathed in a thick layer of pigment for nearly its entire course through the retina; and, finally, in the small size of the motores oculi.

6. The surest evidences of actual degeneration are found, first, in the greatly augmented quantity of pigment in all the parts that are at all pigmented in the normal eye; and, secondly, in the presence of pigment in regions where none is found in the normal eye, as in the hyaloid membrane.

No undoubted instances of degeneration through the breaking down and dissolution of the tissue without the formation of pigment, such as have been described particularly by Looss, have been found, though in a single specimen (the one in which no lens is present) a process of this nature may be taking place.

## THE EYES OF THE BLIND CAT-FISH, AMEIURUS NIGRILABRIS.

All that is known of this fish is contained in the following extract from Cope's paper (Proc. Acad. Nat. Sci., Phila., 1864, p. 231):

> For a knowledge of the first genus of blind *Silurid* from our country, I am indebted to my friend Jacob Stauffer, secretary of the Linnæan Society of Lancaster, an ardent explorer of the zoology and botany of southern Pennsylvania, and who has furnished me with many valuable notes and specimens. This fish, of which specimens have been taken in the Conestoga Creek, a tributary of the Susquehanna, is simply a blind representative of the ordinary type of *Silurids*, characteristic of North America, and is not to be arranged with the exotic groups. * * * The color of the upper surfaces, tail, fins, barbels, and under jaw is black; sides varied with dirty yellow, abdomen and thorax yellowish white. * * * A specimen died in 20 minutes after capture, when put in water, though uninjured; the *Ameiuri*, like other cat-fishes, will live for many hours after complete removal from their element. It is occasionally caught by fishermen, and is supposed to issue from a subterranean stream, said to traverse the Silurian limestone in that part of Lancaster County and discharge into the Conestoga.
>
> Two specimens of this fish present an interesting condition of the rudimental eyes. On the left side of both a small perforation exists in the corium, which is closed by the epidermis, representing a rudimental cornea; on the other the corium is complete. Here the eyeball exists as a very small cartilaginous sphere with thick walls, concealed by the muscles and fibrous tissue, and filled by a minute nucleus of pigment. On the other the sphere is larger and thinner walled, the thinnest portion adherent to the corneal spot above mentioned; there is a lining of pigment. It is scarcely collapsed in one, in the other so closely as to give a tripodal section. Here we have an interesting transitional condition in one and the same animal, with regard to a peculiarity which has at the same time physiological and systematic significance, and is one of the comparatively few cases where the physiological appropriateness of a generic modification can be demonstrated. It is therefore not subject to the difficulty under which the advocates of natural selection labor, when necessitated to explain a structure as being a step in the advance toward, or in the recession from, any *unknown* modification needful to the existence of the species. In the present case observation on the species in a state of nature may furnish interesting results. In no specimen has a trace of anything representing the lens been found.

## THE AMBLYOPSIDÆ.

The Amblyopsidæ are a small family of fishes, first brought to the notice of naturalists by W. T. Craige, who presented a specimen to the Philadelphia Academy in 1842. De Kay, "Natural History of New York" (Reptiles and Fishes, p. 187, 1842), gives a brief description of *Amblyopsis spelæus.* It was followed at once by articles by Wyman (1843 and later, 1850, 1854 *a* and *b*) and other articles by Thompson (1844) and by Telkampf (1844). Renewed interest in the Amblyopsidæ was aroused by Agassiz's discovery of an epigean relative, *Chologaster cornutus*, in the ditches of rice fields in South Carolina.

*Typhlichthys subterraneus* was described by Girard in 1859 from a well near Bowling Green, Kentucky; *Chologaster agassizii*, by Putnam from a well at Lebanon, Tennessee, in 1872; *Chologaster papilliferus*, by Forbes in 1882. In 1898 the present author described *Typhlichthys rosæ*, and a short time afterwards he demonstrated that this species is generically distinct from *Typhlichthys*, naming it *Troglichthys.* More recently (1905) he described *Typhlichthys osborni* and *Typhlichthys wyandotte.*

### RELATIONSHIPS OF THE AMBLYOPSIDÆ.

The Amblyopsidæ are members of the order Haplomi, first characterized by Cope.[1] They have recently been defined by Boulenger, as follows:

> Air-bladder, if present, communicating with the digestive tract by a duct. Opercle well developed. Pectoral arch suspended from the skull; no mesochorochoid. Fins usually without, rarely with a few spines; ventrals abdominal, if present. Anterior vertebræ distinct, without Weberian ossicles.

The order consists of a number of families of which the Galaxiidæ and Aplochitonidæ are found in the fresh waters and occasionally in the oceans of the south temperate zone; the Scopelidæ are found pelagic and abysmal in the ocean, the Kneriidæ in Africa, the Dalliidæ in Alaska and Siberia, the Pœciliidæ in fresh water and along the shores of the tropical and temperate zones, and the Esocidæ in fresh waters of the north temperate zone.

The Amblyopsidæ are distinguished from the other families by the doubling forward of the alimentary tract, the opening of the oviduct and anus being placed close behind the throat, in front of the pectorals.

The genera of the Amblyopsidæ may be distinguished by the following characters:

| | |
|---|---|
| *a.* Ventral fins present; pyloric cœca 2 or 3 | *Amblyopsis* |
| *aa.* Ventral fins absent | |
| *b.* Eye a vestige; pyloric cœca 2 | |
| *c.* Sclera with cartilages | *Troglichthys* |
| *cc.* Sclera without cartilages | *Typhlichthys* |
| *bb.* Eye well developed; body pigmented; pyloric cœca 4 | *Chologaster* |

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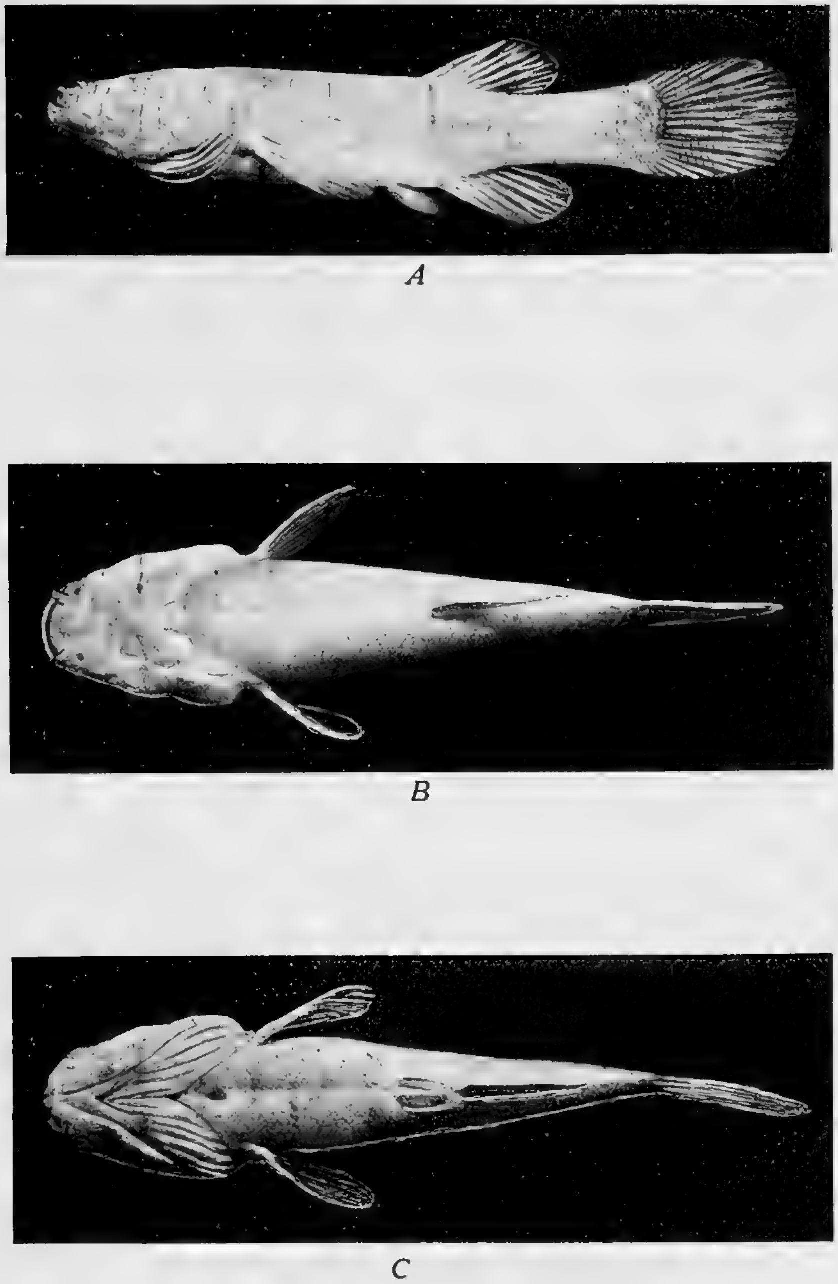
[1] Proc. Amer. Assoc. Adv. Science, Indianapolis, 1872, 328 and 333.



Amblyopsis.
(A) side, (B) dorsal, and (C) ventral views.

## DISTRIBUTION OF THE AMBLYOPSIDÆ.

### **Amblyopsis spelæus De Kay.** Plate 5.

*Amblyopsis spelæus*, De Kay, Nat. Hist. N.Y., Reptiles and Fishes, 1842, p. 187, Mammoth Cave, Ky. — Wyman, Ann. and Mag. Nat. Hist., xii, 1843, p. 298; Amer. Jour. Sci. and Arts, xlv, 1843, pp. 94 to 96, Kentucky. — Thompson, Ann. and Mag. Nat. Hist., xiii, 1844, p. 112. — Telkampf, Müller's Arch., 1844, pp. 381 to 394, taf. 9 .— Wyman, Proc. Bost. Soc. Nat. Hist., iii, 1850, pp. 349 to 357. — Agassiz, Amer. Jour. Sci. and Arts, xl, 1851, p. 127. —Wyman, Proc. Bost. Soc. Nat. Hist., iv, 1854, p. 395, v, p. 18; Amer. Jour. Sci. and Arts, xvii, 1854, p. 258. — Poey, Mem. Cuba, ii, 1853, p. 104. — Günther, Cat. Fishes Brit. Mus., vii, 1868, p. 2, Mammoth Cave, Ky. — Putnam, 1872, Amer. Nat., p. 30, fig., Lansing, Mich. [p. 20], well near Lost River, Ind. — Cox, Report Geol. Res. of Ind., Rhodes Cave, near Corydon; Gulf of Lost River. — Cope, Report Geol. Res. of Ind., iii and iv, 1871 and 1872 (1872), p. 161, Little Wyandotte Cave, Ind.; Ann. and Mag. Nat. Hist., 1872, Little Wyandotte Cave, Ind. — Jordan, Rept. Geol. Nat. Res. of Ind., vi, 1874 (1875), p. 218, Mammoth Cave. — Cope, Rept. Geol. Nat. Res. of Ind., viii, ix, x, 1876, 1877, 1878 (1878), p. 483, Little Wyandotte Cave, Ind. — Jordan and Gilbert, Synopsis, 1883, p. 324. — Packard, Cave Fauna of N. A., Mem. Nat. Ac. Sci., 1886, p. 14, Hamer's and Donnelson's caves, Lawrence Co., Ind.; Clifty cave; Elrod's cave (p. 127), 4 miles west of Orleans, Ind.; Mammoth Cave, Ky. — Hay, Rept. Geol. and Nat. Res. of Ind., xix, 1894, p. 234. — Jordan and Evermann, Fishes N. A., 1896, i, p. 706. — Blatchley, Rept. Geol. Nat. Hist. Res. of Ind., xxi, 1896, p. 183, Sibert's well cave, a part of Little Wyandotte Cave, and in caves near Mitchell, Ind. — Eigenmann, Proc. Ind. Ac. Sci., 1897(1898), p. 230; Degeneration of the Eyes of the *Amblyopsidæ*, its Plans, Processes, and Causes, Proc. Ind. Ac. Sci., 1899, p. 239 (summary). — Eigenmann and Yoder, Ear and Hearing of the Blind Fishes, Proc. Ind. Ac. Sci., 1898 (1899), p. 242. Eigenmann, Eyes of the Blind Vertebrates of N. A., Archiv f. Entwickelungsmech., viii, 1899, p. 545; Pop. Sci. Mo., lvi, 1900, p. 485; Marine Biological Lectures, 1900, for 1899, p. 113. — Cox, Report Bureau of Fisheries, 1904, p. 392, issued 1905.

Most of the Amblyopsidæ are confined to the caves of the Mississippi drainage basin. *Amblyopsis spelæus* has the widest distribution. It is recorded from the following places: Mammoth Cave, Kentucky; Rhode's Cave, near Corydon; Lost River and one of its "Gulfs"; Elrod's Cave, Orange County; Little Wyandotte, near the southern boundary of Indiana; Hamer's and Shawnee Caves in Lawrence County, Indiana; Clifty Caves, near Campellsburg, Washington County. Vague reports of blind fishes have come from near Milford in northern Indiana; from Lansing, Michigan; and from Hiram, Ohio. None of the alleged specimens from the north had been preserved and none could be secured until recently, when I received a specimen of *Amblyopsis* from near Hiram, Ohio, with a letter to Prof. H. H. Lane, in substance as follows:

> Hiram, Ohio, July 7, 1906.
>
> The fish was brought by a student who resided near the place where it was found. The statement made was as follows: The township of Shalersville built a roadway of logs and earth across a swamp, known locally as the Podunk Swamp. The next spring the roadway sank out of sight and in its place there was a canal of reddish brown water. This fish was said to have been caught out of this water. The swamp I have occasionally visited, but have never seen any fish in the water. After the sinking of the road referred to the county rebuilt it at considerable expense only to have it sink out of sight again as before. It has not been touched since and the same stretch of water across it is there to-day. The swamp is one of the kind common to the glacial area and is surrounded by morainic hills. It was no doubt originally a lake and has been converted into a swamp by the growth of vegetable matter.

This specimen makes the other northern records also probable.

The specimens from Milford, Indiana, were reported to have been caught under circumstances identical with those reported for the Hiram specimen.

This species is thus known to be distributed east of the Mississippi, both north and south of the Ohio River, which divides the cave region, and also far north in northern caves or even in glacial swamps. It is probable that it has a very wide distribution in the ground water. It has become quite rare in and about Mammoth Cave. I have visited this cave several times, also Colossal Cavern, Cedar Sinks, and other caves in Kentucky, but so far have not succeeded in capturing or seeing any specimens south of the Ohio River.

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[1] This cave, plate A, has been variously called Shawnee cave, Donnelson's and Donaldson's cave.

I have visited many caves in the Lost River region of Indiana and others have visited different caves without finding this species.

*Amblyopsis* has been pumped out of a well at Mitchell, Indiana. I have taken it in only three caves; one specimen in Clifty Cave and one in Hamer's Cave. The only place where this species is known to be at all abundant is in the caves of the Donaldson farm of Indiana University.

### **Troglichthys rosæ Eigenmann.** Plate 6, Figs. A, B, C.

*Typhlichthys subterraneus*, GARMAN, Bull. Mus. Comp. Zool., xvii, 1889, p. 232, wells and caves, Jasper County, Mo.; not of Girard. — KOHL, Rudimentäre Wirbelthieraugen, 1892, p. 59.

*Typhlichthys rosæ*, EIGENMANN, Proc. Ind. Acad. Sci., 1897 (1898), p. 231, Sarcoxie, Mo.

*Troglichthys rosæ*, EIGENMANN, Science, N. S. ix, 1899, p. 280, Day's Cave, Sarcoxie, Mo.; Degeneration in the Eyes of the *Amblyopsidæ*, its Plans, Processes and Causes, Proc. Ind. Acad. Sci., 1898 (1899), p. 239 (summary); Eyes of the Blind Vertebrates of N. A., Archiv f. Entwickelungsmech., viii, 1899, p. 573; A Case of Convergence, Proc. Ind. Acad. Sci., 1898 (1899), p. 247. — COX, Report U. S. Bureau of Fisheries, 1904, p. 391; issued 1905.

This species has thus far been collected by Miss R. Hoppin and by myself at Sarcoxie, Missouri. Miss Hoppin found it in Wilson's Cave, Day's Cave, Center Creek, and wells. Her reports were published in full by Mr. S. Garman. I found the fish in the fall of 1898, in a pool just within the mouth of Day's Cave. Judging from the localities where it is said they occur either in wells or in caves, the species is distributed over an area 300 miles long by 100 miles broad.

It has been reported to me as occurring in wells at Cassville, Marionville, and Springfield, Missouri, and somewhere in Arkansas, in a spring in Newtonia, from a cave at Joplin, Missouri, and another near Springfield, Missouri, and from Turnback Cave near Marionville. A specimen from Arkansas is said to be in the United States National Museum.

It is said that 7 miles southeast of Lead Hill, in the left hollow off Cane Sugar Orchard Creek, a half mile below an old mill, there is a cave where blind fishes have been found. These were described in such a way as to leave no doubt of the authenticity of the locality. Mr. C. H. Thompson, of the Shaw Botanic Garden in St. Louis, gave the following account of a cave reported to him:

> In a cave about 13 or 14 miles north of Frederickstown, St. François County, Missouri, there is a stream of water averaging 4 to 6 feet wide and 1 to 3 or 4 feet deep. In these deeper "pools" by feeling under the rocks one will find fish which are blind. The stream does not flow out at the mouth of the cave, but a few rods down the slope of the hill, directly below the cave entrance, a large spring breaks out. This is probably the same stream as that found in the cave. The spring forms the source of Coldwater Creek. By consulting the map the source of Coldwater Creek, as there indicated, is northeast of Frederickstown. Coldwater runs in a northeast direction through St. Genevieve County into the Mississippi. From the map the location of the cave is in all probability the extreme southeast corner of St. François County.

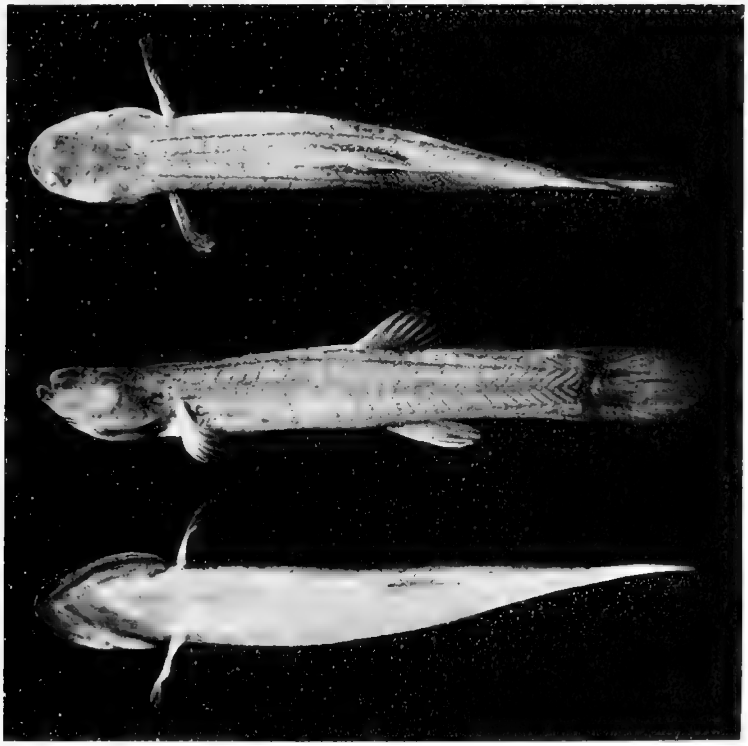
### **Typhlichthys Girard.**

The characters of the three known species of *Typhlichthys* are purely technical and may be summarized as follows:

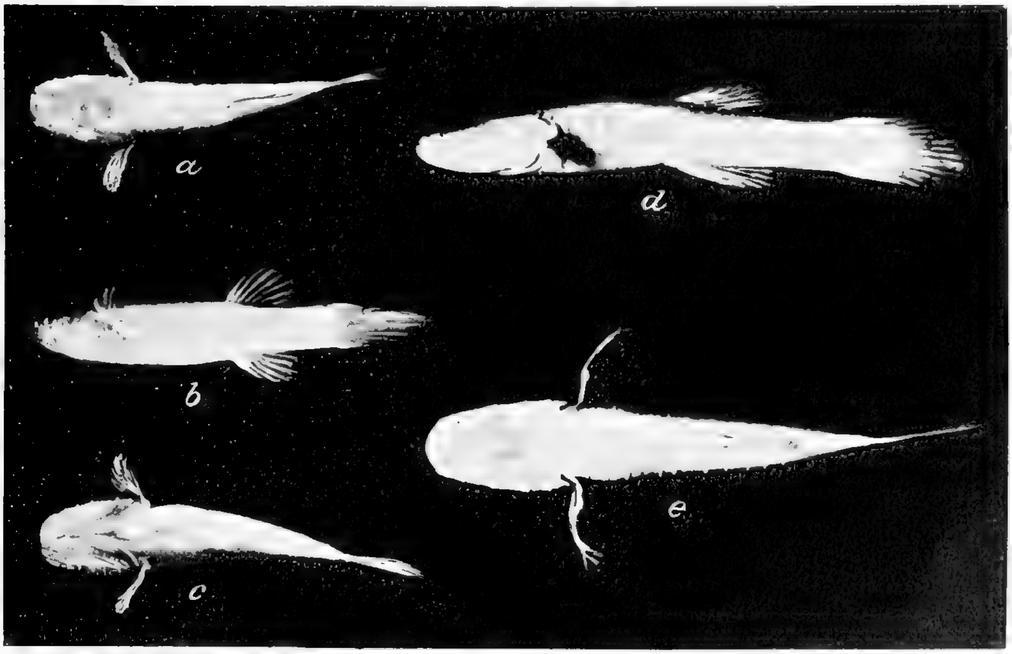
*a.* Width of head more than 6 in length to base of caudal; length of head 3$\frac{2}{3}$; first anal ray nearer base of middle caudal ray than to anus . . . . . . . . *wyandotte*

*aa.* Width of head 5 in length to base of caudal; length of head 3 to 3.4; orbital fat-mass elongate, inconspicuous in life, not projecting; cheeks little swollen; eye on an average 0.16 mm. in diameter, the smallest 0.14 mm. . . . . . . . . . . *subterraneus*

*aaa.* Width of head 4.5 in length to base of caudal; length of head 3$\frac{1}{8}$; orbital fat-mass round and very conspicuous in life, projecting dome-shaped beyond contour of surrounding parts; cheeks much swollen; eye less than 0.10 mm. in diameter . . . . . . *osborni*



Chologaster agassizii. Dorsal, side, and ventral views.



Troglichthys rosæ. (*a*) dorsal; (*b*) side; (*c*) ventral views.
Typhlichthys subterraneus. (*d*) side and (*e*) dorsal views.

**Typhlichthys subterraneus Girard.** Plate 6, Figs. D, E; Text, fig. 28.

*Typhlichthys subterraneus*, GIRARD, Proc. Ac. Nat. Sci. Phila., 1859, p. 62, well near Bowling Green, Ky. — PUTNAM, Amer. Nat., vi, 1872, 17, Mammoth Cave, Ky.; Lebanon, Tenn.; Moulton, Ala. — JORDAN, Rept. Geol. and Nat. Res. of Ind., 1874 (1875), vi, p. 218, Mammoth Cave, Ky. — JORDAN AND GILBERT, Synopsis Fishes N. A., 1883, p. 325. — HAY, Geol. and Nat. Res. of Ind., xix, 1894, p. 234. — JORDAN AND EVERMANN, Fishes North and Mid. Amer., i, 1896, p. 704. — EIGENMANN, Eyes of the Blind Vertebrates of N. A., Archiv f. Entwickelungsmech., 1899, p. 545; Proc. Ind. Acad. Sci. 1898 (1899), p. 239 (summary). — COX, Report Bureau of Fisheries for 1904, p. 389, 1905.

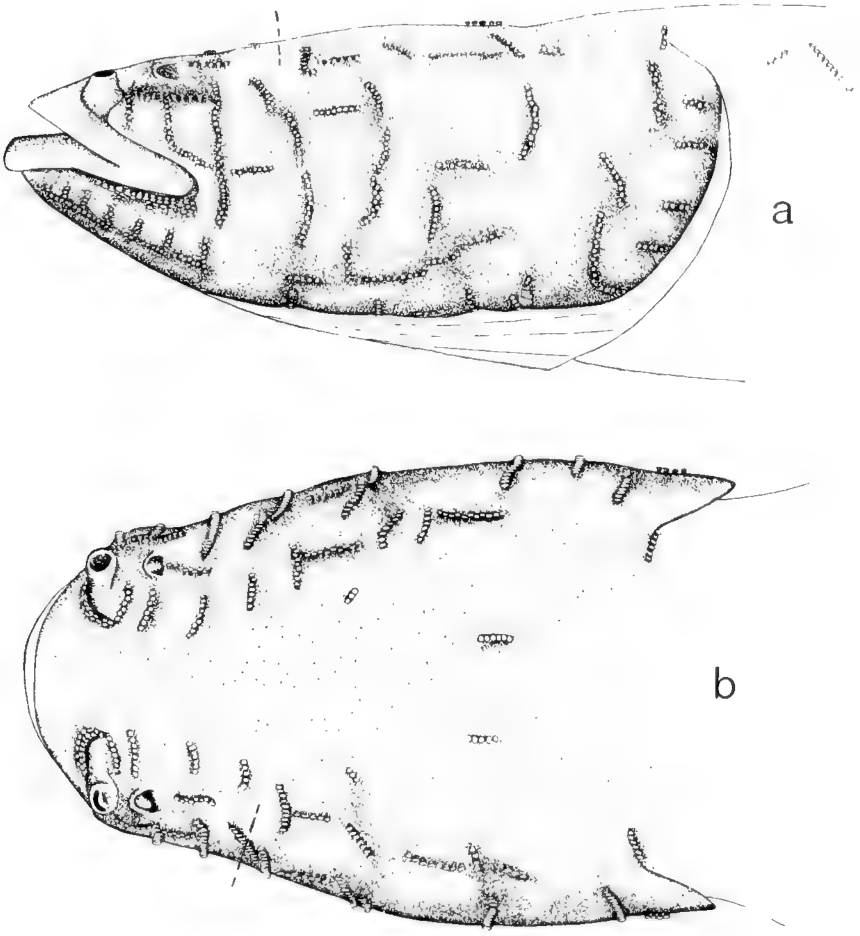


FIG. 28. (*a*) Side and (*b*) Dorsal View of Head of *Typhlichthys subterraneus.*

*Typhlichthys subterraneus* Girard was discovered at Bowling Green, Kentucky, and later found in Mammoth Cave. For a time specimens of this species and of *Amblyopsis* found a ready market at Mammoth Cave, and this probably has had much to do with its later scarcity in this place. It was subsequently caught in other caves, to be sold at Mammoth Cave. The author has taken it in Roaring River of Mammoth Cave, where it was occasionally found swimming freely, but more often under large rocks to be brought out only by tapping the rocks with the net handle or one's foot. The difficulties in collecting this species (as well as other material) in Mammoth Cave arise from the great extent of the cave and the inconvenience of transporting collecting apparatus to the remote places where alone these fishes are to be found.

The author has also taken this species in a small cave at the edge of the town of Glasgow, Kentucky, where it is moderately abundant and easily accessible, but on account of the limited extent of the environment very few were caught on any one trip. One was found under a floating board in this cave. One other specimen was secured after an extensive exploration on foot and on hands and knees in a cave at Cave City, Kentucky.

### Typhlichthys osborni Eigenmann.

*Typhlichthys osborni*, EIGENMANN, Biol. Bull., VIII, p. 63, Horse Cave, Ky.

*Typhlichthys osborni* is known only from Horse Cave, Kentucky. The town of Horse Cave is situated at the junction of two intersecting valleys. Their streams have long ago disappeared from the surface and now flow 185 feet beneath the city. In the heart of the town is a sink or depression with vertical walls which was probably caused by the falling of the roof of a large cavern. At one end of the sink an inclined plane leads into the underground stream, which supplies the city with water. The stream also furnishes the power to light the city. A dam across the cave furnishes the head for the power, but so modifies the conditions above it as to make collecting practically impossible. A convenient break in the dam made

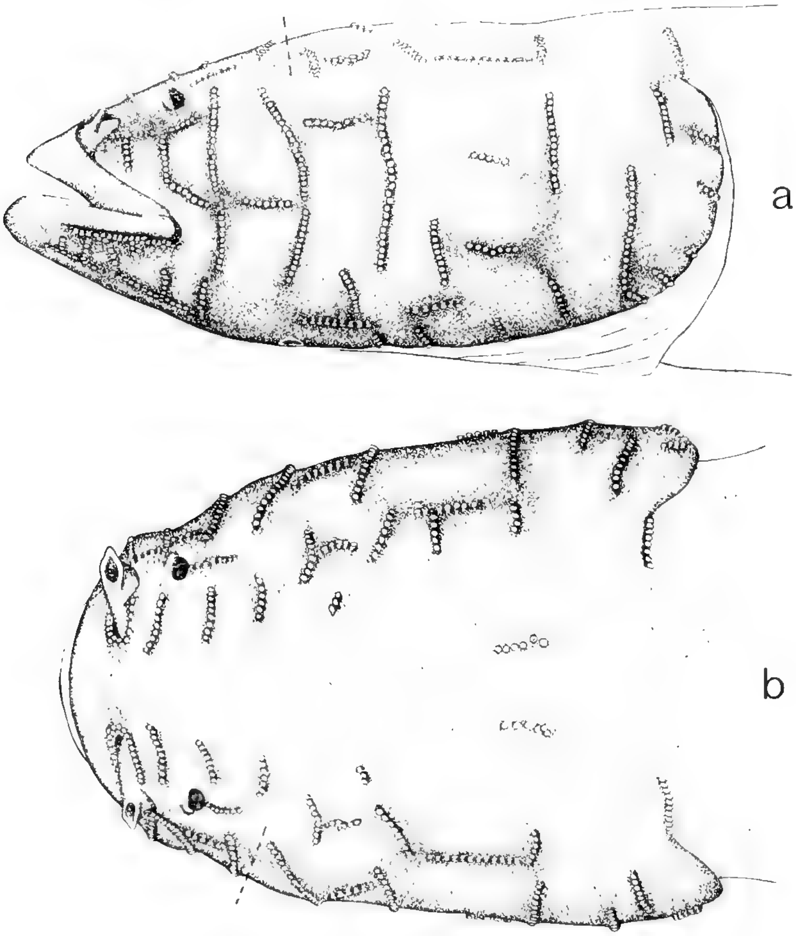


FIG. 29. (*a*) Side and (*b*) Dorsal View of Head of *Typhlichthys osborni*.

it possible, on one of three visits, to ascend the stream to a pile of fallen rocks from under which the water flows and which makes further progress impossible. This stretch is not great. It was noted for the abundance of blind crawfish; no blind fishes were found here. On the right side of this stream, near the entrance, an older, dry channel leads off. At the end of the gallery a small rivulet runs to the left through a series of small pools separated by thin vertical partitions; to the right it expands into a broad stream, quite shallow, but with such a depth of soft mud at the bottom that progress was impossible without a boat. In this expanse *Typhlichthys osborni* was very abundant. In the fall of 1907 this cave was visited again, but no fishes were found where previously they had been abundant.

Below the dam in the main cave the stream is swift and the floor so rock-strewn that progress is difficult and dangerous and fishing unprofitable.

### Typhlichthys wyandotte Eigenmann.

*Typhlichthys subterraneus*, EIGENMANN, Proc. Ind. Acad, Sci. 1897 (1898), p. 230, Corydon, Ind.; not of Girard.
*Typhlichthys wyandotte*, EIGENMANN, Biol. Bull., VIII, Jan., 1905, p. 63.

*Typhlichthys wyandotte* is known from a single specimen from Corydon, Indiana, sent in 1886 by Superintendent Funk of the schools of Corydon to Indiana University. This is the only record of the genus north of the Ohio River. Repeated efforts to secure additional specimens have failed.

*Key to the Chologasters.*

*a.* Eye large, contained 5.5 times in the length of the head.
  *b.* Eye over 1 mm. in diameter; tactile papillæ very small; sides with 3 well-defined longitudinal lines . . . . . . . . . . . . . . . . . . *cornutus*
  *bb.* Eye less than 1 mm. in diameter; tactile papillæ large . . . . . . . *papilliferus*
*aa.* Eye contained 10 times in the length of the head; color very faint . . . . . . *agassizii*

### Chologaster cornutus Agassiz.

*Chologaster cornutus*, AGASSIZ, Amer. Jour. Sci. and Arts, xvi, 1853, p. 135, Ditches of rice fields at Waccama in S. C. — GÜNTHER, Cat. Fishes Brit. Mus., vii, 1868, p. 2. — PUTNAM, Amer. Nat., vi, 1872, p. 30. — JORDAN AND GILBERT, Synopsis Fishes of N. A., 1883, p. 325. — GILBERT, Bull. U. S. Fish Comm., viii, 1888, p. 22, Okefinokee Swamp, Millen, Ga. — JORDAN AND EVERMANN, Fishes North and Mid. Amer., i, 1896, p. 703. — EIGENMANN, Degeneration of Eyes of Amblyopsidæ, its Plans, Processes, and Causes, Proc. Ind. Acad. Sci., 1898, p. 239 (summary); Eyes of the Blind Vertebrates of N. Amer., Archiv f. Entwickelungsmech., viii, 1889, p. 543; Marine Biological Lectures, 1899 (1900), p. 113.
*Chologaster avitus*, JORDAN AND JENKINS, in Jordan Proc. U. S. Nat. Mus., viii, 1888, p. 356, pl. 44, fig. 8, Outlet of Lake Drummond, Dismal Swamp, near Suffolk, Va. — COX, Report Bureau of Fisheries for 1904, p. 386 (issued 1905).

The *Chologasters* have a wide and discontinuous distribution. *Chologaster cornutus* Agassiz has been found in the ditches of rice fields in South Carolina; in the Okefinokee Swamp at Millen, Georgia; and in the Jericho Canal near Suffolk, Virginia, in an outlet of Lake Drummond. Its range is entirely east of the Alleghany Mountains, and it is found in lowland streams only. I visited the locality near Suffolk, but found no specimens.

### Chologaster papilliferus Forbes. Plate 1, Fig. A.

*Chologaster papilliferus*, FORBES, Amer. Nat., March, 1881, and Jan., 1882, Cave spring in southern Illinois. — JORDAN AND GILBERT, Synopsis Fishes N. A., 1883, pp. 325, 890. — JORDAN AND EVERMANN, Fishes North and Mid. Amer., 1, 1896, p. 704. — EIGENMANN, Proc. Ind. Acad. Sci., 1897 (1898), p. 231; Degeneration in the Eyes of the Amblyopsidæ, its Plans, Processes, and Causes, Proc. Ind. Acad. Sci., 1898, p. 239 (summary); Eyes of the Blind Vertebrates of N. A., Archiv f. Entwickelungsmech., 1899, p. 545; Marine Biological Lectures, 1899 (1900), p. 113.

*Chologaster papilliferus* Forbes is known only from cave springs in Clinton and Jackson Counties, Illinois. Most of the specimens have come from a spring in Jackson County, Illinois.

### **Chologaster agassizii Putnam.** Plate 6.

*Chologaster agassizii*, Putnam, Amer. Nat., VI, 1872, p. 22, well at Lebanon, Tenn.; Mammoth Cave, Ky.—Jordan, Rept. Geol. Nat. Res. of Ind., VI, 1874 (1875), p. 218 (reference to Putnam's specimens).—Hay, Geol. and Nat. Res. of Ind., xix, 1894, p. 234.—Jordan and Evermann, Fishes North and Mid. Amer., 1896, I, p. 704.—Eigenmann, Proc. Ind. Acad. Sci., 1897 (1898), p. 230; Eyes of the Blind Vertebrates of N. A., Archiv f. Entwickelungsmech., VIII, 1899, p. 546; Proc. Ind. Acad. Sci., 1898 (1899), pp. 239, 251; Marine Biological Lectures, 1899 (1900), p. 113.

*Chologaster agassizii* Putnam is known only from Lebanon, Tennessee, and caves about Mammoth Cave. I have taken it in the Styx in Mammoth Cave and in Cedar Sinks, near Mammoth. I found the *Chologaster* in only one locality in Mammoth Cave. A short distance after descending the corkscrew the Styx appears on the right. On one visit *Chologaster* was abundant in and around the remains of an old boat, but I secured only a few small specimens on account of their agility and the easily roiled water. They were much more alert than the blind members of the family and made quickly for the lower edge of the wall of the cave, below which many of them escaped. On a subsequent visit the locality had been quite modified, and I secured even fewer specimens than before.

Cedar Sinks, the other locality from which I secured *Chologaster agassizii*, is a highly interesting region. It lies several miles from Mammoth Cave and is reached over a rough road leading, without modifications from the engineer, up and down the steep slopes of the interminable sink holes of the region. Cedar Sinks was formed by the caving in of the roof of an enormous cave room. The vertical walls of the room are still standing. I have been told the bottom of the sink embraces about 4 acres. In the bottom are 2 funnel-shaped depressions holding water. The walls of the funnels are so steep that it is just possible to climb out if one has been foolish enough to slide down. At the base of the highest rock bounding the sink are two openings. One leads to an extensive underground stream which can be followed a very restricted distance; the other, to a stream and cave which must be quite extensive, judging from the inflow of air at the time of one of my visits. Small pools or streams in one of the entrance galleries yielded a few specimens of *Chologaster*.

### THE COLOR OF THE AMBLYOPSIDÆ.

The three species of *Chologaster* are colored with varying intensity from *C. cornutus*, which is darkest, to *C. agassizii* in Mammoth Cave, in which the color is faintest. The color cells are in all cases arranged in a definite pattern. This is determined by the underlying muscles. The pattern consists of three longitudinal bands on the sides following the line where the muscle segments are angularly bent and cross stripes along the line separating successive segments (plate 6, upper figures).

The lower side of the head and the abdomen of *Chologaster papilliferus* are sparingly pigmented and translucent. The underlying liver and gills give the parts a rosy tinge. On the sides and top of the head pigment is abundant. There is a more densely pigmented area extending along the middle of the back, beginning as a narrow stripe at the nape and widening to the dorsal fin behind, where it occupies the entire back. On the sides are 3 narrow stripes, which, owing to the accumulation of pigment in 2 layers, are quite dark. Each stripe has a lighter central band, widest at the middle of the sides. A light band, without the conspicuous bordering dark stripes, runs along the middle of the belly. The sides are thickly covered with a layer of pigment, leaving usually colorless lines where connective tissue separates successive myotomes. On the sides of the tail the pigment is dense on either side of these colorless lines. A dark band extends along the sides of the head through the eye. The top of the head is dark (plate I, fig. A).

The pattern of *Chologaster cornutus* agrees with that of *C. papilliferus*. The longitudinal bands are much darker and wider and without the light central streak. The middle band is much wider than the others and is continued forward to the tip of the snout. The amount of color present varies very greatly with the locality from which the specimens come.

The general color of *C. agassizii* is light gray (plate 6, upper figures). The scales are lighter than the area surrounding them. The color pattern is more striking than in the other species of the genus. Each somite is bordered by a dark line. The lines of successive somites are separated by an almost imperceptible colorless line. A broad, not sharply defined, band extends along the sides. The middle of this is lighter than the margin. Another one extends between the somites and the ventral musculature, another from the nape between the lateral somites and the dorsal muscles, and a diverging one from near the nape to either side of the dorsal fin. Dark areas are caused by the accumulation of pigment along the borders of the small muscles of the fins. Still another dark area is found about the caudal. The ventral surface is white, except the accumulation of pigment along the lines separating the muscles. The fins are uniformly light gray. A light area is found on both the upper and lower part of the caudal peduncle, just within the first short rays of the caudal.

The general color of *Typhlichthys* is cream and pink. It is abundantly pigmented. In younger specimens the pigment is arranged in more definite areas about the head. In the old it is more uniformly distributed, being, however, specially abundant about the brain. The pigment pattern of the body is precisely as in *Chologaster* except that the individual pigment cells are minute and their aggregate not evident except under the lens.

The retention of the color pattern of *Chologaster* in *Typhlichthys* is not less interesting than the retention of similar habits. It is perhaps due to different causes. The color pattern in *Chologaster* is determined by the underlying muscular structure and the retention of a similar pattern in *Typhlichthys* is due to the same underlying structure rather than to the direct hereditary repetition of the color pattern. In *Amblyopsis* the color is much less marked than in *Typhlichthys*. *Amblyopsis* is flesh-colored, ranging to purple in the gill-region, where the blood of the gills can be seen through the overlying structures, and over the liver, which can be seen through the translucent sides and ventral wall. About the head and bases of the fins the color is yellowish, resembling diluted blood. The surface of the body is slightly iridescent and that of the head has a velvety, peach-bloom appearance.

The general pink color of *Amblyopsis* is due to the blood, not to any abnormal development of blood-vessels in the dermis. In the fins where the blood-vessels are near the surface, the general effect is a yellowish color. The surface vessels of the dermis also appear yellowish. It is only on account of the translucent condition of all the tissues, permitting the deeper vessels to show through a certain thickness, that the pink effect is produced. *Amblyopsis* has always been spoken of as white. The term "white aquatic ghosts" of Cope is very apt, for they do appear white in the caves and their gliding motion has an uncanny effect. All alcoholic specimens are white.

The chromatophores in *Amblyopsis* are differentiated and contain color before the yolk is absorbed. The black chromatophores are minute granules, few (15 or thereabout) to the segment. In an older larva the pigment was much more abundant. The eyes are pigmented early, shortly before hatching, and, owing to their pigment, they soon become conspicuous and remain so till the fish has reached 50 mm. in length, when the overlying tissues have become thick. The pigment of the body is lost, or, what amounts to the same thing, does not increase much with age. There is an abundance of pigment cells in the adult, but they are very poor in pigment, and, being in the dermis and covered by the thick layer of epidermis rich in glands, are not apparent. Pigment cells are also abundant in deeper tissues in the adult, so that, while no pigment is visible on the surface, an abundance of chromatophores is present in deeper tissues.

The pigment cells can not be made to show themselves, *i.e.* become greatly pigmented, even by a prolonged stay in the light. The old, if kept in the light, will not become darker; and a young one reared in the light until ten months old not only showed no increase in the pigmentation, but lost its pigment, taking on the exact pigmentless coloration of the adult. Pigment cells appear late in *Amblyopsis.* When the young are two months old pigment is abundant. This pigmented condition is evidently a hereditarily transmitted condition. It disappears with age. In the first instance this disappearance was probably individual. But as in the flounder, the depigmentation has also become hereditarily transmitted, for even those individuals reared in the light lose the color.

Numerous facts and experiments show that, while pigment may be and is developed in total darkness, the amount of color in an individual animal depends, other things equal, directly on the amount of light to which it is habitually exposed.

A number of apparently contradictory observations may be noted:

(*a*) The absence of pigment in pelagic animals or their larvæ, which depend on their colorless condition for their existence, is evidently due to causes entirely different from those preventing the formation of pigment in cave animals. Natural selection has, in pelagic animals, eliminated the color.

(*b*) The migration of pigment granules due to temperature or light and the expanding of chromatophores, when an animal is over a dark background or in the dark, and the contracting over a light background, which may take place at once or at the expiration of several days, is evidently also a different question. The observations of Cunningham, Agassiz, and Semper along this line are of interest.

(*c*) Fischel (A. M. Anat., vol. XLVII, pp. 719–734, plate XXXVI, 1893) has noticed that larvæ of salamanders reared in water at 6° to 7° are dark, while others kept in water from 15° to 58° are light.

(*d*) Flemming (A. M. Anat., vol. XLVIII, pp. 369–374, 1896) found that with uniform temperature in two vessels side by side, the one dark, the other light, the salamander larvæ in the dark vessel develop pigment cells rich in color granules; the larvæ in the white vessels become pale, although the number and character of the pigment cells is not otherwise changed. The difference is entirely due to the character of the vessels, for if the larvæ are taken from the dark to the light vessel, they become light-colored in a few days.

(*e*) Semper ("Animal Life," p. 89) records that "* * * in the tadpoles of our common toads and frogs the pigment is equally well developed in yellow, blue, or red light, and in absolute darkness." This was to be expected, for even in the young of cave animals pigment is, as a rule, well developed.

(*f*) Pouchet (Arch. de Physiol. et d'Anat., 1876, and Rev. Scient., vol. XIII, 1897) has demonstrated that change in color cells, such as are mentioned under (*b*) and (*d*), is brought about by the reflex control of the eye. The section of the great sympathetic nerve puts an end to the changes of color under the influence of light.

The lower and upper surfaces of the flounder, the one protected and the other exposed to the light, give the most striking example, and the argument is clinched here by the fact, noted by Cunningham and McMann, that a flounder whose lower side is for long periods exposed to the light takes on color. Loeb has shown that in the yolk sacs of *Fundulus* embryos more pigment cells are developed if the embryos are kept in the light than when they are kept in the dark. However, in the body, and especially in the eye, the pigmentation was not affected by the absence of light.

The general absence of color in cave animals is conceded. Packard states "as regards change of color, we do not recall an exception to the general rule that all cave animals are either colorless or nearly white, or as in the case of Arachnida and insects, much paler than their out-of-door relatives." Chilton has made the same observation on the underground animals of New Zealand. Similar observations have been recorded by Lönnberg, Carpenter, Schmeil, and Viré.

Hamann enumerates a number of species living both in caves and above ground. In such cases the underground individuals are paler than the others. This confirms similar observations of Packard.

Poulton has mentioned that *Proteus* becomes darker when exposed to the light. This has been verified by others. In *Typhlotriton*, larvæ living at the entrance of a cave are dark, while the adult living farther in are much lighter, but with many chromatophores containing a small amount of color. Epigean fishes found in caves are always lighter in color than their confrères outside.

We have thus numerous examples of colored epigean animals bleaching in caves, and also bleached cave animals turning dark when exposed to the light. We have also animals in which the side habitually turned to the dark is colorless, while the side habitually turned to the light is colored. Finally we have cave animals that are permanently bleached.

Natural selection can not have affected the coloration of the cave forms, for it can be of no consequence whether a cave species is white or black. It could only affect the coloration indirectly in one of two ways: first, as a matter of economy, but since the *individual* is in part bleached by the direct effect of the darkness, there is no reason why natural selection should come into play at all in reducing the pigment as a matter of economy; second, Romanes has supposed that the color disappeared through the selection of correlated structures, a supposition he found scarcely conceivable when the variety of animals showing the bleached condition was considered.

Panmixia can not account for the discharge of the color, since it returns in some species when they are exposed to the light and disappears to a certain extent in others when kept in the dark. Panmixia, Romanes thinks, may have *helped* to discharge the color. In many instances the coloration is a protective adaptation, and therefore maintained by selection. Panmixia might in such instances lower the general average to what has been termed the "birth mean." *Proteus* is perhaps such an instance. But in this species the bleached condition has not yet been hereditarily established, and since each individual is independently affected, "the main cause of change must have been of that direct order which we understand by the term climatic."

Since, however, the bleached condition, which in the first instance is an individual reaction to the absence of light, has become hereditarily established in *Amblyopsis* so that the bleaching goes on even when the young are reared in the light, it is evident that in *Amblyopsis* we have the direct effect of the environment on the individual hereditarily established.

### GENERAL HABITS OF AMBLYOPSIS.

The general impression given by *Amblyopsis* is that of a skinned cat-fish swimming on its back. The largest individual secured by me measured 135 mm. in total length. Individuals as large as this are rare. The usual length of an adult is about 90 mm. At Mammoth Cave I was told of an individual having a length of 200 mm.

*Amblyopsis* is found in pools in the cave streams it inhabits. I have secured as many as 12 from a pool perhaps 10 by 50 feet in size. Very rarely they are to be found in the riffles connecting the pools. I have seen them lying at the bottom, or swimming, rather gliding, through the water like "white aquatic ghosts." In the aquarium they lie at the bottom or at various depths in the water, their axes making various angles with the horizontal, their pectorals folded to their sides. When swimming slowly, it is chiefly by the use of the pectorals. The strokes of the pectoral are lazily given, and the fish glides on after a stroke till its impetus is exhausted, when another stroke is delivered. The fishes frequently roll slightly from side to side at the exhaustion of the result of a stroke. When swimming rapidly, the pectorals are folded to the sides and the locomotion is then similar to that of a salamander, by the motion of the tail. They readily adjust themselves to different depths and are usually perfect philosophers, quiet, dignified, unconcerned, and unperturbed, entirely different from such eyed species as minnows and sun-fishes, which are sometimes found in caves, and which are much more readily disturbed by any motion in the water, making it almost impossible to capture them. The pectorals are also almost exclusively used when quietly rising in the water. At such times the pectorals are extended laterally and then pressed to the sides, beginning with the upper rays. A downward stroke is delivered in this way, not quickly, but with apparent lazy deliberation. In swimming forward the pectorals are brought forward, upper edge foremost. The center of gravity seems to be so placed in regard to their various axes that the fish does not lose its balance whatever its position. It floats horizontally in the water without any apparent effort to maintain its position. It floats with the main axis inclined upward, with the snout sometimes touching the surface of the water, apparently lifeless. Once one was seen resting on its tail in a nearly vertical position, and one while quietly swimming leisurely turned a somersault and swam on undisturbed. At another time the same individual rolled completely over. When one is kept out of water for a short time, it frequently goes in a corkscrew-shaped path through the water, continually spinning around its long axis. In their quiet floating position it is difficult to determine whether they are alive or not.

### RESPIRATION.

The number of respiratory movements of *Amblyopsis* averaged 19 a minute in 5 observations, reaching a maximum of 30 in a small individual and a minimum of 14 in a large one. This is in strong contrast to *Chologaster*, the number of whose respiratory motions reached an average of 80 per minute in 5 observations, with a minimum of 56 and a maximum of 108 in a small specimen. Loeb has called my attention to the more rapid absorption of oxygen in the light than in the dark; this extended would probably mean the more rapid absorption of oxygen through the skin of light-colored animals, a matter of doubtful value, however, to species living in the dark.

The gill filaments are small as compared with the gill-cavity. In addition to the oxygenation through the gills, oxygenation probably takes place through the skin. Ritter has suggested the same for *Typhlogobius*.

Cutaneous respiration is not unique in *Typhlogobius* and the Amblyopsidæ. In the viviparous fishes of California the general surface, and especially the fins which have become enormously enlarged, serve as respiratory organs during the middle and later periods of gestation. The fins are a mass of blood-vessels with merely sufficient cellular substance to knit them together. There is, however, no pink coloration.

Skin respiration would account for the extreme resistance to asphyxiation in *Amblyopsis* and *Typhlogobius*. About 45 examples of *Amblyopsis* were carried in a pail of water 400 miles by rail with only a partial change of water 3 times during 24 hours. A smaller number may be kept for days or weeks — probably indefinitely — in a pail of water without change. The characteristics of *Typhlogobius* along this line have been set forth elsewhere.

### FEEDING HABITS OF AMBLYOPSIS.

The first speculations on the feeding habit of *Amblyopsis* are those of Cope. He remarks:

> The projecting lower jaw and upward direction of the mouth render it easy for the fish to feed at the surface of the water, where it must obtain much of its food. This structure also probably explains the fact of its being the sole representative of the fishes in subterranean waters. No doubt many other forms were carried into the caverns since the waters first found their way there, but most of them were like those of our present rivers, deep-water or bottom feeders. Such fishes would starve in a cave river, where much of the food is carried to them on the surface of the stream.

The speculations of Cope are entirely erroneous as pointed out by Putnam, and we shall see that the deductions based on them naturally fall to the ground.

Dr. Sloan recorded one *Amblyopsis* which he kept 20 months without food.

> Some of them would strike eagerly at any small body thrown in the water near them, rarely missed it, and in a very short time ejected it from their mouths with considerable force. I tried to feed them often with bits of meat and fish worms, but they retained nothing. On one occasion I missed a small one and found his tail projecting from the mouth of a larger one.

Wyman also found a small-eyed fish in the stomach of an *Amblyopsis*.

Hoppin was also struck by the fact that if not capable of long fasts, *Typhlichthys* (*Troglichthys*) must live on very small organisms that the unaided eye can not discern. Garman found in the stomachs of *Troglichthys*, collected by Hoppin in Missouri, species of *Asellus*, *Cambarus*, *Ceuthophilus*, and *Crangonyx*.

All the specimens of *Amblyopsis* from the Mitchell Caves so far examined by me contained very large fatty bodies, a condition suggesting abundance of food. The stomachs, as far as examined, always contained the débris of Gammarus. One young *Amblyopsis* disappeared on the way home from the caves and had evidently been swallowed by one of the larger fish.

The young *Amblyopsis* reared in the aquarium seemed to feed on the minute forms found in the mud at the bottom of its aquarium. Some *Cæcidotea* placed in its aquarium soon disappeared, and the capture of one of these was noted under a reading glass. The fish was quietly swimming along the side of its aquarium; when it came within about an inch of the crustacean it became alert, and with the next move of the *Cæcidotea* it was captured with a very quick, well-aimed dart on the part of the young fish. Others were captured while crawling along the floor of the aquarium.

Mr. Fernandus Payne has made extensive observations and experiments on the feeding habits of this fish, and his notes follow:

> The following experiments have been made to determine what the blind fishes eat and more especially how they detect the presence of their food. Incidentally some correlated reactions have been observed.
>
> In the laboratory, after the fishes had become accustomed to their new conditions, I had no trouble in getting them to eat isopods, amphipods, young crawfish, and diptera and salamander larvæ. They will also take meat from the end of a thread when it is moved about in front of them or brought in contact with the body. The meat, in nearly all cases, is ejected either before or after it has been swallowed. From these observations it seems that they will eat any small animal which moves about in the water. According to my experiments they prefer amphipods. This may be due to the fact that they are more active than the other animals, and hence their presence is more easily detected. If isopods and amphipods are placed in the same aquarium, the amphipods are eaten first. The young of 25 cm. in length readily eat cyclops, daphnids, and aquatic fly larvæ. I have seen them eat fly larvæ until the stomach was so full that they had difficulty in keeping the larvæ from wriggling out again.
>
> In the caves both variety and quantity of food are limited. *Crangonyx* and *Cæcidotea* appear in considerable numbers, but most of them seem to stay under rocks in running water while the fishes are confined to the quiet pools. Young crawfish are certainly not plentiful, for the adults are not very numerous. Whether *Cyclops* or any other small crustacea are present in any abundance, I am unable to say. I know of nothing else on which the young blind fishes could feed. In July, 1906, I took a number of young from the gill cavity of the mother, put them into a box made of cheese-cloth and sunk the box in a quiet pool of water in the cave. They remained in this place for about a month and were growing nicely. I have no doubt they would have lived here much longer had not the cloth become full of holes and freed them. I put no food into the box, so they must have eaten the small organisms in the water.
>
> Fishes must find their food either by the sense of sight, taste, touch, or smell, or by a combination of two or more of these. In most fishes sight undoubtedly plays the predominant part in locating and seizing food. This factor is excluded in *Amblyopsis*. Herrick has given some excellent experiments bearing upon this question. He finds that practically the whole cutaneous surface of *Ameiurus* is sensitive to both tactile and gustatory stimuli, but that the gustatory stimuli are of the greatest value to the cat-fish in procuring food. The hake (*Urophycis tenuis*) catches its food by sight, only when the food is in motion. Bits of meat, etc., lying on the bottom are usually found by the aid of the free ventral fins. From these and other experiments, Herrick concludes that the hake receives both tactile and gustatory stimuli by means of the free fin-rays and to some extent, doubtless, by other parts of the outer body surface. He was unable to determine whether or not smell played any part. When food is thrown into the aquarium the tomcod (*Microgadus tomcod*) catches it while it is falling through the water. The ventral fins are used in locating sapid substances lying on the bottom. He cut the olfactory nerves to see whether smell played any part in the detection of food and found that the fishes with the nerves cut acted in every respect like normal fishes. The sea

robin (*Prionotus carolinus*), according to Morrill and Herrick, finds its food largely by sight and by the use of the free pectoral fin-rays which are tactile in function. The king-fish (*Menticirrhus saxatilis*) uses sight somewhat, but in the main the tactile organs are used as most of the food was taken by contact, and non-nutritious substances were generally taken. The toad-fish (*Opsanus tau*) did not find concealed bait and seemed to get its food wholly by the visual and tactile senses.

Herrick concludes from his experiments that fishes which possess terminal buds in the outer skin taste by means of these organs and habitually find their food by their use. Fishes which lack these organs in the skin have the sense of taste confined to the mouth. The delicacy of the sense of taste in different parts is directly proportional to the number of terminal buds in these areas.

*Amblyopsis* has terminal buds scattered over the entire head. They are most numerous on the lips and the tip of the snout. I did not determine whether or not they were present on other parts of the body. My experiments indicate that, if they are present on parts other than the head, they are but few in number. While these fishes are without doubt able to taste with the buds on the lips and snout, practically all of their food is found by means of the tactile sense. I am unable to say how the terminal buds compare in number with those of other fishes. The young fishes up to 20 mm. in length do not have terminal buds developed. Since this is the case they have only the tactile sense for finding food, for smell plays only a minor part, if any.

Ritter says that in *Typhlogobius* the tactile sense has not only not increased, but has actually diminished *pari passu* with the diminution of the power of sight. Such is certainly not the case in *Amblyopsis*. Eigenmann says:

"(1) The eyes were degenerating and the tactile organs developing beyond the normal before the permanent underground existence began.

"(2) The eyes continued to degenerate and the tactile organs to increase after the permanent entrance to underground waters."

The tactile organs are arranged in rows or ridges. An examination of the number of individual tactile organs in the same 3 ridges in each of 8 fishes gives the following counts:

| Length of specimen. | Number of organs in ridges. | | | Length of specimen. | Number of organs in ridges. | | |
|---|---|---|---|---|---|---|---|
| mm. | 1 | 2 | 3 | mm. | 1 | 2 | 3 |
| 8 | 1 | 1 | 1 | 44 | 16 | 16 | 10 |
| 8 | 1 | 1 | 1 | 48 | 21 | 26 | 18 |
| 28 | 14 | 14 | 10 | 104 | 47 | 42 | 22 |
| 30 | 16 | 16 | 10 | 105 | 42 | 40 | 28 |

Whether the individual tactile organ is more highly developed in the adults than in the young would be difficult to say. At any rate the above figures show that in the adults tactile organs are much more numerous than they are in the young.

Since, in the blind fishes, the factor of sight is entirely eliminated, we have left the senses of taste, touch, and smell by which they may find their food. In testing which of these is concerned I used about 50 individuals. My best results were obtained by placing the fishes in battery jars (one in each jar) 7 inches in diameter and 8 inches high. The water in these jars was from 4 to 5 inches in depth. This enabled me to eliminate all factors except those which I introduced.

During the summer of 1906 I kept a number of fishes in an aquarium in the cave. I tried to get them to eat meat, but had no success. In September of the same year, I transferred other fishes to the laboratory at Bloomington, where my experiments were made. Some individuals begin to eat in a few days, others not till several weeks after they are confined. The young, from 25 to 40 mm. in length, and the adults seem to become adjusted to their new conditions much more readily than those about half-grown. Those from 60 to 70 mm. in length are much more sensitive to mechanical stimuli than either the young or adults, and further their sense of fear seems more highly developed at this time.

When first brought into the laboratory, I kept the fishes in a dark room so as to have the conditions as nearly normal as possible. As this necessitated a light while making the observations, I abandoned it for a lighted room, but several observations were made in the dark room, where I often tried to feed them meat from the end of a thread. After 4 weeks, I got some of the larger ones to take a few pieces, and one large fish took 5 pieces in as many minutes. The next day I found the meat lying on the bottom of the aquarium. In no case did I get the fishes to take meat before it came in contact with the lips. Once when I was feeding them meat, the thread touched the lips of one of the fishes. It immediately snapped at the thread. Before I could bring the meat in contact with the lips, it snapped at the thread a second time. This seemed to indicate that they do not readily distinguish between edible and non-edible substances. Again, I lowered a pair of forceps into an aquarium where there were 15 fishes. All of them were attracted by the disturbance in the water. Fishes 18 inches away turned and swam in the direction of the forceps. I kept the forceps moving just enough to create slight vibrations in the water. Every fish came up and snapped at the forceps and some of them snapped 2 and 3 times. At the least disturbance on the surface of the water these fishes would swim upward as if expecting something to eat. They are able to follow the disturbance anywhere about the aquarium and do it quickly and accurately, turning at any sort of an angle. This experiment was made with non-edible objects, so taste and smell could have played no part whatever. The tactile organs remain as the only means by which these vibrations were detected, located, and followed.

I suspended pieces of fresh beef in the aquarium to see whether the fishes were able to locate it while it remained stationary, but in no case did they pay any attention to the beef. After I had placed the fishes in the light in individual jars, I had no trouble to get the larger ones to take meat from the end of a thread. I also fastened bits of absorbent cotton to the thread as I had done with the meat, and at first they took the cotton just as readily as they had the meat. The cotton was not swallowed, but ejected as soon as taken into the mouth. The fishes turned if any part of the body was touched, but never snapped until the cotton or meat came in contact with the lips. However, after a few trials with the cotton the snap was not so vigorous, and if continued, the cotton was refused altogether. In the course of 4 hours, I got one fish to take the cotton 11 times, but after that it seemed to be able to perceive the difference and though I kept this individual several months no amount of persuasion could induce it to take another piece of cotton. After this it acted toward the cotton just as it did toward the beef until the cotton came in contact with the lips, when it would refuse it. I did get it to take cotton soaked in beef juice. I tried 2 fishes by placing bits of meat on the bottom of the aquarium. In swimming close to the bottom, the meat touched the ventral surface of the body or the pectoral fins. In each case, the fishes stopped, backed up a little until the lips touched the meat, and then snapped at it. This seems to indicate that this species might, in some cases, take food which was not in motion and that it might locate its food partly by taste. I tried these fishes with cotton in the same manner as I had done with the meat, and they reacted in exactly the same way until the cotton touched the lips, when they refused to take it. One fish did snap up one piece of cotton.

I also tested their ability to taste by squirting beef juice on various parts of the body. I got no reaction that I could not get with pure water. I dropped beef juice, a drop at a time, on the surface of the water. The fishes were attracted by the vibrations, came to the surface, and snapped at the drop, but they also reacted in the same manner toward drops of water. They are not able to locate the center of disturbance as readily when the drop falls behind them as when it falls on the side or in front. This experiment again shows how sensitive these fishes are to vibrations in the water and how accurately they are able to locate them.

I might add that slight disturbances, such as the dropping of amphipods into the water, often cause the fishes to sink gradually to the bottom and remain quiet for several seconds, after which they begin to swim slowly about. At this time the swimming is accomplished mostly by the use of the pectoral fins. By a backward stroke, the fins are brought against the body, and then, as the fish glides forward, they are allowed to float out at right angles to the body, the filamentous edge dragging on the bottom. We might term this the "seeking reaction." Amphipods which touch the fins or any other part of the body at this time are snapped up immediately.

I mentioned before that the fishes were confined to the quiet pools. It seems to me that their manner of getting food accounts, in part, for their habitat. They eat living animals, and these animals are found by the vibrations which they make in swimming. In running water the fishes could not detect these vibrations.

A few observations on the memory of *Amblyopsis* may be placed on record in this connection. When the fishes are first brought into the laboratory, they are very sensitive to mechanical stimuli. If kept in a place where they are constantly subjected to stimuli, they soon pay much less attention to them. I kept some fishes in battery jars on my table. At first, when I struck the table lightly, they always responded by springing upward. After a few weeks they responded much less often, and after several months they paid very little attention to jarring of any kind.

It was mentioned before that one fish, in the course of 4 hours, took 11 pieces of cotton from the end of a thread and after that refused to take it again, although the fish was kept for several months. In this case, then, it learned to discriminate within a very short time, and remembered the difference between the cotton and the meat. It took the meat, if brought in contact with the lips, after it refused the cotton.

Another fish was tested by dropping water on the surface of the aquarium. The fish came to the surface and grabbed at the drop. I tested the fish once every day for 12 days, and on the twelfth day it refused to grab, but came up near the surface, poised as if ready to grab, and then sank slowly toward the bottom. The thirteenth day it responded, but not very readily. For the next 8 days I tested it every day and got no attempt at grabbing, although it came near the surface every day. I did not test it again for 3 days, when it again snapped at the drop. It came up to the surface at the first few drops, but sank gradually toward the bottom. Upon continuation of the dropping, it came up again and grabbed. I then left it undisturbed for 5 days before testing and again it grabbed. This was the twenty-ninth day of the experiment. I then started with an interval of 1 day and increased it by 1 day each time, thus making the intervals 1, 2, 3, 4, and 5 days. It did not snap at the drop until after the interval of 5 days. This was the forty-fourth day of the experiment. I again waited 5 days before testing the fish and got no response further than that the fish came near the surface. On account of the lack of time the experiments were discontinued. Whether the fish would eventually have learned not to snap at the drop, I can not say, but that memory plays some part in its reactions is evident from my observations.

The conclusions reached are as follows:

(1) Sight is as a matter of course excluded from food seeking.
(2) The olfactory sense, if any, plays a very minor part in detecting food.
(3) The sense of taste enables them to discriminate between things in contact with the snout.
(4) The tactile sense is the one by which they find and precisely locate their food.

## THE HABITS OF CHOLOGASTER.

The following extract, from a letter from Mr. E. B. Forbes, is of interest:

> Doubtless you have received the little *Chologaster* which I sent you yesterday. The spring in which they are found is in an almost inaccessible part of Jackson County and I drove 17 miles from Cobden, Illinois, in a wagon to this place. The spring is a very large one, flowing from the bottom of a 250-foot cliff of flint and limestone. The little fishes were found under stones at the edges of the spring, very close to the bluffs, and when disturbed they swam back under the cliff. After the rough drive home they were still alive and seemed vigorous when handed over to the expressman. I found this species in other springs than the large one mentioned and have no doubt that it is rather widely distributed. None were found at any considerable distance from the face of the cliff.

I found that *Chologaster agassizii* acts similarly in the River Styx in Mammoth Cave. As soon as my net touched the water they darted in under the ledge of rock at the side of the little pool in which I found them.

The *Chologaster* in general make-up is like *Amblyopsis*, but somewhat more elongate. It sits with its pectorals extended. When it moves horizontally for some distance the pectorals are usually pressed to the sides, the propelling being done largely by the tail, very much after the manner of a salamander, which it resembles. In swimming toward the surface it uses its pectoral fins chiefly, and the fish usually sinks to the bottom as soon as its efforts to raise itself are stopped.

Individuals kept in aquaria with one end darkened either collected in the darkened area, floating about, or under leaves or sticks in any part of the aquarium. They are frequently found under a floating board where they float with the tops of their heads in contact with the board, their bodies slanting downward.

*Typhlichthys*, living in total darkness, has retained the habit of staying under floating boards and sticks and under stones. Miss Hoppin noticed that *Troglichthys* swims with its back to the aquarium, and I have repeatedly noted the same in the young of *Amblyopsis* up to 50 mm., and the still younger *Amblyopsis* frequently hides under rocks.

*Chologaster papilliferus* detects its food entirely by the sense of touch. Two which were kept in an aquarium for over a year were starved for a few days. They became very nervous, continually swimming along the sides of the aquarium. Some individuals of *Asellus* were introduced. These, though quite near, produced no effect if moving in front of *Chologaster*. The moment one came in close proximity to a fish from any direction, by a flashlike motion it was seized. None of them were swallowed. The fishes became very alert after the introduction of the sowbugs and when swimming forward would strike at a part of a leaf if it came in contact with the head of a fish. It seemed evident that the eye gave no information of the character of the object. As the *Asellus* was not altogether to their taste, *Gammarus* was introduced. One of these, swimming rapidly toward the chin of the *Chologaster* from behind and below, was instantly seized when it came in contact with the fish. The eye could not have located the *Gammarus* at all. The action is in very strong contrast to the action of such a fish as *Lepomis*, which detects its food by sight. It is undoubtedly this peculiar method of locating and securing food which has enabled the Amblyopsidæ to establish themselves in caves.

On March 20 the eyes were removed from 7 living specimens of *Chologaster papilliferus* with the following results:

Within half an hour after removing the eyes, examples of *Asellus* were introduced into the aquarium, which were readily detected and captured. In capturing them the chologasters were not as accurate as fishes might be expected to be that do not ordinarily depend on their eyes to help in locating prey. It may be borne in mind, however, that the eyes were removed from the surface and that in addition to the removal of the eyes some of the tactile organs were probably disturbed or destroyed.

A rod held in the hand was readily perceived by the blinded fishes, who avoided it with as much dexterity as an *Amblyopsis* would, except that their actions in avoiding the rod were very much quicker than the action of an *Amblyopsis*. The latter, if approached from in front, will back water with its pectorals and then, if the rod comes nearer, it will turn to one side or another, frequently with lazy deliberation. Chologasters, on the other hand, would turn tail with a flashlike motion when the stick was approaching them. They could be approached from the back more readily than from other regions.

The action of the blinded fishes was in this respect precisely like that of an unblinded one in the same aquarium. Removing the eyes makes no appreciable difference in the appearance of the fish, and a number of colleagues were asked whether the fishes were detecting the rod by sight (with the eyes) or by tactile sensation. Not knowing that the eyes had been removed, the verdict, in the majority of cases, was in favor of the eyes; in the other cases it was doubtful. There was no general disturbance of the fishes in the aquarium when the rod was introduced. Only the ones immediately concerned responded.

On April 4 I was able to touch each of 5 blinded chologasters on the snout with a glass rod before it made any attempt to get away. The same is true of some which had not been blinded.

The blinded chologasters readily swim about in the aquarium, regardless of protection or of contact with the sides of the aquarium. They not infrequently lie at the bottom, but the general tendency is to swim about freely. One of them lived for 2 years after the operation.

At 10 a. m. of one day the blinded fishes were removed from the large aquarium and replaced by a number with eyes. These at first remained at the bottom, but on the following morning they were swimming about as the blinded ones had been. The general conclusion from these experiments is that the *Chologaster papilliferus* with comparatively well-developed eyes can get along without them just as well as with them.

### REACTIONS TO LIGHT.[1]

A long series of observations and experiments was made to determine the reaction of *Chologaster* and *Amblyopsis* to white and monochromatic light. Incidentally other characteristics were brought out.

Some previous experiments on blind or blinded vertebrates may be recalled. Dubois (Compt Rend., t. cx, pp. 358–360) and Semper (p. 79) record that *Proteus*, the blind salamander of Europe, is sensitive to diffuse light. Graber records that blinded salamanders prefer dark chambers to light ones. Korang (Centralblatt f. Physiol. VI, pp. 3–6) notes that concentrated light deprived of heat rays thrown upon the leg of a frog whose brain had been laid bare and covered with extract of beef, caused it to respond each time with reflex movements.

That *Amblyopsis* avoids the light, even the diffuse daylight of a room, is without question. An aquarium was divided in the center by a black partition; one end of the aquarium was covered and the sides painted black, and a small opening was left in one of the lower corners of the partition to enable the fishes to move readily from one chamber to the other. The fishes had no difficulty in finding this opening, and at the beginning of the experiment, before the fishes had quieted down from the excitement incident to moving them, they swam back and forth from one chamber to the other as rapidly as it was possible to note the changes. The following are some of the results obtained at separate times:

*Experiment I:* Observation on 6 individuals placed in the above aquarium, May 12, 1906, gave, between 9.43 a. m. and 10.20 a. m., 104 events in the dark, and 220 in the light.

This would indicate that the fishes have a preference for the diffuse daylight of the room over that of the dark chamber. But these specimens had been in the light several days, so the light-perceiving or light-reacting organs may have been fatigued. Subsequent events and tables indicate the opposite in such a striking way that the evidence is conclusive. A rapid moving of different individuals from one chamber to another was due to the excitement caused by preparing the aquarium, and the preference shown for one or the other conditions of illumination was entirely overcome by the excitement produced.

*Experiment II:* Conditions as in the first experiment with the same 6 individuals in the afternoon of the same day, the aquarium placed so that sunlight entered the lighted end of the aquarium. Result, 114 events in the light, 204 in the dark.

The second experiment shows that there is an inclination to seek the dark rather than sunlight. That the fishes had not gotten into a normal condition is evidenced by the rapid changes of different individuals from light to dark and *vice versa.* Toward evening as the direct light was excluded the fishes began to go over to the lighted compartment.

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[1] For further studies see Payne, Biol. Bull. XIII, pp. 317–323.

*Experiment III:* On May 13 the same 6 specimens were used under the same conditions as in experiment I. The aquarium had been quiet since 5 p. m. the evening before.

| Time. | In the dark. | In the light. | Time. | In the dark. | In the light. | Time. | In the dark. | In the light. | Time. | In the dark. | In the light. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A. M.<br>h. m. s. | | | A. M.<br>h. m. s. | | | A. M.<br>h. m. s. | | | A. M.<br>h. m. s. | | |
| 9 21 0 | 3 | 3 | 9 27 50 | 4 | 2 | 9 35 00 | 5 | 1 | 9 45 3 | 6 | 0 |
| 9 21 20 | 4 | 2 | 9 28 50 | 3 | 3 | 9 36 15 | 4 | 2 | 9 45 15 | 5 | 1 |
| 9 21 30 | 5 | 1 | 9 29 30 | 4 | 2 | 9 37 0 | 5 | 1 | 9 46 15 | 4 | 2 |
| 9 21 35 | 4 | 2 | 9 29 50 | 5 | 1 | 9 38 0 | 4 | 2 | 9 48 0 | 5 | 1 |
| [1]9 22 20 | 4 | 2 | 9 30 0 | 4 | 2 | 9 42 0 | 5 | 1 | 9 48 40 | 4 | 2 |
| [2]9 22 30 | 5 | 1 | 9 32 20 | 3 | 3 | 9 42 30 | 4 | 2 | 9 49 0 | 5 | 1 |
| 9 24 20 | 4 | 2 | 9 32 50 | 4 | 2 | 9 43 30 | 5 | 1 | [4]9 50 15 | 4 | 2 |
| [1]9 25 30 | 4 | 2 | 9 33 0 | 5 | 1 | [1]9 43 50 | 5 | 1 | 9 50 30 | 5 | 1 |
| [3]9 26 10 | 4 | 2 | 9 33 50 | 4 | 2 | 9 44 30 | 4 | 2 | [1]9 51 0 | 5 | 1 |
| 9 26 50 | 3 | 3 | [1]9 34 10 | 4 | 2 | 9 45 0 | 5 | 1 | | 169 | 165 |

[1] Two exchanged.

[2] One other came out, went back.

[3] Two exchanged, the one last out returning.

[4] One came out, but went back at once.

*Experiment IV:* On May 13, from 2 to 3 p. m., during the period corresponding to the time when records were made the day before, the fishes stayed in the dark chamber except occasionally when one would come into the light only to quickly turn and swim back into the dark.

*Experiment V:* On May 15 the fishes remained in their dark chamber nearly all day except during the excitement caused by changing the water, when they swam freely into the light. It is evident that the incessant changing during the first observations recorded was due to excitement caused by the change of water and aquarium.

A small opening was made in the front of the dark chamber, through which observations were made. A few individuals on this occasion came out.

*Experiment VI:* On May 17 no blind fishes were in the light chamber between 8.30 a. m. and 9.20 a. m. Through an opening in the top of the dark chamber several were observed to come to the opening between the two chambers but quickly to withdraw. The sides of the light chamber were painted with a wedge-shaped dark area the better to protect the dark chamber from oblique rays.

*Effect of jarring.* — The aquarium was moved slightly in order to note the effect of jarring. While no fishes had been in the light chamber during the morning, 4 were now out in a few moments; these returned and during 7 to 10 minutes the changing to and from the dark chamber was kept up.

At $9^{h}$ $30^{m}$ 17 approach opening of dark chamber without going out. 16 approach corner above the opening.
At $9^{h}$ $37^{m}$ drew off 2.5 inches of water to 0.5 inch from level of top of opening.
At $9^{h}$ $45^{m}$ one came to opening and returned; 7 went through opening. Evidently still some disturbance.
Left the observations at $9^{h}$ $48^{m}$.

After the fishes had become quiet it was seen that while they were constantly moving past the opening it was rare that one passed out into the light chamber, and then they invariably showed signs of uneasiness, frequently turning sharply round and reëntering the dark chamber, at other times making a complete circuit; this at a time when there was no direct sunlight.

At 12 m. a dark tunnel was constructed by leaning a black pane of glass against the dark partition, leaving an opening at the side of the aquarium opposite to that in the opening of the first partition. For some time after this was done the fishes stayed in the light chamber in which they had been put, without being able apparently to find their way out. After a day, however, all had collected in the dark chamber and it was rare that any of them came out into the light chamber. They remained in the dark chamber for days without coming out, except occasionally at night. On May 24 the blind fishes remained in the dark compartment until night, when all collected in the light compartment, only to be found back again in the dark the next morning.

Everything indicates that they readily perceive light, even the diffuse light of a room, and that they individually react negatively to light.

Four *Amblyopsis* which had been kept for a day in a vessel painted black and covered to exclude the light were experimented upon as follows: a ray of light from a microscope mirror about 2 inches in diameter was thrown on each successively. After from 1 to 5 seconds the fishes became uneasy, the uneasiness giving place to discomfort, the fishes making vigorous efforts to get out of the ray.

Another jar, not painted, containing both *Amblyopsis* and blind Cambarus, was placed where light could be reflected upon them from the mirror of a microscope. The Cambarus, if in motion, came suddenly to a halt; if quiet, it backed or moved off at once. The fishes also responded to the light but it took several times as long for them to do so.

Bright sunlight appears to be irritating; if exposed to it, the fishes swim about uneasily. A shadow passed suddenly across them when in the diffuse light of a room does not affect them, nor do they, when swimming, seem disturbed by a ray of light entering the dark chamber through a small hole in the paint made for the experiment.

Two examples kept in a pail in my cellar were quietly floating, but when a lighted match was held above them the fishes at once darted to the bottom and sides of the pail. The heat could not have been a factor in this case; the reaction to the light of the match was quick and violent.

A similar observation was made on 40 individuals in two aquaria. They were captured one morning, and the observation made the second night after. They had been kept in the dark during most of the intervening time. A lighted match, held near the aquaria, produced a very general and active movement among all the individuals.

Even more striking than this was the action of a colony of *Amblyopsis* in an open pool. During the bright part of the day the fishes remained under the rocks at the bottom. Occasionally a nose could be seen poking out from under a rock; perhaps one of the fishes came out at times during the day. In the morning and evening and at night, they could be seen swimming in various parts of the pool.

The following experiments make it evident that the direction of the light does not influence the actions of these fishes, but that their behavior is due to a perception of difference in the intensity of light. A large box, covered at its southern end, was sunk into the ground where the water of a spring flowed through it. Throughout the lighter parts of the day the fishes stayed in the shade of the southern part of the aquarium. It was only in the evening, in the morning, and at night that the fishes ventured forth. A similar box 2 × 4 × 8 feet, divided in the middle by a partition running to near the bottom, had lids hinged so that either or both compartments could be covered and darkened. Within a short time after one of the compartments was darkened all of the individuals would be found in the darkened compartment, irrespective of the direction of the sun's rays.

Mr. F. Payne has made further studies and found that their negative heliotropism is sufficient to overcome their positive geotropism if an 800 candle-power arc lamp is used 16 inches from the aquarium. He also found that the young fish to an inch in length react more strongly to light than older ones, even if their eyes are destroyed, and that one part of the body is as sensitive as another to a pencil of strong light.

The 7 blinded chologasters mentioned previously were placed at 9 a.m. in an aquarium which was dark at one end and light at the other, but with no partition between. In the bottom of this aquarium, extending from the lighted into the darkened area, was placed a plate of glass propped up at one edge so as to enable the fishes to get under it. The conditions in the two parts of the aquarium were as nearly alike as possible except as to light. The blinded chologasters collected in the darkened half of the aquarium and remained there. The reaction was quite positive. No sunlight entered the aquarium — only the diffuse light of the room. The same reaction took place when sunlight entered the aquarium.

Later, the pane of glass was taken from the bottom of the aquarium and placed against its sides, and the fishes collected behind it in the dark end. A number of normal chologasters in another aquarium had the same habit of squeezing themselves in between the sides of the box in which they were and the small glass aquarium placed in it. It is evident that *Chologaster* is also negatively heliotropic and positively stereotropic.

A series of observations was made to determine to what rays, if any, *Amblyopsis* reacts most vigorously. For this experiment a glass jar 3 feet long and 8 inches in diameter was divided into 6 compartments by 5 partitions. Each partition had a vertical slit extending half-way up from the bottom to enable the fishes to swim freely from one compartment to another. The compartments were thus all connected. A cap was screwed tightly over the end of the jar, which was placed horizontally in a window-sill where each compartment would have an equal amount of light.[1] The jar was surrounded with bands of tissue paper in several layers of violet, blue, green. orange, and pink so that each compartment was lighted by one series of rays, Three *Amblyopsis* were used for these observations; they were selected for their size and named, A, the smallest, B, the middle-sized, C, the largest. These fishes had been in confinement some time, but had been transferred from the cave, with as little exposure to light as possible, to a dark room where they were very seldom exposed to the light. Observations were made as opportunity presented itself.

It was found that some compartments were visited by a certain fish without any definite regard for color. During January, for instance, fish C moved out of the pink and orange compartments but once; fish A remained almost exclusively in yellow, visiting pink once, orange once, and green 4 times. Fish B, on the other hand, remained mostly in the violet, visiting blue 7 times and green 3 times. From this we must conclude either that different individuals react differently or that one color does not produce a stronger reaction than another, and the latter seems the more reasonable conclusion. (See table on page 91.)

To determine whether the apparatus had anything to do with the distribution and also whether widely separated elements of the spectrum would cause the fishes

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[1] For over a month these fishes were sealed in this jar without change of water.

*Series of Observations to determine to which Rays Amblyopsis react most Vigorously.*

| Date. | Time. | Violet. | Blue. | Green. | Yellow. | Orange. | Pink. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| 1896 | h. m. | | | | | | | |
| Dec. 16 | 9 30 | A, B | .. | .. | C | .. | .. | .. |
| | 10 | B, C | .. | .. | .. | A | .. | .. |
| | 2 | A | C | B | .. | .. | .. | .. |
| 17 | 9 30 | .. | .. | .. | A | .. | B, C | .. |
| | 12 30 | B | .. | A, C | .. | .. | .. | .. |
| 18 | 10 30 | A, B | .. | .. | C | .. | .. | .. |
| 21 | 9 | A | .. | B, C | .. | .. | .. | .. |
| | 12 | B | .. | .. | .. | A, C | .. | .. |
| | 1 | B | .. | .. | C | .. | A | .. |
| 22 | 10 | B | .. | .. | A, C | .. | .. | .. |
| 23 | 2 | A | .. | B | C | .. | .. | clear |
| 26 | 1 | B | .. | A | .. | .. | C | cloudy |
| 1897 | | | | | | | | |
| Jan. 4 | 10 | .. | B | A | .. | C | .. | cloudy |
| 5 | 10 | B | .. | A | C | .. | .. | snowing |
| | 12 | B | .. | .. | A | C | .. | snowing |
| | 4 | .. | B | .. | A | C | .. | snowing |
| 6 | 4 | B | .. | .. | A | .. | C | .. |
| 7 | 4 | .. | B | .. | A | C | .. | clear |
| 8 | 4 | B | .. | .. | A | C | .. | clear |
| 9 | 12 | .. | B | .. | A | .. | C | clear |
| 11 | 10 | .. | .. | B | A | .. | C | broken |
| | 4 | B | .. | .. | A | .. | C | clear |
| 12 | 10 | .. | B | .. | A | .. | C | clear |
| | 12 | B | .. | .. | A | .. | C | cloudy |
| | 4 | .. | B | .. | A | C | .. | cloudy |
| 13 | 10 | .. | B | A | .. | .. | C | cloudy |
| 14 | 12 | B | .. | .. | A | .. | C | cloudy |
| | 4 | B | .. | .. | A | C | .. | cloudy |
| 15 | 10 | B | .. | .. | A | .. | C | cloudy |
| | 4 | B | .. | .. | A | .. | C | cloudy |
| 18 | 10 | .. | .. | A, B | .. | .. | C | clear |
| | 12 | B | .. | .. | .. | A | C | clear |
| | 4 | .. | .. | B | .. | .. | A, C | clear |
| 21 | 1 | B | .. | .. | A | C | .. | cloudy |
| | | 26 | 8 | 14 | 24 | 12 | 18 | |
| Fish No. C | | 1 | 1 | 2 | 6 | 9 | 15 | |
| B | | 20 | 7 | 6 | | | 1 | |
| A | | 5 | 0 | 6 | 18 | 3 | 2 | |
| Total | | 26 | 8 | 14 | 24 | 12 | 18 | |

to react positively or negatively, they were put into a rectangular aquarium impervious to light, except at the ends, and divided by a median partition. The ends were covered with translucent celluloid film, care being taken, of course, to have each end equally light. Random observations taken through 20 days show: A, once in the blue compartment and 34 times in the red; B, 6 times in blue and 39 times in red; C, 27 times in blue and 18 times in red; a total of 34 times in the blue and 91 times in the red.

If only A and B had been used, we would have been justified in concluding that *Amblyopsis* is positively tropic toward the red end of the spectrum as against the blue. If only C had been used, we would have been justified to draw the opposite conclusion. The fishes in the red compartment had become nervous and were swimming near the red window, that is, on the side opposite the opening between the compartments. Their proneness to remain in the same compartment may have been partly due to this nervousness, the cause for which was not apparent.

Four specimens of *Chologaster* were placed in the apparatus having 6 different colored compartments. Between January 26 and February 4 rather irregular observations were made.

The number of specimens for each compartment on a purely chance distribution would have been 12.6, leaving out of consideration the element that the end compartments contained but one opening, only one compartment bordering each. A strong positive reaction toward violet is indicated, and a strong negative reaction toward pink and blue. The totals were: violet, 25; blue, 6; green, 11; yellow, 13; orange, 14; pink, 7.

To test these results, the second aquarium, with but two compartments and three specimens, was used. The specimens are marked A smallest, B medium sized, C largest. In series I, 20 out of 24 events occurred in the red. The windows were interchanged, transposing colors, when, in series II, out of 24 events 13 occurred in the red. This indicates a decided positive reaction toward the red. In series III, 16 out of 20 events occurred in the red. A new aquarium was substituted, with the windows side by side, looking toward a west window. Out of 17 events (series IV) 13 occurred in the red. The colors were then interchanged, so the fishes would be compelled to change compartments in order to be in the same light. In this series 27 out of 29 events occurred in the red. These series give conclusive evidence that the affinity of these fishes is strongly in favor of the red. It may also be noted that the smallest specimen was most frequently found in the blue.

### BREEDING HABITS OF AMBLYOPSIS.

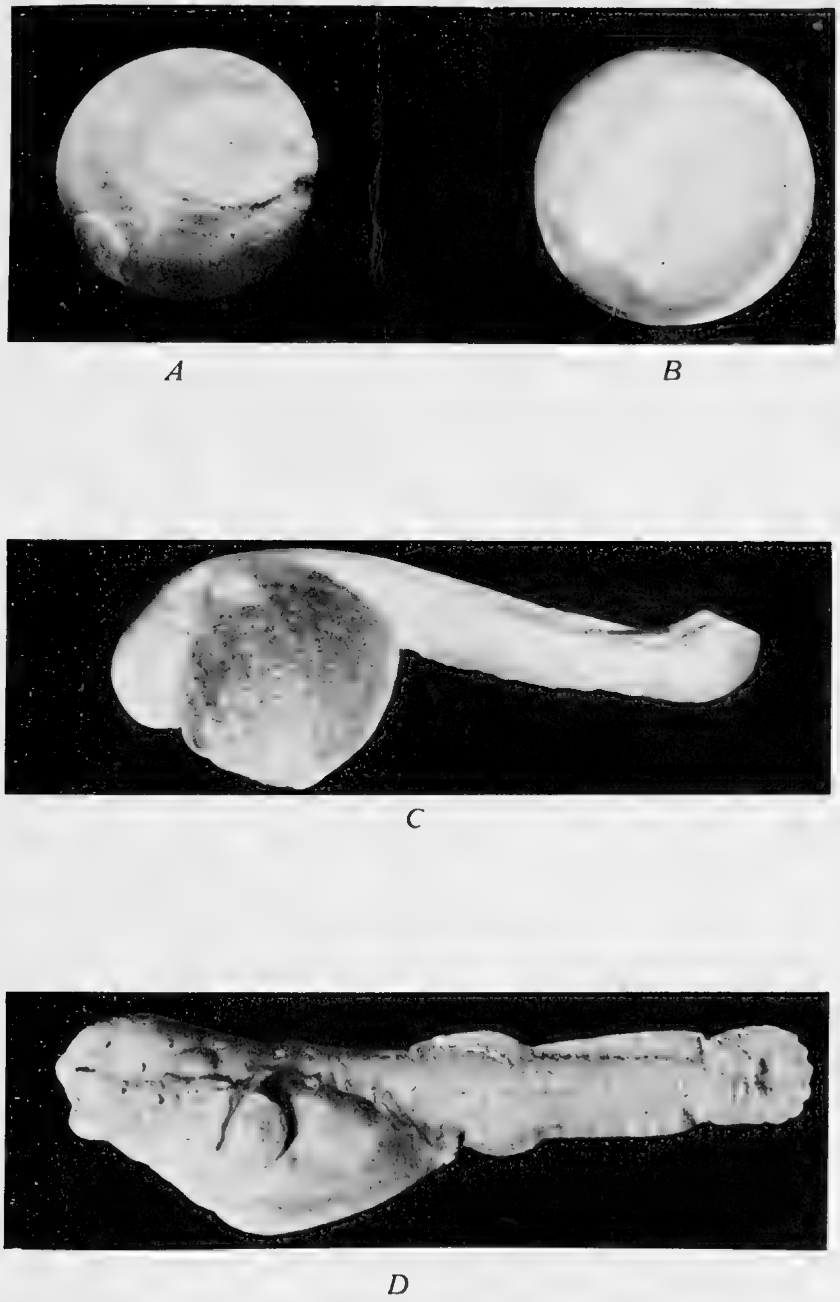
The eggs are laid by the female, to the number of about 70, into her gill chamber. Here they remain for perhaps 2 months, till the yolk is nearly all absorbed and the young fish has attained a length of about 10 mm. If at any time a female with young in her gill pouches is handled, some of them are sure to escape. This was observed and gave rise to the idea that this species is viviparous.

We owe the first observations on the breeding habits of *Amblyopsis* to Thompson, who states that a fish "was put in water as soon as captured, where it gave birth to nearly 20 young, which swam about for some time, but soon died * * * they were each 4 lines in length." It is unfortunate that the highly interesting supposition of Thompson that they were viviparous has gained common currency.

Putnam adds to the above, judging from some data in his possession, that the young are born in September and October, and further along remarks that they are "undoubtedly" viviparous.

The first young I obtained were secured on May 9, 1896. The little fishes could move actively for a few moments, but as they were encumbered with much yolk, they soon settled to the bottom and remained quiet. A large number of old ones were in the water in which the young were found, and the mother of this lot was not identified with certainty. Another lot of young obtained on September 5 of the same year were much farther along in their development. Some were preserved and others placed in various aquaria, where one lived to be 10 months old. As before, the parent was not with certainty determined, simply because it was taken for granted that they were viviparous and the ovaries only were examined. Two other lots of young were obtained on June 5, 1897. One of these lots was in the stage of the first lot obtained, with a large amount of yolk still present, while in the other lot the yolk had almost entirely disappeared. These had been carried in the gill cavity of the mother, and it became evident either that the fishes were not viviparous at all, or that their viviparity was not nearly of the pronounced character hitherto supposed.

On March 11, 1898, 29 individuals were captured. Four were females with eggs in their gill cavities. The youngest stage among these was at the end of segmen-



Views of Amblyopsis, in the early stages.

A and B. Embryos on egg, (A) younger stage, (B) older stage.
C. Larva at time of hatching.
D. Older larva.

tation, the oldest was a gastrula covering but one-third of the yolk. The eggs had not been developing more than 5 days, probably not more than 2 at the utmost, and decided beyond a doubt that these fishes are oviparous and not viviparous. In one individual 61 eggs were found, in another 70. The exact number in the other two, I can not give, but the number does not differ greatly from the above. From one side of one I took 35 eggs, from another individual an uncertain number. The remaining eggs were left in the gills to develop, but all that were not subsequently preserved finally died.

The female with eggs can readily be distinguished by her distended gills, and since dead eggs become opaque, such can readily be distinguished through the translucent opercles and branchiostegal membrane. Dead eggs are retained in the gill cavity till they disintegrate.

I have never secured as many young from any female as the eggs enumerated above. This may have been either on account of the dying of many eggs or the liberation of the young during the struggle of capture.

Emphasis need be laid on the fact that *Amblyopsis* is *not viviparous* and that its breeding period extends at least from the first of March to November and probably throughout the year. A female with nearly ripe eggs was secured on September 9, and since these would have been carried either as eggs or young for about 2 months longer, November is a safe limit. During March the spawning season is evidently at its beginning, and it is during this month and April and May that the early stages may be looked for with the greatest confidence.

No eggs were deposited in the laboratory. Females with eggs in the gill cavities had to be sought for in the caves. To secure embryological material when a female containing favorable stages was captured, she was isolated in a small aquarium and the number of eggs needed freed from the gill cavity by gently raising the edge of the operculum. The rest of the eggs were permitted to remain in their natural surroundings until another lot was wanted. During the early stages of development the edges of the operculum are closely pressed to the neck and there is no danger of freeing more eggs than are wanted unless the fish is roughly handled. During the later stages of development the tension of the operculum is relaxed and eggs or larvæ can be much more easily removed, but there is a correspondingly greater danger of liberating more young than are wanted. If the female is disturbed or confined during the latest stages of brooding, some or all of the young will escape. The eggs freed from the gill cavity will continue their development uninterruptedly, but the gill cavity of the female offers such a unique and self-regulated hatchery that they were usually left in it.

## RIVALRY OF MALES AND SECONDARY SEXUAL DIFFERENCES.

In an aquarium containing six specimens of *Amblyopsis*, two took a great antipathy to each other and engaged in vigorous contests whenever they came in contact. Frequently they came to have a position with broadside to broadside, their heads pointing in opposite directions. The fight consists in quick lateral thrusts toward the antagonist to seize him with the mouth. The motion is instantly parried by a similar move by the antagonist. This blind punching may be kept up for a few seconds, when, by their vigorous motions, they lose each other and jerk themselves through the water from side to side, apparently hunting for each other. At this time they are very agile and move with precision.

When the belligerents meet, one above the other, the snapping and punching is of a different order. While jerking through the water, just after a round, if one of the belligerents touches one of the neutrals in the aquarium, it frequently gives it a punch, but does not follow it up, and the unoffending fellow makes haste to get out of the road, the smaller ones most quickly. If, after an interval of a few seconds, a belligerent meets a neutral, they quietly pass each other without paying any further attention; whereas if the two belligerents meet again, there is an immediate response. Whether they recognize each other by touch or by their mutual excitability, I do not know. In another aquarium I saw one belligerent capture the other by the pectorals. After holding on for a short time it let go, and all differences were forgotten. The thrust is delivered by a single vigorous flip of the tail. These fights were frequently noticed, and, as far as determined, always occurred between males.

The absence of secondary sexual differences in the cave fishes is a forcible argument in favor of sexual selection as the factor producing high coloration in the males. The absence of secondary sexual differences in caves opposes the idea of Geddes and Thomson, that the differences are the external expression of maleness and femaleness.

### THE EGG AND GENERAL DEVELOPMENT OF AMBLYOPSIS.

The eggs are large, measuring 2.3 mm. in diameter. The yolk is translucent, of various tints of amber. The yolk measures 2 mm. in diameter and contains a large protruding oil-sphere 1 to 1.2 mm. in diameter. When the egg is deposited, the yolk is flabby and composed of yolk-spheres of various sizes loosely put together. After the egg has been in water for some time, the yolk forms a tense rounded mass. The egg is heavier than water. The oil-sphere lies uppermost in the egg, and the germinal disk forms at the side of the egg. Attempts at artificial fertilization have not been successful beyond obtaining well-developed germinal disks.

The rate of development will probably be found to vary considerably with the temperature of the water. In a series of eggs in which the gastrula covered half the yolk when observations began, the blastopore was reduced to the size of the oil-sphere in 9 hours, when the embryo encircled about a third of the yolk; 16 hours later the blastopore was closing. The rate of development of the series of eggs taken in May was as follows, the mother containing the eggs having been kept in a small aquarium without change of water and at the temperature of an ordinary living room. The temperature of the water in the cave is 12° C., that in the room was 22° C.

On May 4, 9 p. m., the gastrula covered approximately half the yolk. It lies eccentric, neither below nor at the side, the germ being evidently heavier, the oil-sphere at the top.

May 5, 6 a. m., the embryo surrounds about a third of the egg, the blastopore is about as wide as the oil-sphere, 1.2 mm., and the latter seems to fully fill it. At 2.30 p. m. the embryo is 1.6 mm. long and has 4 protovertebræ. At 6 p. m. the blastopore has narrowed considerably and invariably lies at one side of the oil-sphere, the embryo lying oblique to the vertical axis of the egg. This eccentric position becomes more and more evident as the blastopore closes toward 10 p. m. The embryo is 1.76 mm. long, with 6 protovertebræ. At 10 p. m. the eyes and brain are shaped like the ace of spades, the eye lobes evidently not yet narrowly separated from the brain by a narrow stalk, the blastopore closing, the embryo 1.92 mm. long, and with 10 protovertebræ. On May 6, at 6 p. m., the embryo lies horizontal around the margin of the yolk; the cavity of the central nervous system has appeared; a large piece has been eaten out of the yolk; the lens is just beginning to develop. There are 12 or 13 proto vertebræ. At 8 a. m. the embryo is 2.4 mm. long; at 11 a. m. no marked change is seen; at 6 p. m. tail is beginning to bud out; embryo, 3 mm. long, encircles half the yolk; 17 protovertebræ present.

There is a regular change in the position of the embryo with development. The blastoderm is formed at the side of the yolk. When the gastrula covers half the yolk, the egg has rotated so that the gastrula covers more of the lower than of the upper surface of the yolk. Still later, some hours before the closing of the blastopore the latter structure lies to one side of the yolk-sphere, which always occupies the upper pole of the egg; the embryo extends from this region obliquely over the yolk. After the formation of the tail the embryo is always found coiled about the upper half of the yolk. The period spent in the egg lasts about a month. In the laboratory some embryos hatched in about 28 days, but in the cold cave streams this period would probably be several days longer. The yolk has been but little affected at the time of hatching, measuring 1.8 mm., the oil-sphere about 1 mm.; and since the yolk is all absorbed before the young are freed from the gill membrane, probably another month is spent under the gill membrane.

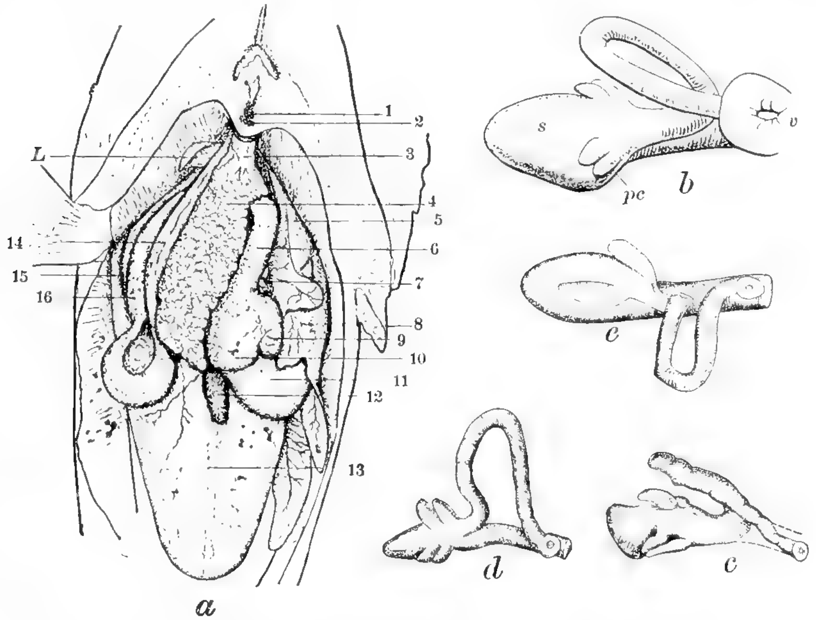


FIG. 30. (*a*) Internal Anatomy of *Amblyopsis spelæus*. 1, anus; 2, opening of oviduct; 3, oviduct; 4, ovary, which is single; 5, liver; 6, duodenum; 7, gall sac; 8, pectoral fin; 9, one of pyloric cæca; 10, cæcum; 11, stomach; 12, spleen; 13, air bladder; 14 and 16, intestine; 15, pancreas; *l.*, liver.
(*b*) Alimentary Canal of *Chologaster cornutus*. *pc.*, pyloric cæca; *s.*, stomach; *v.*, vent.
(*c*) Alimentary Canal of *Chologaster papilliferus*.
(*d*) Alimentary Canal of *Chologaster agassizii*.
(*e*) Alimentary Canal of *Typhlichthys subterraneus*.

The young, on hatching, are about 5 mm. long and lie on their sides. The motion of the tail produces no effect other than to cause them to spin around with the yolk for a pivot. The metamorphosis of the larva into the definitive fish is completed before it leaves the gill cavity of the mother. The longest individuals I have secured from the gill cavity measure about 10 mm.

### THE MIGRATION OF THE ANUS.

Certain structures gain an entirely new significance in the light of the breeding habits. These are the enlarged gill cavities with the small gills, the closely applied branchiostegal membrane, and the position of the anus and sexual orifices.

The anus in all of the species has undergone a curious translocation. The primary cause of the transposition probably lies in the ovary and oviduct, and not in the alimentary canal. The opening of the oviduct has moved forward until it lies in front of the pectorals and it has carried the anus forward with it. In newly hatched individuals the anus has its normal position behind the ventrals. When the fish has reached a length of 25 mm., the anus has reached a point in front of the ventrals, but it is still nearer the ventrals than the pectorals; with a length of 35 mm. the anus has moved forward to just below the insertion of the pectorals. In mature specimens it lies considerably in advance of the pectorals (plate 5, fig. c). The forward movement of the sexual orifice takes place in both sexes.

Nothing is definitely known of the advantages of the location of the opening of the oviduct. They can be inferred from the habit of *Amblyopsis* in carrying its eggs in the gill cavity. Located as it is, the oviduct may be covered by the gill membranes of the 2 sides alternately, or, if the fish takes an oblique position in the water with the head down, the eggs may flow directly into the gill cavities, being carried downward by gravity and held in the groove in front of the anus by adhesion.

It is difficult to imagine even a formal explanation of the origin of the position of the sexual orifice in the Amblyopsidæ. The anus was probably carried forward as the result of the forward movement of the sexual orifice, and it is this that demands explanation. Very probably the habit of carrying the young in the gill pouches antedates the present position of the anus. The eggs may have been allowed to flow into the gill openings, the female occupying a position with head downward during oviposition. If this were the case, then, while the individual skill would count for much in transferring the ova, a variation or mutation which lessens the distance between the sexual orifice and the gills would be of distinct advantage and would probably be transmitted by natural selection. The actual transfer of the ova into the gill cavity has not been observed.

### THE TACTILE ORGANS.

The tactile organs are among the most important in the consideration of the blind forms. Their minute structure will form the basis of a separate paper. The prominent tactile organs about the head of *Amblyopsis* have been mentioned by nearly every writer, and they have been figured by Putnam-Wyman and Leidig; but the figures of the distribution of the ridges are worthless. The description by Professor Forbes of *Chologaster papilliferus* is the only systematic enumeration of the ridges that has appeared. The accompanying figures (32 and 33), drawn by me with the camera lucida, verified and copied by Mr. U. O. Cox, give the exact extent and position of the ridges in *Amblyopsis*, *Typhlichthys*, and *Chologaster papilliferus*. It will be seen that in the number and distribution of the tactile area the three forms agree very closely, the eyed form having the same number and distribution of ridges or rows that the blind forms have. In *C. papilliferus* most of the ridges are much less prominent than in the blind species, being sunk into the skin. About the nose and chin, however, the ridges are as prominent

FIG. 31. Tactile Organ in Head of Larvæ *Amblyopsis*. See also Plate 70 just above Yolk. Larva was placed in weak osmic acid which brought out outlines of structure. 8 mm. + 4 ocular.

as in the other species. In *Chologaster cornutus* there are no distinct ridges at all, the tactile organs being arranged as in other species of fishes. In specimens of the same size the papillæ are not more prominent in *papilliferus* than in *cornutus*. It is only in the oldest of *papilliferus* that the papillæ become prominent. The number of individual papillæ in each tactile ridge differs considerably with age (size), so that an exact comparison between the large *Amblyopsis* and the much smaller species of *Chologaster* and *Typhlichthys* can not be made. From a number of counts, Professor Cox found that ridge No. 6 contains in *Chologaster papilliferus*, 6 organs; in *Typhlichthys*, 11; in two specimens of *Amblyopsis*, respectively 3.33 and 4.25 inches long, 12 and 20. The tactile ridges in the head of *Amblyopsis* are shown in plate 8, figures A and B. The outermost layer of skin has been removed from a small area over the right eye of A, showing the numerous taste buds. Figures C and D show head of *Chologaster papilliferus* under slightly greater magnification. Figure D shows especially the tactile organs about the mouth. The skin passes over the eye without a free orbital rim, and the eye does not show well.

FIG. 32. (*a, b, c*) Distribution of Tactile Ridges in *Amblyopsis;* lateral, dorsal, and ventral views.
(*d, e*) Distribution of Tactile Ridges in *Typhlichthys subterraneus;* dorsal and side views.

Aside from the tactile organs in ridges there are many solitary ones not evident from the surface in *Amblyopsis*. When the epidermis is removed by maceration, the dermal papillæ on which they rest give the whole head a velvety appearance.

In the young, at least, of *Amblyopsis*, each of the tactile organs of the ridges is provided with a club-shaped filament abruptly pointed at the end (fig. 31). They wave about with the slightest motion in water and are so numerous as to give the whole head a woolly appearance.

Tellkampf has remarked:

> The blind fish is found solitary and is very difficult to be caught, since it requires the greatest caution to bring the net beneath them without driving them away. At the slightest motion of the water they dart off a short distance and usually stop. * * * During my stay at Mammoth Cave I observed that the *Amblyopsis* * * * remained motionless while I moved a burning lamp around them, but they were disturbed by a slight motion of the water, proving that the light made no impression upon their optic nerve, while their sense of touch was acute.

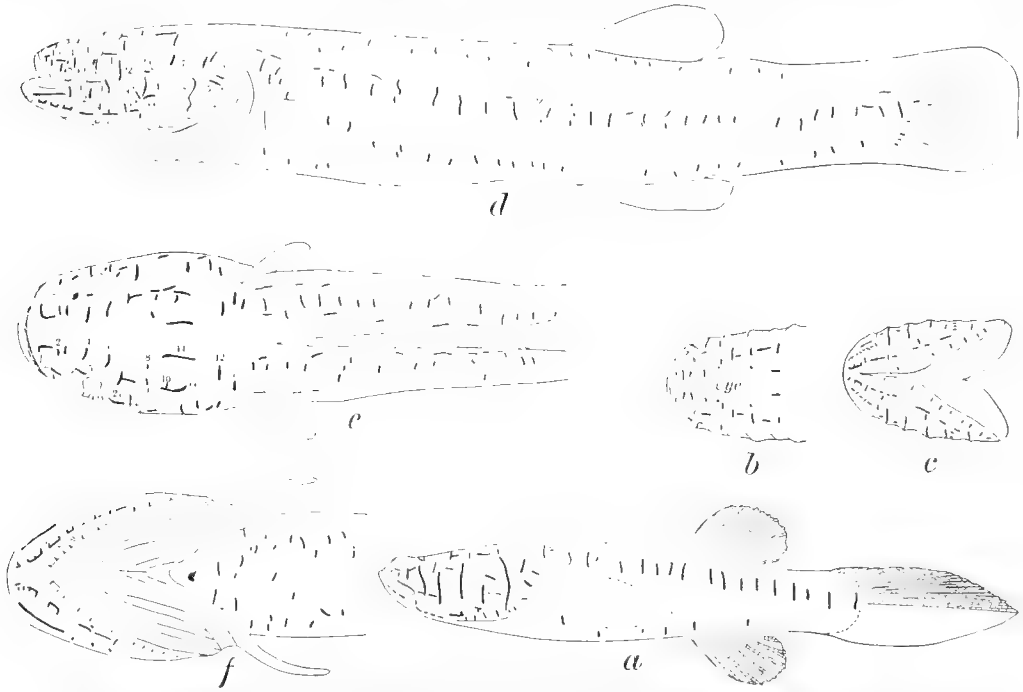


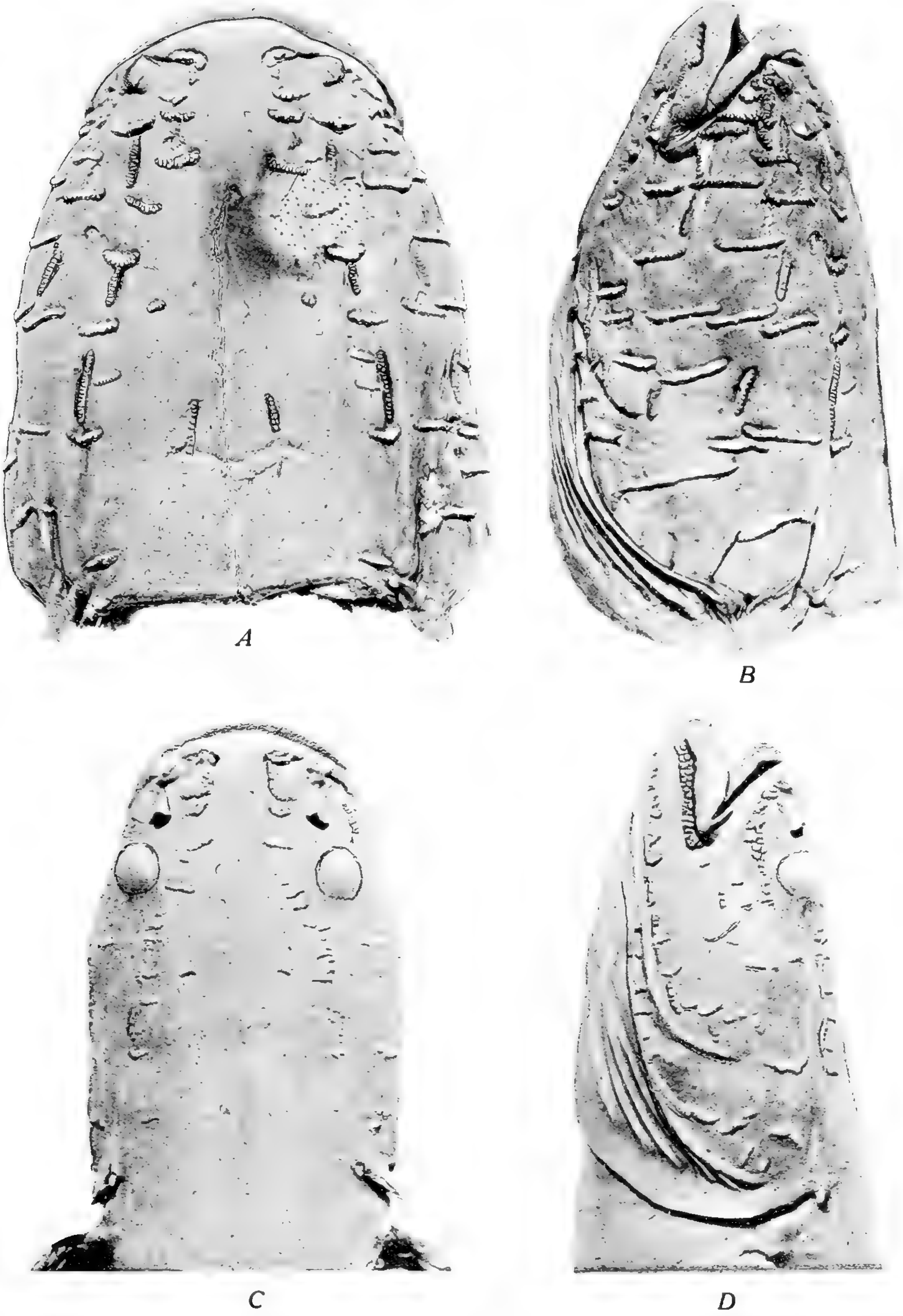
FIG. 33. (*a*, *b*, *c*) Distribution of Tactile Ridges in *Troglichthys*. Side view of entire fish, dorsal and ventral views of head. (*d*, *e*, *f*) Distribution of Tactile Ridges in *Chologaster papilliferus*. Side view of entire fish, dorsal and ventral views of anterior part of body.

Dr. John Sloan in Packard, 1887, wrote:

> We carried our lighted candles within a few inches of them when near the surface, but they seemed wholly insensible to their existence; but if a drop of tallow fell in the water near them, they would swim rapidly away. I brought home 12, as many as could live in my bucket. Of these 12 caught in September none died until next June, when the water became warmed to near 70°, when several of them died with *tetanic convulsions* (?). I put the remainder in my cellar, where the temperature ranged from 45° to 60°, where one, "Blind Tom," lived 11 months, making 20 months of existence without having taken any visible food. While in my aquarium they manifested total indifference to light and sound. * * * They manifest great sensibility on the back and sides to any approaching body, but do not notice an attack from below. It is not possible to capture one by a side sweep of the net, but by passing it under him a considerable distance below and bringing it up slowly there is no difficulty in taking them. In their native pools and in the aquarium when disturbed they do not strike the bottom or sides of their surroundings, but seem to have a sense of resistance (if the term is pardonable) which protects them.

Miss Hoppin in Garman remarked:

> I am very sure they [cray-fishes], as well as the white-fish [*Troglichthys*] have the tactile sense developed in an unusual degree. At the least touch upon the water they dart away. * * * Numerous tests convince me that it is through the sense of touch, and not through hearing, that the fish is disturbed. * * * If I strike the vessel so that the water is set in motion, he darts away from that side through the mass of water, instead of around in his usual way. If I stir the water or touch the fish, no matter how lightly, his actions are the same.



Photographs of the tactile organs of Amblyopsis and Chologaster.

A. Head of Amblyopsis from above, showing tactile ridges.

B. Same head from side. Tactile organs especially numerous about mouth.

C. Head of Chologaster papilliferus, from above, under slightly greater magnification than A.

D. Same head, from side, especially showing tactile organs about mouth.

Blatchley states:

> * * * the least movement of the water frightened them, and they darted rapidly away, usually at right angles to the course they were pursuing. The sense of touch, rather than that of hearing, is, in my opinion, the one which has been intensified by long residence in the dark and silent recesses of the caves.

I have not found the slightest difficulty in capturing *Amblyopsis* with a small dip net, either from a boat or while wading through the subterranean stream, and I have caught one in the hollow of my hand. At such a time any amount of noise I was capable of making did not affect the fishes found swimming in the water. Frequently they were taken in the dip net without apparently taking any note of the vibrations produced in the water until they were lifted out of it; very rarely a fish became noticeably scared. Such a one would dart off a few feet or a few inches and remain on the *qui vive.* If not pursued, it soon swam off quietly; if pursued, it not infrequently escaped by rapidly darting this way and that; when jumping out of the water, often an abrupt turn in the opposite direction from which it started would land it in the net, showing that their sense of direction was not very acute. At other times, if disturbed by the waves produced by wading, one or another individual would follow a ledge of rock to the bottom of the stream, where it would hide in a crevice. But very frequently, much more frequently than not, no attention was paid either to the commotion produced by the wading or by the boat and dip net. In general it may be said that the fishes in their natural habitat are oblivious to disturbances of the water until frightened by some very unusual jar or motion, probably a touch with the net, when they become tensely alert. The fact that they are not easily frightened suggests the absence of many enemies, while their frantic behavior if once scared gives evidence that occasional enemies are present and that they are very dangerous, or that the transmission of the instinct of fear is as tenacious as the transmission of physical characters.

Contrary to Sloan's observation, that they detect the presence of a solid substance in their path, I have never noticed that the fishes in confinement became aware of the proximity of the walls of the aquarium when swimming toward them. Instead, they constantly use the padded, projecting lower jaw as bumpers. Even an extremely rapid dart through the water seems to be stopped by the projecting jaw without serious inconvenience.

Sticks, straws, etc. are never avoided by the fishes, even when the fishes had not been disturbed for hours. By this I mean that they are never seen to avoid such an object when it is in their path. They swim against it and then turn. An object falling through the water does not disturb them even if it falls on them. Gently moving a pencil in front of them does not disturb them much, but if the pencil is held firmly in the hand it is always perceived and the fish comes to a dead halt half an inch before it comes in contact with it. On the other hand, they may be touched on the back or tail before they start away. They glide by each other, leisurely and dignified, and if they collide, as they sometimes do, they usually display no more emotion than when they run against a stick. But this indifference is not always displayed, as was noted under the head of breeding habits.

A number kept in an aquarium having a median partition in which there was a small opening were readily able to perceive the opening, swimming directly for it when opposite it. This observation is in direct contrast to their inability to perceive solid substances in their path. A sharp tap on the sides of an aquarium in which 6 blind fishes were swimming, where they had been for a number of days undisturbed in a dark room, caused nearly all of them to dart rapidly forward. A second tap produced a less unanimous reaction. This repeated on successive days always brought responses from some of the inmates of the aquarium. Those responding were not necessarily the nearest to the center of disturbance, but sometimes at the opposite side of the aquarium or variously distributed through it. After a few days the fishes took no notice of the tapping by any action observable in the artificially lighted room.

Such tapping on a well-lighted aquarium containing both *Chologaster* and *Amblyopsis* was always perceived by the *Amblyopsis*, but the only response from these imperturbable philosophers was a slight motion of the pectorals, a motion that suggested that their balance had been disturbed and that the motion was a rebalancing. The *Chologaster*, on the other hand, invariably darted about in a frantic manner. One individual of *Amblyopsis* floating on the water was repeatedly pushed down by the finger without being disturbed; but if touched on the side, they always rapidly dart away.

From everything observed it is quite evident that *Amblyopsis* is not keener in perceiving objects or vibrations than other fishes, and ordinarily pays much less attention to them. Mr. Payne's observation on the feeding habits leads one to conclude that they possess greater power of discrimination between vibrations. Some observations on young *Amblyopsis* are of interest in this connection.

The young with a large amount of yolk still attached show a well-developed sense of direction. A needle thrust into the water near their heads and in front of them causes a quick reaction, the young fishes turning and swimming in the opposite direction. They will do this two or three times, then, becoming exhausted, will remain at rest. Sometimes an individual will not move until it is actually touched by the needle. The needle must come within about an eighth of an inch of the fish before it is noticed. Then, if the needle produces any result, it causes the fish to quickly turn and swim some distance, when the fish falls to the bottom again and remains at rest. If the needle be placed behind the fish, it will swim directly forward; if at the side or about the middle, it swims directly forward or turns and swims in the direction opposite the origin of the disturbance. Younger specimens have no power over the direction of their progress — the wiggling of the tail simply produces a gyration, with the yolk as pivot.

A young blind fish, 6 months old, swims about in a jerky manner, chiefly by use of its pectoral fins. It keeps close to the side of the vessel, usually with its back to the glass. (The aquarium was a cylindrical jar 300 mm. in diameter and 300 mm. high.) From whatever direction it may be approached it perceives a stick thrust toward it as readily as a seeing fish can, and will invariably dart away a short distance, sometimes making sharp turns to avoid the stick and always successfully. It can be approached from the top nearer than from the sides or from in front. It does not avoid the sides of the aquarium, which it frequently strikes.

## THE EAR OF AMBLYOPSIS.

Anatomically considered, the ear of *Amblyopsis* is normal. Numbers of ears together with the brains have been dissected out. These were treated with Flemming's strong solution or with Hermann's fluid, either of which stained the nerve matter black. In the first place, the three semicircular canals are present and each has its ampulla fully developed. The three ampullæ and the sinus utriculus superior communicate with the utriculus in front, behind, and above. Below, the utriculus communicates with the sacculus, which terminates posteriorly in an appendage, the lagena. The three ear bones are present, one in the recessus utriculi, one (the largest) in the sacculus, and the other in the lagena.

The auditory nerve divides into two branches, the ramus anterior and the ramus posterior. The ramus anterior divides into three branches — the ramulus ampullæ anterioris, which extends to the anterior ampulla; the ramulus ampullæ externæ, which extends to the external ampulla; the ramulus recessus utriculi, which extends to the recessus utriculi. The ramus posterior gives off a heavy branch, the ramulus sacculi, which extends to the sacculus. The rest of the ramus posterior divides into the ramulus lagenæ, which extends to the lagena; and the ramulus ampulla posterioris, which extends to the posterior ampulla. Another branch, the ramulus neglectus, which is normally given off where the ramus posterior divides into the ramulus ampulla posterioris and ramulus lagenæ, has not been identified.

The normal fish ear has seven auditory spots — the macula acusticus recessus utriculi, three cristæ acusticus ampullarum, macula acusticus sacculi, papilla acusticus, and the macula acusticus neglecta. In *Amblyopsis* all of these auditory spots are present.

## DOES AMBLYOPSIS "HEAR"?

(By Fernandus Payne.)

Until the time of Bateson and Kreidl, it was generally taken for granted that fishes could hear because they had ears. Bateson concluded from his observations on congers, flatfishes, pouting, etc., that fishes perceive the sound of sudden shocks, but do not seem to hear the sounds of bodies moving in the water. Kreidl was the first to make experiments to test the hearing of fishes. He experimented on the gold-fish (*Carassius auratus*) and concluded that gold-fishes do not hear with the ear, but that they do react to sound waves by means of sense-organs in the skin. Lee's observations supported Kreidl's results, and he further concluded that the sole function of the ear in fishes is equilibration. Parker was the first to get positive evidence against the conclusions of Kreidl and Lee. His experiments were based on *Fundulus heteroclitus*. He used three classes of fishes; first, normal, that is, unmaimed, ones; second, fishes with the auditory nerves cut; and third, fishes with the skin rendered non-sensitive but with the ears intact.

His apparatus consisted of a heavy aquarium with a slate bottom, two glass sides, and two slate ends, one of which he replaced by a piece of deal board to serve as a sounding board. To the middle of one edge of the sounding board he attached a stout beam of wood so that it stood out horizontally about 1 m. in the plane of that end. He stretched a bass-viol string from the free end of the beam over a bridge in the center of the sounding board to its opposite side. When the string was plucked or bowed, it produced about 40 vibrations per second. The fishes to be experimented upon were placed in a small cage suspended from a cord attached at its ends to the walls of the room. The end toward the sounding board was covered with coarse cloth.

He subjected 10 normal fishes each to 10 tests, and from the 100 tests he got 96 pectoral-fin responses. Fishes with auditory nerves cut responded only 18 times in a total of 100 trials, and Parker thought these 18 times were in part accidental occurrences and in part due to the slight movements of the aquarium caused by the vibrating string. Instead of the vibrating string he substituted an electric tuning-fork which vibrated 128 times per second. With the tuning-fork, where the vibrations of the aquarium could be eliminated, he got no responses with the earless fishes. Fishes in which the skin was made insensitive, but with the ears intact, responded to sound 96 times in a total of 100. These fishes reacted almost exactly as the normal ones did. From these results Parker concludes that the killifish hears. Although his conclusion, that a fish hears, is contrary to Kreidl and Lee, he does not say that the observations of these men are entirely wrong, for the ears in different fishes may function differently. In fact, Parker found no evidence of hearing in the smooth dog-fish (*Mustelus canis*) when he subjected it to the same experiments as the killifish. Bigelow used Parker's methods of experimenting and reëxamined the gold-fish. He concludes that the gold-fish hears.[1]

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[1] Since writing the above Körner in Lucae's Festschrift, 1905, reviewed the evidence advanced to show that fishes can hear, and concludes that while they react to rapidly repeated tone-vibrations such as are produced by a tuning-fork or an electric bell, it is not proven that they perceive this with their ears. He used 25 species of fishes and found that in no case did any of these 25 species react in any way to a single sharp click. He concludes from these experiments that fishes do not hear.

From the evidence at hand it is very probable that some fishes hear and that others do not. The following experiments have been made on the blind fish *Amblyopsis spelæus*. Various opinions have been expressed about the hearing of this fish.

Wyman states:

> It is said that the blind fishes are acutely sensitive to sounds as well as to undulations produced by other causes in the water. In the only instance in which I have dissected the organ of hearing (which I believe has not before been noticed), all its parts were largely developed.

The following words of Professor Cope are frequently quoted:

> If these *Amblyopsis* be not alarmed, they come to the surface to feed and swim in full sight, like white, aquatic ghosts. They are then easily taken by the hand or net, if perfect silence is preserved, for they are unconscious of the presence of an enemy except through the medium of hearing. This sense is, however, evidently very acute; at any noise they turn suddenly downward and hide beneath stones, etc., on the bottom.

Subsequent writers have generally disagreed with Cope. Dr. Sloan (in Packard, 1884) wrote:

> I tested their hearing by hallooing, clapping my hands, and striking my tin bucket when they were in easy reach and near the surface. In no instance did they change their course or notice the sound.

Miss Hoppin (Garman) failed to get any response from *Troglichthys* as long as noises only were resorted to. She says:

> I may scream or strike metal bodies together over him, as near as possible, yet he seems to take no notice whatever.

Blatchley states that noises do not attract them.

Eigenmann's observations (Proc. Brit. Ass. A. Science, Toronto Meeting) on *Amblyopsis* confirm those of Miss Hoppin on *Troglichthys*. No ordinary noises produced had any effect on *Amblyopsis*. Whistles, tuning-forks, clapping of hands, shouting in the reverberating caves, were alike disregarded.

*Amblyopsis*, since it is blind, does not require precautionary methods to exclude sight as a possible disturbing element.

If there are sounds in the water of the caves that concern the blind fishes and the ears are sound-perceiving organs, we might expect the ear to be better developed along with the tactile organs as a compensation for the loss of sight. But if there are no sounds, we might expect them to degenerate along with the eye unless the function is something else than sound perception. *Amblyopsis* has few, if any, enemies in the caves. There are certainly none that make sounds, so the ears of the fishes would not be kept on the alert for them. There is less variety of sounds in the air of the caves than on the outside. This may make but little difference, as sound generated in the air does not penetrate readily into the water. Rippling of the water is certainly perceived more readily by the tactile organs than by the ear. Besides, the fishes are confined to the quiet pools.

My methods of experimenting were practically the same as those of Parker and Bigelow. I used a heavy slate-bottomed aquarium, 24 inches long, 14.5 inches high, and 12.5 inches wide. I removed the glass from one end and substituted a board 2 inches thick. This served as a sounding board. The fishes were confined in a smaller aquarium (4 × 5 × 8 inch) suspended in the larger. The end of the smaller aquarium was covered with cheese-cloth toward the sounding board.

The large aquarium rested on a masonry pedestal, which eliminated all vibrations of the floor. The small one was suspended by cords running from one side of the room to the other.

After various trials with small tuning-forks which produced vibrations between 100 and 512 per second, which gave negative results, I used a large fork 12.5 inches in length vibrating 100 times per second and which produced a large volume of sound.

I used (*a*) unmaimed blind fishes and (*b*) others whose auditory nerves had been cut. I also attempted work with fishes whose lateral line nerves and nerves to the skin had been cut, but the specimens either died or did not revive sufficiently to get normal reactions.

(*a*) Unmaimed blind fishes when placed in the aquarium invariably dart to the bottom and remain there for a short time, after which they begin to swim about rather rapidly. They soon become more quiet if nothing further disturbs them, but continue swimming about in a leisurely way, stopping now and then for a few seconds at a time. After they have been in the aquarium for 12 or 24 hours, these stops are more frequent and longer. The fishes strike various attitudes during these stops, but they seldom rest upon the bottom. Instead they are usually poised as if ready to dart away. The body seems so well balanced that they have no trouble in maintaining any position they may happen to take. During these stops the tail always projects straight backward and the pectoral fins stand at right angles to the body. If at this time the sounding board is caused to vibrate, the fish responds either with a quick movement of both the tail and pectoral fins or by the pectoral fins alone. Twenty fishes were each subjected to 5 tests, and out of the 100 trials there were 97 responses and 3 failures.

(*b*) *Fundulus*, with the auditory nerves cut, acts as normal blind fishes do in swimming slowly or in resting, but when stimulated, loses entire control of its equilibrium. Parker suggests that in resting or swimming slowly the fish depends upon the eye for orientation, but in quick movements the ear comes into play. The reactions of *Amblyopsis* seem to support this suggestion, for with both auditory nerves cut they have no control of their orientation. When resting, they lie on the side or back, either at the surface or on the bottom. In swimming slowly they sometimes move forward in irregular lines, but when they attempt rapid locomotion, they move in irregular spirals about the long axis of the body and make no progress one way or the other. With only one auditory nerve cut the movements are quite different. The fish is able to move forward, but it goes in a corkscrew-like path, turning over on its axis as it swims along. The same result was obtained by Eigenmann by thrusting a pin into one of the auditory organs.

The operation of eliminating the ear is a comparatively easy one to perform. Of those operated on, more than half recovered. They generally lived for 2 or 3 weeks, and some even longer. The observations were made from 1 to 2 days after the operation. With these fishes three kinds of responses were obtained. If they were perfectly quiet when the sounding board was caused to vibrate, they either responded by a slight movement of the pectoral fins or by a movement of both caudal and pectoral fins. If, on the other hand, they lay with the body quiet and with the pectoral fins moving slowly when the sounding board was caused to vibrate, they responded by stopping the fin movements. Ten fishes were each subjected to 10 tests, and out of 100 tests there were 96 responses. This result differs very little from the reaction of fishes not operated upon. Since the ears have been eliminated, there is only one conclusion to reach and that is, that blind fishes detect vibrations with a frequency of 100 per second by means of sense-organs in the skin. As stated, I have not been able to eliminate the skin and lateral-line organs, and so can not say definitely whether or not the ears play any part in the reactions of normal blind fishes. Since the reactions are the same, ear or no ear, the part the ear plays in sound-wave perception, if any, is certainly small.

Using the word "hearing" in the sense in which Kreidl and Parker used it, that is, if we define hearing to be the sensation received through the ear and caused by vibrations either in the air or water, the experiments cited do not enable one to conclude definitely whether the blind fishes hear or not. If they do hear, their power in this direction is very limited.

The rssults show conclusively that they detect waves of 100 vibrations per second by means of sense-organs in the skin.

## THE BRAIN OF AMBLYOPSIS.

(By E. E. Ramsey.)

A comparison of the microscopic appearances of the brain of a normal fish and that of the blind fish, *Amblyopsis spelæus* De Kay, discloses a number of interesting conditions. The optic lobes and the optic tracts are measurably degenerate. The hemispheres are larger in *Amblyopsis* than in the average of normal brains. The brains of *Campostoma anomalum*, *Percina caprodes*, *Eupomotis gibbosus*, and *Amblyopsis* were measured with regard to the comparative widths of the optic lobes and the hemispheres. Five fishes of the same length were taken of each species. The averages obtained are as follows:

| Species. | Optic Lobes. | Hemispheres. | Comparative widths. |
|---|---|---|---|
| | mm. | mm. | p. ct. |
| Campostoma anomalum . . . | 5 | 2.8 | 56 |
| Eupomotis gibbosus . . . . . . | 5 | 3.7 | 74 |
| Percina caprodes . . . . . . . . | 6.4 | 3.5 | 54 |
| Amblyopsis spelæus . . . . . . | 3.2 | 4 | 125 |

It is thus seen that the hemispheres are relatively larger in the blind fish than in the more normal forms, and that the optic lobes are relatively much smaller in the former.

There is no noticeable variation in the cerebellum. In length there is a marked shrinkage, chiefly in the optic lobes, as shown by the position of the cerebellum which lies directly on the lobes. In the normal brain the cerebellum is situated well back, hardly reaching the lobes. The following table gives an idea of the length of the brain, as compared with the length of the fish. The brain length is measured from the tip of the olfactory lobes to the posterior part of the cerebellum:

| No. | Amblyopsis. | | | No. | Campostoma. | | |
|---|---|---|---|---|---|---|---|
| | Length of body. | Length of brain. | Per cent. | | Length of body. | Length of brain. | Per cent. |
| | mm. | mm. | | | mm. | mm. | |
| 1 | 92 | 5.5 | 6 | 1 | 88 | 8.5 | 9.6 |
| 2 | 80 | 5.3 | 6.6 | 2 | 103 | 9 | 8.7 |
| 3 | 90 | 5.5 | 6 | 3 | 72 | 7.5 | 10 |
| 4 | 88 | 5.8 | 6.6 | 4 | 68 | 7 | 10 |
| 5 | 80 | 5.2 | 6.5 | 5 | 58 | 6.3 | 10 |
| 6 | 100 | 6 | 6 | | | | 9.8 av. |
| | | | 6.3 av | | | | |

The result shows the brain of *Amblyopsis* to be only two-thirds as long as that of *Campostoma*. This shrinkage in width and length is great enough to show itself in the extent to which the cranial cavity is filled. A great depth of fatty tissue covers the dorsal surface of the brain. The only other external modification of any note is the absence of either optic nerves or optic chiasma.

The optic lobes are normally composed of 7 layers, which from outside to inside are as follows:

(1) A peripheral zone.
(2) An optic fiber layer from the optic nerve.

(3) An optic cell layer.
(4) A deep cell layer. According to Krause this layer contains in its outer part the cells which serve as terminal stations for the optic nerve, and in its inner sublayer the end stations for the fifth layer (Marklager).
(5) A deep fiber layer.
(6) A granular layer.
(7) The ependyma and its epithelium, which lies next to the ventricle of the lobes.

The optic lobes of *Amblyopsis* show a marked degeneration. The dorsal walls are not more than half or two-thirds as thick as those in the normal brain. Its contour is so flattened that the ventricle is almost obliterated (16 in fig. 34 *b*). The torus longitudinalis, which in the normal brain is suspended in the ventricle in the median line entirely below the layers of the lobes, is *between* the lobes and on nearly the same level with them. The torus thus forms a commissure connecting the lobes. The band of fibers connecting them dips downward in the normal brain and crosses to the opposite side through the torus; in the degenerate lobe they cross from one side to the other in almost a straight line (15 in fig. 34 *b*). The shrinkage in length is shown in the fact that the hypophysis is crowded forward to the anterior level of the lobes.

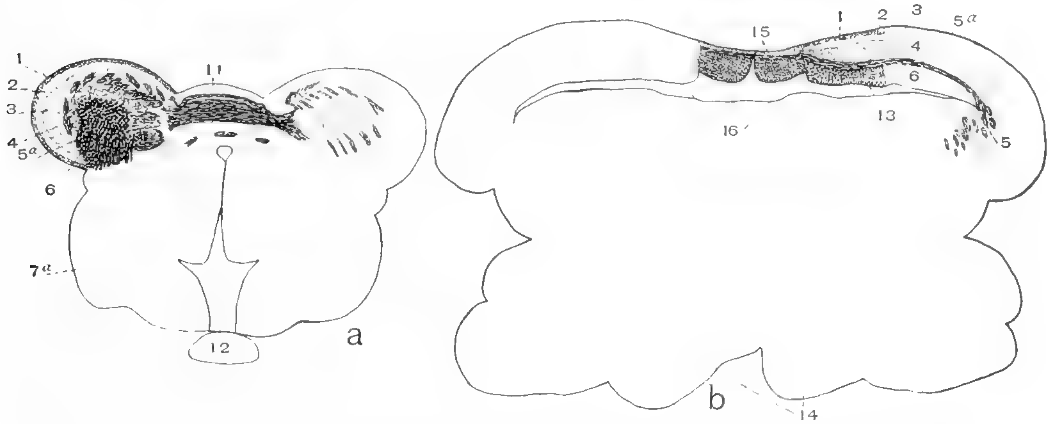


FIG. 34. (*a*) Cross-section of Brain. *Amblyopsis spelæus* near Anterior part of Optic Lobes. Specimen 77 mm. long.
(*b*) Cross-section through Middle of Optic Lobes of *Amblyopsis spelæus*. Specimen 77 mm. long. 1, first layer of optic lobe; 2, degenerate optic fiber layer; 3, optic cell layer; 4, deep cell layer; 5, deep fiber layer; 5 *a*, diagonal fibers of deep fiber layer; 6, granulated layer; 7 *a*, optic tract region; 13, ependyma; 15, torus longitudinalis; 16, ventricle.

The optic nerve of the normal brain is derived from the second and fourth layers of the lobes. The fibers of the second layer pass downward on both sides of the lobes, and the inner ones cross over at the ventral surface, where they join the fibers of the same layer from the other side. They then continue forward and downward to the optic chiasma as the optic tracts. The fifth layer is composed of diagonal fibers and descending fibers. These latter nerves pass downward and become a part of the optic tract.

As has been said, the wall of the optic lobes of *Amblyopsis* has undergone considerable shrinkage in thickness. The outer layer is not changed. The second layer, which is derived from the optic nerve, is entirely wanting. The optic nerve is represented by a small bundle of tissue, which is probably the remnant of the neurilemma. In the brain where the second layer should be, there is a narrow space containing practically no tissue. The third layer is unchanged. The fourth layer consists normally of two sublayers; the outer one has both nerve fibers and nerve cells — the latter according to Krause being the terminal stations of the optic nerve — and the inner sublayer has the terminal stations of the fifth layer in it. The outer sublayer is entirely atrophied in the lobes of the blind fish; and the inner one, if at all present, is indistinguishable from the third layer (3 and 4 in fig. 34 *b*).

The fifth layer is reduced to diagonal fibers. The descending fibers which join the optic tracts are atrophied. The diagonal fibers are more apparent than in the normal brain. These fibers form a broad commissure in the torus longitudinalis, which runs laterally to the outer edge of the lobes, where it turns back into the substance of the brain just beneath the ventricle and becomes diagonal. Cross-sections of fibers arising from various levels of the lobes are shown (5 in fig. 34 *b*).

The sixth layer is a granular layer. Its thickness is less than in the normal brain. No other change is noticeable. The thickness of the seventh layer, ependyma, is not more than half that of a normal brain. The cells show some shrinkage.

The differences in the lobes thus appear to be: first, in the atrophy of the second layer; second, the outer sublayer of the fourth layer is entirely gone; third, the descending fibers of the fifth layer are wholly wanting; fourth, the granular layer is not so thick and the ependyma is not only thinner but reduced in the numebr of its cells.

The optic tracts, that part of the nervous tissue which lies between the optic lobes and the optic chiasma, are entirely wanting. The space occupied by these tracts in the normal brain is in this brain partially occupied by tissue in which I have not been able to make out any structure. All the stains that have been tried have failed to reveal any cells. These tracts do not take the stains with the same readiness and in the same degree that those in normal brains do when subjected to exactly the same treatment. Three fishes, *Amblyopsis*, *Campostoma*, and *Eupomotis*, were killed and the heads placed in Fohl's mixture for the same duration of time. The brains were removed from the skull as soon as they were sufficiently hardened and were placed in the same bottle in order that the conditions might be alike. The three were embedded in the same block and sectioned side by side. The tissue of the tracts of the brains of *Campostoma* and *Eupomotis* differentiated very well — but the degenerate brain showed no structure.

In the dissections of the head of the blind fish, I have been unable to find any indications of optic nerves leaving the lobes. In both the dissections and the sections which have been made of the entire head and brain, there seems to be no break in the enveloping membranes on the anterior ventral surface of the lobes where the optic nerves originate. The vestiges of the optic nerve can be followed backward from the eye for a short distance. The only tracts leading away from the lobes are those which connect them with cerebral hemispheres and cerebellum. Those which pass forward to the hemispheres are from the diagonal fibers of the fifth layer. These pass laterally, but before reaching the lateral aspect of the lobes, turn downward through the granular and epithelial layers, and then course forward toward the ventral surface of the hemispheres.

## CONCLUSIONS ON THE AMBLYOPSIDÆ.

1. *Amblyopsis spelæus* is found from Mammoth Cave north to Michigan. It is the only blind species occurring on both sides of the Ohio.
2. No direct comparison of specimens from south and north of the Ohio has been made.
3. There are 3 species of *Typhlichthys* occurring in 3 different localities, one of them north of the Ohio.
4. *Troglichthys* is confined to the caves of southwestern Missouri.
5. The 3 species of *Chologaster* are found in 3 disconnected areas.
6. The color pattern of *Chologaster* is controlled by the underlying musculature.
7. *Amblyopsis* has been permanently bleached so that even individuals reared in the light do not acquire color. Its colorless condition is due to the transmission of the environmental adaptation in past generations of cave-dwellers.
8. Respiration is probably in part carried on through the skin.
9. *Amblyopsis* is a bottom and pelagic (ubiquitous) feeder on living, moving animals.
10. *Chologaster* does not depend upon its eyes for detecting and securing prey, or for avoiding a rod held in the hand.
11. *Amblyopsis* is negatively phototactic. It seeks the dark regardless of the direction or wave length of the rays of light.
12. In well-lighted, open pools *Amblyopsis* hides under rocks during daylight.
13. *Chologaster* when deprived of its eyes is negatively phototactic, and positively stereotropic. They are positively tropic to red as against other rays of the spectrum.
14. *Amblyopsis* probably breeds during the entire year, but more individuals carry developing eggs between March and May.
15. *Amblyopsis* is *not viviparous,* but the eggs to the number of about 70 are carried in the gill chamber of the female from fertilization till the larvæ are about 10 mm. long. The eggs hatch in about a month, having a length of about 5 mm.
16. There are few, if any, secondary sexual characters which argues in favor of the origin of these through sexual selection as against Geddes and Thompson's explanation that they are the result of maleness.
17. In newly hatched *Amblyopsis* the anus is in the normal position, behind the ventrals. When the fish reaches a length of 25 mm., the anus has reached a point in front of the ventrals; when 10 mm. longer, the anus has moved forward to between the bases of the pectorals. In mature specimens it lies anterior to this point.
18. The heads of the Amblyopsidæ are provided with tactile ridges, rows of tactile organs regularly and definitely arranged.
19. These fishes are not keener in perceiving vibrations than other fishes. They may have greater power of discrimination between vibrations.
20. The ear of the *Amblyopsis* is normally developed. These fishes do not "hear" in the ordinary sense of the word.
21. The external peculiarities of the brain of *Amblyopsis* are the absence of optic nerve and chiasma; the hemispheres are relatively larger than in other fishes and the optic lobes are much smaller.
22. The dorsal walls of the optic lobes have only half the normal thickness, the differences being due to (*a*) the atrophy of the second layer; (*b*) the outer part of the fourth layer has disappeared; (*c*) the descending fibers of the fifth layer are wholly wanting; (*d*) the granular layer is thinner than normal and the ependyma is thinner and has fewer cells; (*e*) the optic tracts are wanting.

## THE EYES OF THE AMBLYOPSIDÆ.

The Amblyopsidæ offer exceptional facilities for the study of the degeneration of eyes. They furnish gradations in habits from permanent epigean species to species that have for ages been established in caves. The eyes of the following are considered:

1. *Chologaster cornutus* Agassiz. Locally abundant in the lowland streams and swamps in the South Atlantic states from Virginia to Florida. Maximum length about 55 mm.
2. *Chologaster agassizii* Putnam. Found in the underground streams of Kentucky and Tennessee. It is rare. Maximum length 62 mm.
3. *Chologaster papilliferus* Forbes. Found under stones in the springs of Southwestern Illinois, in Union and Jackson counties. Maximum length 55 mm.
4. *Amblyopsis spelæus* De Kay. Widely distributed in the caves east of the Mississippi both north and south of the Ohio River. Maximum length 135 mm.
5. *Typhlichthys subterraneus* Girard. Found with the latter species in the caves east of the Mississippi, but confined as far as known to the south side of the Ohio River.
6. *Troglichthys rosæ* Eigenmann. Found in the caves west of the Mississippi River. Maximum length 55 mm.

The first two species mentioned live, as far as known, altogether in terranean streams; the others, altogether in subterranean streams. *Chologaster* has well-developed eyes, the others mere vestiges. We have thus two epigean species with well-developed eyes, one subterranean species with well-developed eyes, and three subterranean species with greatly degenerate eyes. The three latter species are descended from three distinct terranean ancestors. *Amblyopsis* is the only member of the family possessing ventral fins, and *Troglichthys* has scleral cartilages which are not found in the other members except *Amblyopsis*.

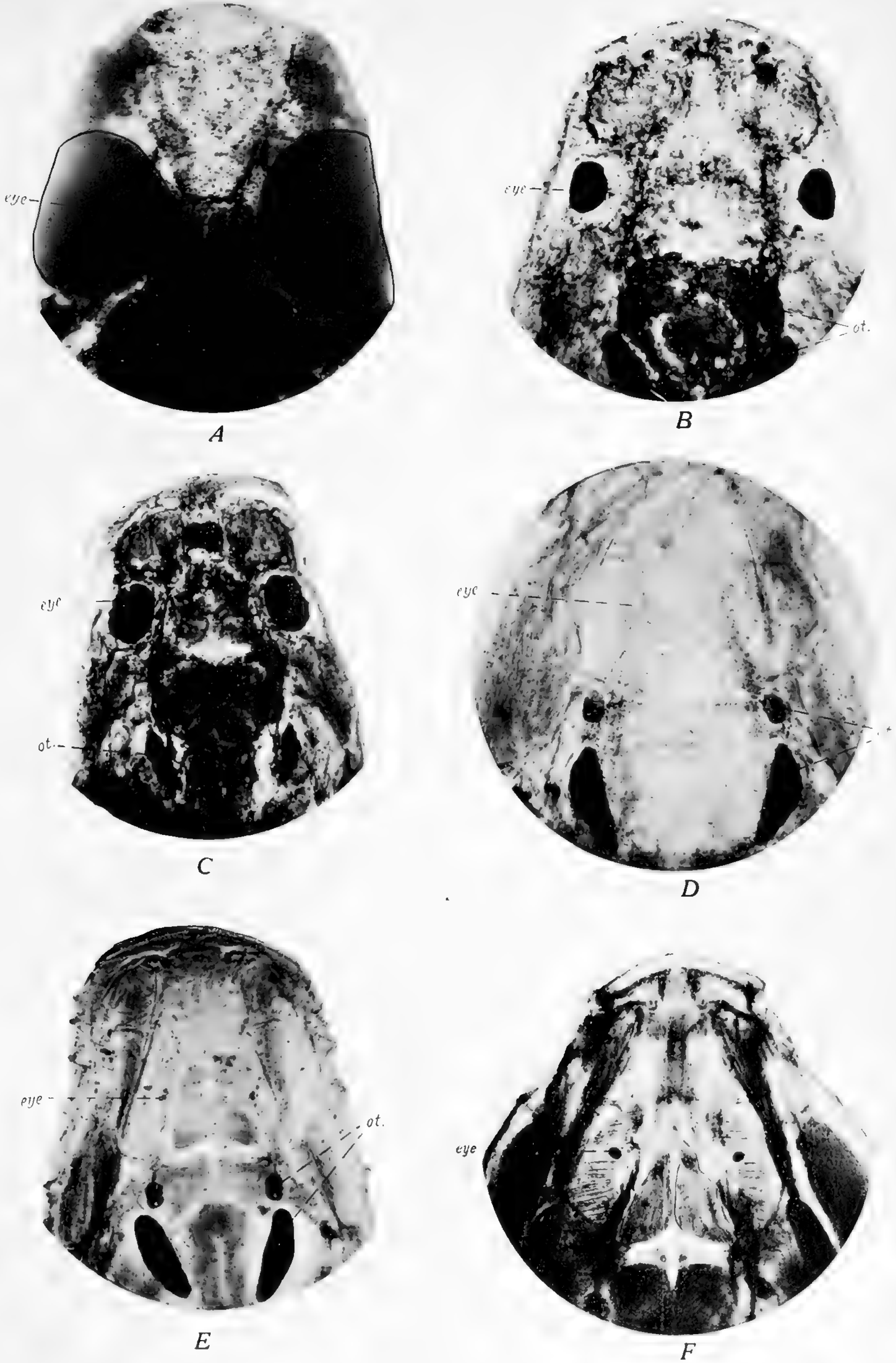
It must be apparent that an experiment on a vast scale has been conducted by nature, leaving us but to read the results. Moreover the experiment is one in evolution without the assistance or intervention of natural selection.

### CHOLOGASTER PAPILLIFERUS.

The only account of the eyes of *Chologaster papilliferus* Forbes, aside from the measurements in the description of the species, is a note by Wright. Professor Wright obtained his specimen from Prof. S. A. Forbes, and therefore had *C. papilliferus*. He announced that the pigment is absent in the pigmentary layer of the retina of this species. But this condition was unquestionably either accidental or due to the reagents employed. Chromic acid partly or wholly removes the pigment, leaving the cells in good condition.

The vertical diameter of the eye in a specimen 39 mm. long is 640 μ; in a specimen 55 mm. long (the largest secured), 960 μ. The distance from the point of entrance of the optic nerve to the front of the cornea is 560 μ and 900 μ, respectively, in the two specimens. The distance from the point of entrance of the optic nerve to the front of the epidermis over the eye is 600 μ in the smaller specimens, the lens about 360 μ in diameter. For further measurements see the table, page 120.

The eye is small when compared with that of other fishes of the same size, and especially so when compared with the eyes of *Zygonectes*. It is located high up on the side of the head, its upper surface being nearly on a level with the top of the head. It is directed outward and forward. In a specimen 35 mm. long it is 1.44 mm. from the tip of the snout and 0.88 mm. long. The distance between the eyes is 1.60 mm.



Heads seen from above and showing the relative sizes of the eyes of:

A. Zygonectes notatus;
B. Chologaster agassizii, 41 mm. long;
C. Chologaster papilliferus, 35 mm. long;
D. Typhlichthys subterraneus, about 35 mm. long;
E. Troglichthys rosæ, 38 mm. long;
F. Amblyopsis spelæus, 35 mm. long.

The dermis over the eye is thinner than elsewhere and devoid of pigment. The epidermis passes directly over the eye without any free orbital rim. It is much thinner, 24 $\mu$ in specimen 39 mm. long, than elsewhere about the side of the head (50 to 60 $\mu$) and consists solely of epithelial cells; those at the base are columnar, those at the free end of the epidermis are flat. All the other elements of the epidermis — goblet cells and mucous cells, very abundant all about the eye — are totally absent over it (fig. 35 *a*).

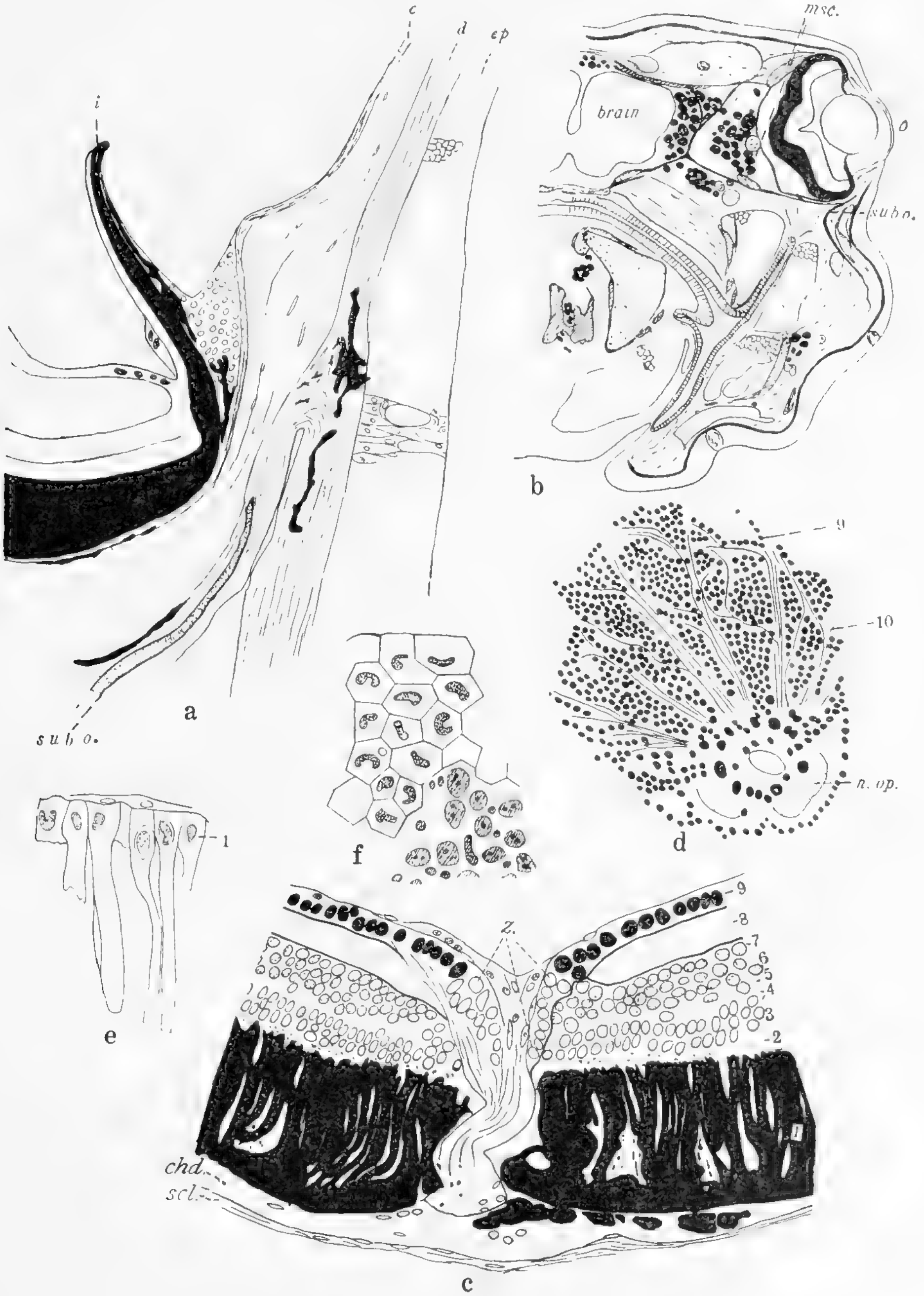


FIG. 35. (*a*) Section through Lower Left Half of Iris of *Chologaster papilliferus*, seen from in front. *i*, iris; *c*, cornea; *ep*, epidermis; *d*, dermis; *sub. o.*, suborbital.
(*b*) Section of Right Half of Head of *Chologaster papilliferus*.
(*c*) Section through Retina at Entrance of Optic Nerve.
(*d*) Inner Surface of Retina nearly tangential at Entrance of Optic Nerve.
(*e*) Vertical Section of Pigment Cells of Retina, depigmented with Chromic Acid.
(*f*) Tangential Section through Pigment Cells. Upper part of figure passes through nucleated part of cells, middle through processes of cells, and lower through cones only.

The 6 normal eye muscles are present in *Chologaster*. The 4 rectus muscles arise near a common point just behind the point of exit of the optic nerve from the skull. The M. rectus superior passes from this point outward, upward, and forward. The M. rectus inferior passes nearly horizontally outward and forward. The M. rectus externus passes nearly straight out at right angles to the axis of the body to the posterior face of the bulb. The M. rectus internus is probably the longest, passing outward and forward to the anterior face of the eye.

The two oblique muscles originate near a common point well in front of the exit of the optic nerve and are inserted near the insertion of the M. rectus superior and inferior. There is nothing remarkable about any of these muscles and they are mentioned solely as a basis of comparison with the condition found in *Amblyopsis.* The space from the wall of the brain case outward about the eye muscles and eye is bounded by a connective tissue capsule. Within this capsule, the space between the muscles and the posterior part of the optic pit and the eye is filled with fat. Above this capsule lies another mass of fat and below it still another (fig. 35 *b*). The supraorbital does not help to protect the eye, which lies entirely lateral from it and extends above it. The suborbital bones are thin, hollowed sheets of bone backing the suborbital mucous canal. Their number, etc., has not been determined, but their location is of importance in view of a statement made by Kohl concerning their absence in *Troglichthys.*

The sclera is represented by a thin fibrous capsule which is sometimes widely separated from the eye by reagents. In the largest specimen it is but 4 μ thick. It is continued over the front of the eye in contact with the dermis as a thin cornea (fig. 35 *a*). This is much more compact than the rest of the sclera. It readily separates from the dermis. The sclera is never at any place cartilaginous. I was at some trouble to demonstrate the absence of cartilage, even in the largest specimen, in order to detect if possible the homologues of the cartilages in *Amblyopsis* and *Troglichthys rosæ,* and can state positively that no cartilage is found associated with the eye of *Chologaster papilliferus* or in fact with the eye of any of the species of *Chologaster.*

The choroid is very thin. Just within the sclera is a homogeneous, sometimes excessively thin, layer containing a few nuclei, the suprachoroidal lymph space. If the eye contracts through reagents, the choroid which clings to the eyeball is separated from the sclera by the widening of this space. Pigment is not abundant except over the iris and below and at the sides of the entrance of the optic nerve. About the entrance of the optic nerve a mass of pigment is prominent, being especially conspicuous in the largest specimen (fig. 35 *c*). A mass of pigment which may be homologous with this has been described by Ritter in *Typhlogobius*, who found no cellular structure in the pigmented mass in *Typhlogobius* and identified this pigmented mass as the choroid gland. A choroid gland or the rete mirabile is not found. A processus falciformis is not present. Blood-vessels are not numerous and it was impossible to separate a distinct vascular layer of the choroid. In the largest specimen the choroid is much richer in blood-vessels ventral of the pigmented mass at the entrance of the optic nerve than elsewhere. The capillary layer reaches here a total of 9 μ in thickness. A layer of excessively thin pigment cells lies close to the pigmented layer of the retina. It is so thin and so closely applied to the pigmented layer of the retina that it is only in a few tangential sections that this part of the choroid becomes evident.

The optic nerve enters the retina as a single strand. It spreads out in all directions as soon as it has passed the pigmented part of the retina (fig. 35 *c*). Some of the fibers pass behind the ganglionic cells just within the entrance of the optic nerve, a condition of importance in the interpretation of the distribution of the optic nerve in the blind members of the family. The diameter of the nervous opticus at the entrance into the pigment layer is 32 μ in the largest specimen. The nerve is not spread out over the ganglionic layer, but is distributed in well-defined tracts between the nuclei. There is no nerve fiber layer proper (figs. 35 *c*, *d*). These strands of fiber not only entirely displace the ganglionic cells along their track, but also plow into the granular layer.

The pigment layer of the retina is very thick, as compared with the other layers, a condition recalling that described by Ritter for *Typhlogobius* and usually to be found in degenerate eyes.

For a comparative statement of the thickness of the various layers of the retina, see table on page 120.

The pigmentary layer is half the total thickness of the retina in the smaller specimen, while in the largest it is still thicker, measuring 104 of the 168 μ of the retinal thickness.

About one-eighth of the outer part of this layer usually appears as a solid mass of pigment where the margins of the cells touch. Just within this is a region where the cells are contracted, there being large, open, pigmentless spaces; at the innermost part there is again an accumulation of granular or rod-shaped pigment granules which obscure almost everything else in the ordinary sections. (See fig. 35 *c*.) Specimens preserved in chromic acid lose most or all of their pigment, which becomes brownish or disappears. The nuclei of the pigment cells are very irregular in outline (fig. 35 *f*), appearing to have no more definite shape than those of white blood corpuscles. Hollow processes extend from the cell body downward to near the external limiting membrane (figs. 35 *e* and *f*).

About the bodies of the cones the pigment is in thin strands, of which there are 8 to 12 to each twin cone; farther out it forms a complete ring about them. The cones are twins, rarely triplets. The twins are nearly all arranged in such a manner that the line, which may be termed the axis, connecting the centers of the components of a twin are nearly parallel and form approximately part of an equatorial circumference of the eye (fig. 36 *b*). There is, therefore, no resemblance to the condition found in *Coregonus* and *Zygonectes* even if we omit for the present the consideration of the rods (or single cones). The cones consist of an outer segment (80 μ long in the largest specimen) with a tendency to become oblique near their outer ends. In chromic preparations these readily split into disks. They stain faintly but evenly. They are joined by a translucent interval to the body of the cone, an ellipsoid body 5 μ + 10 μ taking on a deep stain (fig. 36 *a*). These rest apparently on a membrane cylinder extending from their base to near the external limiting membrane, a distance of 10 μ. Here they rest on a deeply staining cone-shaped cell body which pierces the external limiting membrane and is extended as a less deeply staining, nodulated process to the outer reticular layer, where it spreads out into a cone-shaped base.

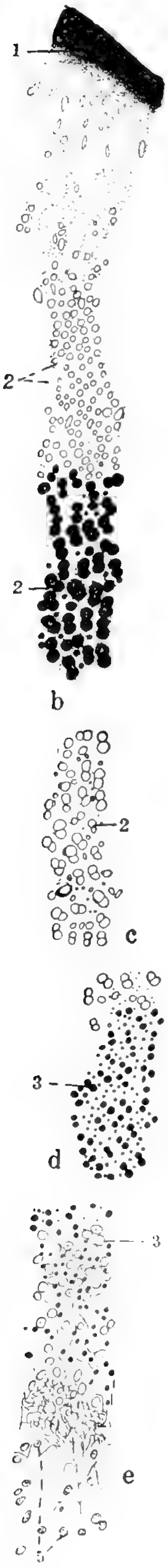
The rods or single cones are very much fewer in number and not regularly arranged. They are much fewer than the number of nuclei in the outer unclear layer exclusive of the twin cone nuclei. But extending just without the external limiting membrane a large number of short processes are seen between the cone nuclei (fig. 35 *e*). Whether these are degenerate rods, I am unable to say.

The outer nuclear layer differs materially in the younger, *i.e.*, smaller (29 mm.), specimens and in the largest specimens. In the younger specimens it consists of several layers of cells exclusive of the cone cells, which in this case can be counted with the layer of rods and cones. In the larger specimens this is reduced to a single layer of nuclei less densely packed, with occasionally a horizontal nucleus near the base which is less granular, staining a more uniform color.

In the largest specimen the outer nuclear layer makes up about 7 per cent of the total thickness of the retina, in the smaller specimens it is slightly thicker, forming 10 per cent of the total thickness.

The outer granular layer differs also materially in the largest and smallest specimens. In the largest it forms a thin layer entirely free from nuclei and with a total thickness of but 2 or 3 $\mu$.

In a specimen 39 mm. long this layer is 5 $\mu$ thick, distinctly granular, contains a few round nuclei — not differing from those of the inner nuclear layer. (See fig. 35 *c*.) These are probably members of the layer of fulcrum cells. The latter are not separable from the underlying bipolar cells in other regions. In tangential sections they appear in groups of two, but are much fewer in number than the twin cone cell. The inner layer of the inner nuclear layer is composed of distinctly larger cells in the largest specimen and separated from the rest by a slight interval. The



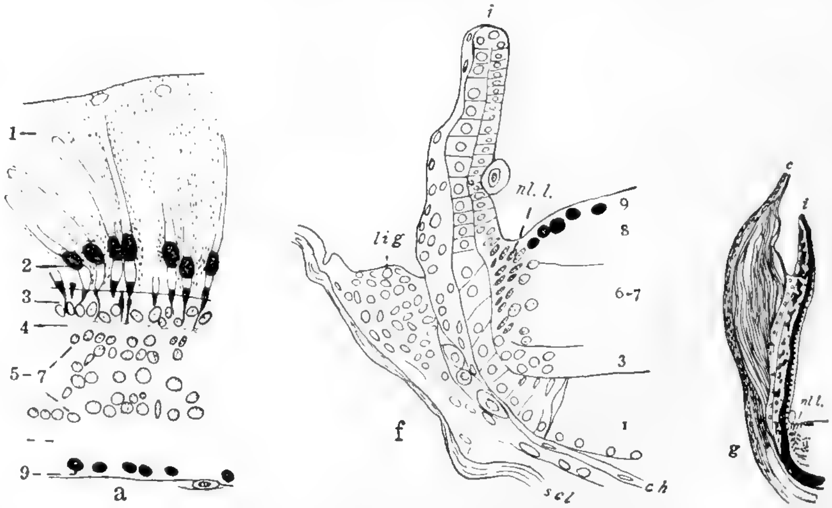


FIG. 36. (*a*) Vertical Section through Retina of *Chologaster papilliferus.* (*b*) Section from Outer Margin of Retina to Base of Cone Bodies. (*c*) Section through Cone Nuclei. (*d*) Section through Basal Segments of Cones. (*e*) Section through Outer Limiting Membrane, Outer Nuclear Layer with Cone Cell Processes, outer Reticular Layer and Outermost Layer of Outer Nuclear Layer. Nuclei in last layer are frequently in pairs, but do not correspond to and are much less numerous than twin cones. (*f*) Section through Lower Half of Left Eye seen from behind. The eye was depigmented. Oval nuclei *nl. l.* at ora serrata. *scl.*, sclera; *ch.*, choroid; *i.*, iris; (*g*) Lower part of Iris of *Zygonectes notatus.*

cells here form a distinct row in section, while the rest for the most part are irregularly placed. The difference in size is especially noticeable near the entrance of the optic nerve. The nuclei are mostly spherical. A few nuclei are found more elongate and with their longer axis at right angles to the retina (Müller's fiber nuclei). The largest spherical nuclei measure 5 μ in diameter. The inner granular layer varies in thickness and contains few cells.

The ganglionic layer consists of a single layer of nuclei, rather irregularly placed. The nuclei measure 6 μ in diameter. For reasons explained in a previous paragraph a distinct nerve-fiber layer is not present. A thin nucleate membrane, the hyaloid membrane, containing the blood-vessels, lies directly on the ganglionic layer (fig. 35 *c*).

It is quite evident from the foregoing that the retina is very much simplified as compared with that of *Zygonectes*. The point of greatest degeneration lies between the outer nuclear and inner reticular layers. The horizontal nuclei are all but entirely eliminated. The bipolar cells are, in the adult, reduced to two layers of nuclei, and the spongioblasts are reduced to a single layer of cells. Even this distinction and differentiation is only seen in the largest individuals. Twin cones are abundant and apparently not lacking in number and structure, but are arranged in a different manner. Rods are much fewer in number than in either *Coregonus* or *Zygonectes*.

The chief difference between the youngest and oldest specimens of *papilliferus* examined lies in the thickness of the pigmented layer and the outer nucleated and the outer granular layers. The relative thickness of the pigmented layer increases very much with age.

The irideal region needs a few words since its structure helps to explain certain conditions in the blind fishes. The epithelial part is composed of two layers of cubical cells, of which the outer are the larger. The outer cells are normally filled with pigment to such an extent that their outlines can not be made out, the inner cells are free from pigment. The outer layer passes directly over into the pigmented layer of the retina. Where the inner layer of the iris merges into the inner layers of the retina it is composed of a group of cells with elongated nuclei (fig. 36 *f*, *nl. l.*). The uveal part of the iris is composed of a thin layer of cells with irregular nuclei, and the pigment cells of this layer are much thinner than the epithelial pigment. The ligamentum ciliary does not contain many muscle fibers, but is abundantly supplied with granular nuclei. The things of greatest importance are these granular nuclei, the epithelial pigment and the oval nuclei at the ora serrata. As compared with the same region in other fishes the shortness of the section of the iris is at once striking (fig. 36 *g*). The absence of ciliary muscles and the insignificance of Decemet's membrane are also notable.

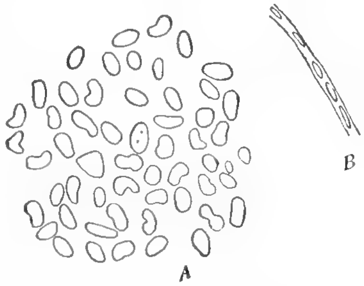


FIG. 37. Nuclei of Epithelial Layer of Lens of *Chologaster*.

The lens offers no peculiarities. The shape of its epithelial nuclei may be seen in figure 37.

### CHOLOGASTER AGASSIZII.

Only a single specimen of this species appears to have been put on record. Putnam described it from Lebanon, Tenn. The present account is based on five specimens secured by me in the river Styx in Mammoth Cave and in Cedar Sinks.

The eye of *Chologaster agassizii* Putnam is much smaller than that of *C. papilliferus.* In a specimen 41 mm. long it is placed 2.08 mm. from tip of the snout, the eye measuring 0.72 mm. in diameter. The distance from eye to eye is 2.72 mm. It is elliptical in outline, with the lateral face depressed. It is directed outward. The optic nerve, which, at its origin, is surrounded by pigment for a distance of 2.4 mm., extends almost straight inward. The dermis over the eye is essentially as in *papilliferus.* The epidermis is less simplified. It is thinner than in the surrounding tissue, but goblet cells are found in it, although they are much smaller and much less numerous than elsewhere. The sclera and choroid are as in *papilliferus*, including a pigment mass below the exit of the optic nerve just within the sclera. The optic nerve measures 24 $\mu$ at its point of entrance into the pigment layer of the retina, and it is thus one-fourth smaller in diameter than in *papilliferus.*

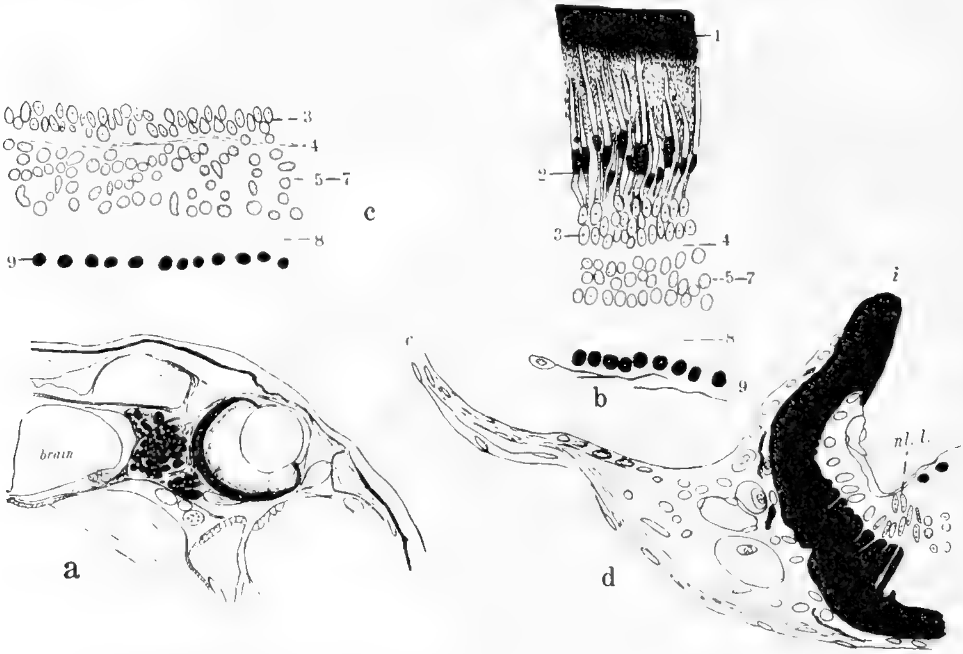


FIG. 38. *Chologaster agassizii.*
(*a*) Cross-section of Part of Head.
(*b*) Vertical Section through Retina of a Specimen 38 mm. long.
(*c*) Vertical Section through Retina of a Specimen 62 mm. long. Rods, Cones, and Pigment Layers omitted.
(*d*) Lower part of Iris of the Same Specimen, 62 mm. long.

The proportionate thickness of the retinal layers as compared with the layers of *papilliferus* is seen in the table. The maximum thickness in the largest specimen is but 130 $\mu$ as compared with 166 $\mu$ in *papilliferus.* This difference is almost entirely due to the thickness of the pigment layer, which is 74 $\mu$ in the largest *agassizii* and 104 $\mu$ in the largest *papilliferus*, leaving a difference of but 6 $\mu$ in the other layers. The pigmented layer is, on an average, much thinner than in *papilliferus*. Yet the per cent of the total thickness of the retina in pigment is larger than in normal fishes. The nuclei of the pigmented epithelium are irregular in outline. The part of the pigment layer about the nuclei forms a mass of pigment in which cell boundaries can not always be made out. The pigment about the nucleus is in granules; farther in, about the cone bodies, it is in prisms. I have not been able to make out rods. The cones are irregularly elongate so that the cone bodies are at various heights. The pattern of the twin cones has, therefore, not been made out.

The outer nuclear layer consists of nuclei conical in shape, partly outside the outer limiting membrane as in *papilliferus*, and a number of oval nuclei forming a double series within these in the younger, a single series in the older specimens.

The outer reticular layer is distinct to the iris. Horizontal cells could not, with certainty, be identified. Some of the cells lie without the inner nuclear layer in the outer reticular layer and may be fulcrum cells. The inner nuclear layer is three to four series of cells deep. Müllerian nuclei are present. If artificial splitting should take place, the innermost series of nuclei separates from the outer layers; these probably correspond to the spongioblast cells of other retinas. The inner reticular layer is well defined and contains very few cells. The ganglionic layer consists of a single series of nuclei. A distinct optic fiber layer is not present.

The iris is much as in *Chologaster papilliferus*, much shorter in section than in *Chologaster cornutus*. The inner cells of the retinal part are pigmented around the margins of the pupil, while in *papilliferus* only the outer cells carry pigment.

## CHOLOGASTER CORNUTUS.

The eye of *Chologaster cornutus* Agassiz is much larger than that of the other species of the genus. The retina on the other hand is simpler. The details of the measurements are given at the end of the account of this eye. But two specimens were available for examination; they were preserved in alcohol and respectively 27 and 43 mm. long. The very remarkable retina deserves much fuller treatment than is possible with the limited material available.

Leaving out of consideration the accessory structures of the eye as choroid, sclera, muscles, etc., which are scarcely if at all different from the same structures in *papilliferus*, the retinal characters may be briefly described.

The pigment layer is very thick as compared with the rest of the retina, forming over 60 per cent of the total thickness. The pigment cells form a sheath common to any pair of the twin cones.

Connections between the cones and the outer nuclei could not be made out. There are apparently fewer cones than nuclei. For the relation of the cones to the underlying cells and of the latter to the nuclei of the inner nuclear layer, see figures 39 *c* and *d*.

The outer nuclear layer consists of a series of nuclei closely packed together with their longer axes vertical. Occasionally a fainter staining nucleus is found among the bases of these cells with its longer axis horizontal (figs. 39 *a* and 40 *b*).

The outer reticular layer is well developed. Its boundary is irregular on the side of the inner nuclear layer, but more regular on the side of the outer nuclear layer.

Horizontal cells are very few and widely separated, if, indeed, this layer is represented at all. A few cells horizontally placed are present on the inner face of the outer reticular layer (fig. 39 *a*).

The inner nuclear layer is represented in the smaller specimen by two series of small rounded nuclei (fig. 39 *a*, 5–7). In the larger specimen a single irregular series represents this layer (figs. 40 *b*, *c*, 5–7). Besides the rounded nuclei there are a few irregular-shaped ones and other elongated ones. Some of the latter lie in the plane of this layer, others at right angles to it. The latter are probably Müllerian nuclei.

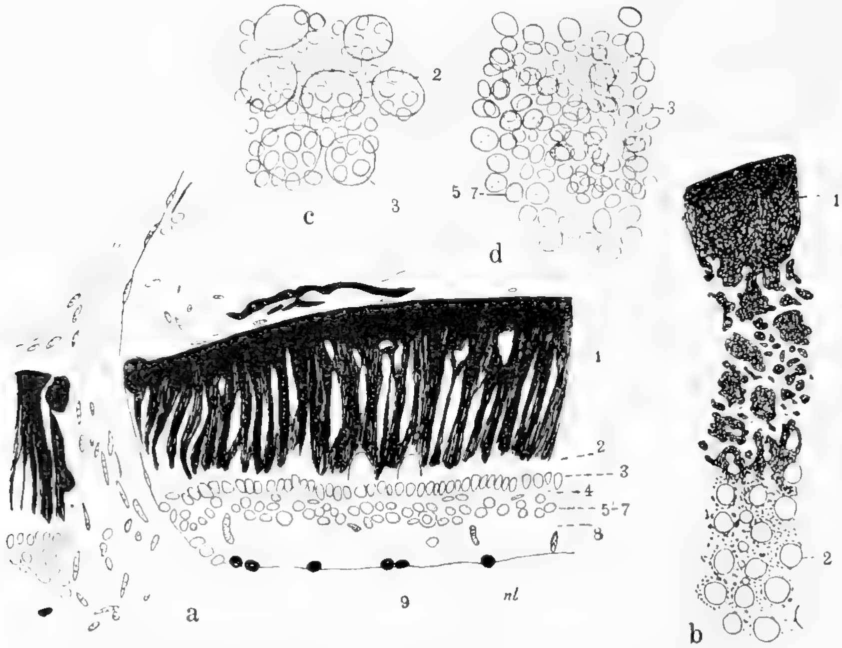


Fig. 30. *Chologaster cornutus* from a Specimen 27 mm. long.
(*a*) Entrance to Optic Nerve and Part of Retina, 2 mm. and 6.
(*b*) Oblique Section through Pigment Layer to near Outer Nuclear Layer, 2 mm. and 4.
(*c*) Bases of Cones and Underlying Nuclei of Outer Layer. Nuclei, in black, are in deeper focus, 2 mm. and 8.
(*d*) Nuclei of Outer Nuclear Layer and Deeper-lying Nuclei of Inner Nuclear Layer, 2 mm. and 8.

The inner reticular layer is well developed and contains a few round nuclei, as in *papilliferus*. In addition, it contains some vertically elongated nuclei at times reaching through half the thickness of the layer. These are also evidently Müllerian nuclei. Some of them extend from the ganglionic layer outward, others from the inner nuclear layer inward (fig. 40 *b*).

The ganglionic layer is very imperfect, being represented by scattered nuclei embedded in the inner layer of the reticular layer. In this layer we have a decided degeneration by a reduction of the number of elements (fig. 40 *a*, 9).

A nerve-fiber layer is not evident in cross-section.

The pigmented layer has not been decreased nor have the reticular layers degenerated materially beyond *Chologaster papilliferus*. The nuclear layers, on the other hand, have been very materially affected. The outer layer has been much reduced. But this need not necessarily imply degeneration. The inner nuclear layer has been reduced one-third and more from the lowest point in *papilliferus*. There is no longer any definite difference between the inner spongiose and outer bipolar cells of this layer, a difference that is usually well marked and is still evident in *papilliferus*. An equally marked change has unquestionably occurred in the ganglionic layer where a layer of cells, continuous but for the strands of the n. opticus passing between them, has dwindled to irregularly scattered cells.

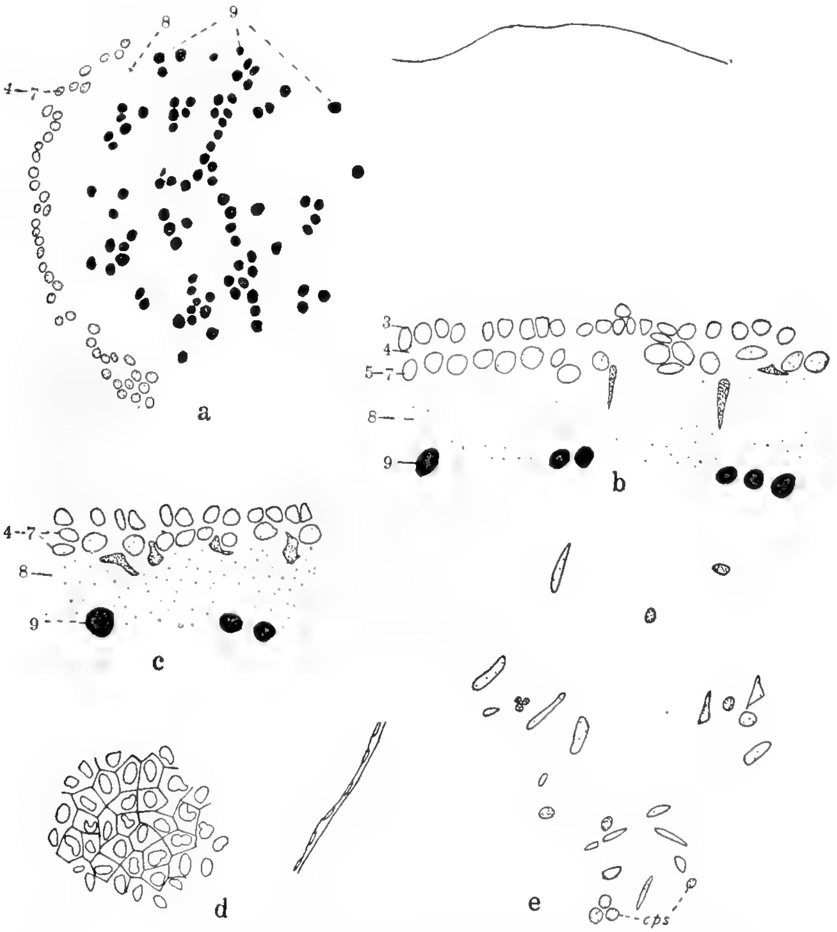


FIG. 40. (*a*) Section Tangential to Ganglionic Layer, showing Distribution of Ganglionic Nuclei, 9. On Left, 4-7, Row of Nuclei of Inner Nuclear Layer, 2 mm. and 4.
(*b*) Section of Retina through Old Individual (47 mm. long). Pigmented Layer left Blank. All Nuclei as seen in one Focus except Vertical Müllerian Nucleus, which is from Another Section. 2 mm. and 4.
(*c*) Fragment of Same Retina at Another Point.
(*d*) Cells of Lens Epithelium, Surface and Tangential, 2 mm. and 4.
(*e*) Cells and Blood Cells from Hyaloid Membrane.

The position of the Müllerian fiber nuclei is also unique in this retina.

The eye is in some respects more degenerate than that of *Typhlichthys subterraneus*. The dioptric arrangements in this eye and the cones are better developed and the layers in general are better differentiated than in *T. subterraneus*, but the nuclear layers are in the latter species composed of more series of cells. A section of the iris is much longer than in either of the other species of this genus. Since the differences in the eye and retina of the species of *Chologaster* are largely a matter of measurements, the following tables are added:

*Measurements of the Eyes of Chologaster in Groups.*

[Treated alike.]

| | Vertical diameter. | Longitudinal diameter. | Medio-lateral diameter with the lens. | Pupil. | Lens. |
|---|---|---|---|---|---|
| | μ | μ | μ | μ | μ |
| *a.* Eye dissected out and measured directly: | | | | | |
| C. cornutus Agassiz, 32 mm. long | 960 | 1,120 | 752 | ... | 544 |
| 45 mm. | 1,200 | 1,360 | 688 | ... | ... |
| C. papilliferus Forbes, 32 mm. long | 832 | 888 | 816<br>(720 without lens) | 320 | 390 |
| C. agassizii Putnam, 39 mm. long | 720 | 800 | 560 | 304 | 336 |
| *b.* Head mounted in balsam, the eyes measured from above: | | | Medio-lateral without the lens | | |
| C. papilliferus Forbes, 35 mm. long | ... | 880 | 640<br>(612 without lens)<br>(688 with cornea) | .. | ... |
| C. agassizii Putnam, 41 mm. long | ... | 720 | 486<br>(576 with cornea) | ... | ... |
| *c.* Heads sectioned in paraffine: | | | Medio-lateral | | |
| C. cornutus Agassiz, 27 mm. long | 720–800 | ... | 672 | ... | 480 |
| C. papilliferus Forbes, 39 mm. long | 640 | 805 | 560 with cornea | ... | 260 |
| C. agassizii Putnam, 38 mm. long | 536 | 738 about | 520 with cornea | ... | 296 |

*Measurements of the Retina of Species of Chologaster.*

[Only averages from two to nine measurements are given in each case.]

| | C. cornutus. | | C. papilliferus. | | C. agassizii. | |
|---|---|---|---|---|---|---|
| | 27 mm. | 43 mm. | 29–39 mm. | 55 mm. | 38 mm. | 62 mm. |
| | μ | μ | μ | μ | μ | μ |
| Pigment | 47 | 52.5 | 64.5 | 102 | 48.5 | 74 |
| Outer nuclear | 4 | 4.5 | 13.5 | 13 | 14 | 10 |
| Outer reticular | 2 | 1.5 | 3 | 2 | 2 | 3 |
| Inner nuclear | 6 | 7.5 | 18 | 19.5 | 20 | 22 |
| Inner reticular | 9 | 13 | 15 | 17 | 14.5 | 16 |
| Ganglionic | 5 | 4.5 | 8 | 9 | 8 | 5 |
| | 73 | 83.5 | 122 | 162.5 | 107 | 130 |

It is seen that the retina of *agassizii* differs from that of *papilliferus* almost altogether in the decrease of the thickness of the pigment epithelium. The retina of *cornutus* differs from that of *agassizii* in the reduction of the layers inside of the pigment epithelium.

## TYPHLICHTHYS SUBTERRANEUS.

The eye of *Typhlichthys subterraneus* has not heretofore been made the subject of study. The following account is based on 3 specimens, 20, 25, and 45 mm. long respectively, from a small cave in the town of Glasgow, Kentucky, and a number of specimens of various sizes, the largest 54 mm., from Mammoth Cave, Kentucky. These were all collected by myself in the early part of September, 1897.

The eye of this species is in general less degenerate than that of *Amblyopsis*. The accessory structures are, on the other hand, much more degenerate than in *Amblyopsis*. The eye can not be seen from the surface. The region of the eye is, however, more conspicuously apparent than in *Amblyopsis* on account of the thinner tissues of this smaller species through which the orbital fat-mass can be seen. The eye can not be seen even in heads cleared with oil on account of the almost total absence of pigment about the eye and its total absence in the eye itself.

The eye is surrounded by a large mass of fat through which connective tissue cells are scattered. A distinct separation of the orbital fat from the other fatty tissues in this neighborhood by connective tissue membranes such as are found in *Amblyopsis* is not noticeable in this species. A few pigment cells are found scattered through the fat-mass. They are nowhere massed together so as to become evident to the naked eye. In one eye not a single pigment cell is found about its surface, in another three are found on the surface of the connective tissue surrounding the eye. In no case is the pigment about the eye of any significance, for it is as abundantly found throughout the fatty tissue surrounding it.

No trace of eye muscles are present. Scleral cartilages are entirely absent, a condition in striking contrast to that found in *Troglichthys rosæ*, with which this species has been confounded.

*Sclera and Choroid.* — The sclera and choroid coats are not separable in this species. In specimens up to 40 mm. in length the eye is surrounded by a very thin membrane containing here and there a nucleus, and in the region of the choroid fissure and near the exit of the optic nerve a few capillaries. In the oldest specimen, 54 mm. long, the tissues about the eye are distinctly more fibrous, but even here I have not been able to separate the layers. From the front of the eye a strand of tissue similar to that surrounding the eye extends outward. A blood-vessel reaches the eye with the optic nerve, and a few capillaries are found on the surface of the eye and in the hyaloid membrane, but the details of their distribution I have not made out. This primitive condition of the outer layers of the eye is not so striking as at first appears when the conditions in *Chologaster* are taken into consideration, for even in *Chologaster* the choroid and sclera are insignificant.

*The Eyeball.* — The eye is on an average 1.68 mm. in diameter and has reached this size when the individual has reached 25 mm. in length. In specimens of this length the cells of the retina are still undergoing division. In a specimen 20 mm. long it has a diameter of 1.42 mm. Its maximum differentiation is not reached at the time it first reaches its maximum diameter. The eye is probably potentially functional throughout life as a light-perceiving organ. A minute vitreal cavity, remnants of the hyaloid with its blood-vessels, outer and inner nuclear as well as inner, and usually also the outer reticular layers are well differentiated, and the optic nerve is certainly still connected with the brain at a time when the fish has reached a length of 40 mm.

The position of the eye is not fixed, so that in different series of sections, presumably cutting the head in the same planes, the choroid fissure occupies various positions and the eyes are cut in various directions. With this general sketch the various layers may be taken up in detail.

*Pigment Layer* (1 in figs. 41 *a*, 43 *c*). — No pigment granules are present in the eye, a condition in great contrast to that in either *Amblyopsis* or *Chologaster*, where the pigment is least affected by the degeneration processes. The absence of pigment in this eye is indeed unique among vertebrates. Whether pigment is developed in earlier stages and disappears I have not been able to determine. In the specimens 40 mm. and less in length the pigment layer consists of a series of cells, but little separated from the underlying outer nucleated layer. The separation between the layers is greatest near the exit of the nerve and at the iris. In older individuals a considerable space is formed between the pigment layer and the outer nucleated layer on the dorsal and proximal parts of the eye, but since in all of the cases under consideration a good share of this space is attributable to reagents, a more detailed description is useless. However, in these regions delicate protoplasmic processes extend inward to the nucleated layer. The nuclei of the pigmented layer stain much more faintly than those of the rest of the retina with Biondi-Ehrlich, but just as deeply as the others with hæmalum. The cells of the pigment layer are in one series, but occasionally a cell is found below the level of the rest. A few cells very elongate in section may be mentioned here. They were found (fig. 41 *a*) on the inner face of the pigment layer. These are important in the interpretation of the structure of the eye of *Troglichthys rosæ*, where they are also found. Their origin and significance are not known.[1]

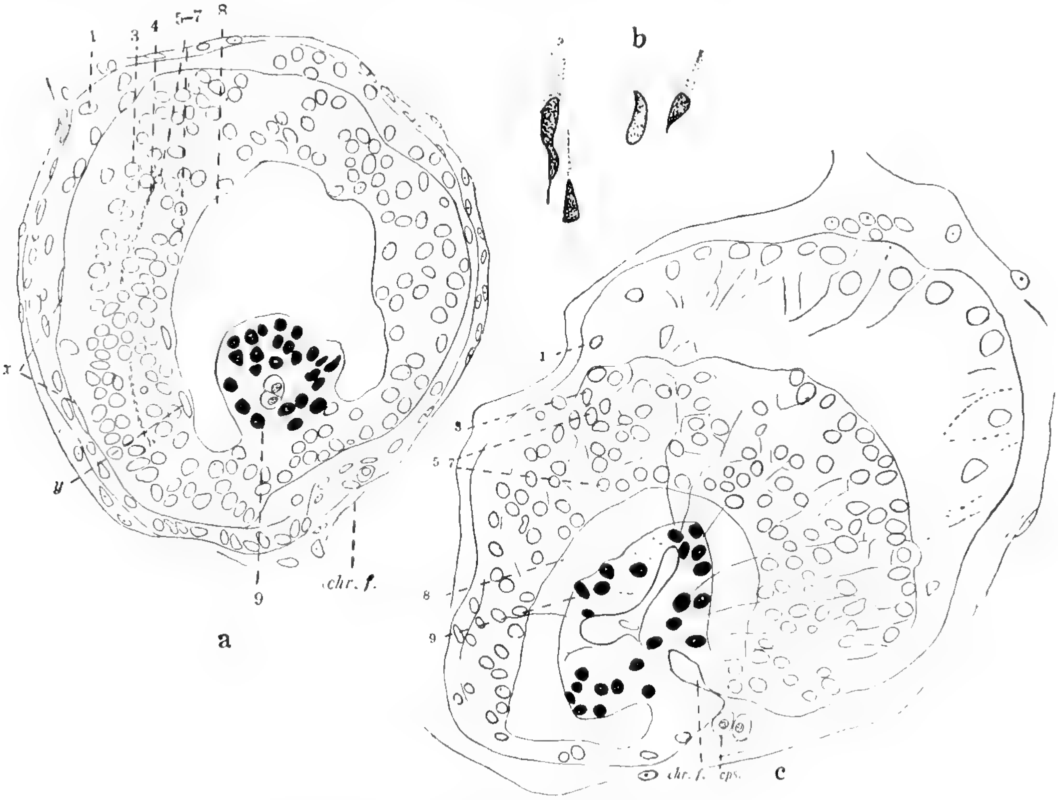


FIG. 41. (*a*) Sagittal Section through Right Eye of *Typhlichthys subterraneus*, 25 mm. long.
(*b*) Müllerian Nuclei (?) from Retina of Individual 25 mm. long.
(*c*) Horizontal Section of Eye of Individual 40 mm. long.

*Rods and Cones with their Nuclei.* — While the outer nuclear layer is very well developed indeed, the rods and cones are not definite. In the most highly developed eye there is a distinct outer limiting membrane. Without this are filmy processes continuous with those from the pigment cells. Very rarely one sees an elliptical, slightly granular body which may or may not be a cone body. The outer nuclear layer is in some cases quite distinct, consisting of a compact series of outer (cone?) nuclei, irregularly elliptical in outline, below which are a few cells of a second series (rod nuclei?) sometimes with their longer axes parallel with those of the outer layer, sometimes horizontally disposed.

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[1] See also *Rhineura*.

Cells of bizarre appearance were noted near the iris in one of the younger individuals (fig. 41 *b*). Some of these are long, club-shaped, with rounded end turned inward, others the reverse, still others with long, elliptical outer segments and smaller inner segments.

The cones are certainly less developed than in *Amblyopsis*, while the reverse is the case with the nuclei belonging to them.

The outer nuclear layer seems but little more degenerate than in *Chologaster* as far as differentiation is concerned, being of course very much more limited in extent.

*Outer Reticular Layer* (4 in the figures). — A distinct break between the outer and inner nuclear layers, of varying thickness, where nuclei are absent or few and far between, is present. A distinct boundary line for this layer does not exist, and a reticulate appearance is only to be seen in short stretches, otherwise the layer is only distinguished in the preparations by the absence of nuclei. In the younger specimens examined this layer is not differentiated, the nuclear layers forming one continuous structure. It is quite evident from this that tissue differentiation is not completed in the eyes of this species till very late.

*The Inner Nuclear Layer.* — The nuclei of the inner layer are of two sorts, larger, granular, more faintly staining ones, and smaller, more homogeneous, deeper staining ones. In one individual they are seen to be surrounded by a comparatively large cell body whose outlines are made distinct by the branches of the Müllerian fibers. In thickness this layer exceeds both the nuclear layers in *Amblyopsis*. It was not possible to identify nuclei belonging to the Müllerian fibers as such. Supporting fibers can be followed in some individuals from the ganglionic layer through the inner reticular and the inner nuclear layers, in which they branch to send processes between the regular cells (fig. 41 *c*). Once peculiar horizontal nuclei were noticed on the inner face of this layer. They are marked *y* in figure 41 *a*.

*The Inner Reticular Layer.*—Horizontal cells are not present in the inner reticular layer. Otherwise the layer offers no peculiarities. Owing to the persistence of the union of the lips of the choroid fissure and the consequent merging of the ganglionic into the outer layers at this point, the inner reticular layer appears horseshoe-shaped in a vertical longitudinal section (fig. 41 *a*, 8). In a section going through the plane of the choroid fissure (fig. 42 *a*, 8, and plate 3, fig. D, of *Rhineura*) it appears as a central area in the eye, free from nuclei. This condition, which is seen in all but the eyes of the oldest individuals, is of importance in interpreting the conditions seen in *Troglichthys rosæ*. In the older individuals the nuclear layers become thin on either side of the choroid lips and the reticular layer approaches the pigment layer (fig. 41 *c*). The layer is well developed. Its relative thickness may be gathered from the comparative table.

*The Ganglionic Layer.* — There is no distinct optic fiber layer. The ganglionic layer consists of a single layer of cells irregularly disposed about the vitreal cavity where this is present and forming a solid core of cells behind the vitreal region inclosing blood-vessels and hyaloid nuclei. Some of the cells appear to send fibers into the inner nuclear layer in the older retinas. These may be Müllerian nuclei, since in *Chologaster cornutus* such are found in this layer. The total number of nuclei counted in one example as belonging to this layer is 100, not very greatly different from the number noticed in specimens of *Amblyopsis*. In specimens up to 40 mm. in length the choroid fissure is a well-marked structure. The pigment layer and inner layers merge into each other here, and the ganglionic layer is continuous with the pigment layer. As stated above, the inner reticular layer does not surround the ganglionic layer at this point. A vertical longitudinal section of the eye has the general appearance of a section through a Graafian follicle (fig. 41 *a*). The ovum would correspond in position to a cell in the ganglionic layer, the stalk of the ovum to the lips of the fused choroidal fissure, the outer follicular cells to the nuclear layers, and the interior cavity of the follicle to the inner reticular layer of the eye.

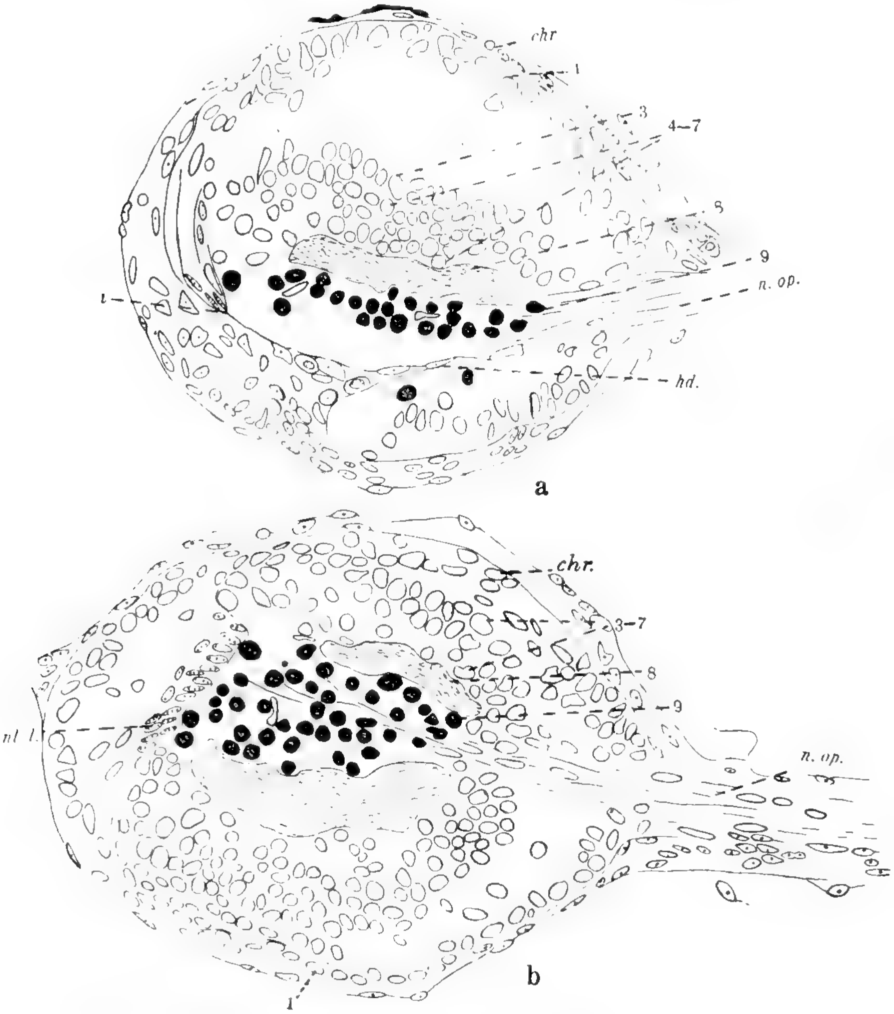

FIG. 42. (*a*) Vertical Section through Left Eye of Individual 25 mm. long.
(*b*) Vertical Section through Left Eye of Specimen 40 mm. long. Inner Layer of Cells of Uvea shows well as a Series of Elongated Nuclei, *nl. l.* Section, while passing through Ganglionic Layer, does not pass through Pupil.

*Optic Nerve.* — The optic nerve is not as distinct at its exit from the ganglionic layer as in *Amblyopsis*, but in specimens even 40 mm. long there is no difficulty in tracing it to the brain. In specimens of the latter size it has a diameter of 9 $\mu$. It contains many elongated nuclei, some of which are also seen with the optic fibers within the eye (fig. 42 *b*). The covering of the optic nerve partakes of the same indefinite nature as that of the eye itself, with which it is continuous. No pigment accompanies the nerve as a distinct layer, but here and there, as in the covering of the eye, a pigment cell may be seen, while about its entrance into the brain cavity some pigment cells are also found.

*Epithelial Part of the Iris.* — The pigment cells, as in *Amblyopsis*, decrease in height toward the irideal portion of the retina, where they become a series of pavement cells with rounded nuclei directly continuous with a layer of cells with elongate elliptically nucleated cells forming the inner layer of the iris. The homologues of the elliptically nucleated cells are found in the iris of *Chologaster* in the region of the ora serrata. At the junction of the outer and inner layers of the iris the cells are sometimes heaped up, making the irideal margin quite thick (fig. 43 *b*). There is in some cases a distinct free pupil (fig. 43,) while frequently the opening is directly continuous with the choroid fissure which may remain open in this region (fig. 41 *c*).

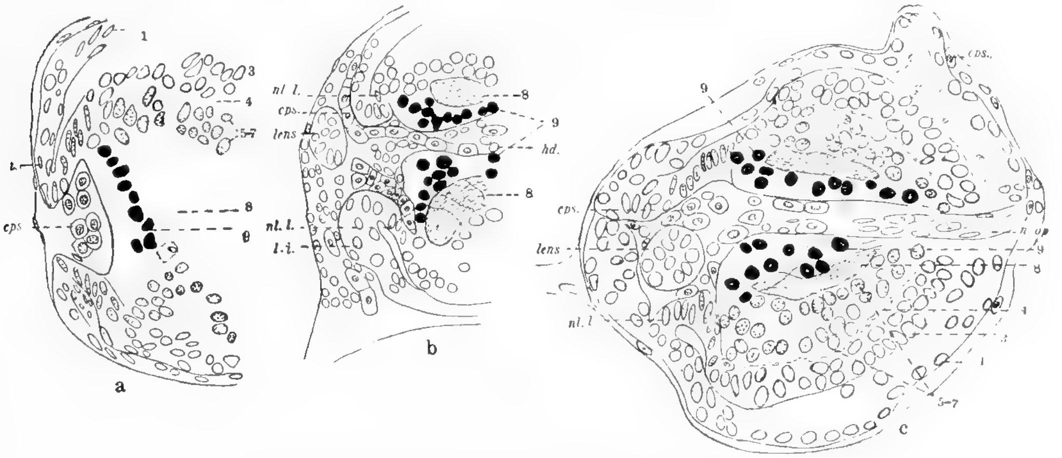

FIG. 43. (*a*) Iris of Eye shown in 42 *a*.
(*b*) Section through Iris and Lens of Right Eye of *Typhlichthys* 42 mm. long.
(*c*) Median Vertical Section of Left Eye of Same Individual.

*Lens.* — The lens was not found in all eyes; when present it is situated at the anterior end of the choroid fissure or behind the iris. It consists of but very few cells. These cells are undifferentiated. No fibers or other signs of differentiation are at all evident. The lens cells are not distinguishable from the neighboring cells, and only the faint lines seen to surround the group serve to distinguish them.

*Vitreous Body and Hyaloid.* — The choroid fissure is distinctly evident in specimens at least 42 mm. long, not as a distinct fissure, except in front, but as a line along which the various nucleated layers of the retina are merged. In the distal part of the retina the fissure is not entirely closed, and it here leaves an opening into the vitreous cavity which is more distinct and larger in the large specimens than in the smaller ones (fig. 41 *c*). The vitreous cavity, when present at all, is confined to a very narrow region just behind the lens. Here a few oval nuclei and an abundant supply of blood-vessels are to be found (figs. 41 *c*, 43 *a*, *b*), the latter communicating with the exterior through the open part of the choroid fissure. The vitreal body or cavity does not extend far into the eye, and in the core of ganglionic nuclei, where the vitreal cavity does not extend, the hyaloid membrane is represented by blood corpuscles and by a few cells with elongated nuclei whose longer diameters are parallel with the optic nerve.

*Measurements of the Eyes of Typhlichthys subterraneus.*

| Length of fish. | Diameter of eye, axial. | Diameter of eye, vertical. | Pigment layer. | Nuclear layer. | Reticular layer. | Ganglionic layer. |
|---|---|---|---|---|---|---|
| mm. | μ | μ | μ | μ | μ | μ |
| 20 | 142 | 120 | 24 | 28 | 22 | 7 |
| 25 | 160 | 142 | .. | .. | 32 | .. |
| 25 | 180 | 160 | 40 | 36 | 20 | .. |
| 40 | 180 | 144 | 12 | 36 | 18 | 12 |
| 42 | 160 | 120 | 20 | 28 | 16 | 8 |
| 42 | 160 | 142 | 16 | 32 | 12 | 28 |
| Averages | 162.66 | 128 | 22.4 | 32 | 20 | 13.75 |

## TROGLICHTHYS ROSÆ.

In December, 1889, Garman published an account of cave animals collected by Miss Ruth Hoppin in Jasper County, Missouri. Among them were a number of what were supposed to be *Typhlichthys subterraneus* Girard. A comparison of the eyes of two of the specimens collected by Miss Hoppin with the eyes of specimens of *Typhlichthys subterraneus* from Mammoth Cave showed that the western specimens represented a distinct species, and that Kohl must have based his account of the eye of *Typhlichthys* on specimens from Missouri.

In the spring of 1897, I visited the caves examined by Miss Hoppin, at Sarcoxie, Missouri, but as my stay was limited and the caves were full of water I did not succeed in getting any additional material. In September, 1898, through a grant from the Elizabeth Thompson Science Fund I was enabled to make another and this time successful effort to secure this highly interesting material.

Kohl described the eyes of *Typhlichthys*, basing his account on two specimens respectively 36 and 38 mm. long. Dr. Mark informed me that at least one of these specimens came from Missouri, and Kohl's account was certainly drawn from Missouri specimens only.

He found that the bulbus is nearly spherical, with a diameter of 0.04 mm. The orbit is a very flat cavity that offers little protection to the eye. Suborbitals are totally wanting and in their place is a cartilaginous protecting capsule, placed over the bulbus dorsally and laterally, and made up of several cartilaginous plates 0.02 mm. thick. Between the plates the connective tissue frequently contains thick and large nuclei which are sometimes united into groups. One such mass he thinks has been taken for the lens by Wyman (Putnam, fig. 5). It lies 0.195 mm. from the outer surface of the epidermis. All tissues covering the eye show absolutely no difference from neighboring parts. Eye muscles are not found, but sometimes there are stiff connective tissue strands connecting the cartilaginous bands with the tissues immediately surrounding the eye. The eye in the specimens examined he considers in the stage of the formation of the secondary eye vesicle. There is still a large cavity present representing the primitive eye cavity which is only being encroached upon by the invaginating outer cells, which in part are precociously ganglionic, sending each a process to the optic stalk. The optic stalk no longer shows a cavity, which he assumes became obliterated by the direct ingrowth of nerve fibrils and not in the usual way. The invagination of the inner layer may have progressed farther in one eye than in the other, but there is always a considerable space still left between the inner and the outer layers of the primitive eye vesicle. The elements of the inner layer, the ganglionic cells, he found to send their processes directly inward. They must have gradually revolved, since in the normal eye the nerve processes are directed outward. Some of the fibers cross each other on their way to the outlet for the nerve. Not all of the invaginated cells send processes. Among those that do there are smaller, round cells without a trace of fibers. From these the rest of the nervous parts of the retina, including of course other ganglionic cells, would probably have arisen. The outer layer of the secondary eye vesicle is also single-layered. The cells are elongate, with oval nuclei, and without a definite arrangement. They are connected with the few cells of the optic stalk that still remain. Connective tissue cells are found in the nervus opticus. They are probably mechanically active in degeneration by separating the elements. He found no sheath to the optic nerve, as described by Wyman. The lens he found to be a spherical cell heap 0.01 mm. in diameter in the distal pole of the eye. It lies just within the sclera and the cup of invagination. The sclera is made up of several layers of very fine fibrillæ. Nuclei are not found in it, but nuclei are found on its outer surface. No vessels are found in the choroid, which consists of connective tissue cells more numerous on the dorsal than on the ventral surface. The *Typhlichthys* eye is "absolut pigmentlos." The surrounding tissues are rich in pigment, which, however, is not related to the eye. There are pigment masses found here and there, but especially between the bulb and the cartilaginous capsule.

It is hard to arrive at the proper explanation of the structure of this highly degenerate eye even with an abundance of material, and it is probably not to be wondered at that Kohl in the work outlined above did not see the eye muscles, mistook the sclera for suborbitals, parts of the retina for the choroid, interpreted the pigmented epithelium of the eye as an extra optic pigment mass, mistook the inner reticular layer for the primary optic cavity, the nuclear layers for the pigment epithelium, etc., and arrived at a thoroughly erroneous idea of the general structure of the eye and based his theories on the degeneration of eyes in general on his conception of the structure of this eye. The invaginating cells of the primary optic vesicle are supposed to have been directly converted into the ganglionic cells, which are usually among the very last products of the histogenesis of the retina.[1]

By supposing that the eye was arrested at the beginning of the invagination, and that the invaginating cells rotated on their axes and were converted directly into ganglionic cells, Kohl derived the nucleated layers from the outer pigment-producing layer of the primary vesicle, at the same time ruling the pigment layer out of the eye.

The eye is very small and situated so deep that it is impossible to see it from the surface (fig. 44 *a*). In the upper half of a head cleared in xylol it is just evident to the naked eye as a minute black dot (figs. 44 *b*, *c*). As in *Typhlichthys* and in *Amblyopsis*, it is surrounded by a fat-mass filling the orbit. It is not at all

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[1] The mistakes of Kohl, especially as far as they are the result of criticising work done on *Amblyopsis* while he was working on another species, seem to me to point a moral. A certain species must not be too readily taken as an exponent of a family, order, or class, and a knowledge of related species and geographical distribution is not altogether to be neglected.

uniform in shape in different individuals or even the two sides of the same individual. It can be located and seen in cleared heads solely on account of the pigment which is always abundant over the distal face of the eye. It is located so far beneath the surface as to occasionally lie in contact with the brain case nearly opposite the posterior end of the olfactory lobe. It has thus been withdrawn much farther than in the other blind species.

It is very much smaller than the eye of either *T. subterraneus* or *Amblyopsis*. Its size is, however, quite variable, measuring 40, 49, 56, 64, 54 by 96, 56 by 120 $\mu$ in different instances, exclusive of choroid and sclera.

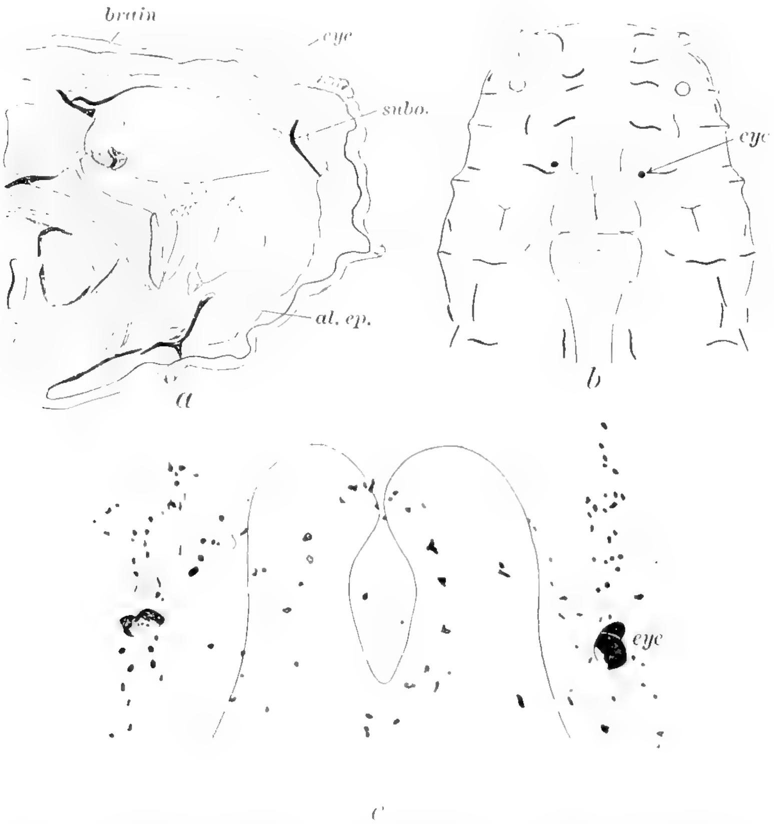

FIG. 44. (*a*) Cross-section of Part of Head of *Troglichthys*, 25 mm. long, showing Position and Proportions of Eye.
(*b*) Head of *Troglichthys* from above, showing Relative Positions of Tactile Organs and Eyes.
(*c*) Part of Same Head, showing Eyes with their Peculiar Pigmentation and Distribution of Pigment Cells in Surrounding Tissues.

The muscles of the eye were in no case normal. I have not found more than two rectus or more than one oblique muscle belonging to any one eye. They can best be made out from horizontal sections. In cross-sections it is very difficult to identify or follow them.

The best-developed rectus was found in a specimen 35 mm. long. It is composed of a number of normal fibers forming a bundle 20 $\mu$ in thickness, and from its origin to its insertion it is 256 $\mu$ long. The remarkable peculiarity of this muscle is that 100 $\mu$ of this is a tendon 4 $\mu$ in thickness (fig. 46 *b, msc. r.*). The tendon spreads into a cone-shaped mass of fibers attached to the proximal face of the eye. Traces of two muscles were made out connected with the right eye of another individual.

The oblique muscle is attached by a tendon to the face of the eye opposite that of the attachment of the rectus (fig. 46 *a*, *msc.*). In the best-developed condition it was found to be but 9 μ in diameter, taking its origin at a point on the level of the lower surface of the olfactory nerve where the latter pierces the ethmoid and 160 μ laterad from it. The muscle itself is in this instance about 200 μ in length and is attached to the eye by a tendon of equal length. The rectus in the same individual is 208 μ long.

In all the cases enumerated above the muscles of the opposite side were not nearly so well developed. In the one with the well-developed rectus the oblique was indistinct, while in the one with the well-developed oblique the rectus is also well developed, but the striations are not distinct.

The scleral cartilages form one of the striking features of this eye. They are quite variable, forming a more or less complete covering for the eye. In some they are several times as long as the eye and in such cases extend much beyond the eye. In one eye 49 μ in diameter the length of one of the cartilages reaches 160 μ (fig. 45 *a*). They have not kept pace in their reduction with the reduction of the eye in size. As a consequence individual cartilages either extend beyond the eye or are bent at acute angles in their endeavor to apply themselves to the shrunken eye (fig. 46 *a*, *scl.c.*). These cartilages were mistaken for the suborbital bones by Kohl. There is absolutely no ground for this supposition. The suborbitals are present (fig. 44 *a*, *subo.*) and widely separated from these cartilages. Further, the eye muscles are attached to the cartilages and to similar ones in *Amblyopsis*.

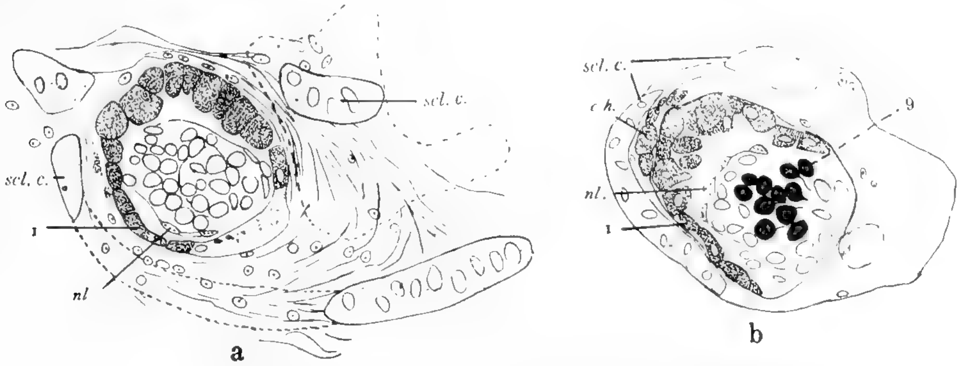

FIG. 45. (*a* and *b*) Two Cross-sections of Eye of Specimen preserved in Alcohol, 38 mm. long. Sections show Variable Extent of Pigment, Choroidal (*ch.*) Pigment, and Scleral Cartilages. Extent of latter represented by dotted lines in figure *a*.

The presence of these large cartilages is the more remarkable when we consider that none are found in *Typhlichthys subterraneus*, and in the species of *Chologaster*, which in other respects resemble *Typhlichthys* in all but the development of the eye and the color. It is quite evident that *Troglichthys* and *Typhlichthys* are not derived from a common ancestor (except, of course, remotely). Their present superficial resemblances are the result of converging development under similar environments. A species similar to *Chologaster agassizii* gave rise to *Typhlichthys subterraneus*. What the ancestry is of *Amblyopsis* and of *Troglichthys* is not known. The cartilages are bound together by an abundant fibrous connective tissue containing a few corpuscles. (These I have found nowhere as abundantly as represented by Kohl.)

The choroid, in so far as this layer can be distinguished from the sclera, consists of a dense layer of fibers closely applied to the eye. Over the distal surface it is split into two layers between which there are a greater or smaller number of pigment masses (fig. 45 *b*, *ch.*). These would prove effective to prevent the performance of the natural function of the eye were it functional. Pigment cells are much more sparingly found in other parts of the choroid. Blood-vessels are very few in number, a condition to be expected in such a minute organ. This layer was mistaken for the sclera by Kohl.

The eye proper of *Amblyopsis* differs very greatly in different individuals, but in general it maintains a certain degree of development from which the many individual variations radiate. The eye of *Troglichthys rosæ* has similarly a general type of structure which is maintained, but with many variations. This type is more degenerate than that of either *Amblyopsis* or *Typhlichthys subterraneus.*

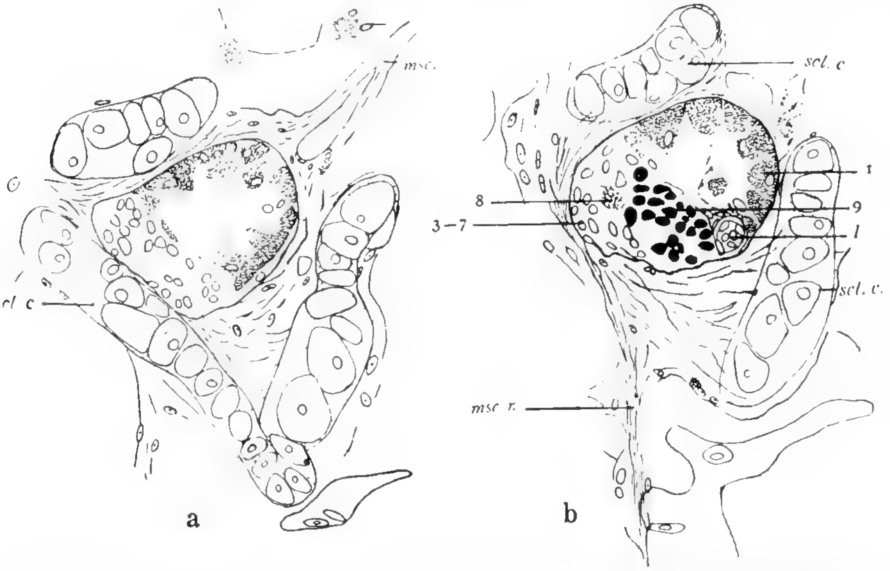

Fig. 46. Two Horizontal Sections through Eye, showing Extent of Scleral (*scl.*) Cartilages and Tendons of Oblique (*a*, *msc.*) and Rectus Muscles (*b*, *msc.r*). Fig. *a* represents section just above Fig. *b*, from an individual 34 mm. long. Drawn under magnification of 560 diameters.

The eye of *Troglichthys* has been derived from an eye like that of *Amblyopsis* by the disappearance of pigment from the posterior part of the retina and the reduction of the central mass of ganglionic cells to the vanishing point. In the most highly developed eye of *T. rosæ* (9, fig. 47) I found but three of these cells. Both in size and in structure the eye of *T. rosæ* is the most rudimentary of vertebrate eyes so far known, except that of *Ipnops* which is said to have vanished.

The vitreous cavity and the hyaloid membrane have vanished. The eye has collapsed, the margins of the iris have probably fused, and the pigmented and inner layers of the iris separated from each other. With this general sketch the elements of the eye may be taken up in detail.

The pigment layer is variously developed (1 in figs. 46 and 47) and may be quite different on the two sides of the head. One peculiarity is practically always present and very striking. The layer forms a covering over the distal face of the eye where, *a priori*, there ought to be no pigment, and is thinnest or absent over the proximal face where it ought to be most highly developed. Kohl has cut the Gordian knot by excluding this pigment from the eye entirely by the choroid (sclera), but there is certainly no such membrane intervening between this pigment and the rest of the eye as Kohl has figured. On the contrary the choroid very clearly surrounds it, and from its own epithelial structure there is no room for doubt as to its nature. As said, its extension over the sides and back part of the eye differs materially in different eyes. In a number of instances no pigment cells are present either on the sides or at the proximal surface; in others the sides are well covered. If by any means the tissues of the eye are separated from each other, the space is always formed between the pigmented layer and the rest of the eye. Processes are at such times seen to extend down from the pigment cells toward the rest of the retina. The cell boundaries and nuclei of the pigment cells are for the most part distinct. The cells are deepest over the distal pole of the eye and from this point they decrease in size to the proximal pole. Toward the upper face, where the pigment epithelium approaches the lens, the densely pigmented cells are transformed into much thinner pigmentless cells. These are probably the homologues of the pigmentless cells over the distal face of the eye of *Amblyopsis*, and, if so, are all that is left of the outer layer of the iris.

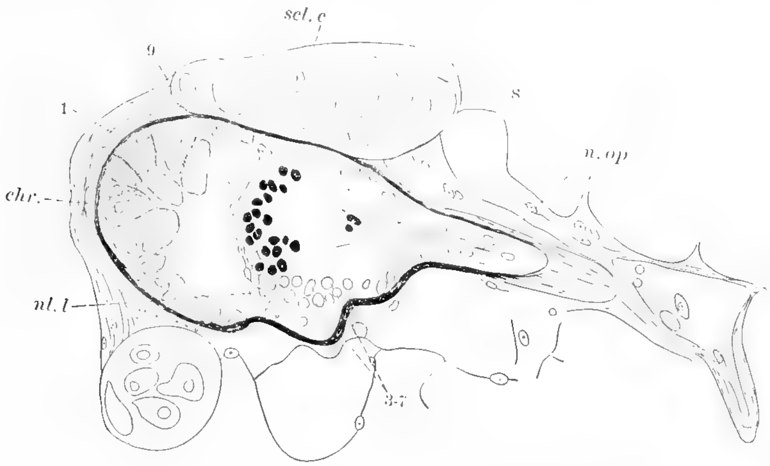

FIG. 47. Horizontal Section through only Eye with Central Ganglionic Cells.
From an Individual 34 mm. long.

The explanation of the condition of the pigment epithelium in this eye presents more difficulties than any other structure. In the eye of *T. subterraneus* no pigment is developed, but the pigment epithelium is normally developed. In this eye pigment is formed in the cells that are present, but the epithelium has anything but a normal structure. The pigment cells in the proximal face of the eye have either disappeared or been displaced. The only other alternative, that they are present but without pigment and indistinguishable from the cells of the outer nuclear layer, while possible, is scarcely probable, for in many eyes there is but a single layer of cells representing all of these structures, and in other cases even these have vanished. The objection to the idea that the cells have vanished is to be found in the fact that they are so well developed over the distal face. This point can only be settled by a study of the development of the eye, but one other suggestion may not be out of place. A comparison of this eye with that of *Amblyopsis* will suggest the homology of the anterior cell mass in the latter case, with the pigment cells always present between the retina and the irideal pigment layer in the former species. This correspondence is further strengthened by the fact that frequently the pigment in *T. rosæ* over the front of the eye is in more than one layer of cells. Since, however, I was unable to arrive at an entirely satisfactory explanation of the origin of this pigment mass in *Amblyopsis*, it will not help us much, should the two structures be homologous.

Attention may be called here to the fact that both in *Amblyopsis* and in the present species the lens — and therefore the lost pupil — are not situated at the distal pole of the eye, but above this point, and that both in regard to the pupil and the eye in general the location of the pigment masses in the two species is the same.

The pigment is granular, not prismatic.

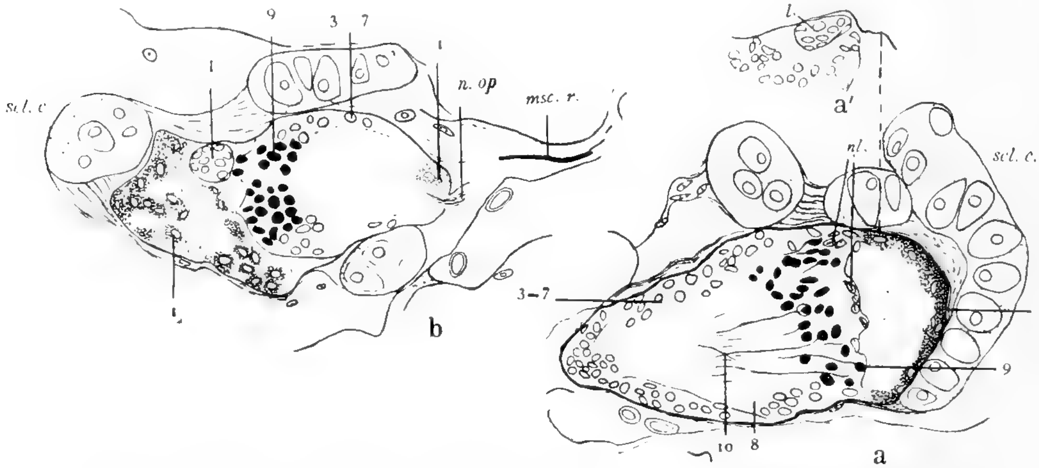

FIG. 48. Cross-sections through Right and Left Eye of an Individual 25 mm. long. Sections *bc* pass through Lens. Fig. *a* is a Composite from 3 Sections. Fig. *b* represents one Section, but the "Lens" is from the Next Section.

The lens is the only structure of the eye concerning which Kohl has not made any mistake.[1] It is a small group of cells closely crowded together and about 10 or 12 μ in diameter (figs. 48 *a′* and *b*, *l*). There are no signs of fibrilation or the result of any other histogenic process; it appears as an aggregation of indifferent cells. On its surface there are at times cells that are evidently of an epithelial nature, being flattened so that their sections appear much longer than deep. It lies at the upper outer face of the eye at the margin of the pigment mass described in the last section. It is not covered by pigment or other retinal substance. Kohl considered this condition a primary one. The lens, however, does not lie in an incipient secondary optic cavity, the vitreal cavity, as Kohl supposed, but in the remains of such a structure. Under the circumstances it is doubtful whether the uncovered condition is primary. It seems more probable, considering the condition in *Amblyopsis*, that the lens was inclosed by the closing of the pupil over the eye, and that the present naked condition is the result of the subsequent degeneration of the iris over it. That the latter is the phylogenetic origin of its present condition there is no doubt.

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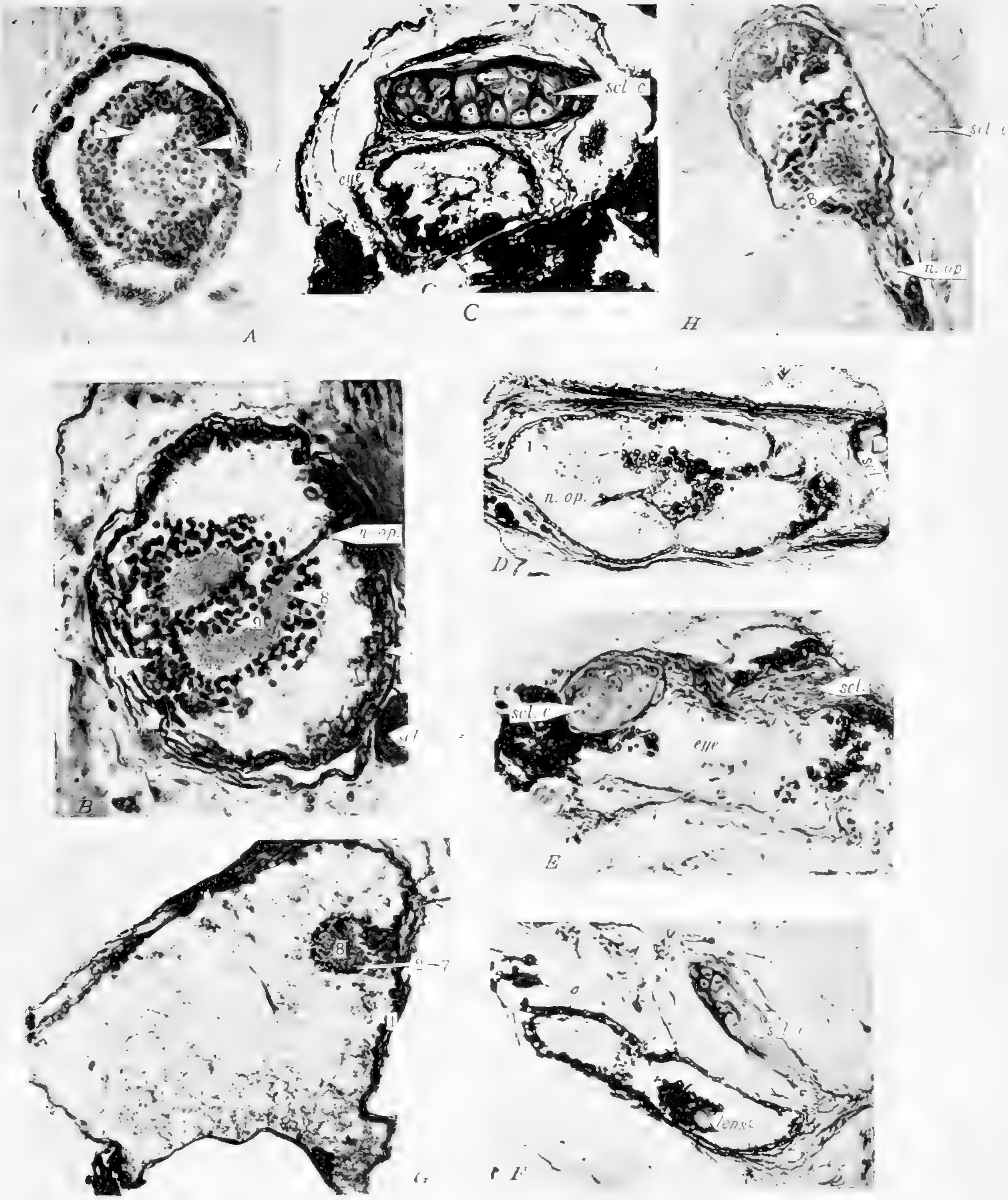
[1] Considering the history of the lens in *Amblyopsis*, I am not sure now whether Kohl was or was not mistaken about these cells.



A to G, Photographs of the eyes of Amblyopsis; H, eye of Troglichthys.

A. Horizontal section of right eye of fish 9.5 mm. long.

B. Dorsal face of horizontal section of left eye of fish 25 mm. long. Optic nerve directed forward and inward.

C. Cross-section of left eye of fish 100 mm. long.

D. Anterior face of transverse section of left eye of fish 123 mm. long.

E. Transverse section of left eye of fish 130 mm. long. No definite structures are distinguishable aside from scleral cartilage.

F. Transverse section of right eye of fish from which E was taken.

G. Cross-section of right eye of fish 105 mm. long, showing large vesicle formed by pigment epithelium and remainder of retina as small nodule on its distal face.

H. Eye of Troglichthys rosæ showing large scleral cartilages and different layers of the eye.

*The Retina:* The elements of the retina proper, *i.e.* the ganglionic, nuclear, and reticular layers, form a vesicle arranged so that the cellular elements surround a central (the inner) reticular layer. These may be taken up seriatim. The cellular elements are of three sorts.

(1) Behind the lens and behind the pigment layer, sometimes also over the side of the retina, lie a few cells with elongated nuclei (*nl,l*, in figs. 45 *a*, *b*, 46 *b*, 49 *a*) and so arranged as to suggest an epithelial covering for the underlying structures. Some of these cells were supposed by Kohl to represent the choroid, with which they have absolutely no connection. It is possible that some of these lateral cells are modified pigment cells, but even this seems doubtful. I am unable to refer the cells of this nature situated laterally over the retina to any structure in the normal retina. Such cells are, however, found in the eyes of *T. subterraneus* between the pigment epithelium and the nuclear layers (fig. 41 *a*), and whatever their origin the two structures are unquestionably homologous in the two eyes. It is probable that the cells with elongated nuclei to be found behind the lens are of different origin and significance. They may be the remains of the elongated cells found in the inner surface of the iris of *Chologaster,* cells which are still present in both *Amblyopsis* and *T. subterraneus.* It is also possible that they are the remains of the hyaloid nuclei.

(2) The ganglionic cells, which in *Typhlichthys* are arranged around the vestige of the vitreal cavity and in *Amblyopsis* form a central core and are distributed over the front of the retina, are in this species practically confined to the latter location. All there is left of the central core of ganglionic cells in *Troglichthys rosæ* is three cells in the most highly developed eye found (fig. 47 and plate 10, fig. 11). In the other eyes no indication of these cells was detected. If these cells come to be formed at all in the present eye, they migrate forward, where they form the anterior wall of cells surrounding the inner reticular layer. The fibers of the ganglionic cells extend directly from the ganglionic cells through the reticular layer to the exit of the optic nerve. The cells must, as Kohl has suggested, have undergone a rotation on their axes to send their fibers directly to the optic nerve, unless only the lineal descendants of those ganglionic cells immediately surrounding the entrance of the optic nerve in *Chologaster* are here represented, a supposition not to be entertained. The ganglionic nuclei are occasionally notably larger than the nuclei of the rest of the retina, but they are by no means always so.

(3) The cells of the nuclear layers join those of the ganglionic layer. The cells of the inner and outer nuclear layer and the horizontal cells are indistinguishable from each other. They form, in the most highly developed condition, figure 47, 3–7, a layer three cells deep covering the sides and the proximal surface of the inner reticular layer. In some cases the layer is reduced to a single series of cells, and even these are occasionally absent. There is no sharp distinction between the nuclei of this layer and those of the ganglionic layer, so that the boundary between these cells and the ganglionic cells is not marked. In some instances these cells appear to be directly continuous with the cells surrounding the origin in the optic nerve. This condition led Kohl to imagine that the primary optic stalk had become filled with nerve fibrils.

Of the reticular layers the outer (8, in fig. 47) is not developed. The inner reticular layer forms, with the optic fibers traversing it, the spherical or pear-shaped central mass of the retina. No cells are developed in the reticular layer. The optic fibers appear to pass directly through the reticular layer. This condition is probably apparent rather than real. First the vitreous cavity disappeared, bringing the ganglionic cells and the optic fiber layers together in the center of the eye. This condition has just been reached by *T. subterraneus* and *Amblyopsis*. In the present species the ganglionic cells have disappeared from the center, and only the optic fiber layer remains. This is represented by the individual fibers passing from the ganglionic cells to the exit of the optic nerve. They do not form a compact nerve, but the fibers pass individually to the exit in the most direct route from their respective cells.

I have been unable to trace the optic nerve for any distance beyond the eye. In one case it leaves the eye as a loose bundle 12 μ in diameter; in another case it is more compact, being but 4 μ in diameter. It is surrounded by a sheath of varying thickness and complexity. In one case there are a few cells about the nerve, and these are covered by the tendon of the rectus muscle, which forms a complete covering.

Measurements in μ: The scleral cartilages vary from 18 to 40 in thickness. The distance from the distal face of the retinal pigment to the ganglionic cells varies from 30 to 40. The pigment cells have a maximum depth of 14, dwindling from this to 2 or 3 on the sides. The nuclear layers reach a maximum thickness of but 10. The inner reticular layer, including the optic fiber layers, is about 40 in all directions, reaching a proximo-distal length of 70. The lens measures from 10 to 15.

## AMBLYOPSIS SPELÆUS.

The eyes of *Amblyopsis* have been described by Tellkampf, Wyman, and Putnam. These authors gave general accounts of the eyes as far as this could be done without serial sections, and their accounts are far from satisfactory. It is therefore unfortunate that Kohl, who had less material of a supposed *Typhlichthys* from Missouri, should have based a criticism of the facts observed by Wyman in *Amblyopsis* on what he saw, especially since scarcely a statement made by Kohl corresponds to a condition found in *Amblyopsis*, or even the *Typhlichthys subterraneus* from Mammoth Cave. An abstract of Kohl's result are given under *Troglichthys*.

Tellkampf first pointed out the presence of rudimentary eyes and states that these can be seen in some specimens as black spots under the skin by means of a powerful lens. The statement that the eyes are externally visible in some specimens, which was afterwards thrown in doubt by Kohl, is perfectly correct. The eye of *Amblyopsis* can be seen as a black spot with the unaided eye in specimens up to 50 mm. in length.

Wyman, in Putnam, figured the optic nerve, a lens, and muscular bands attached to the exterior of the globe, but did not recognize them as homologues of the muscles of the normal eyes of fishes. In a four-inch fish Wyman found the eye to be one-sixteenth of an inch in its long diameter. A nerve filament was traced to the cranial wall, but farther it could not be followed. The eye is made up of (1) a thin membrane, the sclera; (2) a layer of pigment cells, the choroid, which were most abundant about the anterior part of the eye; (3) a single layer of colorless cells larger than the pigment cells, the retina; (4) just in front of the globe, a lenticular-shaped transparent body, the lens; (5) the whole surrounded by loose areolar tissue.

Wyman was mistaken in his identification of Nos. 2 and 4, and part of 3.

Of this species I have had an unlimited supply of fresh material from the Shawnee Caves in Lawrence County, Indiana. I shall first give the histology of the eyes of fishes from 25 mm. long to their maximum size, 135 mm. The details of the development of the eye will follow.

In well-fed adult specimens of *Amblyopsis* there is no external indication of an eye. In poor individuals the large amount of fat surrounding the eye and collected in a ball-shaped mass becomes apparent through the translucent skin. In young specimens, before they have reached a length of 50 mm., the eyes are perfectly evident from the surface. By this I do not mean that they are conspicuous, for the minute eyes would not be conspicuous were they situated just beneath the skin. The skin is not modified in the region over the eyes, but has the same structure it possesses in the neighboring regions. This condition is in strong contrast to the conditions described for *Chologaster papilliferus*. The position of the eye can be determined from the surface in older individuals by certain tactile ridges, being between a long longitudinal ridge (supraorbital) situated caudad of the posterior nares and two vertical (suborbital) ridges. They can also be approximately located by the mucous canals, being situated above the middle of the suborbital canal forward from the fork of the suborbital and rostral canals. The exact location in relation to these ridges differs, however, to some extent in different specimens.

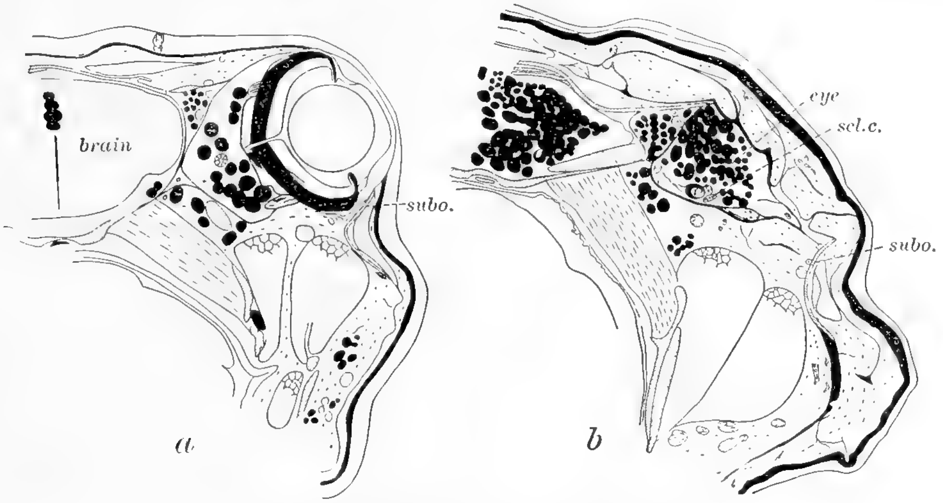


FIG. 49. (*a*) Section of Right Half of Head of *Chologaster*, through Eye.
(*b*) Section of Right Half of Head of *Amblyopsis*, through Eye.

The skull is surprisingly little modified, there being deep orbital notches, large enough to accommodate a large eye. The maintenance of this skull structure long after the eye has dwindled is significant in the consideration of the causes of degeneration and will be referred to again.

The change in the relation of the eye to surrounding tissues as well as the relative size can best be gathered from the accompanying figures or cross-sections of *Chologaster* and of *Amblyopsis*, drawn with the same magnification, but from different sized individuals (figs. 49 *a*, *b*).

Beneath the dermis (black in the figures) a thick layer of connective tissue has developed in *Amblyopsis*. The large fibrous capsule occupied by the eye, eye muscles, and orbital fat in *Chologaster* has in *Amblyopsis* become largely filled with fat. There is no indication of fatty degeneration; it is simply the accumulation of fatty cells in the eye cavity. The eye is very small and lies on the floor of the optic capsule. The infraorbital and supraorbital fat-masses described for *Chologaster papilliferus* are also large in *Amblyopsis* and form especially large masses in front and behind the optic capsule. In *Chologaster* the brain extends forward beyond the front of the eye, while in *Amblyopsis* the brain does not extend as far forward, the anterior portion of the brain cavity being filled with fat. Attention may also be called here to the presence and position of the suborbital bones which Kohl says are represented in *Troglichthys* by the cartilaginous masses forming a hood over the front of the eye. These cartilages (*scl.*, fig. 49) are present in front of the *Amblyopsis* eye, and it can readily be seen that they have nothing to do with the suborbital bones (*sub. o*).

The adult eye of *Amblyopsis* with its appurtenances may now be taken up seriatim. The eye occupies the lower part of the eye cavity. It is surrounded by loose connective tissue, which is so associated with the eye that if contractions occur through reagents, as frequently happens, a space is left between the eye with its connective tissue and the septum forming the lower floor of the eye cavity. Above the eye with its connective tissue is the large accumulation of fat mentioned previously. From the eye to the inner wall of the orbit extends a continuation of the connective tissue surrounding the eye. In this continuation of the connective tissue the optic nerve and eye muscles extend. In the longest individual, 135 mm. long, the eyes were 5 mm. from the surface of the epidermis.

The shape of the eye together with the pigment variously scattered in the connective tissue associated with it is very variable, differing from subspherical in the smaller individuals to long spindle-shaped in the old. Considerable difference is found in the shape of the eye itself. See table of measurements, page 144.

Pigment is found in very variable quantity and variously scattered in the connective tissue surrounding the eye. The amount of this pigment seems to vary inversely with the amount of pigment in the eye itself and to increase with age.

As Wyman has stated and figured, eye muscles are present in *Amblyopsis*, but, contrary to his statement, they are the homologues of the normal eye muscles. Not all preparations are equally good for tracing the muscles. They are best demonstrated in heads treated entire by Golgi's method and sectioned in celloidin. While the muscles have been noted in a variety of preparations the description will be drawn from those treated by Golgi's silver method and stained at times with hæmalum or Biondi-Ehrlich's 3-color stain.[1]

In one individual the upper rectus and upper oblique muscles are inserted together on the upper median surface of the eye, or more exactly on the upper posterior angle of the upper scleral cartilage. The lower oblique is inserted opposite this place. From these places the oblique muscles extend inward and forward. The origin of the lower oblique is 0.72 mm. in front of its insertion, while the larger upper oblique extends a little farther forward, being inserted 0.85 mm. behind its origin. It takes its origin in the projecting angle of a cartilage above and in advance of the origin of the lower oblique. In the inner part of the orbit a small muscle

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[1] Golgi's method did not give the desired results for nervous structures, but by staining with the above methods the material was found excellent for general purposes.

extends from the inferior oblique horizontally backward, taking its origin with the rectus muscles. This muscle in its posterior extent has the characteristics of the inner rectus. But whether or not its fibers reach the eye, I was unable to determine. If they do, they reach it with the fibers of the lower oblique.

The rectus muscles arise from the lateral margin of the bone forming the brain case, just behind the anterior end of the brain, the upper rectus taking its origin behind the others. They extend as four bundles forward in a connective tissue tube. Before leaving this tube they are reduced to three bundles by the union of a small bundle situated above the others in the tube with the largest bundle situated nearest the outer margin. One of these is the lower rectus. The largest one is the upper rectus and the one joining it, in all probability, the external rectus. The external rectus, if I am correct in the identification, is not distinguishable from the latter during the rest of its course nor in its insertion in the sclera. The entrance of the rectus muscles into the connective tissue sheath occurs 0.5 mm. behind their insertion in the eye. In this eye we have the two oblique muscles, the upper rectus, the lower rectus, a small bundle of fibers following for the most part the course of the upper rectus, the external rectus, and a small bundle of fibers extending from the origin of the rectus muscles forward to the lower oblique which may be the inner rectus. We have at least five, probably all six, of the muscles normal to fish eyes. But that this is not always the case is very strikingly emphasized by the fact that the eye of the opposite side of the same individual lacks the upper oblique.

In another individual the superior rectus and superior oblique are the only muscles present on the left, while on the right the upper rectus is the only muscle present. The preparations of this individual are particularly favorable for tracing the muscles. They are stained with Mayer's hæmalum and indigo carmine. The muscles are stained an intense blue, while the connective tissue through which they pass is light purple.

In still another specimen both the oblique muscles are present on the left and three of the rectus muscles, one of which, the interior, extends forward in the inner part of the orbit and joins the lower oblique as in the first individual described. No fibers of this muscle reach the eye. On the right side of the same individual the upper rectus and but one oblique muscle are present. In still other individuals not suitable for tracing the muscles, their fluctuating number has been noted, and their varying method of attachment to the eye is also a matter frequently noticed.

Inside of the loose connective tissue surrounding the eyes there is a more compact sheath. This is thickest in front of the eye where it contains spherical nuclei and holds one to three compact cartilages which usually are disposed to form a hood over the front of the eye. These cartilages, described by Wyman in this species and by Kohl in *rosæ*, and taken by the latter as the remnants of suborbital bones, have nothing whatever to do with the latter structures. Their nature can be understood from their close association with the eye, by the fact that they are closely bound together by the scleral connective tissue, and by the fact that some, at least, of the eye muscles are attached to their outer surfaces. They are unquestionably scleral elements (*scl.c.* in figs. 49 to 52). There may be some hesitation in accepting this view of the nature of these cartilages since no cartilage whatever is found in the sclera of *Chologaster*. Their position, usually in front of the eye, is also anomalous if they are scleral cartilages. It may be stated, however, that the eye of *Amblyopsis* is not simply a miniature normal eye. The whole eye has collapsed with the disappearance of the vitreous humor, and looked at in this light there is no difficulty in the position of the cartilages which have fallen together over the front of the eye. The presence of granular nuclei in front of the eye over the region of the iris has been noted by Kohl in *Troglichthys* and observed by me. These nuclei are probably the homologues of the nuclei found in the ligamentum pectinatum of *Chologaster.*

In shape, number, and size the scleral cartilages differ very much. In one instance cartilages extend continuously from the exit of the optic nerve more than half-way over the side and around the front of the eye. In another a single cartilage lies directly in front of the eye, and on the opposite side of the same individual a single cartilage lies behind the eye. The sclera is much more developed than in *Chologaster*, consisting, aside from the cartilages, of an abundant fibrous tissue.

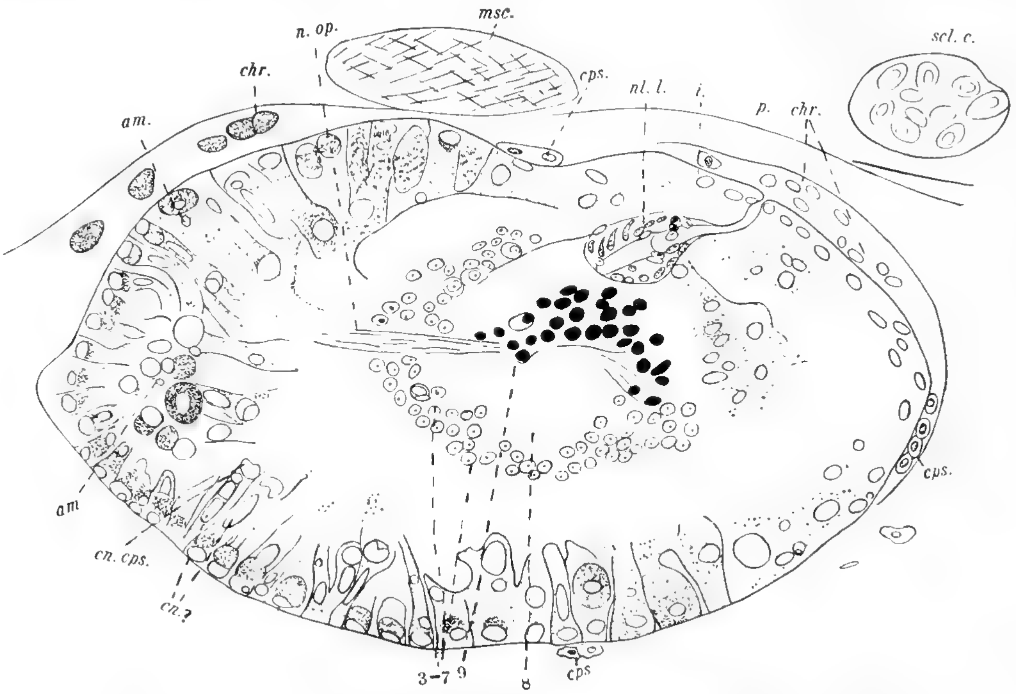

Fig. 50. Section through the Eye of *Amblyopsis spelæus* 75 mm. long, killed with Chromic Acid and stained with Biondi-Ehrlich's three-color mixture. This is the most highly developed eye seen, 2 mm. and 4.

The choroid is a thin membrane closely applied to the eye. It contains a few oval nuclei parallel with the surface of the eye. Pigment cells are few, irregularly scattered, and not at all uniform in different eyes. The pigment cells are rounded masses usually much thicker than the whole of the choroid in regions devoid of pigment. About the entrance of the optic nerve is frequently a large accumulation of pigment corresponding with the increase in the amount of choroidal pigment in *Chologaster* at the same place. Even this mass is not uniformly present. Sometimes granular masses interspersed with pigment are found here, which give one the impression of a degenerating mass. An especially large accumulation of pigment is found in the eye represented by figure 53. Blood-vessels are present in the choroid. They are apparently as great in relative capacity as in *Chologaster.* In an individual with the vascular system injected, a vessel, 0.01 mm. in diameter, approaches the eye with the optic nerve, but it does not enter the ball with the latter.

It breaks up into smaller vessels distributed in the choroid. A vessel is usually found in a groove of the pigment layer of the retina. This groove extends along the dorsal wall of the eye — otherwise it might be taken for the choroid fissure (fig. 50, *cps.*).

A somewhat larger vessel than at other points is found near the iris, where this structure appears to be continuous with a deep choroidal groove (fig. 51 *a*, *cps.*). In the young a blood-vessel enters the hyaloid cavity at this point.

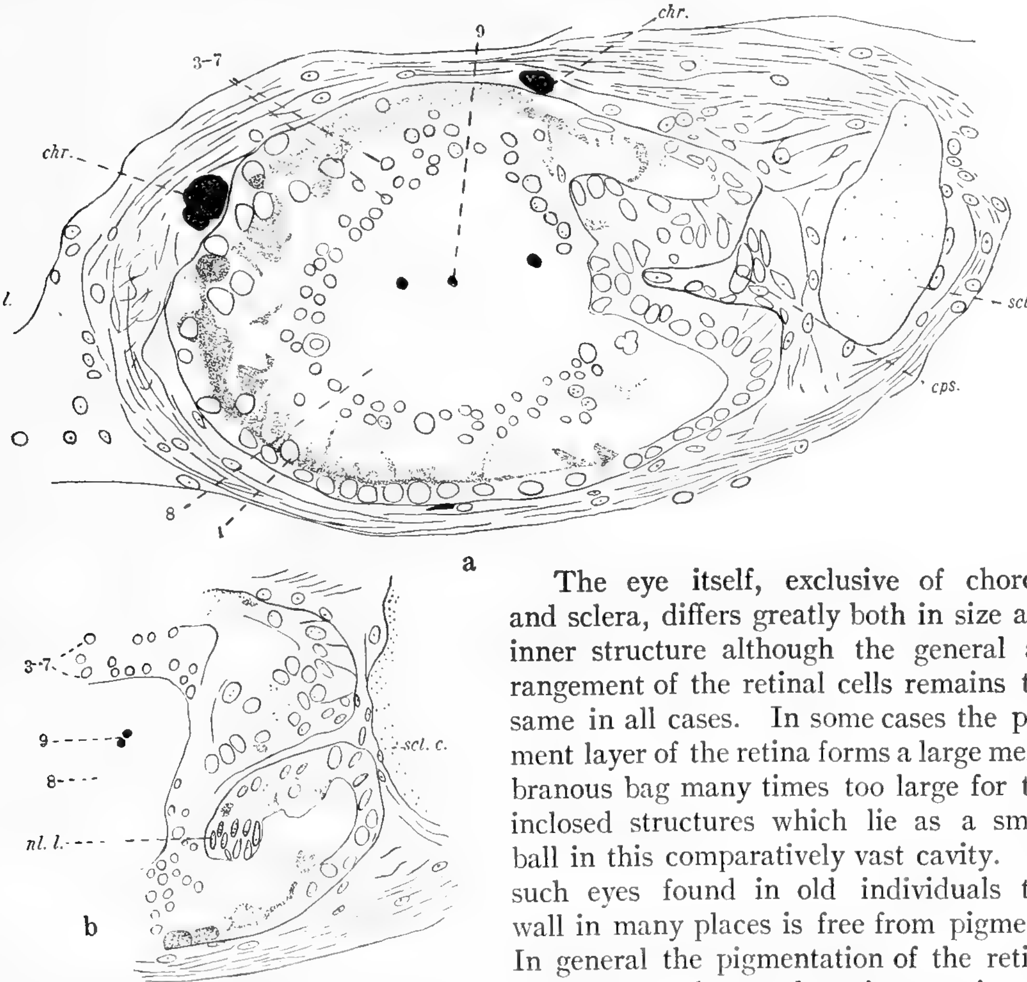

FIG. 51. From *Amblyopsis* 95 mm. long killed in Picric Acid and stained with a Mixture of Hæmalum and Indigo Carmine. Figures made with Bausch and Lomb $\frac{1}{12}$ Immersion and 4 Eyepiece.
(*a*) Section of Right Eye. Choroidal Groove with one of Scleral Cartilages in front of Eye. Nuclear Layers thinner than usual. Densely Pigmented Segments of Pigment Cells form a Conspicuous Layer just below Pigment Nuclei.
(*b*) Next Section after 51 *a*, showing Group of Elongate Uveal Cells.

The eye itself, exclusive of choroid and sclera, differs greatly both in size and inner structure although the general arrangement of the retinal cells remains the same in all cases. In some cases the pigment layer of the retina forms a large membranous bag many times too large for the inclosed structures which lie as a small ball in this comparatively vast cavity. In such eyes found in old individuals the wall in many places is free from pigment. In general the pigmentation of the retina varies inversely as the pigmentation of the choroid. In other individuals the eye forms a compact mass of cells (fig. 53). To anticipate somewhat, the vitreous cavity with the hyaloid membrane and its blood-vessels have entirely disappeared, the ganglionic cells have in large part been brought together into a solid mass, and the irideal opening has usually become closed.

*Pigmented Layer and Cones.* — The pigment cells as they appear in the best preparations of the better-developed eyes may be described first (fig. 50). The cells are longest near the entrance of the optic nerve. They possess an outer segment, not determinable in all cells, which is free from pigment. They have a homogeneous, vesicular, ellipsoidal nucleus situated near the outer end of the cell. This nucleus is strikingly different in shape and constitution from the same structure in *Chologaster*. It stains but faintly and then homogeneously. Just within the nucleus there is a well-defined mass of dense pigment forming a cap over the inner side of the nucleus and at times encroaching on the rotundity of its inner outline. This pigment mass evidently has its counterpart in *Chologaster* where a solid band of pigment is found just within the nucleus. In depigmented cells this pigment cap is seen as a deeper-staining, more dense protoplasm than the rest of the cell. From this pigment segment a prolongation, much poorer in pigment and containing a central uniformly staining core, extends toward the interior of the eye. This core, which in reality extends also into the pigmented section, occupies the position of the cones in *Chologaster*. In no case have I been able to trace any connection between these bodies and the outer nuclear layer. They are sometimes in several esgments or in a number of spherical bodies, and occasionally two are seen side by side in the same cell in tangential section. In position they certainly suggest cones, and this suggestion is heightened by the presence in the inner end of some of the cells of a vesicular structure very similar to the nucleus, but frequently with an angular indentation on the surface. These occupy the relative position of the cone bodies, they are by no means found in all eyes. The evidence seems to point most strongly in favor of the supposition that they are cones. One of the cells measures as follows: diameter of cell, 0.007 mm.; nucleus, 0.007 by 0.007 mm.; deeply pigmented mass, 0.007 mm.; total length of cell, 0.036 mm. No rods have been found.

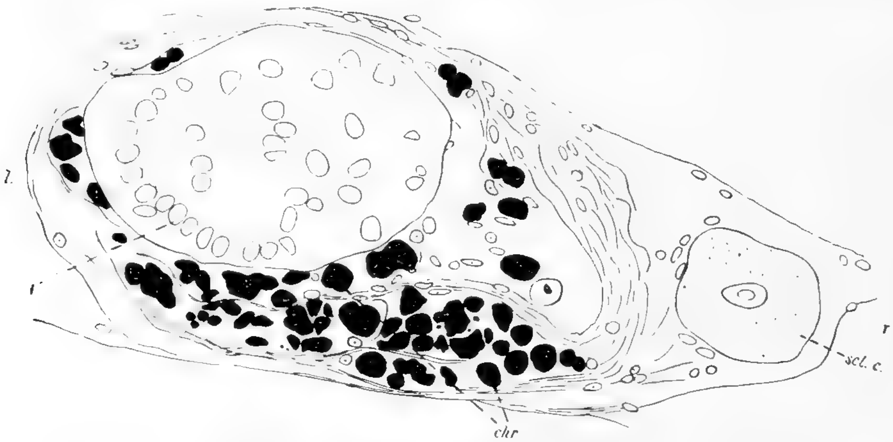

Fig. 52. Section near Posterior Face of Left Eye of Small Individual, showing particularly Position of one of Scleral Cartilages behind Eye and Thick Choroid filled with more or less Angular Mass of Granular Pigment. This Eye shows one of the Largest Accumulations of Pigment noticed.

In many individuals, and strikingly so in two specimens 25 mm. (fig. 53, *am.*) and 35 mm. long (fig. 54 *b*) respectively, deeply staining spherical bodies, much smaller than the nucleus and staining much deeper, are present in the pigment cells. Those stained with hæmalum are quite dark and give the appearance of a large centrosome. These I take to be myeloid bodies noted in the pigment cells of the frog and other forms.

In most individuals the high development of the pigmented region, above described, is not found. In some individuals the pigmented layer is composed of flat pavement cells, forming a large vesicle (plate 10, figs. D and G). In others the pigment is either entirely absent or very sparingly developed. As mentioned above, the pigmentation of the eye seems to vary inversely with the pigmentation of the surrounding structures.

The pigment is in all cases granular and differs in this respect from the prismatic pigment of the eye of *Chologaster*.

*Iris.* — The pigment cells decrease in height toward the irideal part of the eye, where they are replaced by a layer of pigmentless cells forming a thin membrane (fig. 50). The nuclei of these cells stain darker than the bodies of the cells, which is the reverse of the conditions seen in the pigmented cells. In individuals up to 35 mm. long similar cells extend along the line of the vanishing choroid fissure (figs. 54, *a* and *f*).

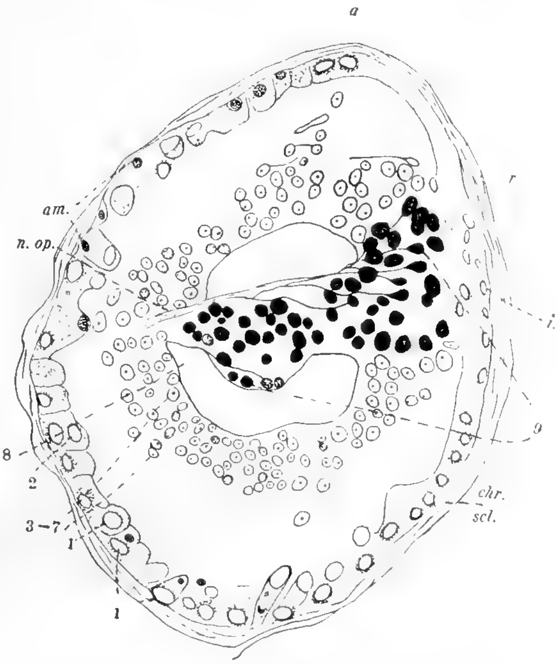

FIG. 53. Horizontal Section through Right Eye of Specimen, 25 mm. long from above. A Large Branch of Optic Nerve is seen to pass in front of Cone of Ganglionic Cells. This is not Constant, and in Left Eye of Same Individual the Largest Strand passes behind Number of Ganglionic Cells lying in front of Inner Reticular Layer and the Central Ganglionic Mass.

The pigmentless membrane is apparently the relic of the outer pigmented layer of the iris. If so it has undergone greater changes than the rest of the pigmented layer, for it is well pigmented in all the species of *Chologaster*.

The inner layer of the iris is frequently entirely separated from the outer layer and not infrequently is entirely obliterated. (A few rounded pigment masses are always found within the eye at this point.) In other individuals a minute opening is still present and the outer layer of the iris is continuous with the inner, which contains some of the elongate nucleated cells found in the region of the ora serrata in *Chologaster*. These are much more regularly present in *Typhlichthys subterraneus*. These nuclei are variously grouped in different eyes, as is represented by the figures 50, 51 *b*, 54 *b*, *d*, *e*. The exact significance of the various structures about this region in the eye can not always be determined owing to their presence or absence in different individuals and their great variability when they are present. In this region are sometimes a few cells with elongate nuclei that can not be identified with any of the structures considered. These may represent all that is left of the hyaloid. Blood-vessels are usually not found in the eye of the adult.

Between this pigmentless membrane and the rest of the retinal structures, *i.e.* within the pigment epithelium, there is in the majority of the adult eyes an irregular mass of pigmented cells. I am entirely at a loss to account for this mass unless with the shrinking of the eye as the result of the loss of the vitreous body and lens and the consequent closing of the pupil, the margin of the iris is rolled inward and some of the pigmented cells of the outer layer of the iris come to lie within the eye after the closing of the pupil. The iris is seen to be rolled in the way imagined in many sections of *Chologaster* and the method of the closing of the pupil in *Typhlomolge* is as I have suggested.

*The Nuclear Layers.* — Within the pigment and cone layer lies a nuclear layer made up of about four series of cells (3 to 7 in figs. 50, 54 *e*). The nuclei reach from 2.5 to 3.5 $\mu$ in diameter. Rarely I have succeeded in staining the smaller nuclei different from the larger. They are, in such cases, more refringent, the large nuclei being granular. The larger nuclei may be the spongioblasts. In a young individual this difference was well marked. Here the smaller cells were confined to the proximal part of the eye (fig. 53). A separation of the nuclear layer into an inner and outer with an intervening outer reticular layer I have noticed but once. In this eye a slight separating space was found on one side, and here there were one or two cells that may be fulcrum cells. If so, it is the only indication of this layer in all the preparations made. The suppression, partial or total, of the separation into an outer and inner layer, has also been noted by Ritter in *Typhlogobius.*

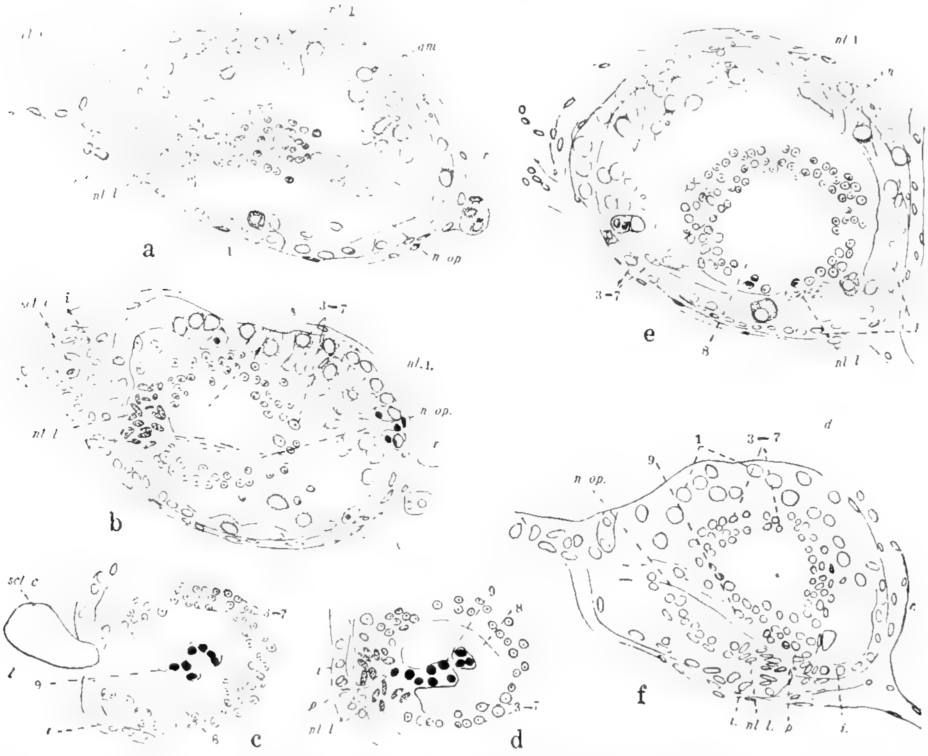

FIG. 54. From an Individual 35 mm. long killed in Perenyi's Fluid and stained with Mayer's Hæmalum.
(*a*) Outer Nuclear Layer in Center, Choroidal infolding on Left. Lower Part of figure passes through Choroidal Fissure Area and Pigment Cells are here undifferentiated, quite different from those of the Dorsal Part of Same Section.
(*b*) Further Forward and shows Strands of Optic Nerve (*n. op.*) and Elongated Nuclei of Inner Layer of Iris irregularly arranged (*nl. l.*). Choroid and Sclera can not be separated from each other except where Latter is differential as Cartilage, in front of Eye.
(*c, d*) Surface and Deeper Focus of Section passing through Iris and Central Ganglionic Cells. In fig. *d* Irideal Structure with Elongated Inner Nuclei is well shown.
(*e*) Passes near Center of Eye. Choroidal Fissure Epithelium seen below and Irregular Mass of Section through Elongated Irideal cells (*nl. l.*).
(*f*) Passes through Optic Nerve and Pupil of Same Eye as fig. *e*.
Figs. *a* to *d* are from Left Eye, *e* and *f* from Right Eye. All under Lenses 2 mm. and 4.

*The Inner Reticular Layer.* — This layer is always well developed; occasionally a few nuclei extend partially in from the outer nuclear layer. It is frequently thicker on the dorsal half of the eye (fig. 54 *f*) than on the ventral half, but sometimes the reverse. In figure 50 the ventral half is but 0.012 mm. Nuclei have but once been found in this layer, and I have not been able to identify Müllerian nuclei as such either in this or the nuclear layers. The ganglionic layer forms a compact mass of nuclei, somewhat funnel-shaped, with the narrow end toward the exit of the nerve (9 in figs. 50–54 *e*). I have found from 60 to 125 nuclei in this mass. At the wide end of the funnel this mass of cells is directly continuous with the cells of the nuclear layers. The cells in this intermediate layer are of the large type, and as they give off fibers to the optic nerve, they may be classed as ganglionic or possibly as cells belonging to the spongioblasts.

*Optic Nerve and Lens.* — The optic nerve is always evident in the eye itself except in very old individuals. It passes as a compact thread through the pigmented layer into the ganglionic layer. Here it breaks up into smaller bundles, the fibers of which pass in part to the cells within the ganglionic core, while the greater part pass to the large cells at the outer rim where the ganglionic cells pass over into the cells of the granular layers. The fact that these large cells give off the greater part of the optic fibers suggests whether or not these cells are really the ganglionic cells, while the cells forming the core are such cells as are seen at the entrance of the optic nerve in *Chologaster* (*z* in fig. 35 *c*) and there form a plug around which the optic fibers pass directly to the ganglionic cells. The bundles of fibers passing to the anterior cells never pass through the mass of core cells but at one side of this mass. In the right eye of an individual 25 mm. long they pass out in front of the mass; in the left eye of the same individual, behind them.

Outside the eye itself the matter of following the optic nerve becomes a much varying task. In very young, and up to 25 mm., there is no difficulty in tracing the optic nerve to the brain. In newly freed individuals (about two months old) the optic nerve passes nearly obliquely down and in, while in an individual 25 mm. long it passes horizontally back and in toward the foramen for the optic nerve. In the latter individual the nerve leaves the eye, not as might be expected at the posterior inner face, but at the anterior inner, making a sharp turn as it leaves the eye. Its compact nature is entirely lost after leaving the eye, forming a loose bundle several times as thick as the optic nerve within the eye. It is here surrounded by a very thin film of pigment, which in its turn is surrounded by layers of fibrous tissue.

In individuals much more than 25 mm. long it is usually no longer possible to follow the nerve to the brain. It can be followed some distance, but usually disappears before reaching the optic foramen. In but one instance did I succeed in following it into the brain cavity in an adult specimen. The structures surrounding the optic nerve are as variable as those surrounding the eye. In one case it is surrounded by various layers of pigment, while in others scarcely any pigment is found with it.

The most highly differentiated lens[1] was found in an individual 130 mm. long, *i. e.*, a very old one. The lens in this case consists of a few nuclei about which there are concentric layers of a homogeneous tissue (fig. 54 *f*). In other individuals structures approaching this condition were found (fig. 55 *a*), in one a large cell, in another a cell with concentrically arranged lamellæ. The lens, in an individual 25 mm. long, could not be found at all, and in another 35 mm. long could

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[1] It is certain that this is not the lens. The name "secondary lens" may be applied to it. Similar structures are found occasionally in *Rhineura* and *Lucifuga*.

not be determined with certainty. The relative development of the lens is not dependent on age. The lens described by Wyman was undoubtedly one of the scleral cartilages, for these cartilages are frequently nodular in this species and one usually lies in front of the eye.

The supposition of Wyman that one of the scleral cartilages is the lens need not be criticised too severely. The structures described above as the lens are considered such, more because they could not be identified as anything else, and because nothing else that could with certainty be considered a lens could be found aside from these structures, rather than on any direct evidence. The development of the eye would indeed lead one to suppose that the lens is actually placed entirely outside the optic cup, and in that case none of the structures here described can be the lens. With as much variation as is found in all the structures it is not improbable that the lens may, in some individuals, be found within the optic cup, and in others outside of it.

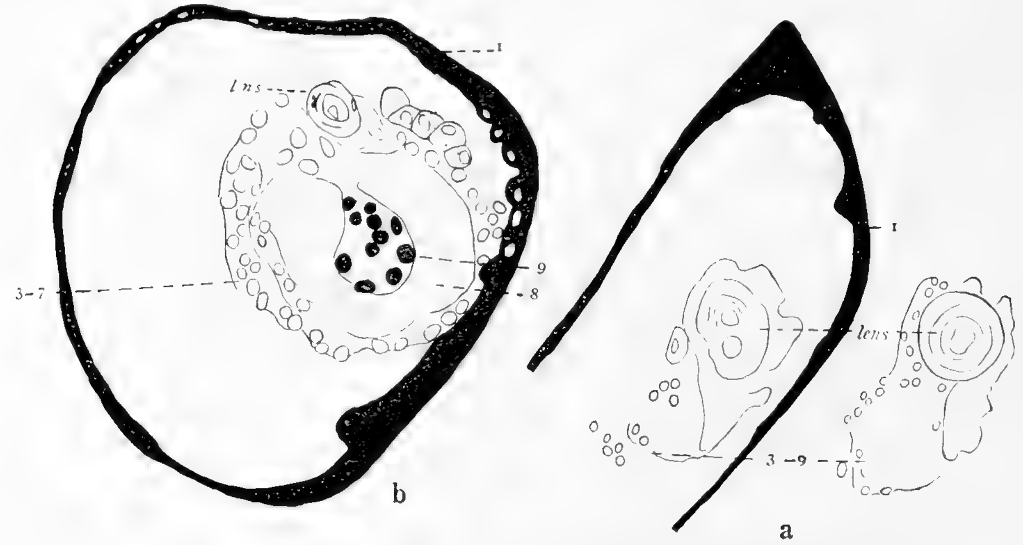

FIG. 55. (*a*) Two Successive Sections through Right Eye of very Old Individual 130 mm. long, showing a Lenslike Body.
(*b*) Outline Section of Left Eye of Individual 108 mm. long, showing highly developed Lenslike Body.

The progressive ontogenetic degeneration of the eye after maturity will be given in the section dealing with its ontogenetic history.

*Measurements of the Eye of Amblyopsis in μ*

| Length of Fish. | Diameter of Eye, Axial. | Diameter of Eye, Vertical. | Pigment Layer | | Nuclear Layer. | Granular Layer. | Ganglionic Layer. |
|---|---|---|---|---|---|---|---|
| | | | Posterior. | Anterior. | | | |
| mm. | | | | | | | |
| 25 | 160 | 160 | 28 | ? | 16 | 24 | 12 |
| 60 | 144 | 108 | 36 | 20 | 16 | 24 | |
| 65 | 96 | 108 | 28 | 12 | 12 | 28 | |
| 75 | 56 | 142 | 56 | 4 | 12 | 24 | 12 |
| 85 | 204 | 108 | 16 | 4 | 12 | 22 | |
| 108 | 200 | 142 | 56 | 8 | 10 | 54 | |
| 108 | ? | 84 | 52 | 4 | 13 × 120 | | |
| Averages | ..... | ..... | 39 | $8\frac{2}{3}$ | 13 | 24 | 12 |

## SUMMARY OF THE EYES OF THE AMBLYOPSIDÆ.

1. There are at least 8 species of "blind fishes," Amblyopsidæ, inhabiting North America; 3 with well-developed eyes and 5 with mere vestiges.

2. The 5 species with vestigial eyes are descended from 3 generically distinct ancestors with well-developed eyes.

3. The genera can be more readily distinguished by the structure of their eyes than by any other characteristic.

4. The most highly developed eye is much smaller and simpler than the eye of normal-eyed fishes.

5. The structure of their eyes may be represented by the following key to the genera and species of *Chologaster:*

*a.* Vitreous body and lens normal, the eye functional. No scleral cartilages. Eye permanently connected with the brain by the optic nerve. Eye muscles normal. No optic-fiber layer. Minimum diameter of the eye 700 $\mu$. *Chologaster*
  *b.* Eye in adult more than 1 mm. in longitudinal diameter. Lens over 0.5 mm. in diameter. Retina very simple, its maximum thickness 83.5 $\mu$ in the old; the outer and inner nuclear layers consisting of a single series of cells each; the ganglionic layer of isolated cells. Maximum thickness of the outer nuclear layer 5 $\mu$; of the inner layer 8 $\mu$. . . . . . . . . . . . . *cornutus*
  *bb.* Eye in adult less than 1 mm. in longitudinal diameter. Lens less than 0.4 mm. Outer nuclear layer composed of at least 3 layers of cells; the inner nuclear layer of at least 3 layers of cells, the former at least 10 $\mu$ thick, the latter at least 18 $\mu$.
    *c.* Pigment epithelium 65 $\mu$ thick in the middle-aged, 102 in the old . *papilliferus*
    *cc.* Pigment 49 $\mu$ thick in the middle-aged, 74 in the old; 24–30 per cent thinner than in *papilliferus*. Eye smaller . . . *agassizii*

*aa.* The eye a vestige, not functional; vitreous body and lens mere vestiges; the eye collapsed, the inner faces of the retina in contact; maximum diameter of eye about 200 $\mu$.
  *d.* No scleral cartilages; no pigment in the pigment epithelium; a minute vitreal cavity; hyaloid membrane with blood-vessels. Pupil not closed. Outer nuclear, outer reticular, inner nuclear, inner reticular, ganglionic, and pigment epithelial layers differentiated. Cones probably none. No eye muscles. Maximum diameter of eye 180 $\mu$. Eye probably connected with brain throughout life . . *Typhlichthys*
  *dd.* Scleral cartilages; pigment in the pigment epithelium; vitreal cavity obliterated; no hyaloid membrane. Pupil closed. Some of the eye muscles developed. No outer reticular layer. Outer and inner nuclear layers merged into one. Eye in adult not connected with the brain.
    *e.* Pigment epithelium well developed; cones well developed; ganglionic cells forming a funnel-shaped mass through the center of the eye. Pigment epithelium over the front of the eye without pigment. Maximum diameter of eye about 200 $\mu$ . . . . . . . *Amblyopsis*
    *ee.* Pigment epithelium developed on distal face of the eye, rarely over the sides and back. No cones. Nuclear layers mere vestiges; the ganglionic layer restricted to the anterior face of the eye just within the pigmented epithelium. Maximum diameter of eye about 85 $\mu$ . *Troglichthys*

6. The steps in degeneration are seen in figure 66, page 176.

7. The structure of the vestigial eyes differs much in different individuals.

8. The eye of *Chologaster* is an eye symmetrically reduced from a larger, normal fish eye.

9. The retina in *Chologaster* is the first structure that was simplified.

10. Later the lens, and especially the vitreous body, degenerated more rapidly than the retina.

11. The eye of *Typhlichthys* has degenerated along a different line from that of *Amblyopsis*, its pigmented epithelium having been most profoundly affected.

12. The eye muscles have disappeared in *Typhlichthys*.

13. *Troglichthys* shows that the steps in the degeneration of the muscles were in the direction of lengthening their attaching tendons, finally replacing the muscles with strands of connective fibers.

14. The scleral cartilages have not kept pace in their degeneration with the active structures of the eye.

15. The lens in the blind species, if present, is, for the most part, a small group of cells without fibers; in *Amblyopsis* it disappears early.

16. The proportional degeneration of the layers of the retina is shown in figure 67, page 179.

17. With advancing age the eye of *Amblyopsis* undergoes a distinct ontogenetic degeneration from the mature structure.

18. The phyletic degeneration does not follow the reverse order of development. None of the adult degenerate eyes resemble stages of past (phyletic) adult conditions.

19. The degenerate eyes do not owe their structure to a cessation of development at any past ontogenetic stage, *i.e.*, at any stage passed through in developing a normal eye.

20. Cessation in development occurs in the reduction of the number of cell generations produced to form the eye and in histogenesis, not in cessation of morphogenic processes.

21. In some cases (*Typhlichthys*) there is a retardation in the rate of development, the permanent condition being reached later in life than is usual in fishes. (It is possible that the pigment of the pigment epithelium never comes to develop at all. It is, however, impossible to assert this until the embryos of this species are examined. It is possible that the pigment degenerates before the stages that I have examined are reached.)

## DEVELOPMENT AND LATER HISTORY OF THE EYE OF AMBLYOPSIS.

The present chapter describes the developmental stages of the eye of the blind fish *Amblyopsis spelæus* and gives the history of the eye during growth, maturity, and old age. Questions of special interest in the history of this very degenerate organ are:

1. Do the rudiments of the eye appear as early as usual or later?
2. How much does the eye grow from the time of its appearance?
3. When does each part of the eye reach its maximum (*a*) in size, (*b*) in morphogenic development, (*c*) in histogenic development?
4. When does the eye as a whole reach its maximum development?
5. Are there evidences of a slowing down of the rate of the developmental processes: (*a*) cell division, (*b*) cell arrangement, (*c*) cell differentiation?
6. Are there evidences of a cutting off of late developmental stages, that is, are there any parts of the normal eye that are not developed?
7. Does the eye develop directly toward the condition of the adult or does it follow palingenetic paths and then retrograde to the condition found in the adult?
8. What parts of the eye degenerate first?
9. What is the comparative rate of the ontogenetic degenerative modifications of the various parts of the eye, and how does their rate compare with the rate of phylogenetic degeneration implied by the structure of the adult eye of *Amblyopsis* and the different stages of degeneration reached by other members of the family?
10. Is there any evidence for or against the dictum of Sedgwick that structures which have disappeared from the adult organization are retained in the embryo only if the organ was of use to the larva after it had ceased to be of use to the adult?

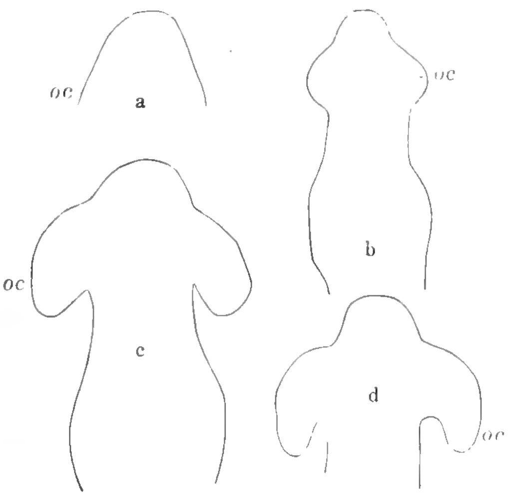

FIG. 56

(*a*) Outline of Head of Embryo between 1.3 and 1.5 mm. long.
(*b*) Outline of Brain and Optic Thickening in Mounted Embryo 1.6 mm. long, with 4 Protovertebræ (2.30 p.m., May 5).
(*c*) Outline of Brain and Optic Thickening in Living Embryo 1.92 mm. long with 10 Protovertebræ (12 p.m., May 5).
(*d*) Outline of Brain and Optic Vesicle of Living Embryo 2.4 mm. long with 10 Protovertebræ (12 p.m., May 5).

### EARLIEST STAGES TO A LENGTH OF THREE MILLIMETERS.

The development of the eye has been followed in several series of living embryos and in sections of these embryos. The earlier stages of the eye as they were observed in the series obtained on May 4, 1901, will be described.[1] Where advisable other series will be described also. The first indications of the eye are seen in living specimens when the embryo is about 1.5 mm. long, at about the time of the formation of the first protovertebra. This size was reached in the present series in 2.5 to

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[1] For an account of the general development of this series see p. 95.

3 days from fertilization. The degree of development when the eye begins to form is exactly as in fishes with normal eyes.

At 11 a. m., May 5, 1901, the head was slightly raised so that its outlines appeared definite and clear, while the remaining outlines of the embryo were hazy. It was not possible at that time to distinguish eyes (fig. 56 *a*). At $2^h\ 30^m$ p. m., when the embryo has reached a length of 1.6 mm., the eyes form prominent lobes on either side of the brain. The lobes are distinguishable in living embryos, but stand out much more prominently in embryos mounted entire. In an embryo prepared in this way, a camera outline of which is reproduced (fig. 56 *b*), the eye protuberance (*oc.*) has a length of 80 $\mu$ and projects 36 $\mu$ beyond the lateral margin of the brain. Sections of embryos at this stage of development show the brain to be still joined with the ectoderm. There is no indication of any cavity in the central nervous system at this time and the eye lobes are solid, symmetrical, lateral protuberances with their anterior margins but 48 $\mu$ from the tip of the brain. At 6 p. m. the embryo had reached a length of 1.76 mm. and 6 protovertebræ had been formed. The eye was no longer a symmetrical swelling on the side of the brain, but its outer, posterior angle was now distinctly farther back than the posterior inner angle. In other words, the lobes had grown laterad and were bent backward. The lateral projection of the eye beyond the contour of the brain amounts to 48 $\mu$ and has a longitudinal extent of 100 $\mu$ (fig. 56 *c*). The greatest diameter — measured from the anterior inner angle of the eye to the posterior outer — was 116 $\mu$. Sections show the nervous system, including the eye, to be still a solid mass of cells, which anteriorly is still continuous with the ectoderm. Histologically there is no difference between the cells composing the optic lobes and those composing the brain. There is a slight indication in the arrangement between the two optic lobes suggesting a lateral traction of the cells. At 9 p. m. the characters of the eye shown at 6 p. m. had become intensified without other material change. The embryo had reached a length of 1.92 mm. and 10 protovertebræ had been formed. The optic lobe was still broadly united with the brain, but its lateral growth was largely represented in the lobe extending back. There was no cavity as yet in the nervous system. A little later the canal of the central nervous system made its appearance, for at 12 p. m. it was well formed. There was probably some fluctuation as to the rate of growth in length and the degree of differentiation

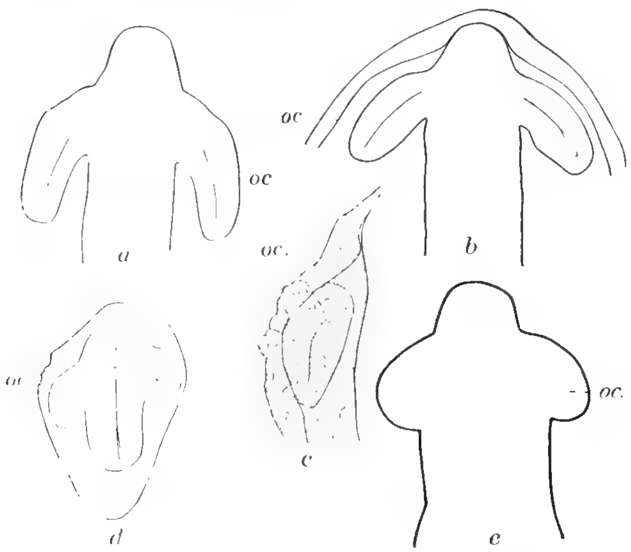

Fig. 57.

(*a*) Outline of Brain and Optic Vesicle of Living Embryo between sizes of those shown in figs. 56 *d* and 57 *b* (5.50 a.m., May 6).
(*b*) Outline of Brain and Optic Vesicle of Living Embryo 2.4 mm. long, with 12 or 13 Protovertebræ (8 a.m., May 6).
(*c*) Horizontal Section through Left Eye of Embryo about 2.44 mm. long, 2 Sections Ventrad of one represented in fig. 56 *d*.
(*d*) Horizontal Section through Head of Same Individual, showing Optic Vesicle (11 a.m., May 6).
(*e*) Outline of Brain and Optic Vesicle of Embryo 1.68 mm. long, with 5 Protovertebræ from Living Specimen.

the tissues reach, for, in embryos of another series, some individuals had a well-developed canal, while others of the same size did not. At 12 p. m. the embryos had reached a length of 2.4 mm. (fig. 56 *d*). At $5^{h}$ $30^{m}$ a. m., May 6, the eyes had become a pair of flaps lying along the sides of the brain or diverging from near its anterior end and connected only in front by the contracted optic stalk (fig. 57 *a*). The split in the optic lobe which separates it into an outer and an inner layer had developed to such an extent that it could readily be made out in living embryos. At 8 a. m. some of the embryos were still only 2.4 mm. long and 12 to 13 protovertebræ had been formed (fig. 57 *b*). The changes in the eye from 12 p. m., May 5, to 12 noon, May 6, were not very great, and consisted chiefly in the constriction of the optic stalk and the consequent gradual separation of the optic lobe from the brain. The skin had not yet begun to thicken to form the lens (figs. 57 *c*, *d*).

The changes from noon till 6 p. m., May 6, when the last embryo of this series was preserved, consisted largely in the shifting of the optic vesicles as the result of the development of the olfactory pits. Seventeen protovertebræ had developed and the embryo was about 3 mm. long.

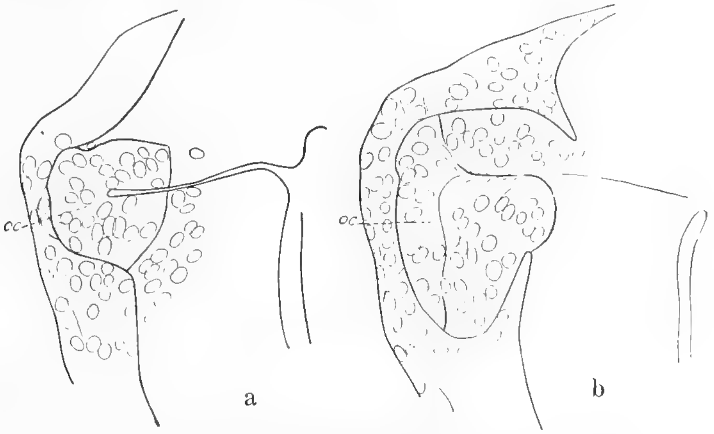

Fig. 58. Horizontal Sections through Optic Stalk (fig. *a*) and Optic Vesicle (fig. *b*) of Embryo of Second Series.

For later stages I am compelled to draw on another series of embryos which I also observed through the earlier stages described above. They were taken from a female that was captured March 11, 1898, and that contained eggs in the early stages of gastrulation.

The eyes had reached a stage seen at about 2.5 to 3 days from the beginning of development. An outline of the development may be given to connect this series with that just described. The rate of development was considerably slower than in the preceding series. Figure 57 *e* (March 13, 10 a. m.) was taken from a living specimen, showing 5 protovertebræ. Sections demonstrated that at the stage represented by figure 57 *e* the neural tube was still a solid structure. The distance from edge of eye to edge of eye measured 164 μ.

About a day later the larvæ were 2 mm. long. The neural canal had been formed and extended out into the now well-formed vesicle through a distinct optic stalk. Sections showed that the epidermis was still unmodified over the eye, with no indication of a thickening to form the lens.

Figures 58 *a* and *b* show horizontal sections through the base of the optic stalk and through the middle of the optic vesicle respectively. The embryo is 2 mm. long and in about the same stage of development as those 2.8 mm. long of first series.

During the next 24 hours the embryo grew to a length of 2.4 mm. At this stage the tail was free for 0.4 mm. of its length. Embryos 24 hours older than the last were found to be 2.5 to 2.8 mm. in length. The latter, while not longer than the oldest embryos of the first series described, are evidently farther along in the development of the eyes. In all of these specimens (figs. 59 *a*, *c*) the eyes have become greatly modified. The secondary optic vesicle has been formed by the thickening of the skin to form the lens. The retinal wall of the vesicle is three series of cells deep, while the wall destined to form the pigment epithelium has become thin and is composed of a single series of cells. The eye, at this stage, does not differ materially from that of a *Cymatogaster*[1] larva about half as long. (Compare figs. 59 *c*, *d*.)

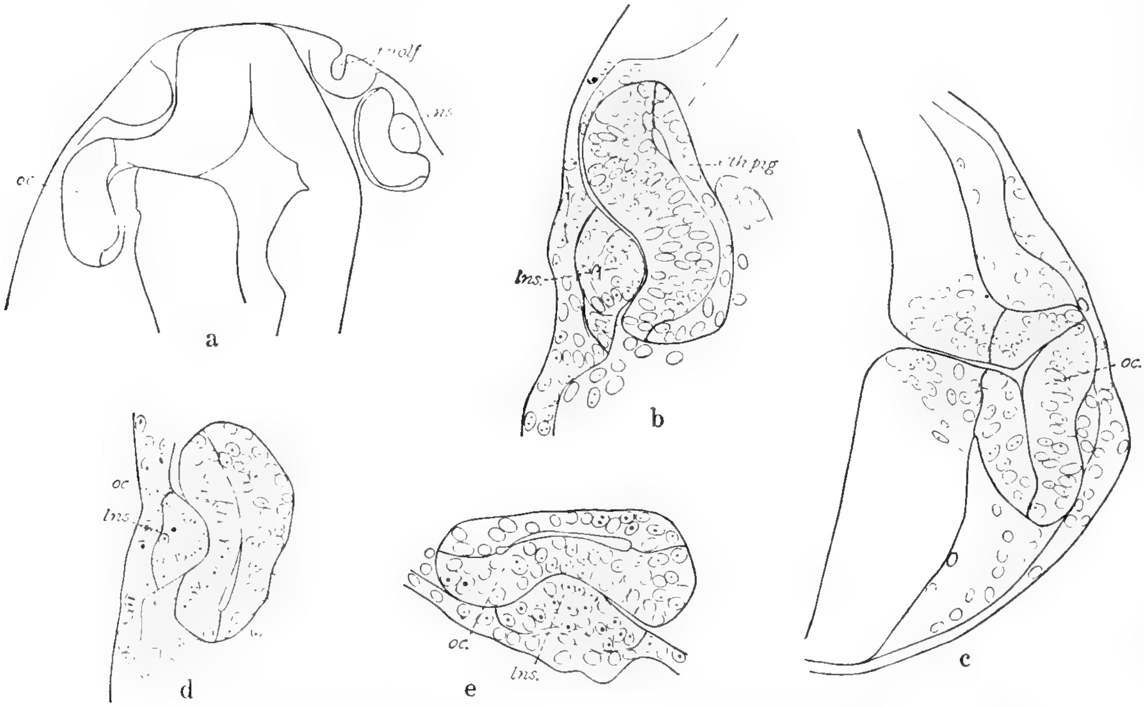

FIG. 50. (*a*) Horizontal Section of Head of Embryo 2.5 mm. long, two Sides at Different Levels.
(*b*) Left Eye of Same Embryo as that from which fig. 59 *a* was taken, showing First Indication of Lens.
(*c*) Transverse Section through Dorsal Part of Optic Stalk of Embryo 2.7 mm. long.
(*d*) Optic Vesicle and beginning of Lens in another Specimen 2.7 mm. long.
(*e*) Transverse Section of Optic Vesicle and beginning of Eye of a *Cymatogaster* larva, 1.5 mm. long.

There is no indication of a differentiation of an iris. The secondary cup is a shallow, bowl-shaped structure, the depression being entirely filled by the thickening of the skin which is giving rise to the lens (figs. 59 *b* and *d*).

### FOUR-MILLIMETER STAGES.

In specimens 4.4 mm. long the eye had become a deeper cup than it was during the 3-mm. stage. The lens, which no longer fills the entire cavity, has become

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[1] *Cymatogaster* is a teleost with large and well-developed eyes. Figures 60 *a*, *b* (*Cymatogaster*) should be compared with figures 60 *d*, *e* (*Amblyopsis*).

a spherical mass of cells, solid in some cases (fig. 60 *d*) but with a cavity in others. It is still connected with the skin. In one case the lens was a vesicle with a distinct epithelium bounding the cavity (fig. 60 *e*). In the other cases there seemed to be no regularity in the arrangement of the lens cells.

The pigmented layer has become very thin compared with the thickness of the rest of the retina. Its thickness increases toward the margin of the cup. The retina is very thick, with about 5 layers of nuclei; these are crowded except at the free margin of the retina, which is free from nuclei. There is no histological difference between the different cells of the retina unless there is an appreciable elongation in the cells at the margin of the cup.

Optic fibers are not yet developed.

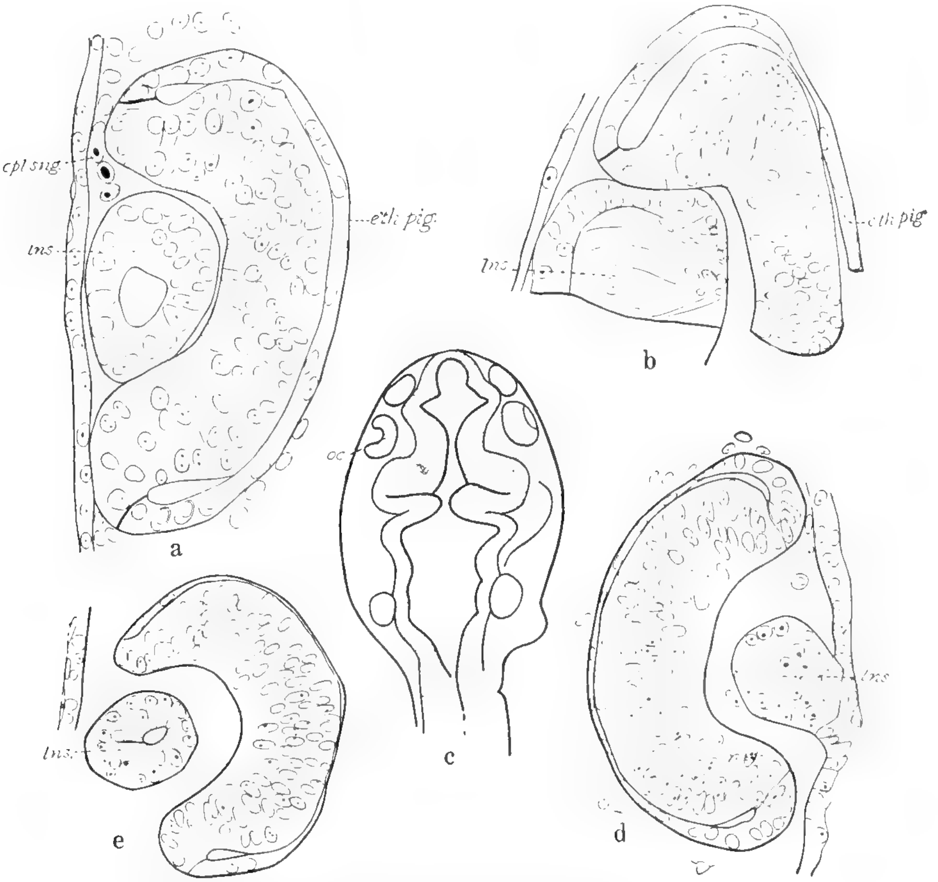

Fig. 60. (*a*) Transverse Section of Eye of *Cymatogaster* larva, 3.2 mm. long.
(*b*) Transverse Section of Eye of *Cymatogaster* larva, 4.5 mm. long.
(*c*) Transverse Section of Eye of *Amblyopsis* embryo, 4.4 mm. long.
(*d*) Section of Right Eye of Larva, 4.4 mm. long. Nuclei all drawn without a change of focus.
(*e*) Vertical Section of Eye of another Larva, 4.4 mm. long.

## FIVE-MILLIMETER STAGES.

The embryo is hatched at the beginning of this period. The least differentiated eye of this stage is represented in vertical section in figures 61 *a* and *b*. The secondary vesicle has become more definitely formed. The vitreous cavity is reduced in size and the retina has become distinctly thicker, but shows as yet no differentiation into different layers.

In a larva 5 mm. long the eye is still in contact with the epidermis on one side and the incipient dura mater on the other. The epidermis is distinctly thinner over the eye, reaching an extreme thinness of 16 μ as compared with a thickness of 40 μ at a distance of 100 μ below the eye and of 0.24 μ at 100 μ above the eye.

The lens lies directly beneath the skin. In this particular eye (fig. 62 *a*) it is an ellipsoid, 30 μ by 38 μ (36 by 28 in another eye). It is entirely separated from the skin and takes on a deeper stain. The cells of the lens are not very regularly grouped, but apparently they are arranged about a median point or space. The lens lies entirely outside of the eye in contact with the outer face of the dorsal part of the iris. The eye proper is a subspherical solid mass with only a shallow depression below the lens representing the vitreous cavity and choroid fissure. In the eye more particularly described here the depression is filled largely with blood corpuscles (fig. 62 *a*, *cpl.sng.*).

The pigmented layer is not more than 4 μ thick, and is very sparingly pigmented over the posterior face of the eye. At the iris and the lower margin of the choroid fissure it is continuous with the inner layers of the retina through cells whose nuclei are distinctly elongate. The retina proper, from the pigmented layer to the vitreous cavity, is 64 μ thick.

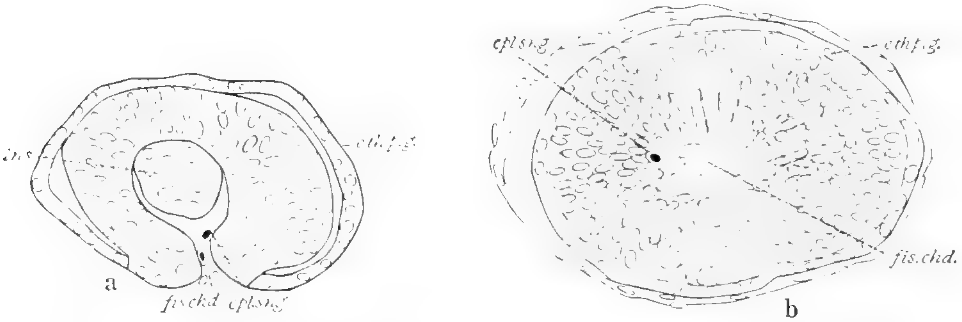

Fig. 61. Two Vertical Sections of Eye of Individual about 5 mm. long. Fig. *a* taken through Lens, Vitreous Cavity, and Choroid Fissure. Fig. *b*, Second Section Proximal to that from which fig. 61 *a* was drawn and passes through Innermost Part of Vitreous Body. Layers of Retina have not yet begun to be differentiated.

It is differentiated into a nuclear layer (the outer and inner together) and the ganglionic layer, separated by the incomplete inner reticular layer. The ganglionic layer is composed of two sorts of cells. Those nearer the vitreous cavity have much more distinct nucleoli than those nearer the reticular layer. Cell multiplication is still going on.

The optic nerve is well developed, forming a solid strand of fibers, 12 μ in diameter, readily traceable to the brain.

The muscles are represented by strands of cells closely crowded. No striation is evident.

### SIX-MILLIMETER STAGES.

In embryos 6 mm. long the cells giving rise to the oblique muscles and those for at least 2 of the recti can be distinguished. Scleral cartilages are not yet formed.

In 3 of the specimens sectioned there was no indication of a lens. In others it was well developed. Cell division was still going on in the retina.

The optic vesicle was very shallow. The rim of the vesicle was wide and still continuous with the choroid fissure, which showed as a shallow groove along the ventral surface. The choroid fissure, instead of leading into a central secondary optic cavity, led to the mass of ganglionic cells (fig. 62 *c*). This condition of the choroid fissure and its relation to the interior of the eye leads me at this point to say a few words concerning the general structure of the eye. In the description of the eye of the adult I considered that the central ganglionic mass was the result of the collapsing of the eye with the disappearance of the vitreous body and cavity. I was justified in this conclusion by the process of degeneration going on in the eye of *Typhlomolge*, *Typhlichthys*, and *Typhlogobius*. Whatever may have been the phylogenetic process in *Amblyopsis*, it is evident that ontogenetically the mass of cells does not arise as imagined. It appears from the embryos that the condition of the adults arises more as the result of a contracting of the retinal area without a corresponding decrease in the size of the eye as a whole than as the result of the collapsing of a vesicle followed by the coalescence of the walls brought together by the collapse. Sagittal sections of the eye (fig. 62 *c*) show the lips of the choroid fissure drawn apart with the contraction of the retina, only the dorsal two thirds of the eye reaching full development. From a study of the embryos of this size the point

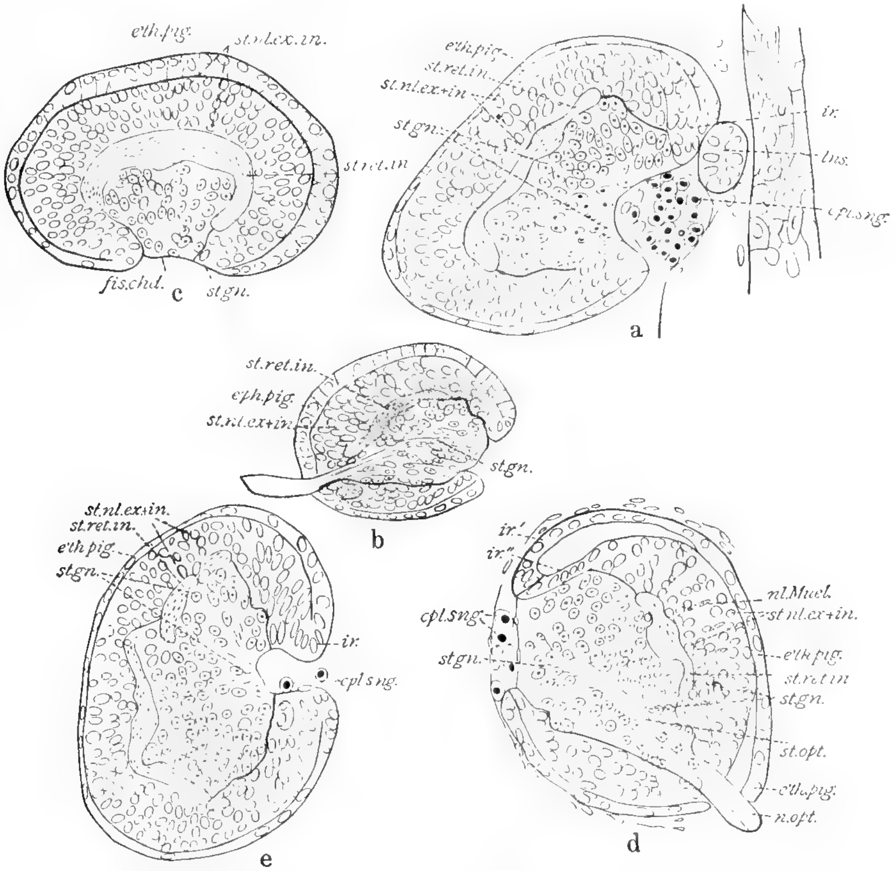

FIG. 62. (*a*) Anterior Face of Transverse Section of Left Eye of Larva 5 mm. long. Sections run obliquely in such a way that Right Eye is cut first, series beginning in front. Divergence from Spherical Outline is due to Pressure of Brain on Proximal Face and Epidermis on Distal Face.
(*b*) Anterior Face of Transverse Section of Left Eye of Larva 6 mm. long. No Lens in connection with this Retina.
(*c*) Parasagittal Section of Eye of Larva 6 mm. long, showing Ventrally Choroid Fissure represented by space between Pigmented Layers and Vitreous Cavity represented by Shallow Depression on Ventral Face. Retina differentiated into Ganglionic, Inner Reticular, and Nuclear Layers.
(*d*) Anterior Face of Transverse Section through Right Eye of Larva, 7.5 mm. long.
(*e*) Horizontal Section through Middle of Eye of Larva, 7 mm. long, showing Choroid Groove.

of exit of the optic nerve which marks the proximal end of the choroid slit alone gives evidence that potentially, at least, we have to do with an eye from which a central cavity has disappeared, *i. e.*, in which it does not develop.

The optic nerve is well developed, arising apparently from the ventral cells of the ganglionic mass, that is, those immediately lining the potential optic cavity.

The pigment cells are well developed and have a varying depth in different parts of the eye. They are low and without pigment over the front of the eye and the ventral surface near the choroid slit.

The retinal layers proper are differentiated into the ganglionic layer or mass which occupies the central and lower part of the interior of the eye. Apparently only the more centrally placed cells of this mass give rise to fibers. The inner reticular layer surrounds the ganglionic mass above and partly on the side, not at all below. The nuclear layers are well developed, without a differentiation into outer and inner layers or any indication of an outer reticular layer. The latter structure is apparently never formed at all.

### SEVEN-MILLIMETER STAGES.

The variability in the rate of development of the eye is well seen in a series of specimens about 7 mm. long and whose eyes are little if any beyond the stage of development reached in other specimens only 5 mm. long taken from another female. In the former the eye is in contact with the dura proximally, but is withdrawn from the epidermis by 36 μ or more. A strand of cells extends from the eye upward and outward to the thinnest part of the epidermis. The epidermis is distinctly thinner over the eye than in neighboring regions.

The eyeball is subspherical, with a shallow groove along its ventral surface representing the choroid slit (fig. 62 *e*).

In half of the specimens of this size examined no lens could be detected. In one the lens was a comparatively large pear-shaped structure whose cells were undergoing degeneration, if the numerous dark granules in them were indicative of degeneration. In one individual in which no lens could be found on one side, that of the other side was probably represented by a small group of cells lying between the eye and the skin (*lns.* 63 *c*). The cells were breaking apart and the outline of the structure as a whole was irregular. In all cases the lens lies outside the iris, and in fact the entire vitreous space is not large enough to hold the lens in such eyes as still show this structure.

The pigment layer is pigmented over the dorsal part of the eye. In vertical sections no pigment appears below the entrance of the optic nerve. The iridian part of the layer is, as usual, without pigment. The ganglionic cells, as in the last stages described, are exposed to the exterior through the choroid fissure, or where this is not evident there is no differentiation into different layers along the line of the choroid fissure. The ganglionic cells placed at the distal face of the eye give off fibers to the optic nerve. Fibers have not been definitely traced to the cells of the same series occupying the proximal or middle position. The optic nerve reaches a thickness of 20 μ and breaks up into bundles a short distance within the eye. These bundles radiate, forming an incomplete funnel-shaped structure. The incomplete inner reticular layer only partially separates the ganglionic and the nuclear layers. The relative development of the pigment layer and the inner reticular layer both show a less degree of differentiation than the same layers in the eyes of another series of larvæ only 6 mm. long. This is due to the individual variation in the rate of development, not to degeneration since the last stage.

Dividing cells are found in the nucleated layer. In the nuclear layers some nuclei elongated in a vertical direction are probably the nuclei of the Müllerian fibers.

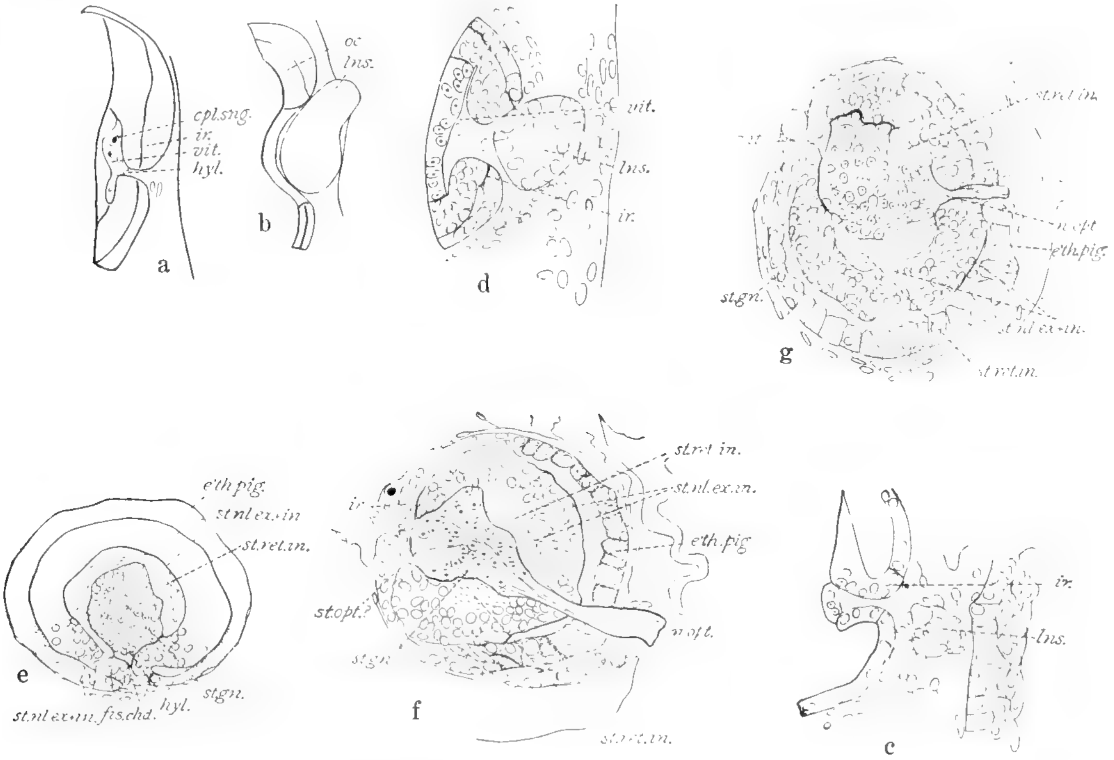

FIG. 63. (*a*) Horizontal Section 10 $\mu$ Dorsal to that given in fig. 60 *e*, and showing Iris and Vitreous Cavity.
(*b*) Outline of Lens of Same Eye as that shown in figs. 60 *e* and 61 *a* but at a Level Dorsal of fig. 61 *a*.
(*c*) Region between Eye and Epidermis of Larva 7.5 mm. long, showing Degenerating Lens.
(*d*) Lens of Larva about 7 mm. long.
(*e*) Vertical Section near Center of Right Eye of Fish 0.5 mm. long.
(*f*) Anterior Face of Transverse Section through Eye of Fish 0.5 mm. long.
(*g*) Horizontal Section through Left Eye of Fish 0.5 mm. long.

### NINE TO TEN-MILLIMETER STAGES.

In larvæ 9 to 10 mm. long the eyes lie from 60 to 100 $\mu$ removed from the epidermis and in contact with the brain capsule or but little separated from it. Their average measurements are: longitudinal diameter, 114 $\mu$; antero-posterior, 98 $\mu$; vertical, 106 $\mu$ (figs. 63 *e*, 64 *b*).

The epidermis over the eye has assumed the thickness found over neighboring regions, and from now on till death by old age there are no external modifications to indicate the former position of the cornea.

The pupil is still open, and also the choroid fissure in the region of the pupil (figs. 63 *e*, *g*). In the proximal portions the choroid fissure is indicated by the absence of pigment along the ventral line (fig. 63 *f*). The vitreous cavity is a shallow depression in the distal face of the eye with a very narrow slit, sometimes a line, separating the iris from the solid mass of cells representing the retina. The vitreous cavity formed by the ventral invagination, that is, proximal of the iris, is obliterated in some individuals except in so far as the absence of pigment along a median line and in the union of the ganglionic layer with the pigmented layer along this line indicates its presence. The choroid fissure has been noted in an individual over 100 mm. long, so that evidently in some cases it may not close. Blood-vessels are still present in the vitreous cavity as far as it is developed. The distance from the exit of the optic nerve to the ventral margin of the pupil is considerably less than the distance between the exit of the optic nerve and the dorsal margin of the pupil.

A few nuclei, probably the remnants of the hyaloid membrane, lie over the distal face of the retina.

In 10 specimens sectioned, all of them from 9.5 to 10 mm. long, the lens has disappeared without leaving any trace.

The pigmented layer increases in thickness from the iris to the exit of the optic nerve. Its pigmentation also increases from the iris to the optic nerve. Within any one cell the pigment is uniformly distributed. In the dorsal part of the eye the pigment reaches to the iris, while in the ventral it does not reach so far, and in fact in a line from the optic nerve to the iris very few (only about 3) cells are pigmented. The maximum thickness of this layer is 12 $\mu$.

The inner cells of the iris have taken on their elongate shape which distinguishes them in the eye of the adult, where the region of the iris and pupil can not otherwise be distinguished.

The layers of the retina are now well developed except that the ganglionic mass of cells occupying the center of the eye is continuous with the outer nuclear and the pigmented layers along the ventral line. The outer and inner nuclear layers are represented by about 4 rows of nuclei immediately within the pigmented layer. The cells represented by these nuclei are not separable into an outer and an inner layer histologically, nor is there any break indicating the presence of any outer reticular layer. The cells form a compact layer of approximately uniform thickness. There are no indications of cones in any of the eyes examined.

The inner reticular layer is well developed except along the region of the choroid fissure, where, as has been said above, the nucleated layers of the retina meet. There is possibly one exception to this in one of the eyes, in which the reticular layer surrounded the optic nerve at its entrance to the eye (fig. 63 *f*).

The space ventral to the central axis of the eye is occupied by the mass of ganglionic cells. This mass is irregularly trumpet-shaped, with the narrow end of the trumpet at the entrance of the optic nerve and the wide end at the distal part of the retina, where its cells are continuous with those of the nuclear layers. In the distal face of the trumpet, in what would be its hollow end, there is a distinct conical area free from cells and abundantly supplied with fibers (fig. 63 *g*). It is possible that this represents the optic-fiber layer. The optic nerve is well developed, but its fibers seem to go to their respective cells directly without first going to this apparent optic-fiber layer. The outer nuclear layers measure about 20 $\mu$, the inner reticular about 8 $\mu$, and the ganglionic layer about 32 $\mu$ in thickness. The changes taking place between 10 and 25 mm. are insignificant.

### THE EYE OF THE ADULT.

The eyes of adult individuals from 25 to 75 mm. long were fully described in a previous chapter, and the eyes of very old individuals were mentioned briefly. The most highly developed eye found was that of an individual 75 mm. long. This eye is much above the average in the development of its pigmented layer, etc. Perhaps 25 mm. represents the stage at which the eye as a whole reaches its maximum development.

### GROWTH OF THE EYE FROM TIME OF ITS APPEARANCE.

The question of the rate and amount of growth of the eye from the time it appears can best be answered by the following table of measurements of the eyes of successive sizes of embryos. Attention should be called to the great variability of the size of the eye in any one stage or in successive stages of development.

It is seen from this table that the eye reaches the full vertical and longitudinal diameter of the adult when the embryo is only 2 mm. in length. Since the eye does not make its appearance till the embryo has reached a length of 1.5 mm. and the lens does not begin to develop until 1 mm. has been added to the length attained by the embryo after the eye has reached its full size, that is, not until it has reached a length of 2.5 mm., it is apparent that from the beginning the eye is in longitudinal and vertical diameter equal to the full adult eye.

*Table of Measurements of the Eye from the Time of First Appearance to Maturity.*

[All measurements are given in micra, except lengths of embryos, which are in millimeters.]

| Condition of embryo; living, or if preserved, direction of the sections. | Length of embryos. | Number of embryos measured. | Longitudinal diameter. | Vertical diameter. | Axial diameter from cornea to optic nerve. | Diameter. | Diameter of optic nerve. |
|---|---|---|---|---|---|---|---|
| Alive | 1.6 | 1 | 80 | ... | 36 | ..... | .. |
| Alive | 1.76 | 1 | 100 | ... | 48 | ..... | .. |
| Alive | 2 | 3 | 135 | ... | ... | ..... | .. |
| Alive | 2.5 | 2 | 190 | ... | 100 | ..... | .. |
| Alive | 2.8 | 1 | 170 | ... | ... | ..... | .. |
| Alive | 4 | 1 | 200 | 150 | 100 | ..... | .. |
| Alive | 5 | 7* | 144 | 134 | .... | 16 to 48 | .. |
| Sagittal | 6 | 1 | 136 | 88 | .... | ..... | .. |
| Transverse | 6 | 1 | ... | 70 | 100 | ..... | .. |
| Horizontal | 6 | 1 | 136 | ... | 80 and 108 | ..... | .. |
| Mounted entire | 6.5 to 7 | 1 | 160 | 160 | ... | ..... | .. |
| Transverse | 5.5 to 7 | 3 | ... | 126 | 99 | 16 to 36 or none | 15 |
| Horizontal | 6.5 to 7 | 3 | 152 | ... | 115 | 18 to 50 or none | 17 |
| Sagittal | 9 to 9.5 | 1 | 108 | ... | 88 | ..... | .. |
| Transverse | 9 to 9.5 | 1 | 108 | 106 | 90 | ..... | 11 |
| Horizontal | 9 to 9.5 | 1 | 114 | ... | 98 | ..... | 12 |
| Sagittal | 10 | 1 | 120 | 112 | .... | ..... | .. |
| Transverse | 10 | 2 | ... | 108 | 109 | ..... | 12 |
| Mounted entire | 10 | 1 | 135 | 130 | ... | ..... | .. |
| Horizontal | 25 | 1 | 120 | ... | 128 | ..... | .. |
| Transverse | 25 | 1 | ... | 160 | 160 | ..... | .. |
| Horizontal | 35 | 1 | 192 | ... | 144 | ..... | .. |
|  | 60 to 108 | .. | ... | 115 | 139 | ..... | .. |

* The following gives the individual measurements of the eyes of the seven specimens whose average is here noted :

|  | No. 1. | No. 2. | No. 3. | No. 4. | No. 5. | No. 6. | No. 7. |
|---|---|---|---|---|---|---|---|
| Longitudinal diameter | 176 | 160 | 136 | 172 | 160 | 160 | 128 |
| Vertical diameter | 144 | 128 | 112 | 160 | 144 | 128 | 128 |
| Lens | 48 | .... | .... | 16 | .... | .... | .... |

### THE HISTORY OF THE LENS.

The lens begins to develop when the embryo is about 2.5 mm. long (fig. 59 *b*). It forms as a thickening of the skin where the optic vesicle is in contact with it. It is still connected with the skin when the embryo has reached a length of 4.5 mm. (Compare figs. 59 *b*, 59 *c*, 60 *d*, 60 *e*, 63 *c* with figs. 60 *a*, 60 *b*, the latter representing the development of a normal lens.) The history of the lens after this stage is somewhat uncertain. It is well established that the cells composing it never lose their embryonic condition, that they are never differentiated into fibers. In many eyes, certainly in all in which a lens could be detected in later stages, the lens becomes separated from the skin (fig. 60 *e*). The separation is completed when the larva has reached a length of 5 mm. (fig. 62 *a*). From this stage on, the lens begins to be resorbed; in some 6-mm. larvæ it could no longer be found (fig. 62 *b*). In 7-mm. larvæ exactly half the eyes were without a lens (figs. 63 *b*, *c*, *d*), and in 9 to 10-mm. larvæ no trace of a lens could be detected. The history of the lens is completed. Judging from this rapid and universal disappearance of the lens in the young I am inclined to the opinion that the structure described in the adult eye as a lens is not a lens.

The lens is the first organ to stop developing, the first to begin to degenerate, and the first to disappear.

### THE HISTORY OF THE SCLERAL CARTILAGES.

Attention was called to the variation of the scleral cartilages. A study of the development of the cartilages has enabled me to detect perhaps a greater degree of uniformity of plan, if not of structure, in these cartilages than I was able to make out from a study of the adult alone. It would seem that there are normally two cartilaginous bars of variable shape developed. One or both of them may be replaced by two or more smaller cartilages. One of the cartilages is found over the distal face of the eye and the other on the posterior face caudad of the optic nerve. The earliest stages at which cartilages were noticed were 9.5 to 10 mm. (figs. 63 *g*, 64 *a*, *b*) long. In one fish 10 mm. long there were in the right eye about 10 cartilage cells, all directly over the pupil and iris. In the left eye there were about 22 cells, all over the dorsal part of the iris, none of them in front of the pupil. There were no traces in these eyes of scleral cartilages elsewhere. The cartilage cells were still for the most part isolated, not bound together into a definite cartilage.

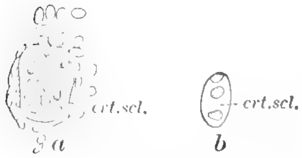

FIG. 64. (*a*) Scleral Cartilage of Right Eye of Same Fish as that from which fig. 63 *g* was taken.
(*b*) Scleral Cartilage of Left Eye of Another Fish of Same Size.

In another fish 10 mm. long the cells were definitely bound together into a small cartilage in each eye, that of one side encroaching on the pupil, that of the other side not.

In a fish 25 mm. long there were two cartilaginous masses in each eye. One of these was over the distal face of the eye, the other over the caudal face of the eye caudad of the exit of the optic nerve (plate 10, fig. B). The one over the distal face curved ventro-caudad.

In a fish 30 mm. long the cartilages were confined to the caudal half of the eye and were developed in such proportions that they encroached on the eye.

The development of these cartilages to such unexpected size indicates that these cartilages are self-determining and not conditioned by the stimulus to growth by the eye with which they are in contact. In the right eye of this fish there were two cartilages in close contact with each other over the distal face. A third cartilage lay on the dorsal surface of the proximal part of the eye. The larger one of the two distal cartilages measures 63 by 32 by 65 μ, with a maximum diameter of the eye of 12 μ.

In a fish 33 mm. long there were no cartilages on the proximal faces of the eye. In the right eye there was a cartilage 128 μ long by 40 μ thick, curved along the ventral part of the distal face. In the left eye there were two much smaller cartilages on the distal face of the eye.

In a fish 35 mm. long there were two cartilages in the left eye placed as in the fish 25 mm. long, but they were larger. In the right eye the distal cartilage was represented by two cartilages in contact with each other.

From the above it is seen that the distal cartilage arises first (10 mm. stage), the proximal ones not till much later (25 to 30 mm. stage). The cartilages do not reach their maximum size till later.[1]

The distal cartilage in older fishes is frequently nodular and lies in front of the eye, where it was taken to be the lens by one of the earliest observers. In a specimen 90 mm. in length a globular cartilage 62 μ in diameter lay just over the pupil of the eye, which had a total diameter of 84 μ. One or the other cartilage not infrequently encroached on the general outline of the eye.

In the left eye of an individual 105 mm. long there were no traces of a scleral cartilage; the right eye was not examined. In the right eye of an individual 108 mm. long there was a single large cartilage, 134 μ by 208 μ, lying at one side of the center of the distal face of the eye. In the right eye of an individual 123 mm. long a minute cartilage was found on the proximal face of the eye. It was not determined whether one occurred over the distal face. In the left eye of the same fish a large cartilage lay over the distal face (plate 10, fig. D).

In the left eye of the largest fish a single large cartilage 64 μ by 96 μ in section occupies the region to one side of the distal face (plate 10, fig. D). In the right eye (plate 10, fig. F) the distal cartilage measured 48 μ by 160 μ in section, and two smaller proximal ones were also present, one of them 24 μ by 32 μ in section.

The scleral cartilages are the last structure to appear in the development of the eye; they grow during the greater part of life and retain their structure to the end.

### THE HISTORY OF THE OPTIC NERVE.

The details of the formation of the optic nerve have not been followed. No indications of it were seen in the eyes of the embryos 4.4 mm. long. In the eyes of embryos 5 mm. long it is well developed, forming a solid strand of fibers 12 μ in diameter which is readily traceable to the brain. The optic nerve increases but little, if any, after its formation. Its development is rapid. In subsequent stages it is not always traceable from all the cells forming the ganglionic mass. In the 6-mm. larvæ its fibers were distinctly traceable from the cells nearest the choroid fissure, while in later stages they were more distinctly traceable from the

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[1] In the original the words "and there is no evidence of degeneration in them even in the oldest fish" completed the sentence. This is not strictly true and is omitted.

distal cells of the ganglionic group. The optic nerve can be followed to the brain in all the larval stages and in the young fish up to 25 mm. in length (plate 10, fig. B). The optic nerve is evident within the eye in older stages up to about 100 mm.; in the very oldest ones it could not be found. In individuals much more than 25 mm. long it was not possible to follow the nerve to the brain, though it could usually be followed for some distance from the eye. The fibers are never medullated, and so far I have not been able to give them a differential stain.

### HISTORY OF THE DEVELOPMENT, MATURITY, AND DEGENERATION OF THE EYE.

The history of the eye may be divided into four periods:

The first period extends from the appearance of the eye till the embryo reaches 4.5 mm. in length. This period is characterized by a normal palingenetic development except that cell division is retarded and there is very little growth.

The second period extends from the first till the fish is 10 mm. long. It is characterized by the direct development of the eye from the normal embryonic stage reached in the first period to the highest stage reached by the *Amblyopsis* eye; its latter half is further characterized by the entire obliteration of the lens.

The third period extends from the second period to the beginning of senescent degeneration, from a length of 10 mm. to about 80 or 100 mm. It is characterized by a number of changes, which, while not improving the eye as an organ of vision, are positive as contrasted with degenerative. There are also distinct degenerative processes taking place during this period.

The fourth period begins with the beginning of senescent degeneration and ends with death. It is characterized by degenerative processes only, which tend to gradually disintegrate and eliminate the eye entirely. It is questionable whether these changes should be called senescent. It may be urged that they are the result of disuse in the individual, or that the end product of these degenerative changes is the typical structure of the eye of *Amblyopsis*.

*First Period.* — During the first period the eye arises as a solid outgrowth from the solid central nervous system when the embryo is about 1.5 mm. long. The outgrowth increases rapidly in size during the next 0.5 mm. of growth in length. The solid lateral outgrowth is bent back along the side of the brain, and its connection with the brain becomes constricted into the optic stalk. A cavity approximately central arises in the optic lobe at the same time that a cavity appears in the central nervous system, which occurs when the embryo is about 2 mm. in length. The two layers of the optic vesicle formed by the appearance of the cavity are of about equal thickness. A little later the secondary optic vesicle is formed by the thickening of the skin over the eye and the consequent cupping of the distal face of the eye. The process reaches its culmination when the embryo has a length of 4.4 mm. The lens is still connected with the skin, and the two layers of the secondary vesicle have become very different, the proximal one being one-layered, the distal one several-layered. The details of the changes of this period have been given in the preceding pages.

At any time up to this length the eye might, as far as its structure is concerned, give rise to a perfect eye in the adult. The eye so far follows phylogenetic paths with the reservation that no adult ancestor is supposed to have had eyes like these embryonic stages.

*The Second Period.* — The development during the second period is direct and leads to the condition obtaining at the end of that period. Some of the processes are palingenetic, some are of purely ontogenetic significance, while still others (if I may make the distinction) are degenerative.

The optic nerve develops at the beginning of the period in an undoubted phylogenetic way. As in the case of the eye as a whole, the nerve develops directly into its full size. The details of its history are given under the head of the optic nerve. The latter half of the history of the lens belongs entirely to this period. Its history is also given under another head. The changes the lens undergoes during this period are all katagenic, and some time before this period closes the lens has disappeared.

The direct development of the optic vesicle of the beginning of this period into the eye as found at the end of this period is very difficult to interpret satisfactorily.

A comparatively very narrow marginal part of the secondary optic vesicle is converted into the epithelial part of the iris. The lens is almost always entirely excluded from the optic cup when the iris develops. The extreme shallowness of the optic cup and the comparative thickness of the retina would lead one to expect the choroid fissure proper to be a very short structure. The shallow cup develops into the adult eye by processes like those that take place in normal eyes. These purely palingenetic processes operating on so deficient material give rise to conditions that are not palingenetic. In the closing of the choroid fissure of the normal eye the thing of chief concern is the union of the infolded margins of the optic cup from the margin of the pupil to the point of exit of the optic nerve and the closing in of the retina around the optic nerve at its exit from the eye. In *Amblyopsis* the former process has become insignificant, and the latter the prominent process. This is further complicated by the fact that the vitreous cavity has ontogenetically disappeared nearly as much as phylogenetically, so that, while the processes of changing the optic cup into the eye are palingenetic, the material operated upon being quite different from that normally obtaining in fish embryos, the resulting stages of the eye are not palingenetic.

The choroid fissure, which is distally a distinct slit leading into what remains of the optic cavity, becomes proximally a groove in a solid mass of cells. The closing of this groove takes place at various times, or it may remain permanently open. This condition has undoubtedly been brought about by a contraction of the area of the retina and the consequent heaping up of cells, either concomitantly with, or as the result of, the obliteration of the optic cavity. The funnel-shaped mass of cells in the center of the *Amblyopsis* eye is thus the result of the phylogenetic rather than the ontogenetic disappearance of the optic cavity.

I must confess that an easier way of explaining the developmental stages would be reached by assuming that the central mass of cells, through which the optic nerve passes, is not really ganglionic — that only the distal cells of the mass are ganglionic — and that the proximal ones are the homologues of the cells found at the point of entrance into the eye of *Chologaster* (fig. 65, *z*). This would imply that a cavity has not disappeared from the center of these cells (because there never was one), and that the entire vitreous cavity has been reduced to that now found in the embryo, and that no part of the cavity has disappeared *in toto*. This interpretation is especially suggested by figure 62, *c*. This would account for the fact that the optic nerve does not form a central strand through the funnel of ganglionic cells, but passes through it in several strands as it does through the mass of cells at the entrance of the optic nerve (fig. 65). The objection is that it would not account for the position of the exit of the optic nerve, which should, according to this view, be at the proximal end of the choroid fissure. The second objection is found in the phylogenetic stages of degeneration indicated in different eyes, notably that of *Typhlomolge*. Furthermore, it would not account for the groove that is undoubtedly found along the ventral side of the larval eye, nor would it account for the presence of the inner reticular layer around the optic nerve. It would, moreover, make it necessary to assume that the cells found about the entrance of the optic nerve in *Chologaster* have been retained in *Amblyopsis* out of all proportion to the other structures of the eye. These objections seem to me fatal to this second supposition.

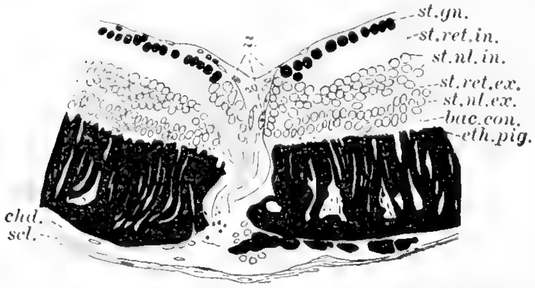

Fig. 65. Exit of Optic Nerve from Eye of *Chologaster papilliferus*, showing Ganglionic Cells and (z) cells at Entrance of Optic Nerve.

During this period the differentiation of the several layers of the retina also takes place. At the beginning of the period the pigmented layer is represented by a layer of thin cells without pigment. At the end of the period it is composed of cylindrical cells 12 $\mu$ high which are markedly pigmented. Pigment granules first make their appearance when the larva is about 5 mm. long. The remainder of the retina is at the beginning of the period several cells deep without any differentiation into layers. The inner reticular layer first appears as a number of irregular spaces separating the ganglionic from the nuclear layer when the embryos are 5 mm. long. These spaces soon unite into a single layer, but this does not occur till the very latest stages of the period when the choroid fissure has been closed for some time, and in fact they may never form a layer entirely around the central ganglionic cells. In earlier stages the layer extends between the dorsal and lateral parts of the ganglionic and nuclear layers. The nuclear layers never become separated into outer and inner ones, nor is an outer reticular layer ever formed. There is no indication of cones such as are seen in some adult eyes. Müllerian fibers are well formed in older individuals at this period.

The development of the scleral cartilages described under another head also takes place toward the close of this period. No dividing cells have been found in the eyes of specimens more than 7 mm. long. The nuclei of the retina in the 10-mm. stage are all granular and measure 4 to 5 $\mu$ in diameter.

*The Third Period.* — This extends from the time the fish has reached a length of 10 mm. till marked senescent changes begin, which take place when the fish approaches 100 mm. in length.

The nuclei of the retina, when the fish has reached a length of 25 mm., are no longer alike. There are two types of cells in all layers: cells with larger granular nuclei, and cells with smaller compact or dense nuclei. The difference is perhaps due less to histogenesis than to the process of degeneration which has already set in. The cells with smaller nuclei are probably degenerate. In the oldest fish only cells of the second type are found.

A number of changes take place during the third period, some of which can be classed neither as progressive nor as retrogressive. As the fish grows, the eyes are farther and farther removed from the surface. In the fish 25 mm. long they are nearly 1 mm. below the skin, and in the largest specimen examined they are as much as 5 mm. beneath the surface of the skin. The scleral cartilages develop progressively probably during the entire period, in some cases encroaching on the regular outline of the eye. Other processes which are progressive nevertheless do not tend to make the eye a more perfect organ of vision. The pupil, for instance, becomes closed in many cases, or reduced to a very minute opening. The vitreous cavity, which was still evident, becomes, concomitantly with the closing of the pupil, entirely obliterated. The pigmented layer becomes a variable structure, the pigment granules being in many cases entirely absent. Rarely the pigment layer changes to a high columnar epithelium. The stages of this period have not been successively observed as in the younger period, and the genetic relationship of different stages is not always apparent.

*The Fourth Period.* — This extends from the time the fish has reached a length of about 100 mm. to the end of its life. There are distinct features that characterize the eye of this stage (plate 10, figs. C–G).

The fibrous capsule enveloping the eye is distinctly thicker than in younger stages. The scleral cartilages are as well developed as at any time.[1] The eye-muscles, as far as present, show no indication of degeneration and their striation can readily be made out in all individuals.

The most marked changes take place in the size of the eye itself. The pigmented layer becomes distended to form a thin-walled vesicle of two or three times the diameter of the eye in previous stages (plate 10, figs. F and G). This development of the pigmented layer beyond the requirements of the retina has also been seen in the eyes of *Rhineura* and other blind vertebrates. The cells of this layer become spherical or attenuated and the columnar epithelium converted into a thin epithelium thickened in places. Within this vesicle, whose sides may be compressed, as in figure F, the rest of the retina forms an insignificant little ball of tissue. In an eye of an individual 105 mm. long whose pigmented epithelium forms a vesicle 320 $\mu$ in diameter, the rest of the eye forms a small sphere 60 $\mu$ in diameter in contact with the iridian part of the pigment (plate 10, fig. G). The elements composing this little ball and representing the retina have also undergone a marked senescent modification. The optic nerve is no longer evident.[2] The ganglionic cells no longer form a compact mass, but are either unidentifiable or irregularly scattered. The cells of the outer nuclear layer are also less regular. While in the second period and up to 95 mm. in length two sorts of nuclei are distinguishable, some of them small and dense, others larger and granular. In these later stages they are all small and dense, no granular ones being present, and their outlines are less well defined than in the young.

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[1] In the left eye of a specimen 105 mm. long no cartilages were found. It is not possible to say whether they had disappeared or were never developed. Because of the irregularity in the development of these cartilages and their large size in other individuals of this period, I am inclined to think cartilages never appeared in this specimen.

[2] The optic nerve can be traced as a very delicate filament through the pigment layer in an individual 123 mm. long. In this eye the choroid fissure was still open.

In a fish 25 mm. long the smaller nuclei measure 2.5 $\mu$, the larger ones measure 3.5 to 5 $\mu$. In the specimen 123 mm. in length the nuclei measure but 2 to 3 $\mu$. Evidence that the smaller nuclei in the younger specimen are degenerate is furnished by the fact that optic fibers can not be traced to the smaller ganglionic nuclei in a 25-mm. specimen.

The most disorganized eye found is the left one of the largest fish examined, 130 mm. long (plate 10, fig. E). The fibrous sheath (sclera) is thick; the cartilage is large, 64 by 96 $\mu$ in section. The eye itself is a disintegrated mass abundantly provided with granular pigment and without well-defined outline or structure. The right eye of the same specimen is less degenerate (plate 10, fig. F). It is an elongated vesicle 60 by 256 $\mu$ in section, with a large cartilage to one side of its distal half, 48 by 160 $\mu$ in section, and two smaller proximal ones, one of which measures 24 by 32 $\mu$ in section. Associated with the retina of this eye is a structure that I described as a possible lens in my first paper. It consists of a few nuclei about which there are concentric layers of a homogeneous tissue. Considering the fate of the lens in all the young fishes examined, it seems very doubtful, if not impossible, that this structure should be a lens.

That the eyes of these largest individuals belong to the fourth period is seen in the fact that they become distended vesicles whose parts are finally resorbed after undergoing degenerative changes. The scleral cartilages offer an exception to the general fate.

*Summary of the Origin, Development, and Degeneration of the Eye and its Parts.*

| | Earliest appearance or differentiation. | End of cell division. | End of morphogenesis. | End of histogenesis. | Beginning of degeneration. | Disappearance. |
|---|---|---|---|---|---|---|
| | mm. | mm. | mm. | mm. | mm. | mm. |
| Eye | 1.5 | 5–7 | 10 | Before 25 | 25 | Beyond 130 |
| Choroid fissure | 2.5 | — | — | — | — | 10–130 |
| Pigmented layer | 2 | ? | 2.5 | 10 | 100 or before | Beyond 130 |
| Cones | Rarely and then after 10 | — | ? | ? | ? | ? |
| | | 5–7 | — | 10 | Before 25 | Beyond 130 |
| Outer nuclear | 4.4–5 | | | | | |
| Outer reticular | Never | — | — | — | — | — |
| Horizontal cells | Never | — | — | — | — | — |
| Inner nuclear | 4.4–5 | — | — | 10 | Before 25 | 130 mm. and beyond |
| Ganglionic | 4.4–5 | — | — | 10 | Before 25 | 130 mm. and beyond |
| Optic fiber layer or nerve | 4.4–5 | — | — | 5 | 25 | 100 |
| Scleral cartilages | 9–10 | ? | ? | 75 | — | — |
| Lens | 2.5 | 5 | 5 | — | 3 | 6–10 |
| Corneal epithelium | 5 | ? | — | — | 7 | 10 |

? I do not know. — Does not take place.

## COMPARATIVE RATE OF ONTOGENETIC AND PHYLOGENETIC DEGENERATION OF THE PARTS OF THE EYE.

On pages 134 *et seq.* an outline of the probable phylogenetic history of the eye of *Amblyopsis* is given. In the preceding chapter the rate of ontogenetic degeneration and its extent has been found to vary in different parts of the eye. It has also been found that certain parts begin to degenerate earlier than others. We shall now attempt to discuss briefly the ratio between the rates and extent of ontogenetic degeneration and the rate and degree of phylogenetic degeneration implied by the structure of the eye. The discussion is somewhat intangible, but certain definite results can be obtained by it.

In order to compare the ratio between the ontogenetic and phylogenetic rates of degeneration, it is necessary to use some stage in the development of the eye as the point which phylogenetic degeneration has reached. For such a point we shall use the optimum reached by various parts of the eye during their development. It is certain that the phylogenetic stage is below this optimum, that some of the degeneration in individual eyes is due to phylogeny, but since we do not know how much of the descent from the optimum is due to heredity and how much to the peculiarities of the environment and the resulting functionless life of the parts during the life of the individual, it will be best to take the optimum as above indicated.

All phylogenetic time is taken as a unit, although some parts of the eye have been degenerating longer than others. The ontogenetic degeneration leads from the optimum to the vanishing point for most parts of the eye.

Ontogenetically the lens degenerates very rapidly, reaching its vanishing point from its optimum during the period in which the fish grows not more than 5 mm. in length. The rate of its phylogenetic degeneration must have been proportionately rapid, for at its optimum in *Amblyopsis* it is minute and its cells are undifferentiated. In the epigean relatives of *Amblyopsis* the lens is one of the parts least affected, so that it must have degenerated very rapidly in its later phylogenetic history, after the fish had entered the caves.

At its best the vitreous body is so inappreciable in amount that I have not been able to consider its ontogenetic degeneration. Its phylogeny has approached the vanishing point toward which most parts of the eye are heading.

The retina may be considered in its extent and in the degree of the histogenic differentiation of its parts. In the matter of its extent or size there is little change from its optimum until its disintegration in old age. Its ontogenetic changes are slight. Its optimum is comparable with that of the lens and indicates a rapid and great reduction from the lowest retina of epigean relatives. The ontogenetic and phylogenetic rates of degeneration in the extent of the retina differ greatly, the former having come practically to a standstill.

In its histogenic differentiation the retina is not comparable with the lens, for it rises above the embryonic phases. In fact, in its histogenic differentiation the retina rises far above the requirements of the case, and the most highly developed eye of *Amblyopsis* approaches the lowest of its epigean relatives. Over any given area it is doubtful whether the ganglionic and inner reticular layers are more degenerate or as degenerate as the same parts in the eyes of *Chologaster cornutus*. It is certain that in their highest development the parts between the inner reticular and the pigmented layers are below the lowest point reached by the corresponding parts in the epigean species mentioned. The same is true of the pigmented epithelium.

The simplification of the structure of the retina from its maximum to its minimum in ontogeny is of greater extent than its simplification from the lowest differentiated retina found in epigean species to the maximum found in *Amblyopsis*.

From the foregoing we may conclude that there is no constant ratio between the extent and degree of ontogenetic and phylogenetic degeneration, and that the observed rate of ontogenetic degeneration is not necessarily proportionate to the rate of phylogenetic degeneration inferred from the degree of degeneration of the eye at its optimum.

## THE FUTURE OF THE EYE.

There can be no doubt that the phylogenetic fate of the eye, exclusive of connective tissue, sheaths, sclera, etc., is total disappearance. The most degenerate ontogenetic eye indicates as much. There are no relatives of *Amblyopsis* that have reached this condition, but *Troglichthys* has an eye distinctly more degenerate than that of *Amblyopsis*. It may offer a clew as to whether any of the ontogenetically degenerate eyes, such as are found in old specimens of *Amblyopsis*, are prophetic of the condition through which the eye will pass in its route to the vanishing point. The most highly developed eye found in any specimens of *Troglichthys* (plate 10, fig. H) is comparable in a general way with the eyes of the old of *Amblyopsis*. The pigmented epithelium is larger than the requirements of the eye in both cases, and the scleral cartilages are disproportionately developed in both cases. The ganglionic cells extending through the center of the eye of the younger *Amblyopsis* are absent in both cases. Only 3 cells have been found in this region in all the eyes of *Troglichthys* examined. When we attempt a closer comparison, our efforts fail.

We may conclude that if *Troglichthys* indicates one of the steps through which the eye of *Amblyopsis* will pass to its annihilation, the degenerative phases seen in the oldest specimens of *Amblyopsis* indicate only in a general way the phylogenetic path over which the eye will pass in the future.

## RETARDATION AND CUTTING OFF OF LATE STAGES OF THE DEVELOPMENT OF THE EYE.

In my first paper on the Eyes of Blind Vertebrates (Roux' Arch. VIII. p. 596, 1899) I said:

> Cessation of development takes place only in so far as the number of cells are concerned. The number of cell generations produced being continually smaller results in an organ as a consequence also smaller. In this sense we have a cessation of development (cell division, not morphogenic development) in ever earlier stages. That there is an actual retardation of development is evident from *Amblyopsis* and *Typhlichthys* in which the eye has not reached its final form when the fish are 35 mm. long.

I am convinced now that this statement did not go far enough. There is, indeed, a gradual retardation in all processes of development which frequently terminates in a complete arrest of development before the final stages of normal eyes are reached. This is especially true of the lens. In discussing the changes it will be best to keep separate the three groups of processes concerned in development.

The proof of the limiting of the number of cell divisions mentioned has been brought out in the chapters on the development. It has also been seen that the rate of division is very much retarded. In the retina it stops altogether at the time the fish has reached a length of 5 to 7 mm., and very rarely more than two dividing cells are found in any eye. In its first stages the eye is thus about equal in size to the adult eye. Cell division stops earlier in the lens, where no new cells are formed after it is cut off from the skin. The lens is at this time relatively as well developed as the retina. In both the retina and the lens cell division ceases in late stages, and the total number of cell generations is very much limited. The lens is looked upon as phylogenetically a new structure, and we have, by the stopping of its later stages of cell division, a step in the elimination of a phylogenetically new structure. This is, however, of no consequence because it is not differential, for the retina, a phylogenetically older structure, suffers a similar stoppage. There is no evidence, then, that phylogenetically younger structures lose their power of cell division earlier than phylogenetically older ones.

The retardation of the morphogenic processes, cell arrangement, movement, union and separation, etc., is conspicuous in the delay of the closing of the choroid fissure and all that this implies. There is no conspicuous stopping of this process except in the occasional failure of the choroid fissure to close at all.

Histogenic processes are also distinctly retarded, and in conspicuous instances suffer an entire stoppage. While the eyes of 3-mm. specimens of *Cymatogaster* or *Carassius* and *Amblyopsis* are nearly alike, in the former two the tissue differentiation has progressed vastly farther by the time the fishes have reached a length of 10 mm. Histogenesis is carried surprisingly far in many degenerate eyes. In *Rhineura*, for instance, the layers of the retina are differentiated far beyond the requirements of the case. In *Amblyopsis* the process, as far as it can be made out with the methods available, falls short of the normal development.[1] The cells of the lens never lose their embryonic characters; they are never transformed into lens fibers. Cones are rarely if ever developed in the retina, and an outer reticular layer never. In normal development the cones and the outer reticular layers are the last to differentiate, so that we have certainly a cutting off of late ontogenetic stages. The question whether these are also phylogenetically young may be passed over.

The total evidence from the three processes is that none of them proceed with the push and rapidity found in normal structures, and though they are normal, they grow weaker with development and frequently give out altogether. But with all this lack of vigor, while there is more variation in each structure developed than has been noted in normal eyes, the point to which cell division, cell arrangement, and histogenesis are carried, in different individuals, is about the same. The causes leading to the changed development are of approximately equal value in different specimens from the same locality.

## CAUSES OF RETARDATION AND CESSATION IN THE DEVELOPMENT OF THE EYE.

The retardation and arrest in the ontogenetic development of the eye of *Amblyopsis* may be due to one of several possible causes. They are either conditioned by something outside the cells composing the eye, or they are inherent or predetermined in the egg cell from which the eye is ultimately derived. The conditioning factor, if it lie outside the eye, may be a peculiarity in the physical and chemical environment in which the fish lives, or a lack of stimulation or an inhibition exercised by some other part of the body. Unless we assume that the eye of *Amblyopsis* has reacted and does now react differently to the physical and chemical environment from that of some of the relatives of *Amblyopsis*, physical and chemical factors may readily be eliminated as contributing directly to the retardation and cessation.

Although, in discussing the phylogenetic degeneration of the eyes of cold-blooded vertebrates in general, I have insisted that cross-country conclusions must be guarded against, I then saw no objection, and now see none, to considering the different members of the Amblyopsidæ as homogeneous material within the bounds of which we may expect similar causes to effect similar results. The different stages

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[1] The difficulties, for instance, of differentiating with Golgi methods the bipolar cells of an eye whose total diameter falls short of 0.2 mm. can readily be imagined.

(phyletic) of development found in the eyes of the different members of the Amblyopsidæ are all referable to the difference in time in which they have been subjected to their present environment. The only environmental condition surrounding the developing eggs of *Amblyopsis* to which the peculiarities of development might be attributed is the total absence of light.

Temperature, oxygen pressure, chemical composition, etc., of the surrounding medium may be entirely excluded from the possible agents affecting the eye, inasmuch as normal eyes are developed by other fishes in the same water and under all possible fluctuations of the above conditions within the limits of the *possibility* of fish life. But the same objection holds in attributing the lack of development to the absence of light. *Chologaster agassizii*, a member of the Amblyopsidæ, which *always lives in caves* in exactly the same conditions under which *Amblyopsis* lives, has nevertheless normally developed, though small, eyes.

While guarding against the possibility of attributing too much weight to the results obtained in other families of animals, it still may be mentioned that many fishes living perpetually in total darkness develop normal eyes. This is also true of the young of all viviparous animals which develop in more or less complete darkness.

If, then, so closely related fishes as *Chologaster* and *Amblyopsis* are subjected to the same environment which is minus a certain element and both develop their normal parental structure, one developing a normal eye, the other a very abnormal degenerate one, it is scarcely warrantable to say that the abnormal structure in one of them is due to the absence of the one element (light) from the environment. Moreover, if the development is controlled by the absence of light, there is no reason why development should be normal, even to the extent of forming a normal start and should then be arrested or retarded. The fact that the presence or absence of light is not the controlling factor in the retarded development of the eye of *Amblyopsis* does not vitiate the supposition that a certain amount of change may not be produced on the eyes of an individual by rearing it in the light. Such change would, however, stand on a par with the ontogenetic degeneration of the eye with age in the absence of light; that is, it would be a functional adaptation due to use.

Experiments have been in progress to test the effect of light. So far only negative results have been obtained. One young has been reared till it was 6 months old. It was obtained from the caves at a time when it was ready to swim about freely; that is, when the eye was already fully formed. There was no difference in the gross anatomy of the eye of this individual as compared with that of others. The minute anatomy, as the result of an accident, was not available for study. The others examined in earlier stages have not been reared beyond a length of a few millimeters, and the effect of the light, if any, was not appreciable. From the observations on the development of the eyes — which show that some processes are arrested very early — it would seem that the only rational way to determine the effect of light on the total development is to colonize the adults in an outdoor pool where the young can be reared, from the fertilization on, in normally lighted waters.

The lack of development of the eye not being chargeable to any factor in the environment, is there any factor within the fish that inhibits its development, or whose absence fails to furnish the stimulus necessary to the development? If so, this factor must be present or absent at the time the retardation begins or some time before.

The inhibition, if any, might operate through a mechanical crowding on the part of a neighboring organ or the greater selective power in eliminating the food requisite for the development of the eye. The first cause may be eliminated, for there is no evidence whatever of crowding other than that found in normal eyes; in fact, in all stages beyond the earliest, the eye is much smaller than the optic sockets can easily accommodate.

The question of selective food elimination is not so readily disposed of. The ophthalmic artery provides the eyes abundantly with blood, so it is not an absence of this that causes the supposed starving. Indeed if the retardation were due to a lack of blood supply we would be removing the problem one step from the eye without solving it. Besides, Loeb's experiments have shown that the action of the heart may be greatly diminished without affecting the rate of growth of the larval fish. The blood supply being abundant, is there any other organ that may drain it of the nutriment necessary for the proper growth of the eye? Leaving aside the question whether an organ can be starved by having the nutriment requisite for development withdrawn from the blood by another organ, I can think of no organ or set of organs that attain an unusual growth aside from the tactile organs of the skin. This system of organs is undoubtedly very highly developed in the adult and has also attained a remarkable degree of development at the time the fish is 10 mm. long. It is, however, not unusually developed in the earlier stages before hatching and shortly thereafter when the cessation of cell division, the most important element of the stunted optic development, takes place. Besides this, the tactile organs of *Chologaster,* which possesses normal eyes, are very highly, if not so elaborately, developed as in *Amblyopsis.* I have experimentally determined by eliminating the eyes altogether that the tactile organs in *Chologaster papilliferus* are amply developed to enable the fish to live indefinitely without the use of its eyes. The same must also be true of *Chologaster agassizii,* which lives permanently in caves. While not impossible, it seems, therefore, very improbable that the tactile organs affect the development of the eyes in *Amblyopsis* and not in *Chologaster.*[1]

I know of no other organs in *Amblyopsis* whose development differs from that of *Chologaster* in a degree sufficient to make it a successful contestant for a food supply in *Amblyopsis* and not in *Chologaster.*

What has been said concerning organs whose presence might affect the development of the eyes is equally true concerning organs whose absence might deprive the eye of the necessary stimulus to reach normal development. I know of no organ, either in *Amblyopsis* or *Chologaster,* whose absence in the one and presence in the other might account for the difference in the degree of development reached by the eyes in the two fishes.

The conclusion is forced upon us by the above considerations that neither in the environment nor in the fish itself is there a factor sufficient to account for the early arrest in cell division, the retardation of the morphogenic processes, and the stopping of the histogenic processes. We are therefore entirely justified in assuming that the determining cause of the method of development lies in the cells themselves and is inherited. The great development of the scleral cartilages beyond the needs of

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[1] As an example bearing on this subject attention may be called to the tactile apparatus of the Siluridæ, which is certainly in many instances more elaborate than that of *Amblyopsis,* and yet the eyes are normal, though small.

the eye also tend to locate the formative or hereditary power in the cartilages themselves rather than in the stimuli to their development that they receive from their contact with the developing eyes, for they develop entirely beyond the needs of these eyes.[1]

The causes operating in ontogeny and phylogeny that have led to the limited power of development and differentiation I have fully considered in the concluding chapter, which was also published in the Popular Science Monthly.[2] The conclusion is reached that the phylogenetic degeneration, which is equivalent to saying the limited power of development found in the cells entering into the eye of the individual, is the result of functional adaptation during the lifetime of past individuals to the total disuse of the eye. This adaptation, it was concluded, was transmitted to a certain extent to the succeeding generation through the usual vehicles of transmission. There has always been and is yet a serious objection to this conclusion, because the method of the transmission of functional adaptations to the organization of the egg so as to limit or extend its powers is not known.

Recently, while admitting that functionally adaptive structures arise developmentally without reference to function, Driesch has maintained that: "Wer hier von 'Vererbung' früher einmal functionell 'erworbener' Eigenschaften reden will verlässt den wissenschaftlichen Boden, denn wir wissen von solcher Art der Vererbung gar nichts."

Possibly we might find a warrant for the assumption of the transmission of functional adaptation to the germ cells in the writings of Driesch himself, though he might not thank us for it. He maintains that certain developmental results whose proximal cause he is not able to determine may be produced by factors working in a distant part of the embryo. Without entering into a discussion of the validity of these factors working at a distance, if they are really factors and capable of acting, as Driesch imagines, why may not functional modifications effect changes in the hereditary cells in a similar manner?

I conclude that retardation and cessation in development are not due to ontogenetically operating causes, but they are inherent in the fertilized ovum — they are inherited.

## THE EYES OF AMBLYOPSIS AND THE LAW OF BIOGENESIS.

During recent years the law variously termed von Baer's law, Agassiz's law, Haeckel's law, or the law of biogenesis, has been frequently called into question. Its general tenets are: (1) every individual in its development repeats in brief the development of the race; (2) closely related forms have a similar ontogeny, and the nearer two animals are related the longer their embryos are alike; (3) the embryos of high animals pass through stages resembling the adult stages of lower animals; and (4) in every ontogeny there are, among the truly ancestral stages, stages which are adaptive and have been acquired during ontogenetic development.

No objection has been raised to the fourth tenet in so far as its acceptance does not commit to the acceptance of the first. In objection to the first of these propositions Hurst writes:

> I do not deny that a rough parallelism exists in some cases between ontogeny and phylogeny. I do deny that the phylogeny can so control the ontogeny as to make the latter into a record of the

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[1] The same conditions are found in *Lucifuga*.

[2] See the next chapter.

> former — even into an imperfect record of it. * * * Vestiges, and these only, can give any embryological clew to past history which could not be equally well made out from comparative anatomy.

Zittel finds cases in paleontology both in support of and against this first proposition:

> All know that it (development of Antedon) does not in the remotest manner agree with the facts of paleontology. * * * No observations of embryology would warrant our imagining the former existence of graptolites or stromatophores. No stage in the development of any living brachiopod informs us that numerous spine-bearing genera lived in Palæozoic and Mesozoic times. * * * The beautiful researches of Hyatt, Würtemberger, and Branco have shown that all ammonites and ceratites pass through a goniatite stage, and that the inner whorls of an ammonite constantly resemble, in form, ornament, and suture-line, the adult condition of some previously existing genus or other.

Smith finds that "the development of *Placenticeras* shows that it is possible to decipher the race history of an animal in its individual ontogeny."

But it is not the intention to review the numerous expressions of opinion pro and con which have appeared on this subject in recent years. A full discussion of the literature to 1897 has been given by Keibel.

The eye of *Amblyopsis* presents, however, such an excellent opportunity to test an opinion vaguely expressed by Balfour in his "Embryology," and carefully and clearly stated by Sedgwick and reiterated by Cunningham in his "Sexual Dimorphism" and in other places, that the facts presented in the foregoing pages may be reëxamined in their relation to this point.

Balfour says:

> Abbreviations take place because direct development is always simpler, and therefore more advantageous; and, owing to the fact of the fœtus not being required to lead an independent existence till birth, and of its being in the mean time nourished by food-yolk, or directly by the parent, there are no physiological causes to prevent the characters of any stage of the development *which are of functional importance during a free, but not during a fœtal, existence* from disappearing from the developmental history. * * * In spite of the liability of larvæ to acquire secondary characters, there is a powerful counterbalancing influence tending toward the preservation of ancestral characters in that larvæ are necessarily compelled at all stages of their growth to retain in a *functional state* such systems of organs, at any rate, as are essential for a free and independent existence. It thus comes about that, in spite of the many causes tending to produce secondary changes in larvæ, there is always a better chance of larvæ repeating, in an unabbreviated form, their ancestral history than is the case with embryos which undergo their development within the egg.

The most concrete critique of the law of biogenesis has been offered by Sedgwick. After rejecting the second proposition by showing that, while in many cases the adults differ more from each other than the young, in other cases the embryos differ more from each other than the adults, he takes up the main question stated in the first proposition by a consideration of "The Significance of Ancestral Rudiments in Embryonic Development." It is, indeed, around this phase of the subject that the discussion has centered. His views are best given by a series of excerpts from his paper. Thus Sedgwick states that

> * * * The tendency in embryonic development is to directness and abbreviation and to the omission of ancestral stages of structure, and that variations do not merely affect the not-early period of life where they are of immediate functional importance to the animal, but, on the contrary, that they are inherent in the germ and affect more or less profoundly the whole development.
>
> The evidence is of this kind: 1. Organs which we know have only recently disappeared are not developed at all in the embryo. For instance, the teeth of birds, the fore limbs of snakes, reduced

> toes of bird's foot (and probably of horse's foot), the reduced fingers of a bird's hand. * * * 2. Organs which have (presumably) recently become reduced or enlarged in the adult are also reduced or enlarged in the embryo. * * * 3. Organs which have been recently acquired may appear at the very earliest possible stage. * * * The latter arrangement ["ancestral organs have disappeared without leaving a trace"] seems to be the rule, the former the exception.
>
> I think it can be shown that the retention of ancestral organs by the larvæ [embryos?] after they have been lost by the adult is due to the absorption of a larval or immature free stage into embryonic life. A larval character thus absorbed into the embryonic life, its disappearance is no longer a matter of importance to the organism, because, the embryo being protected from the struggle for existence, the pressure of rudimentary functionless organs is unimportant to it. Characters which disappear during free life disappear also in the embryo, but characters which, though lost by the adult, are retained in the larva may ultimately be absorbed into the embryonic phase and leave their traces in embryonic development.
>
> To put the matter in another and more general way. The only functionless ancestral structures which are preserved in development are those which at some time or another have been of use to the organism during its development after they have ceased to be so in the adult. * * * But another explanation is possible, which is that organs which are becoming functionless, and disappearing at all stages, may in some case disappear unevenly, that is to say, they may remain at one stage after they have totally disappeared at another.

The question seems to me not quite so simple as imagined by Sedgwick. Degenerate organs may or may not be better developed in the young than in the adult.

(1) They are better developed in the young if they are still functional in the young after they have become functionless in the adult.

(2) They *may be* better developed in the young, if they *were* of use to the young, after they ceased to be of use to the adult.

(3) They may be well developed in the young after complete disappearance in the adult if the material is used for other purposes in later life.

(4) They are better developed in the young if their presence is essential to provide the necessary stimulus to bring about or to inhibit cell movements or cell differentiation in the development of other organs.

(5) They are supposed to be no better developed in the young than in the adult, if they ceased to be of use to the young when they lost their use in the adult.

The material entering into the formation of the eyes is not used for the building up of other organs, and it is uncertain whether the eyes positively or negatively influence the development of other organs, so that a discussion of numbers 3 and 4 of the above possibilities is not profitable. Inasmuch as both young and adult live permanently in total darkness, and the eye of the young can not be functional under the present mode of existence, the first possibility is also eliminated from the discussion.

In *Amblyopsis*, which carries its young in its gill cavity, we are undoubtedly dealing with an animal in which the eyes are useless in the young as well as in the adult and in which *they became totally useless in the young at the same time that they became totally useless in the adult*, that is, at the time the species took up permanent quarters in the caves. Do the eyes in this case repeat the phylogenetic history of the eye, or have the eyes in the embryo degenerated in proportion to their degeneration in the adult? In this form the question is whether a perfect or better eye is produced to be finally metamorphosed into the condition found in the adult, or whether development of the eye is direct.

We have seen in the preceding pages that the foundations of the eye are normally laid, but that the superstructure, instead of continuing the plan with new material, completes it out of the material provided for the foundations, and that in fact not even all of this (lens) material enters into the structure of the adult eye. The development of the foundations of the eye are phylogenetic, the stages beyond the foundations are direct to the present adult condition of the eyes from which they are now ontogenetically degenerating to the vanishing point.

### CONCLUSIONS.

The study of the development and its related questions shows:

1. The eye of *Amblyopsis* appears at the same stage of growth as in fishes developing normal eyes.
2. The eye grows but little after its appearance.
3. All the developmental processes are retarded and some give out prematurely. The most important of the latter is the cell division and the accompanying growth that provides the material for the eye.
4. The lens appears at the normal time and in the normal way, but its cells never divide and never lose their embryonic character.
5. The lens is the first part of the eye to show degenerative steps and it disappears entirely before the fish has reached a length of 1 mm.
6. The optic nerve appears shortly before the fish reaches 5 mm. in length. It does not increase in size with the growth of the fish and possibly never develops normal nerve fibers.
7. The nerve does not increase in size with growth of the fish.
8. The optic nerve gradually loses its compact form, becomes flocculent, dwindles, and can not be followed by the time the fish has reached 50 mm. in length. In the eye it retains its compact form for a much longer time, but disappears here also in old age.
9. The scleral cartilages appear when the fish is 10 mm. long; they grow very slowly — possibly till old age. They do not degenerate at the same rate as other parts of the eye, if they degenerate at all.
10. The history of the eye may be divided into four periods:
    - (*a*) The first period extends from the appearance of the eye till the embryo reaches 4.5 mm. in length. This period is characterized by a normal palingenetic development except that cell division is retarded and there is very little growth.
    - (*b*) The second period extends from the first till the fish is 10 mm. long. It is characterized by the direct development of the eye from the normal embryonic stage reached in the first period to the highest stage reached by the *Amblyopsis* eye.
    - (*c*) The third period extends from the second period to the beginning of senescent degeneration, from a length of 10 mm. to about 80 or 100 mm. It is characterized by a number of changes which, while not improving the eye as an organ of vision, are positive as contrasted with degenerative. There are also distinct degenerative processes taking place during this period.
    - (*d*) The fourth period begins with the beginning of senescent degeneration and ends with death. It is characterized by degenerative processes only which tend to gradually disintegrate and eliminate the eye entirely.

11. For a summary of the origin, development, and degeneration of the eye and its parts see table, page 164.

12. There is no constant ratio between the extent and degree of ontogenetic and phylogenetic degeneration. The observed rate of ontogenetic degeneration is not necessarily proportionate to the rate of phylogenetic degeneration inferred from the degree of degeneration of the eye at its optimum.

13. If *Troglichthys* indicates one of the steps through which the eye of *Amblyopsis* will pass to annihilation, the degenerative phases seen in the oldest specimens of *Amblyopsis* indicate only in a general way the phylogenetic path over which the eye will pass in the future.

14. Some late stages of development are omitted by the giving out of developmental processes. Some of the processes giving out are cell division, resulting in the minuteness of the eye and the histogenic changes which differentiate the cones and the outer reticular layer.

15. There being no causes operative or inhibitive either within the fish or in the environment that are not also operative or inhibitive in *Chologaster agassizii*, which lives in caves and develops well-formed eyes, it is evident that the causes controlling the development are hereditarily established in the egg by an accumulation of such degenerative changes as are still notable in the later history of the eye of the adult.

16. The foundations of the eye are normally laid, but the superstructure, instead of continuing the plan with additional material, completes it out of the material provided for the foundations. The development of the foundation of the eye is phylogenetic, the stages beyond the foundations are direct.

## GENERAL SUMMARIAL ACCOUNT OF THE EYES OF THE AMBLYOPSIDÆ.

As in all organs no longer of use or hindrance, and therefore no longer under the control of selection, the individual variations in the structure of the eye of *Amblyopsis*, *Troglichthys*, and *Typhlichthys* are very great.[1] There is also a marked change in the eye with age. It is therefore necessary to distinguish between individual variations and stages in ontogenetic and phylogenetic degeneration. The eye of each species has a general structure which is typical for the species. The individual variations have been sufficiently described under the respective species.

### PHYLETIC DEGENERATION OF THE EYE OF THE AMBLYOPSIDÆ.

The steps in degeneration in the Amblyopsidæ are indicated in figure 66. The most highly developed eye is that of *Chologaster papilliferus*. The parts of this eye are well proportioned, but the eye as a whole is small, measuring less than 1 mm. in a specimen 55 mm. long. The proportions of this eye are symmetrically reduced if it has been derived from a fish eye of the average size. The retina is much simpler than in *Zygonectes*. The simplifications in the retina have taken place between the outer nuclear and the ganglionic layers. The pigment layer has not been materially affected. These facts are exactly opposed to the supposition of Kohl that the retina and the optic nerve are the last to be affected, and that the vitreous body and the lens cease to develop early. In *Chologaster papilliferus* (*b*) the latter parts are normal, while the retina is simplified. That the retina is affected first is proved beyond cavil by *cornutus* (*a*). The vitreous body and the lens are here larger than in *papilliferus*, but the retina is very greatly simplified. *Cornutus*, it must be borne in mind, lives in the open. The eye of *Chologaster agassizii* (*c*) differs from that of *papilliferus* largely in size. There is little difference in the retinas except the pigmented layer, which is about 26 per cent thinner in *agassizii* than in *papilliferus*.

If we bear in mind that no two of the eyes represented here are members of a phyletic series, we may be permitted to state that from an eye like that of *cornutus*, but possessing scleral cartilages, both the eyes of *Amblyopsis* and *Troglichthys* have been derived, and that the eye of *Amblyopsis* represents one of the stages through which the eye of *Troglichthys* passed. The eye of *Amblyopsis* (*h*) is the eye of *Chologaster cornutus* minus a vitreous body with the pupil closed and with a minute lens or more probably none at all. The nuclear layers have gone a step farther in their degeneration than in *cornutus*, but the greatest modification has taken place in the dioptric apparatus.

In *Troglichthys* (*i*) even the mass of ganglionic cells present in the center of the eye as the result of the collapsing after the removal of the vitreous body has vanished. The pigmented epithelium, and in fact all the other layers, are represented by mere fragments.

The eye of *Typhlichthys* (*g*) has degenerated along a different line. There is an almost total loss of the lens and vitreous body in an eye like that of *papilliferus*

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[1] This is also true of the eye of *Lucifuga* and *Stygicola*.

without an intervening stage like that of *cornutus*, and the pigment layer has lost its pigment, whereas in *Amblyopsis* it was retained.

The reduction in size from the normal fish eye went hand in hand with the simplification of the retina. There was at first chiefly a reduction in the number of many times duplicated parts. Even after the condition in *Chologaster papilliferus* was reached the degeneration in the histological condition of the elements did not keep pace with the reduction in number (*vide* the eye of *cornutus*). The

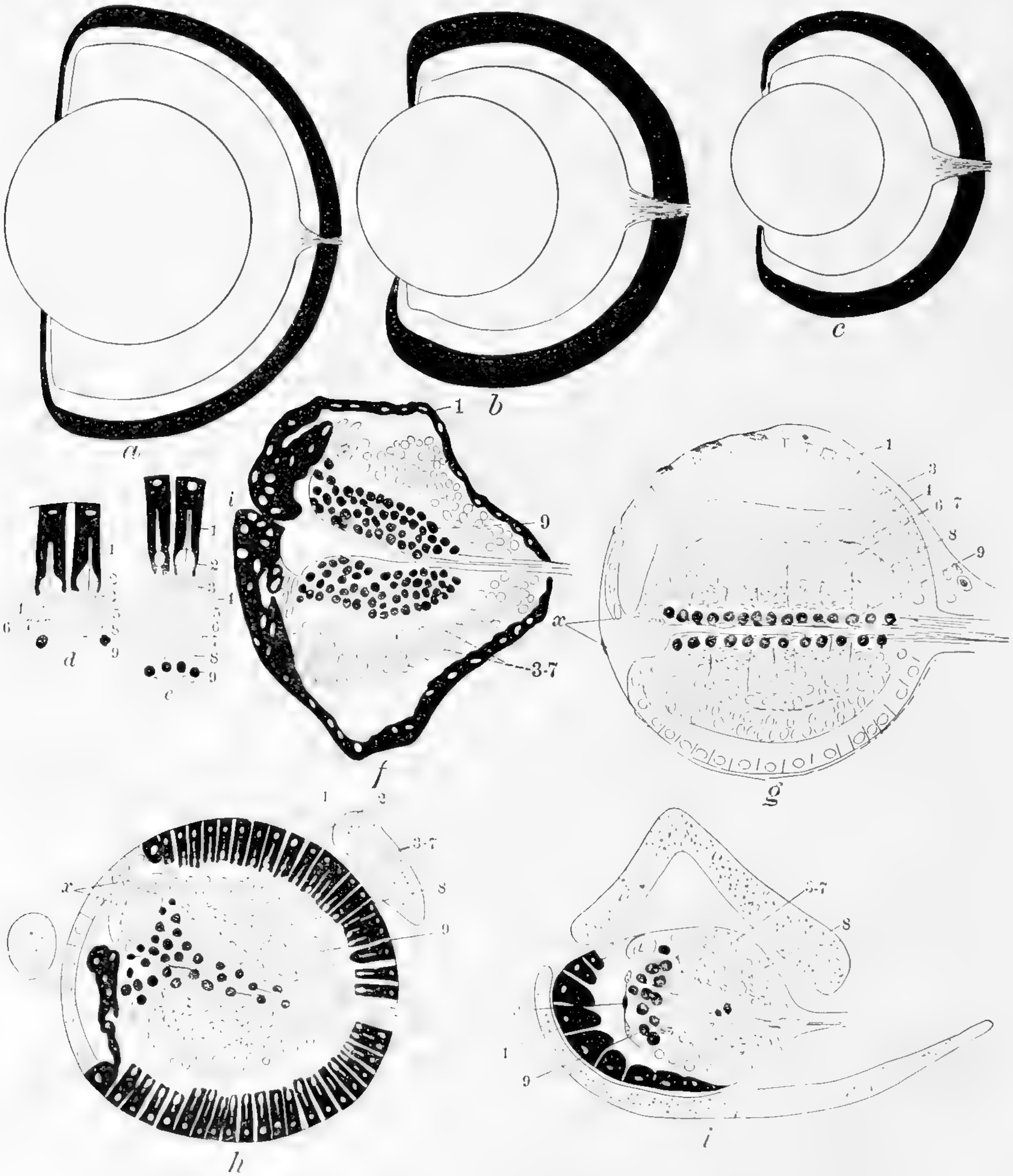

FIG. 66. Diagrams of Eyes of all Species of Amblyopsidæ and *Typhlomolge*, *d*, *e*, *g*, *h*, and *i* drawn under same magnification. (*a*) *Chologaster cornutus*, (*b*) *Chologaster papilliferus*, (*c*) *Chologaster agassizii*, drawn to scale; (*d*) Retina of *Chologaster cornutus*; (*e*) Retina of *Chologaster papilliferus*; (*f*) Eye of *Typhlomolge* under lower magnification than *d–f*; (*g*) Eye of *Typhlichthys subterraneus*; (*h*) Eye of *Amblyopsis spelæus*; (*i*) Eye of *Troglichthys rosæ*.

dioptric apparatus disappeared rather suddenly, and the eye, as a consequence, collapsed with equal suddenness in those members which, long ago, took up their abode in total darkness. The eye not only collapsed, but the number of elements decreased very much. The reduction was in the horizontally repeated elements. The vertical complexity, on which the function of the retina really depends, was not greatly modified at first.

In those species which took up their abode in total darkness the degeneration in the dioptric apparatus was out of proportion to the degeneration of the retina, while in those remaining above ground the retinal structures degenerated out of proportion to the changes in the dioptric apparatus, which, according to this view, degenerates only under conditions of total disuse or total darkness which would necessitate total disuse. This view is upheld by the conditions found in *Typhlogobius*, as Ritter's drawings and my own preparations show. In *Typhlogobius* the eye is functional in the young and remains a light-perceiving organ throughout life. The fish live under rocks between high and low tide. We have here an eye in a condition of partial use and the lens is not affected. The retina has, on the other hand, been horizontally reduced much more than in the Amblyopsidæ, so that, should the lens disappear, and Ritter found one specimen in which it was gone, the type of eye found in *Troglichthys* would be reached without passing through a stage found in *Amblyopsis;* it would be simply a horizontal contracting of the retina, not a collapsing of the entire eye.

The question may with propriety be asked here: Do the most degenerate eyes approach the conditions of the pineal eye? It must be answered negatively.

### RESULTS OF THE PHYLETIC DEGENERATION ON THE DIFFERENT PARTS OF THE EYES OF THE AMBLYOPSIDÆ.

The different structures of the eye may now be taken up in detail.

(*a*) The eye muscles are normally developed in *Chologaster*. They are present to a greater or less extent in *Amblyopsis*. They have been reduced in number in *Troglichthys*, where the half nearest the eye has been replaced by bundles of fibrous tissue. In *Typhlichthys* they have vanished.

(*b*) The scleras of the different members are not comparable on account of the presence of cartilage in some species and not in others. Both this layer and the choroid are insignificant in *Chologaster* and *Typhlichthys*. In *Amblyopsis* cartilages different in size and number are found anywhere about the eye, being frequently present in shape and position to suggest a displaced lens. In thickness the cartilages are disproportionate to the size of the eye. In *Troglichthys* we have a still more evident misfit, for the scleral cartilages are both too long and too thick. Evidently the scleral cartilages have not decreased in size in the same ratio as the eye, or, what amounts to the same thing, they develop beyond the present needs of the eye. (See also *Lucifuga*.)

(*c*) The choroid is thin in all cases except where pigment cells are situated. These are frequently several times as thick as the rest of the choroid. In *Amblyopsis* the pigmentation of the choroid is inversely proportional to the pigmentation of the retina.

(*d*) The lens has already received sufficient attention. It is merely necessary to insist again that, as long as an eye is functional to any extent, the lens — in fact the dioptric apparatus in general — does not degenerate and that when absolute disuse comes, the lens, both phylogenetically and ontogenetically, disappears rapidly. In *Typhlogobius* Ritter found the lens absent in one very old individual, and Cope found that in *Gronias* the lens is sometimes present on one side, while not on the other. In *Amblyopsis* and *Typhlichthys* it has degenerated to a mere vestige, or is gone altogether. Ritter, after considering the structure of degenerate eyes as far as known at the time, came to the conclusion "that the lens disappears before the retina; and that, where degeneration takes place at all in ontogeny, the lens is affected first and most profoundly." With the first part of this statement the more recent observations are in full accord. It is, however, doubtful whether the lens is ever the first part affected; in fact the retina always leads, but certainly the lens, if affected at all, is affected profoundly.

(*e*) There is more variety in the degree of development of the pigment epithelium than in any other structure of the eye. Ritter has found that in *Typhlogobius* this "layer has actually increased in thickness concomitantly with the retardation in the development of the eye, or it is quite possible with the degeneration of this particular part of it. An increase of pigment is an incident to the gradual diminution in functional importance and structural completeness." There is so much variation in the thickness of this layer in various fishes that not much stress can be laid on the absolute or relative thickness of the pigment in any one species as an index of degeneration. While the pigment layer is, relative to the rest of the retina, very thick in the species of *Chologaster*, it is found that the pigment layer of *Chologaster* is not much if any thicker than that of *Zygonectes*, but exception must be made for specimens of the extreme size in *papilliferus* and *agassizii*. In other words, primarily the pigment layer has retained its normal condition, while the rest of the retina has been simplified, and there may even be an increase in the thickness of the layer as one of its ontogenetic modifications. Whether the greater thickness of the pigment in the old *Chologaster* is due to degeneration or the greater length of the cones in a twilight species I am unable to say.

In *Typhlichthys*, which is undoubtedly derived from a Chologaster-like ancestor, no pigment is developed, the layer retains its epithelial nature and remains apparently in its embryonic condition. It may be well to call attention here to the fact that the cones are very sparingly developed, if at all, in this species. In *Amblyopsis*, in which the degeneration of the retina has gone farther, but in which the cones are still well developed, the pigment layer is very highly developed, but not by any means uniformly so in different individuals. The pigment layer reaches its greatest point of reduction in *rosæ* where pigment is still developed, but the layer is fragmentary except over the distal part of the eye. We thus find a development of pigment with an imperfect layer in one case, *Troglichthys*, and a full-developed layer without pigment in another, *Typhlichthys*. In the chologasters the pigment is prismatic; in the other species granular.

(*f*) In the outer nuclear layer a complete series of steps is observable from the two-layered condition in *papilliferus* to the one-layered in *cornutus*, to the undefined layer in *Typhlichthys* and the merging of the nuclear layers in *Amblyopsis*, and their occasional total absence in *rosæ*. The single cones disappear first, the cones long before their nuclei.

(*g*) The outer reticular layer naturally meets with the same fate as the outer nuclear layer. It is well developed in *papilliferus* and *agassizii*, evident in *Cholo-*

*gaster cornutus*, developed in spots in *Typhlichthys*, and no longer distinguishable in the other species.

(*h*) The layers of horizontal cells are represented in *papilliferus* by occasional cells; they are rarer in *cornutus* and beyond these have not been determined with certainty.

(*i*) The inner nuclear layer of bipolar and spongioblastic cells is well developed in *C. papilliferus* and *C. agassizii*. In *cornutus* it is better developed in the young than in the older stages, where it forms but a single layer of cells. There is evidently in this species an ontogenetic simplification. In the remaining species it is, as mentioned above, merged with the other nuclear layer into one layer which is occasionally absent in *Troglichthys*.

(*j*) The inner reticular layer is relatively better developed than any of the other layers, and the conclusion naturally forces itself upon one that it must contain other elements besides fibers of the bipolar and ganglionic cells, for, in *Amblyopsis* and *Troglichthys*, where the latter are very limited or absent, this layer is still well developed. Horizontal cells have only been found in the species of *Chologaster*.

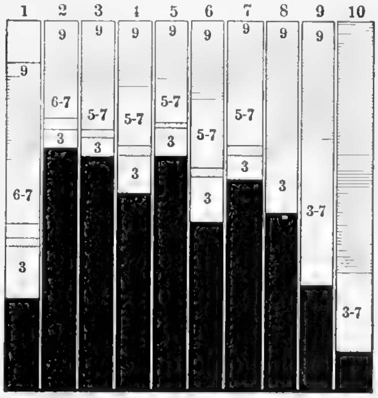

FIG. 67. Diagram showing per cent of Total Thickness of each Layer of Retina in 1, *Zygonectes notatus;* 2 and 3, *Chologaster cornutus*, 27 mm. long and 43 mm. long; 4, *Chologaster papilliferus*, 29 to 39 mm. long, and 5, 55 mm. long; 6, *Chologaster agassizii*, 38 mm. long and 7.62 mm. long; 8, *Amblyopsis;* 9, *Typhlichthys;* 10, *Troglichthys.*

(*k*) In the ganglionic layer we find again a complete series of steps from the most perfect eye to the condition found in *Troglichthys*. In *papilliferus* and *agassizii* the cells form a complete layer one cell deep except where they have given way to the optic fiber tracts which pass in among the cells instead of over them. In *cornutus* the cells have been so reduced in number that they are widely separated from each other. With the loss of the vitreous cavity the cells have been brought together again into a continuous layer in *Typhlichthys*, although there are much fewer cells than in *cornutus* even. The next step is the formation of a solid core of ganglionic cells, and the final step the elimination of this central core in *Troglichthys*, leaving but a few cells over the anterior face of the retina.

(*l*) Müllerian nuclei are found in all but *Amblyopsis* and *Troglichthys*. In *C. cornutus* they lie in part in the inner reticular and the ganglionic layer. Cells of this sort are probably also found among the ganglionic cells of *Typhlichthys*.

We thus see that the simplification or reduction in the eye is not a horizontal process. The purely supporting structures like the scleral cartilages have been retained out of all proportion to the rest of the eye. The pigment layer has been both quantitatively and qualitatively differently affected in different species. There was primarily an increase in the thickness of this layer, and later a tendency to total loss of pigment. The degeneration has been more uniformly progressive in all the layers within the pigment layer. The only possible exception being the inner reticular layer, which probably owes its retention more to its supporting than to its nervous elements. Another exception is found in the cones, but their degree of development is evidently associated with the degree of development of the pigmented layer. As long as the cones are developed, the pigmented layer is well developed, or *vice versa*.

### ONTOGENETIC DEGENERATION.

The simplification of the eye in *cornutus* has been mentioned in the foregoing paragraphs. It may be recalled that the nuclear layers are thinner in the old than in the young. There is here not so much an elimination or destruction of element as a simplification of the arrangements of parts, comparatively few being present to start with.

The steps in ontogenetic degeneration can not be given with any degree of finality for *Amblyopsis* on account of the great variability of the eye in the adult. While the eyes of the very old have unquestionably degenerated, there is no means of determining what the exact condition of a given eye was at its prime. In the largest individual examined the eye was on one side a mere jumble of scarcely distinguishable cells, the pigment cells and scleral cartilages being the only things that would permit its recognition as an eye. On the other side the degree of development was better.

The fact that the eyes are undergoing ontogenetic degeneration may be taken, as suggested by Kohl, that these eyes have not yet reached a condition of equilibrium with their environment or the demands made upon them by use. Furthermore, the end result of the ontogenetic degeneration is a type of structure below anything found in the phlyogeny of the vertebrate eye. It is not so much a reduction of the individual parts as it is a wiping out of all parts.

### PLAN AND PROCESS OF PHYLETIC DEGENERATION IN THE AMBLYOPSIDÆ.

Does degeneration follow the reverse order of development or does it follow new lines, and if so, what determines these lines? Since the ontogenetic development of the eye is supposed to follow in general lines its phyletic development, the above question resolves itself into whether or not the eye is arrested at a certain stage of its morphogenic development, and whether this causes certain organs to be cut off from the development altogether. In this sense the question has been answered in the affirmative by Kohl. Ritter, while unable to come to a definite conclusion, notes the fact that in one individual of *Typhlogobius* the lens which is phyletically a new structure had disappeared. This lens had probably been removed as the result of degeneration rather than through the lack of development. Kohl supposes that in animals placed in a condition where light was shut off more or less, every succeeding generation developed its eye less. Total absence of light must finally prevent the entire anlage of the eye. Time has not been long enough to accomplish this in any vertebrate. Phyletic degeneration is looked upon as the result of a long series of "Hemmungen" which in successive generations appeared in ever earlier time of ontogenetic development in always lower stages of the development of the individual eyes. The eye develops after the vertebrate type. At certain stages the rate of progress is diminished and in most cases finally completely ceases. A retardation has developed which after a shorter or longer period ends in the cessation of all development. The first appearance of the retardation falls in a time of embryonic or post-embryonic development that in the phylogeny corresponds to the moment when the *lack of light* became operative. The period in ontogeny which lies between the first disturbance in development and its cessation corresponds to the phyletic time during which the development of the eye is checked at a continually lower stage of development. The point of cessation in ontogeny corresponds to the time when the eye reached its equilibrium. If in ontogeny there is undoubted degeneration, it is always an indication that the eye has not yet reached the point where it is in equilibrium with its functional requirements.

Cessation of development does not take place at the same time in all parts of the eye. Those not essential to the perception of light are disturbed first. The retina and the optic nerve are the last affected, the iris coming next in the series. Because the cornea, aqueous and vitreous bodies, and the lens are not essential for the performance of the function of the eye, these structures cease to develop early. The processes of degeneration follow the same rate. Degeneration is brought about by the falling apart of the elements as the result of the introduction of connective tissue cells that act as wedges. Abnormal degeneration sometimes becomes manifest through the cessation of the reduction of parts that normally decrease in size so that these parts in the degenerate organ are unusually large.

Kohl's theoretical explanation here given somewhat at length is based on the study of an extensive series of degenerate eyes. He has not been able to test the theory in a series of animals living actually in the condition he supposes for them, and has permitted his erroneous interpretation of the highly degenerate eye of *Troglichthys* to lead him to this theory of the arresting of the eye in ever earlier stages of ontogeny. It has been shown in previous pages that this most degenerate eye is in an entirely different condition from that supposed by him. The mere checking of the normal morphogenic development has done absolutely nothing to bring about this condition, and it could not have been produced by the checking of development in ever earlier and earlier stages of ontogeny, for there is no stage in normal ontogeny resembling in the remotest degree the eye of *Troglichthys*. The process of degeneration as seen in the Amblyopsidæ is in the *first instance* one of growing smaller and simpler — not a cutting off of late stages in the development. The simplified condition, it is true, appears earlier and earlier in ontogeny till it appears almost along the entire line of development, even in the earliest stages. But the tendency for characters added at the end of ontogeny to appear earlier and earlier in the ontogeny is well known, and there is no inherent reason why an organ disappearing in the adult should not eventually disappear entirely from ontogeny. The fact that organs which have disappeared in the adult have in many instances not also disappeared in the ontogeny and remain as so-called rudimentary organs has received an explanation from Sedgwick. For a discussion of this see the chapter on the Law of Biogenesis.

In *Amblyopsis*, where the eye has not been functional at any period of ontogeny for many generations, where degeneration begins at an early period and continues till death, the degenerate condition has reached the early stages of the embryo. It is only during the first hour or so that the eye gives promise of becoming anything more than it eventually does become. The degree of degeneration of an organ can be measured as readily by the stage of ontogeny when the degeneration becomes noticeable as by the structure in the adult. The greater the degeneration, the farther back in the ontogeny the degenerate condition becomes apparent, unless, as stated above, the organ is of use at some time in ontogeny. It is evident that an organ in the process of being perfected by selection may be crowded into the early stages of ontogeny by post-selection. Evidently the degenerate condition is not crowded back for the same reason. How it is crowded back, I am unable to say. A satisfactory explanation of this will also be a satisfactory explanation of the process by which individually acquired characteristics are enabled to appear in the next generation. The facts, which are patent, have been formulated by Hyatt in his law of tachygenesis. Histogenic development is a prolonged process, and ontogenetic degeneration is still operative, at least, in *Amblyopsis.*

Degeneration is not the result of the ingrowth of connective tissue cells as far as I can determine. It is rather a process of starving, of shriveling, or resorption of parts.

From the foregoing it is evident that degeneration has not proceeded in the reverse order of development, rather the older normal stages of ontogenetic development have been modified into the more recent phyletic stages through which the eye has passed. The adult degenerate eye is not an arrested ontogenetic stage of development, but a new adaptation, and there is an attempt, in later ontogeny at least, to reach the degenerate adult condition in the most direct way possible.

# THE CUBAN BLIND FISHES

# THE CUBAN BLIND FISHES.[1]

## HISTORY OF THE WORK.

The Cuban blind fishes were discovered by the surveyor D. Tranquilino Sandalio de Noda. They were described as *Lucifuga subterraneus* and *Lucifuga dentatus* by Poey, in his "Memorias sobre la Historia Natural de la Isle de Cuba," tomo 2, pp. 95–114, 1856. Poey recorded them from the cave Cajio, near La Guira de Melena, La Industria, half-way between Alquizar and Guanimar, the Cave of Ashton, the Cave of the Dragon, on the cattle farm San Isidro, near Las Mangas, La Concordia, a cave near the bee house of the coffee plantation La Paz, and a well near the tavern Frias.

Poey stated that *Lucifuga dentatus* from some of the caves had vestiges of eyes, while those from others were without the least vestige of eyes. Poey later added some notes on their distribution in his "Enumeratio Piscium Cubensium." In 1863 Gill (Proc. Acad. Nat. Sci. Phila., 1863, p. 252) recognized *Lucifuga dentatus* as the type of a distinct genus, which he called *Stygicola*.

No additions were made to the knowledge of these fishes until March, 1902, when I visited Cuba with Mr. Oscar Riddle expressly to secure material for the study of their eyes. We visited several of the caves mentioned by Poey and many others, securing 119 specimens of both species. One of the specimens contained four young, making in all 123 specimens. The discovery that the blind fishes are viviparous, and that the young have fairly well developed eyes, made it seem very desirable to secure a full series of embryos and also if possible to rear some of them in the light.

The expenses of this trip were defrayed in part by a grant from the American Association for the Advancement of Science and in part from subsidiary work on the fresh-water fishes of the western end of the island. The results as far as published are included in an article on the "Fresh-water Fishes of Western Cuba" (Bull. U. S. Fish Com., 1902, pp. 211–236, plates 19–21, 1903).

Grant No. 64 of the Carnegie Institution made additional work in the field possible. It was planned to spend the entire breeding season near the caves and rear young in the light, but for reasons that will appear the grant was exhausted in apparently determining that these fishes do not breed in the places visited.

My trip to Cuba in March, 1902, made it seem probable that the blind fishes give birth to their young in February. Many recently born young of *Lucifuga* were obtained at that time, and one of the females caught contained young nearly ready to be born. The California viviparous fishes, with which I had extensive experience[2] and which give birth to young in a similar degree of maturity, carry their young about 5 months. On these premises I concluded that early stages of the young of the blind fishes should be found during the middle of September. Allowing a month for the probably more rapid development in the tropics, I visited the caves the latter part of October and first part of November.

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[1] The specimens were numbered as they were collected, 1, 2, etc., and when referred to are given by their serial number.

[2] On the viviparous fishes of the Pacific coast of North America, Bull. U. S. Fish Com., 1892, pp. 381–478, 27 plates, 1894.

Aside from obtaining young it was planned to build cages in a well-lighted cave in which the adult would be compelled to carry and give birth to their young in the light. The body walls in the majority of individuals would offer little or no obstacle to the penetration of light to the embryos.

Dr. J. W. Beede, of the Geological Department of Indiana University, acted as volunteer assistant and rendered very valuable aid in collecting fishes, making the cages, and taking the traverse to the various caves in the chief cave region about Cañas. Only a single individual with young was obtained and one other with nearly *mature eggs*. Two cages were built and fishes were confined in them and the cages sunk in the Modesta, a well-lighted cave in which fishes were naturally abundant.

On December 1 a few fishes were collected and sent me by Mr. F. Martinez, of Cañas. Although these promised little better success than the ones collected in October and early November, I started for Cuba again on December 18, 1903, accompanied on this trip by Mr. John Haseman, as volunteer assistant. It was again found that this was not the breeding season, as no fishes with young were found at all. The cages were found intact and received a new supply of fishes.

On May 1 a number of fishes were sent me by Mr. F. Martinez, and as these promised no young the trip planned for May was abandoned. On June 1, when Mr. Martinez was again to send me samples, he was unable to obtain any fishes on account of high water.

Between June and August I could not get away from my routine work, but this period was later covered by Mr. Haseman. On August 15, 1904, I started again for Cuba, accompanied by Mr. Hankinson as volunteer assistant. I returned September 7. On this trip, which was more extensive than the former, I obtained two females with young, one a *Lucifuga* containing 10 young, and one a *Stygicola* containing 1 young. On this occasion I visited two new localities. At one of these, Jovellanos, from which Poey reported *Stygicola*, I obtained nothing. At the other, the Carboneria farm, on the north coast near Matanzas, I obtained my first specimens from the northern slope of Cuba. I am under many obligations to Dr. Felix Garcia, the harbor health officer of Matanzas for the opportunity to visit the Carboneria.

At this time the cages in the Modesta were found to be entirely spoiled, the wire screening having corroded in large pieces. I succeeded in bringing living fishes to Indiana, but it was not possible to bring large numbers. There was great mortality en route on account of the extreme sensitiveness to cool water, which rules entirely out of court the idea of colonizing them in some of our northern caves.

In June, 1905, two of my students, Mr. J. Haseman, who had accompanied me on one of the trips, and Mr. Norman McIndoo, made another tour of the caves, but with no better success as far as embryos were concerned. They secured but one female with young.

The following papers have appeared on material gathered during the various Cuban trips:

1. The Blind Fish of Cuba. Science, N. S., XVI, p. 347.
2. Eigenmann, C. H. The fresh-water fishes of western Cuba. Bull. U. S. Comm. Fish and Fisheries, 1902, pp. 211–236, plates 19–21.
3. The water supply of Havana. Science, N. S., XVIII, pp. 281–282. Aug. 28, 1903.
4. In search of Blind Fish in Cuba. World To-day, V, pp. 1129–1136.
5. Auf der Suche nach blinden Fischen in Cuba. Die Umschau, VII, pp. 365–367.
6. Hay, W. P. On a small collection of crustaceans from the island of Cuba. Proc. U. S. Nat. Mus., XXVI, pp. 429–435, Feb. 2, 1903.
7. Lane, H. H. The ovarian structures of the viviparous blind fishes, *Lucifuga* and *Stygicola*. Biological Bulletin, VI, pp. 38–54, 1903.

Carboneria beach near Matanzas.

Dividing line between naked beach (on right) and sand-filled area (on left). Rift separates the two zones. Bushes on extreme left mark line of older beach.

Cave of the Insurrectos, near the Carboneria, from entrance. Pool of water showing at bottom of cave.

8. Muhse, E. F. The eyes of *Typhlops lumbricalis* (L.), a blind snake from Cuba. Biol. Bull., v, pp. 261–270, Oct. 1903.
9. Pike, F. H. The degenerate eyes in the Cuban cave shrimp, *Palæmonetes eigenmanni* Hay. Biological Bulletin, xi, pp. 267–276, 1906.
10. Payne, F. The eyes of *Amphisbæna punctata* (Bell), a blind lizard from Cuba. Biol. Bull., xi, pp. 60–70, plates i and ii, July 1906.
11. Weckel, A. L. The fresh-water Amphipoda of North America. Proc. U. S. Nat. Mus., xxxii. Describing a new Amphipod, *Gammarus cæcus*, from the Modesta Cave, Cuba. pp. 47–49, 1907.
12. Haseman, J. D., and McIndoo, Norman N. On some fishes of Western Cuba. Proc. Acad. Nat. Sci. Phil., 1906.

### ZOOLOGICAL POSITION OF LUCIFUGA AND STYGICOLA.

*Lucifuga* and *Stygicola* are members of the Brotulidæ, of which Jordan and Evermann say: "These fishes are closely related to the Zoarcidæ. In spite of various external resemblances to the Gadidæ, their affinities are rather with the blennioid forms than with the latter."

They are most closely related to the genera *Brosmophycis* and *Ogilbia*, with which they have a distinct caudal peduncle in contradistinction to the numerous other American genera of the family. *Brosmophycis marginatus* (Ayres) occurs on the coast of California in moderate depth. *Ogilbia ventralis* (Gill) occurs in rocky pools about the Gulf of California and at La Paz. The other member of the genus, *Brosmophycis cayorum*, was taken on a shoal covered with algæ at Key West.

Other members of the family are found at great depths in various parts of the world; one, *Brotula barbata*, occurs about Cuba in water of moderate depth.

The genera *Lucifuga* and *Stygicola* differ from each other in their dentition. *Stygicola* has teeth on the palatines; *Lucifuga* has none. In *Stygicola* the nape is more strongly arched than in *Lucifuga*. The maximum recorded size of *Stygicola* is 152 mm.; of *Lucifuga*, 104 mm.

### PRIMARY AND SECONDARY SEXUAL CHARACTERS.

The male organ of *Stygicola* consists of a conical papilla, two-lobed at the tip and surrounded by a dermal pouch. It reaches to the second or third anal ray, being turned either to one side or the other of the anal. It is pigmentless, but is covered from in front by a pigmented dermal flap.

In color, *Lucifuga* varies from a faint pink to lilac-pink and lilac. There is, in general, an increase of pigment with age. *Stygicola* varies from pinkish lilac to steel-blue, with transparent edges to the fins. There is no regular increase of color with age in this species nor is there any distinction in the sexes. Both black and light-colored individuals are found side by side in caves. It is possible that light-colored individuals have lived in the remote recesses of the cave and that the black ones have remained in the lighted chambers, but there is no direct evidence on this point.

The males of *Stygicola* are distinctly larger than the females. The average length of 137 females caught is 98.2 mm., the largest one being 140 mm. The average size of the 82 males is 107 mm., the largest one being 152 mm. long. In the first lot secured the males were in excess of the females in the ratio of 100 females to 115 males. In all I have 137 females to 82 males. Counting the first 43 specimens secured, there is but an appreciable difference in the average of the fins as far as these could be counted, the average formula for the female being, D. 91.4; A. 74; and for the males D. 91.1; A. 73.3; or the average for the two, D. 91.2; A. 73.6.

Of *Lucifuga*[1] 74 males have an average length of 63.5 with a maximum of 104, and 82 females have an average length of 58 mm., with a maximum of 95. Only specimens over 50 mm. in length were considered.

While the average number of rays differs considerably in the two species, the number in each varies so much that the numbers in individual cases overlap, the individuals of *Lucifuga* reaching as high as 88 dorsal rays, and the individuals of *Stygicola* as low as 87. The same is true with the anal.

### DISTRIBUTION OF STYGICOLA AND LUCIFUGA.

*Stygicola* is known to be distributed from Jovellanos and Alacranes on the east to Cañas. *Lucifuga* is confined to the region from Guira de Melena westward to Cañas. The entire region between Alacranes and Cañas on the southern slope is drained by underground rivers. In the Cañas region, the two species live side by side with apparently no choice, except that while the young of *Lucifuga* are abundant in shallow water among the roots of trees I have not been able to see or secure *Stygicola* shorter than 60 mm. except as larvæ from the mother. Stygicolas are perhaps more abundant in the deeper, darker caves, though they are also found in the shallowest, while lucifugas are more abundant in shallower, more open caves, they in turn being found in the deeper caverns. Blind fishes resembling *Stygicola* or *Lucifuga* have been reported to me from well-like caves at Merida, Mexico. None have been captured. Other blind fishes which may be related to them are said to occur in Jamaica.

### NATURE OF THE HABITAT OF STYGICOLA AND LUCIFUGA.

Within the area over which they are distributed the blind fishes of Cuba live, as far as known, in well-like caves in coralline limestone. The character of the region in which they live can best be understood from an examination of the Finca Carboneria, just outside of the Bay of Matanzas. There is here a coral strand about on a level with high water.

At the point of contact between ocean and land there is an abrupt wall, 5 to 10 feet high, profusely covered with seaweed, the nearly tideless water coming to the top of the wall where there are shallow, panlike pools replenished by waves and spray. Immediately on top of the wall follows a low, naked, jagged mass of rock resembling a huge sponge with its numerous pits and points. This area is inhabited by innumerable mollusks. This low area is separated by a cleft (plate 11, fig. A) forming a sharp line of demarcation from a second zone similar to the first, but in which the pits and depressions in the rock have become filled with sand which gives foothold to tufts of plants. Over this lizards scamper from rock to rock. Following this there is an abruptly sloping beach, the outer half of which is rocky and sandy, partly covered with cactus and other low-growing plants, the inner or land half being covered with shrubs and trees. All of these zones occupy perhaps 100 yards. They are followed by the level, practically treeless, meadow,

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[1] The following account was published of the first 53 specimens of *Lucifuga* secured: The females are distinctly larger than the males. In making the average for the size of the sexes, individuals less than a year old were not considered, because differences in the sexes, if present, could be but very slight, and because in such young the sex could not always be determined with certainty. An examination of all specimens makes it probable that at the end of a year after birth the young are about 50 mm. long. In obtaining the average size of the sexes only those specimens over 50 mm. were considered. The males above this size measure 59.7 mm. on an average, with a maximum of 94 mm.; the females measure 71.1 mm. on an average, with a maximum of 93 mm. Of the specimens over 50 mm. long, 23 were males and 22 females, or 100 females for every 104.5 males. Counting the fin rays of the first 43 specimens over 50 mm. long, we get males, D. 82.1, A. 67.4; females, D. 81.9, A. 68. The average formula for those less than 50 mm. long is D. 83; A. 67.2, or for all together, D. 82.6; A. 67.5.

perhaps 0.75 mile wide and less than 10 feet above sea-level. It is such a beach as is shown in figure A, plate 11, raised to a little higher elevation. There is here but little sandy soil, the underlying rock coming near the surface. The slope of the hill behind this level stretch is composed of bare rocks very similar to those of plate 11, figure A, except that the gnarled roots of the densely growing stunted shrubs and trees twist about the rocks and into the crevices. The character of this area was very well described by my host, who dryly remarked, when I asked him whether I should go on horse to the caves on top of the hills, "No, you will go on your hands and knees." The disagreeable impression that these hills make on one traversing them on foot in the heat of the day is heightened by the innumerable hermit-crabs that lurk in every cranny and scamper over the rocks. At an elevation of about 100 feet is another level stretch of rocks with a thin layer of sandy soil.

Within less than a quarter of a mile from the ocean is a natural well, improved somewhat with the chisel. It is circular, with a diameter of about 6 feet and is less than 10 feet deep. It is evidently situated along the line of an original fissure in the coralline rock such as is shown in plate 11, figure A, for there are openings in opposite sides of the deeper part of the well that have an indeterminable extent. The surface of the water in this well is near sea-level, about 4 feet below the level of the land. The water, over 5 feet deep, is perfectly fresh and blind fishes were more abundant in this well than in any other area of the same extent.

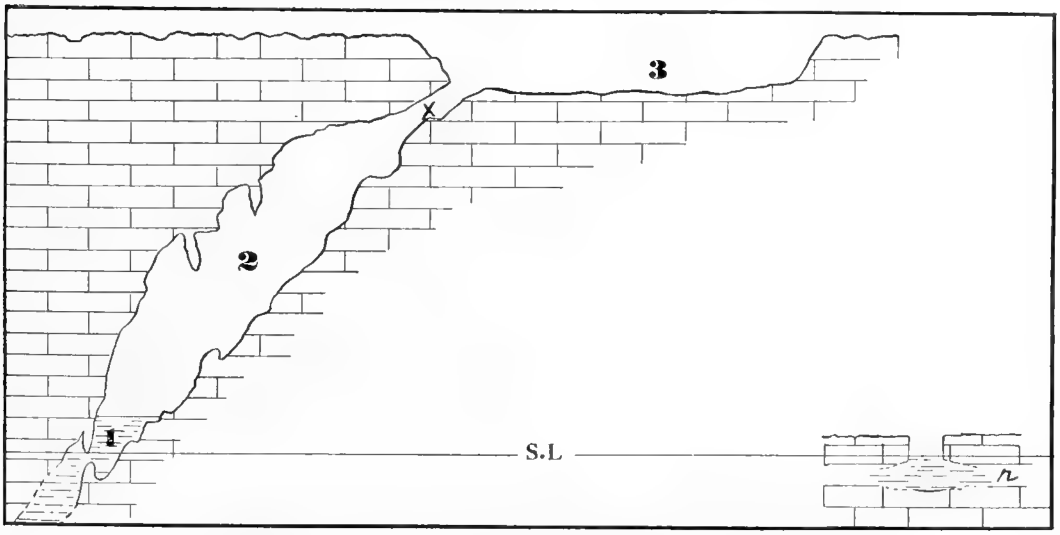

FIG. 68. Diagrams of Cave of the Insurrectos and the Carboneria Well (fig. B, plate 11) taken from X. 3, Depression about Mouth of Cave; 2, Dry Cave; 1, The Pool of Water near Sea-level, S.L, and with Submerged Stalactites and Stalagmites; r, Side Rifts in Carboneria Well.

There are a number of caves on the plateau over the hills and I visited two of these. They are within 4 or 5 miles of the seashore. Their mouths lie at an elevation of about 100 feet (87 and 93 by barometer). In general character these caves are like others visited in Matanzas province, *i. e.*, at Matanzas and at Alacranes, or Alfonso XII. They occur in a level area and from a distance there is nothing to indicate their presence. There is first a slight depression in the level country (fig. 68 (3) ). From one side of this depression a fissure, whose upper and lower surfaces are approximately parallel, extends down at an angle of about 45° or more (plate 11, fig. B). The slope is in all cases very steep, though not always regular. In horizontal section the walls appear to form sections of a circle so that these caves all suggest fragments of hollow cones. At a depth of about 80 feet water is encountered in a crescent-shaped pool. The caves extend down for an indeterminable distance below the water-level. The surface of the water in the caves is near sea-level. Light penetrates to all the recesses of these caves, one of which is called Cueva dos Insurrectos from the fact that a company of Cubans was quartered in it during the Revolution. Figure B, plate 11, gives a glimpse down the Cave of the Insurrectos from the entrance X in figure 68 to the pool of water at the bottom, at a vertical distance of 83 feet. These caves are inhabited by *Stygicola*, but in very much fewer numbers than the well near the seashore. One specimen was secured.

A cave in the side of the hill at the edge of Matanzas shows essentially the same character. The slope is very much steeper and the cave is much smaller. There is the same sort of pool at the bottom as in the Cave of the Insurrectos. I secured no fishes in the Matanzas Cave, though it probably contains them. We were told that into this cave the Cubans, shot during the Revolution, were thrown by the guardians of Matanzas.

On the southern slope of the island, both at Alacranes and westward about Cañas, are formations very much like each other and very much like the condition represented in figure 68, with these exceptions: the territory is farther from the sea; the pockets corroded in the surface rocks are much deeper and larger, and are filled with a stiff red clay.

Bananas are grown in the pockets of soil about the caves at Alacranes. About Cañas most of the territory is still in its primitive condition, covered with manigua, a straight-stemmed, smooth-barked, but irregular-surfaced, sapling that grows in such abundance mingled with other bushes and vines that it obscures the nature of the ground and makes progress through it impossible without the machete.

Frequent clearings made to convert the manigua into charcoal and prepare the soil for seed tobacco reveals the nature of ground to be a series of jagged rocks with pits and depressions filled with the aforesaid red clay. The roads through this region are simply trails along which the manigua has been removed. The rocks are in the natural condition or worn a little by the two-wheeled vehicles which alone are usable here. The wheels of these are so large that they bridge most of the pits between rocks. Traveling over the roads in the manigua in one of the two-wheelers is quite a serious performance. Where the soil is a little thicker, tobacco, casava, and other things are grown. I do not know whether the formation is continuous from Cañas to Alacranes, but it seems quite certain that we have to deal with the same sort of structure in both places. It is a raised coral beach somewhat shattered and with a thin, in many cases interrupted, layer of soil.

The entire southern slope of the area from Alacranes and Union to Cañas is drained by underground streams which, for the most part, are inaccessible. The underground drainage begins further north than the northern edge of the manigua. At San Antonia de los Baños [1] a stream is seen to enter the ground, and a few yards from this place, where the thin limestone roof of the underground channel has given way, the stream can be seen. (See frontispiece.)

For reasons to be mentioned at once the streams are inaccessible. In August of 1904 a very heavy rain caused a small torrent to run in the road leading south from Cañas for a distance of about a mile to the Finca Rosa, where the water spread out over a depression of several acres, so shallow that the depression was

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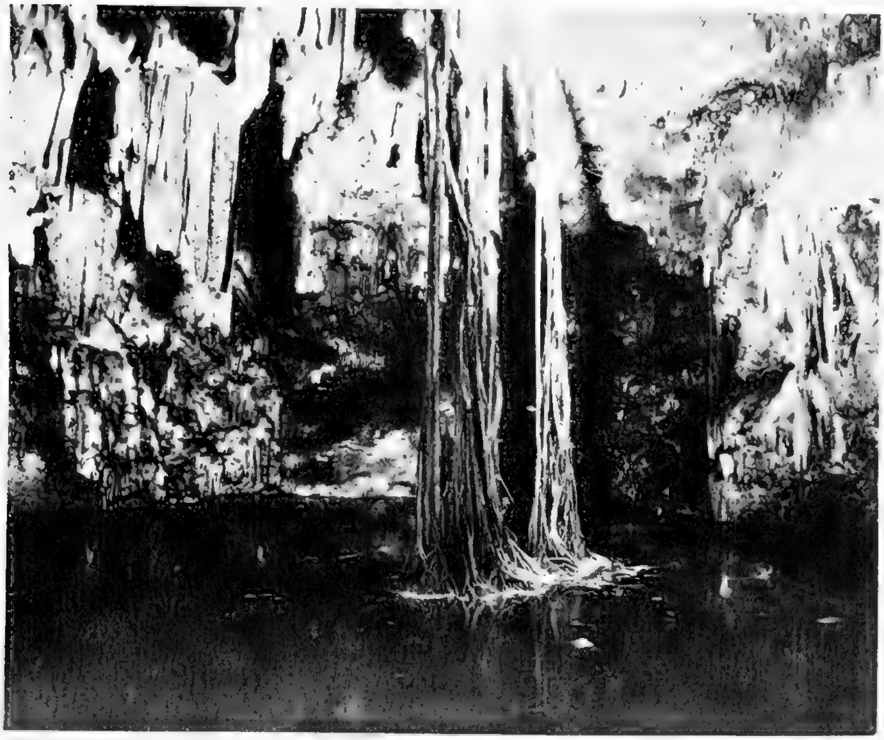
[1] The elevation of the railroad track is 62.92 m.

Root breaking up into rootlets in Ashton Cave. Young of Lucifuga are found among these rootlets.



Cave Isabella, showing group of roots coming through crack in roof. Taken with artificial light.

not perceptible to a casual observer. Mr. Francesco Martinez, who lives within a mile of the place and has been my guide about the caves of Cañas, informed me that the water would all disappear in a day, but that there was no distinct opening to any stream below the surface. Though I have been able to get to the ground-water in many caves about the neighborhood, none of the caves had any intimate connection with an underground stream, for, while the surface water was extremely muddy and abundant and all of it was carried off as rapidly as it would have been in a surface-drained area, the water in the caves to the south, in which direction the drainage flows, remained limpid and showed no appreciable rise. I was told, however, that during an unusual freshet in 1886 the entire region about Modesta Cave became flooded and, naturally, the cave was overflowing.[1] The underground streams come to the surface in a series of "ojos de agua." I visited two of these. One of them is in the Cienaga near the Playa of Guanimar. The water simply rises here in a pool 20 feet across in a swamp and is conducted in an artificial canal by the side of a road to the sea. I did not make extensive observations in this neighborhood, for the Cienaga has a great number of soft places with unknown depth, from which even the highway with a ditch on either side was not altogether free. One of the ditches showed the ground to be permeated with canals up to a foot in diameter. In this Cienaga many of the southward-flowing subterranean streams find their exit, doubtless others have a subaqueous exit in the ocean; two others are found at Batabano on the coast just south of Havana. On the northern slope the most famous of the exits of the underground rivers is the Vento Springs, which supply the city of Havana with water. I have described these in Science (N. S. xviii, pp. 281–282, 1903). I should say that this spring does not yield half as much water as that at Guanimar. Underground streams and tunneled mountains are not rare in other parts of Cuba, though I have not connected them directly with the blind fishes.

I was told a cave passes through a hill west of Matanzas, over which the United Havana Railroad runs. I was also told that at Cardenas, only 10 or 15 miles from the Cave of the Insurrectos, there are underground streams with blind fishes, but this information reached me too late to make a personal inspection.

The most famous of the underground streams and tunneled mountains in all of Cuba is the Sumidero which I visited. This region is half a day's travel by horse from Pinar del Rio. I found no blind fishes here, and it is extremely doubtful whether any occur in the main stream which twice pierces mountains in the course of a mile amid the most impressive cave scenery I have seen.

In the blind-fish area drained by underground streams the surface water reaches the underground streams through sink-holes, fissures, and "caves."

The sink holes are shallow and imperceptible. One at Finca Rosa, I have described above; another is at Aguada on the United Havana Railroad, where, in extreme cases the water rises to stand several feet over the railroad track and then gradually disappears entirely.[2] The difference in the nature of the sink holes of

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[1] Mr. Martinez gave me the following facts: Rain unless protracted makes no impression on the water in the caves—as measured by visual standards. After a rain of 3 days and nights it rises 6 or 8 inches. In 1886, after a long rain of 5 days and nights the water in the well at Isabella rose to within 5 feet of the surface. Ordinarily it is about 50 feet from the surface. In the Modesta Cave in which the water is normally 15 feet from the surface the water rose to the top and over, till it stood 1 foot in the house of Modesta, and between the houses at Isabella and Modesta the water was in places 5 to 6 feet deep. The rain water does not run off in surface streams, but all of it sinks into the ground. At the time of the high water the water disappeared from the surface at Modesta in 2 days, while in the deeper places it did not disappear for 5 or 6 days.

[2] The lowest part of the land at Aguada del Cura is 45.77 m. above the Nueva R. R. station in Havana. The railroad track is 2.82 m. higher.

Cuba and of Indiana seems due to the difference in the thickness of the soil, which, as stated, is extremely thin in this part of Cuba. In the manigua frequent fissures or narrow wells lead down to the groundwater.

There are, finally, the so-called "caves" which also lead down to groundwater.

As stated above, the caves at Alacranes are of essentially the same character as those of the Carboneria. There are several of these. I have visited three, but obtained fishes from only two, the "M" and Donkey.

Into the deeper parts of one of the caves visited, the Pedregales, light does not penetrate; stalactites and stalagmites are clear, tinted rosy, and pure in tone when struck. The usual pool of water did not contain any fish at the time of our visit. An amusing incident occurred at this place. Our guide evidently thought our chief object was to view the marvels of cave formations. When we asked whether there were any caves in the neighborhood with fishes in them, he remarked, "Yes, but the fish don't amount to anything, they haven't any eyes."

The "M" cave consists, first, of the slight depression in the general surface, and second, of the opening at one side of the depression leading down to the water. The slope is here gentle enough for a zigzag path in the shape of the letter "M" and enables cattle to get to the water at a vertical depth of 83 feet. Light penetrates this cave, and indeed the part directly down from the opening is well lighted. The pool of water leads off to the left, so that the remote part of the pool is in perpetual darkness. This condition makes this cave an ideal place to observe the reaction of the blind fishes to light. As in the Cave of the Insurrectos the caves extend down for an undetermined distance below the surface of the water and blind fish could frequently be observed here far below the reach of our 10-foot dip net.

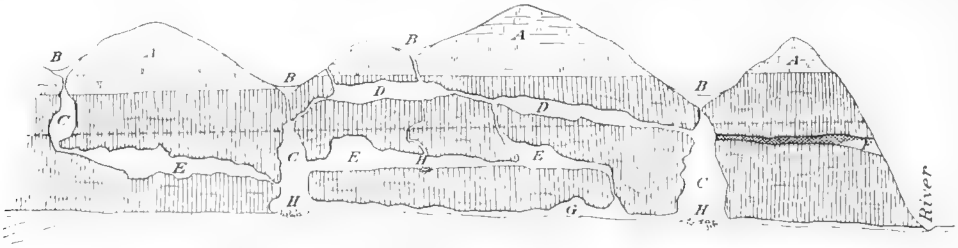

Fig. 68*a*. Diagram of the Kentucky Cave Region, after Shaler. *A*. Sandstone and limestone showing ordinary topography. *B*. Sink holes. *C*. Domes below large sink holes. *D*. Upper line of caverns first formed. *E*. Lower line of caverns. *F*. Cavern filled with stalactite. *G*. Lowest line of caverns filled with water. *H*. Masses of pebbles.

The Donkey Cave is similar to the "M" Cave, but the descent is steeper and there is a large shallow expanse of water on the left of the shaft of light from the opening. The depression at the mouth of the cave is here 9 feet below the general surface and the water is reached at 64 feet below the surface. Water was formerly pumped from this cave for purposes of irrigation.

The caves about Cañas differ from those of the Carboneria and Alacranes. They are cistern-shaped sink-holes rather than caves in the ordinary sense of the word, but on account of the absence of soil there are no funnel-like depressions on the surface to indicate their presence. There is absolutely no general surface indication that one is in a cave country in traveling through it, and it is not until standing at the very brink of one that the presence of a "cave" may be suspected. All of the caves in the Cañas region are modifications of the Modesta type. They are dome-shaped rooms (fig. 69) whose roofs are in different stages of dilapidation and collapse. They have a circular doughnut or crescent-shaped pool of water

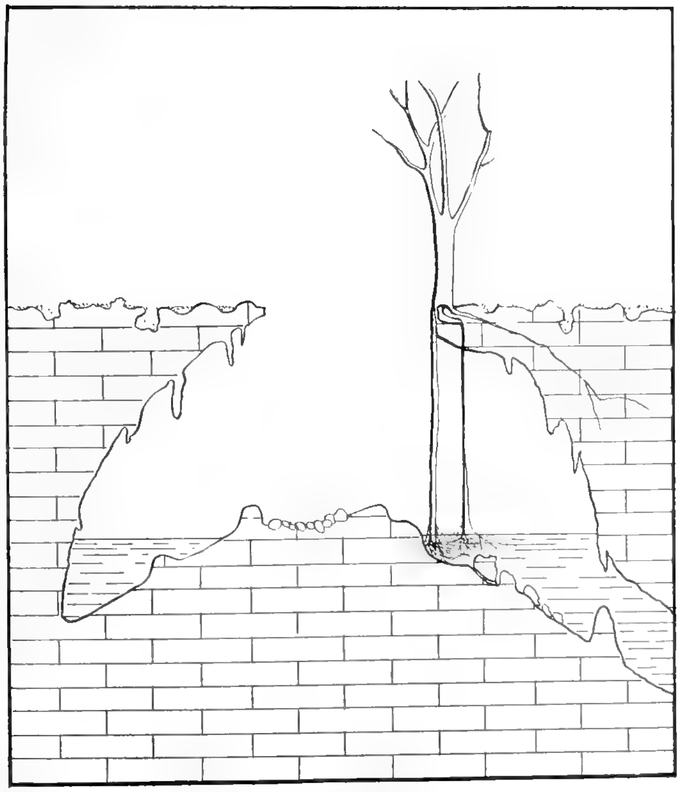

Fig. 69. Diagram of Modesta Cave.

at the bottom. In most cases the roof is very thin; that is, the dome is just beneath the surface, the room being high. More rarely the roof is thick and the cave correspondingly low. In one case the roof is intact and a narrow tunnel slopes down to the cave from the side. In several cases a vertical shaft leads down at the edge of the cave, in other cases a smaller or larger opening or openings occur near the middle of the dome, while not infrequently more than half of the roof has fallen, forming a slope down one side, while at the opposite side the overhanging walls still stand (fig. 70). The latter is the Ashton type found in several of the caves on the Finca Ashton. In all the caves visited there was a pool of water. (There are said to be dry caves, but we had no time to visit them.) In one case the pool forms a simple sheet of water;

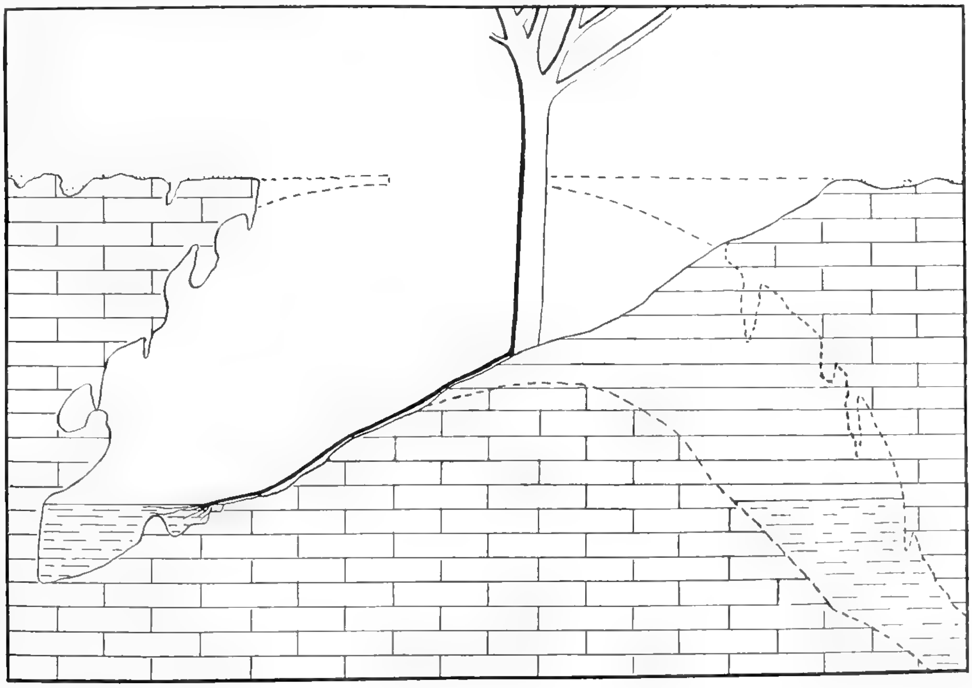

Fig. 70. Diagram of Ashton. Hypothetical Outline of Cave before Fall of Right Part of Roof is indicated by Dotted Lines.

very frequently there is an island in the water beneath the opening in the roof, and in the Ashton type the water has become restricted to a crescent at the base of the wall still standing. It is possible that the Carboneria and Alacranes caves belong to the latter type of caves.

Almost invariably one or more trees (Ficus) stand over the cave and send long roots down through the cave to the water below, where they break up into numberless rootlets (plate 12, fig. A). The roots were very useful in gaining access to the bottom of some of the caves. During my earlier trips, access was gained to most of the dome-shaped caves by climbing down the roots or a bamboo pole. In the later trips the roots were still the most effective ladders to some of the caves, but I substituted a portable rope ladder for the slippery bamboo pole.

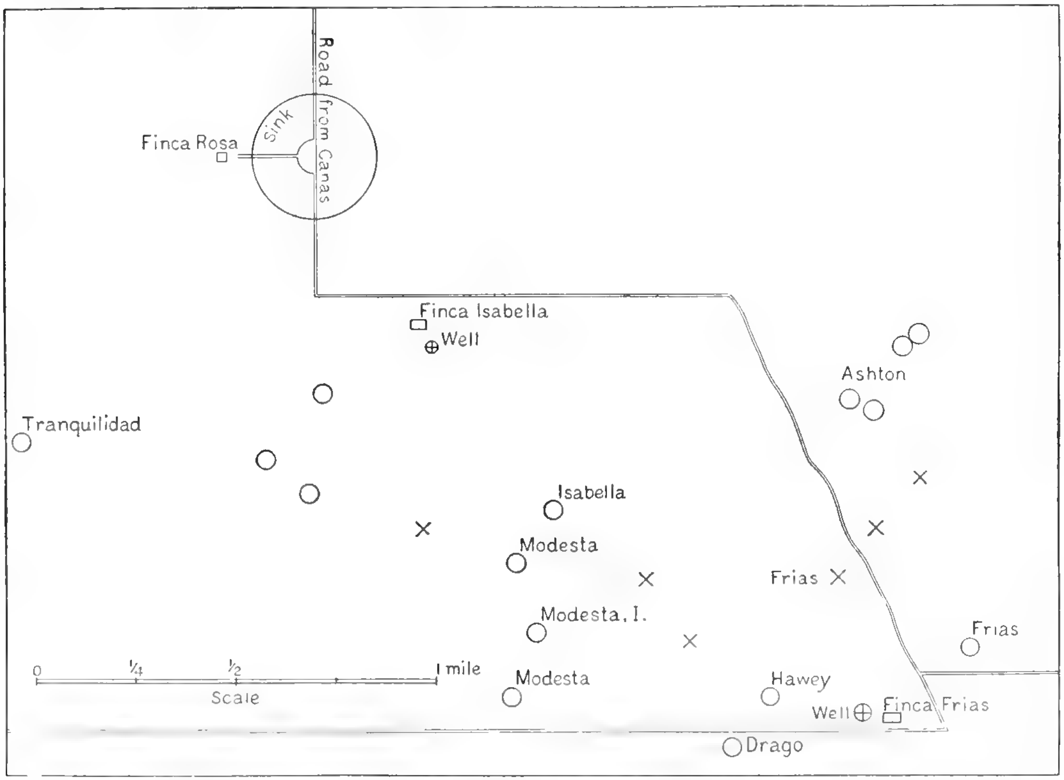

Fig. 71. Partial North and South Section through Cave Region about Cañas, Cuba. Entire area has subterranean drainage. Road from Cañas becomes a stream in heavy rains and sinks within the area inclosed in circle. Caves marked with small circles were located by traverse readings, those marked × were located by guess. There are caves south of area mapped, but land slopes to ocean so that water is found very near surface. There are many others in area covered that are not indicated on this map.

The density of the caves may be gathered from the accompanying sketch of a section extending south from the station Cañas on the Western Railroad but not quite to the southern edge of the cave region (fig. 71). The caves marked with a cipher (o) were located by traverse readings by Dr. Joshua William Beede, of the Geological Department of Indiana University, who volunteered his services on one of the trips. The caves marked with a star (X) were "discovered" on a subsequent visit and located by estimate. Numerous "wells" and other caves are not indicated, but from the number located an idea of the abundance of the caves can be formed. They are about as numerous as sink holes in the cave regions of Indiana and Kentucky. There are caves south of the area mapped, but the land slopes to the ocean 15 miles to the south, so that water is found very near the surface.

An attempt was made to determine the relation of the water in the various caves to a general level of groundwater and to ocean-level. An aneroid barometer was used for this purpose, but although it was of latest pattern and its vernier read to 1 foot, I am afraid that the readings are approximations only, because allowance for barometric changes could not readily be made.

Barometer readings along the line of the Western Railroad compared with the elevations determined by the engineers of the line may give us an approximation to the dependence that may be placed on the respective readings.

| Stations. | Barometer readings. | | | Engineer's elevations. | Stations. | Barometer readings. | | | Engineer's elevations. |
|---|---|---|---|---|---|---|---|---|---|
| | Aug. 22, 1903. | Aug. 26, 1903. | Average. | | | Aug. 22, 1903. | Aug. 26, 1903. | Average. | |
| Cristina | 9 [1] | ... | ... | 9 | Gabriel | 77 | 89 | 83 | 76.65 |
| Pinos | 156 | 164 | 160 | 162.16 | Guira | 47 | 63 | 55 | 52.77 |
| Arroyo Narranjo | 222 | 227 | 224.5 | 210.83 | Alquizar | 47 | 63 | 55 | 57.33 |
| Calabazar | 156 | 160 | 158 | 142.58 | Dagame | 86 | ... | ... | 99.22 |
| Rancho Boyeros | 209 | 215 | 211 | 202.18 | Cañas | 100 | 108 | 104 | 104.53 |
| Santiago de las Vegas | 247 | 266 | 256 | 255.18 | House of Finca Isabella | 61 | 69 | 65 | ..... |
| Rincon | 248 | 262 | 255 | 252.13 | Artemisa | ... | ... | ... | 124.31 |
| Salud | 175 | 187 | 181 | 181.2 | | | | | |

[1] Accepted engineer's determination.

The engineer of the United Havana railroads furnished the following elevations of stations in the cave region. The elevations given are above the Villa Nueva station at Havana, not above sea-level. As the line crosses the Western Railroad at Rincon and the elevation of its rails above sea-level at Rincon is 252.13 feet, I estimate Villa Nueva to be 23 feet above sea-level.

| Locality. | Elevation above Villa Nueva. | Locality. | Elevation above Villa Nueva. |
|---|---|---|---|
| Cienaga station | 16.83 | Guara station | 32.68 |
| Almendares station | 27.06 | Melena station | 29.50 |
| Rio Almendares water-level | 22.07 | Palenque station | 30.64 |
| Rio Almendares water-level to the face of the superior rail | 4.84 | Guines station | 46.21 |
| Vento station | 41.00 | Rio Seco station | 32.04 |
| Mazorra station | 48.44 | San Nicolas station | 20.21 |
| Aguada del Cura station | 49.20 | Las Vegas station | 21.20 |
| Depression of land at Aguada del Cura, height of lowest point | 45.77 | Palos station | 19.23 |
| Height above this point to the face of the superior rail | 2.82 | Bermeja station | 30.45 |
| Rincon station | 69.81 | Union station | 42.25 |
| Bejucal station | 89.55 | Rincon station | 70.05 |
| Buenaventura station | 63.14 | Goven station | 89.37 |
| Duivican station | 44.13 | San Antonio station | 62.92 |
| San Felipe station | 36.31 | Seborucal station | 104.66 |
| Duran station | 39.42 | Saladriyas station | 111.45 |
| | | Seiba del agua station | 120.05 |
| | | Guanajay station | 143.07 |

At Cañas there is a well in the yard of a store about 100 yards from the railroad station. On August 26, 1903, the surface of the water in this well stood very near sea-level, *i. e.*, exactly 100 feet below the surface of the ground.

Mr. A. P. Livesey, general manager of the Western Railroad, kindly furnished me with the depths of 3 wells.[1] Tabulating these and the depths obtained in the

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[1] He wrote: "Regarding the depths of wells along our line, I may say that these vary very considerably, not only in the different localities, but also during the two seasons, viz., wet and dry, but for your information and guidance I give below the average depths of 3 of our company's wells, which are used to obtain water for our locomotives. They are as follows: Salud, 100 feet; Guira, 50 feet; Artemisa, 80 feet."

various caves, together with the elevations of the mouths of the caves, we get the following results:

| Stations. | Elevation of station. | Elevation of surface of groundwater above sea-level. | Stations. | Elevation of station. | Elevation of surface of groundwater above sea-level. |
|---|---|---|---|---|---|
| Salud | 181 | 81 | Isabella Finca caves: | | |
| Guira | 55 | 5 | 5. Open pool at Hawey | .. | 18 |
| Cañas | 104 | 4 | 6. Isabella | 30 | 11 |
| Artemisa | 124 | 44 | 7. Drago | 32 | 18 |
| Isabella Finca caves: | 65 | 16 | 8. Frias | 59 | 19 |
| 1. Modesta | 29 | 14 | 9. Ashton No. 1 | 79 | 52 |
| 2. Miserid | 38 | 8 | 10. Ashton (new) | 50 | 22 |
| 3. Hawey (new) | 26 | 11 | 11. Baños | 79 | 45 |
| 4. Hawey No. 1 | 25 | 18 | 12. San Pedro | 59 | 46 |

The elevations of the water of the caves together with the well at the house Isabella fall into two groups: first, those from numbers 1 to 8 in which the elevation of the water does not vary more than 11 feet. This amount may easily be due to change in barometric pressure during the various readings and to the personal equation. It seems probable that the water in these caves, most of which are south of Finca Isabella, is at a level, and that this level is between 8 and 19 feet above sea-level. The Finca Isabella is about 15 miles north from the coast, or 10 miles from the Cienaga, in which some of the underground rivers rise to the surface.

The second group, from 9 to 12, are east of Isabella; 9 and 11 are near each other; 12 is 2 miles or more east of 9 and 11, and I am not certain about the location of 10. These readings were taken August 25, 1903, in the order: 10, 12, 11, 9. The first reading at the house was at $6^h$ $30^m$ a. m., when the barometer stood at 1,114 feet. The trip consumed all of the morning. About 3 p. m. the barometer stood at 1,179 at the house, so it is very probable that the high elevations may in part be due to the change in barometric pressure.

For comparison we have the data for the caves, Adolfino and Insurrectos, at the Carboneria, near the north coast.

The Cave of the Insurrectos is about 93 feet above sea-level according to barometer. The water is 83 feet below the surface and according to that 10 feet above sea-level.[1]

The top of Adolfino is 87 feet above sea-level, the water is 80 feet below, or 7 feet above sea-level. The surface of the Carboneria well is about 4 feet above sea-level, the surface of the water is at sea-level and more than 5 feet deep.

There is every indication that the water has risen about 10 feet in the caves in very recent geologic times. In all the caves stalagmites are seen to rise out of the water, in some cases from a depth of at least 10 feet. As these could only have been formed on ground free from water it is evident that the water must have risen in the caves. As the water is now near sea-level, this rise is probably due to the subsidence of the western end of the Island of Cuba. This subsidence is general, as stalagmites are found submerged on the northern and southern sides of the island.

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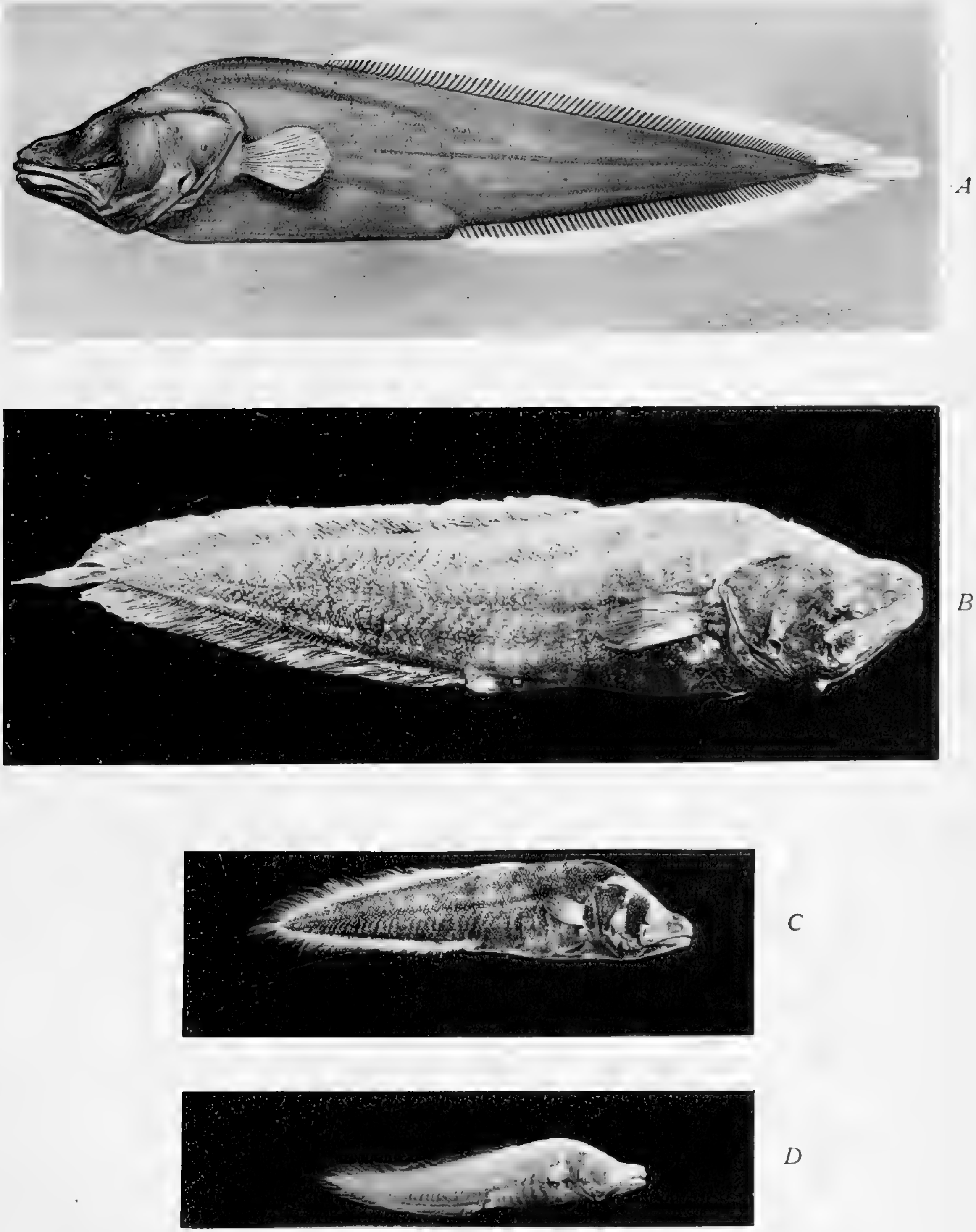
[1] The water in the Donkey and "M" caves, according to barometric readings from the railroad station at Union, is below sea-level. It is not at all probable that this reading is correct, but it indicates that the groundwater level is here again very near sea-level. At "M," according to barometric reading, it is 83 feet below the general level of the surface and at the Donkey it is 73.



A. Drawing of black individual of Stygicola.
B, C, D. Stygicola. Photographs of preserved specimens.

### ABUNDANCE OF STYGICOLA AND LUCIFUGA.

The number of fishes in any cave differs very greatly. They are rare in caves entirely inclosed; in those entirely open and not connected with hidden recesses they are also very rare or absent. They are most abundant in caves with both well-lighted and dark portions and those that are continued subterraneously. The density of the distribution of the fishes evidently varies greatly, directly with the food supply. The food supply itself varies with the openness of the cave to the external world. The question arises whether the caves visited are independent pockets or form part of a continuous underground system of channels, and whether the fauna of the caves visited may be easily exhausted or continuously replenished from the extensive subterranean channels and reservoirs. Collections made in the same caves indicate that there is an undoubted decrease in the numbers and that the decrease is not usually compensated by immigration from the underground reservoirs. It has rarely proved worth while to visit the same cave twice on any of the stays in the cave region. The results of three visits to the "M" and Donkey Caves on October 25, November 2, and December 23 illustrate the point. In 1904, I secured 15 fishes in the Donkey Cave on October 25; 5 on November 2; and 3 on December 23. In the "M" Cave I secured 20 in March, 1902; 19 on October 25; 14 on November 2; and 9 on December 23, 1904. Equal efforts were made on each occasion and an equal amount of time was given to the caves.

On June 24, 1905, Mr. Haseman secured 4 fishes in the Donkey Cave and 7 in the "M." The Donkey thus yielded 15, 5, 3, and 4 fishes respectively, on successive visits; the "M," 20, 17, 14, 7, 7. Both of these caves are with deep recesses in which fishes could be seen but not secured.

### THE ORIGIN OF THE CUBAN BLIND FISHES.

Without doubt the remote ancestors of the Cuban blind fishes lived in the ocean and were adjusted to live in the light and to make use of it in detecting their food, their enemies, and their mates. Equally without doubt, their less remote ancestors became adjusted to do without light and lived in total darkness, either at a depth in the ocean or more probably in the crevices in Cuban coral reefs. If in the former, they entered the subaqueous exits of Cuban rivers; if in the latter, they are older than the rivers themselves, having remained in their original habitat in the crevices of the coral reefs as these were elevated to their present and even greater heights.

The latter seems to me the more plausible theory. The fresh-water blind fishes of Cuba are as old as the parts of Cuba they inhabit. They are part of the result of the formation of the island.

The deeper recesses of the crevices and rents in the naked reef at the Carboneria already described are probably now inhabited by fishes of some sort, possibly by *Ogilvia* among others. Attention has been called to the fact that within less than a quarter of a mile from them, in a coral reef raised only 4 feet above the ocean-level, there is a rift essentially like those found in the naked reef skirting the ocean. This rift contains fresh water, and blind fishes are abundant at a place where a circular opening has been cut to form a well.

It is entirely within the range of probability that the ancestors of these fishes lived in this rift when it was 5 feet lower and contained salt water and that they gradually, as the reef was raised, became adapted to fresh-water conditions. But if this rift with its well contain descendants of its original marine inhabitants, there is no reason why the same should not be true of the wells and caves and rifts of the more elevated coral reefs of Cuba. In other words, there is no reason why the blind fishes should not have developed over the entire area and risen with the entire area over which they are now known to be distributed. *Stygicola* is found from Cañas at least as far east as Jovellanos; *Lucifuga* only west of Guira and at least as far as Cañas. There is, furthermore, no special reason why the blind fishes which have been reported from the natural wells at Merida in Mexico and from Jamaica should not be identical or related to the Cuban species, why they should not have been independently derived in different places from one or more species widely distributed in cracks and crevices of coral reefs.

## PHYSICAL ENVIRONMENT OF STYGICOLA AND LUCIFUGA AND THEIR REACTIONS TO IT.

### LIGHT.

Whatever conditions may have been in the past, at the present light is entirely absent from some of the places inhabited by the blind fishes while others are as well lighted as any stream. In the only cave I entered which light does not penetrate, the pools of water, in every respect similar to those in other caves, contained no blind fishes. On the other hand, in Ashton Cave, parts of which are as well lighted as any stream, blind fishes live side by side with eyed fishes. In a few of the best-lighted caves no blind fishes were found, but in Modesta, where an 11-foot opening in the ceiling lights a space 35 by 45 feet so that pebbles and fishes can be seen with perfect distinctness at a depth of water of 10 feet and more, blind fishes are abundant. The same is true of similar caves, well or partially lighted.

Blind fishes were abundant in Tranquilidad, a dark cave into which light penetrates through a narrow shaft over 20 feet deep and then only illuminates the margin. They were also abundant in the open well at the Carboneria, about 6 feet in diameter and with a total depth of about 10 feet.

It is to be emphasized that blind fishes are abundant in well-lighted caves only when these are connected with underground channels that extend into the dark. Such caves contain many more fishes than caves that are totally dark. The reason for this lies entirely in the much greater abundance of the food supply in caves open to the surface; the lighting of the cave is incidental.

The reaction of the blind fishes to light can be as well studied in the " M " Cave as in any aquarium ideally constructed for the experiment. The pool of water in this cave varies from 5 to about 20 feet across, and from a few inches in depth to many feet — certainly over 10 feet and possibly 50. The pool is probably between 150 and 300 feet long. A direct shaft of light reaches the pool near one end so that the water is well illuminated within this shaft. The right end, near which the shaft of light reaches, is shaded by rocks and is so dark that a lamp is of distinct assistance in exploring its 2 to 3 feet of depth. The other end of the cave is in total and perpetual darkness. Fishes are abundant in this cave. I have seen very few within the shaft of light and most of those were driven there by my movements. In the shade of the rocks to the right, on the contrary, they are abundant, and in the larger dark parts of the cave to the left they are also abundant though relatively less so than on the right. Here we have a very distinct reaction to the light — all the fishes avoiding it. Cattle come down to drink in this cave within the shaft of light. The indirect result of this is a great abundance of blind-fish food. In the movements and distribution of the fishes in this cave we have a clear balance struck between the positive attraction to the food and the negative response to the light.

The same reactions demonstrating perception and tropic relations to light are seen in the Donkey Cave near by. In this cave I have never seen a blind fish within the shaft of light, but have seen and caught them in numbers in the expanse of shallow water in the shadow and total darkness to the left of the shaft of light. While fishing in Ashton in December, 1903, I caught 3 specimens in the lighted part of the cave and about a dozen in the dark recesses to the right of the entrance.

Unfortunately, on account of the difficulty of getting about over the jagged country, I have been able to visit but few caves at night, but the observations in the Carboneria well were exceedingly instructive.

A few bushes growing over the well shade it to a certain degree. As stated elsewhere, poles and fence rails were placed slanting into the water crossing each other and in sufficient number to form a teetering foothold that enabled me to stand waist deep in water. From this position every part of the well was within reach of my net, except pockets in the sides too small for the net and the indefinitely extending side rifts I have mentioned.

On visiting the well about 9 a. m. perhaps as many as 10 stygicolas were seen swimming about or resting on the wood or sides of the well. I entered the well but succeeded in catching only one fish; the others readily escaped either by making for the dark side rifts or by hugging the walls of the well and entering the small pockets where I could not get them. There seemed to be no hesitation in their actions. I again entered this well the same night. Liberally discounting the result for the experience already gained in entering the well and knowledge of the location, the result alone is evidence of a distinct difference in the actions of the fishes at night and in the day — I caught twelve.

Their actions were quite different. While in the daytime they seemed able to locate the dark recesses and make for them with precision, their action at night gave distinct evidence of confusion and lack of ability to readily escape. They could be easily followed with the pencil of light from the lamp and picked up with the net.

## TEMPERATURE.

The fluctuations in the air temperature of caves with small openings are, in a climate like that of Indiana, reduced to a few degrees Fahrenheit, and must be reduced to a minimum in a climate like that of Cuba. The temperature of the water will also fluctuate but little. The air of caves that are open like that of Ashton will, on the contrary, fluctuate to nearly the same extent as that of the epigean neighborhood. The nights of the Cuban winter are cool and the temperature of the water in the open pools of these caves may be reduced a few degrees. No direct observations are at hand on this point.

The temperature of the water in 18 caves containing fishes, taken in June, 1905, showed a total range from 74° to 76.5° Fahrenheit. Only 2 caves had a temperature as low as 74°; 3 of 75°; 5 over 75.5°; 6 of 76°; 2 of 76.5°.

Observations between August 22 and 25 showed slightly higher temperature for open caves, thus: in June, 1905, the temperature at Baños was 75.8°, at Ashton, 75.6°, and on August 25, 1904, it was 77° at Baños and the same at Ashton. In the "M" Cave, a closed one, the temperature was the same, 75° Fahrenheit.

The blind fishes are adjusted to withstand slight fluctuations in temperature. Some were kept in aquaria and the water became distinctly chilled over night and warmed during the day. While they lived for several days in these aquaria, they were always sluggish or numb in the morning. A more distinct reaction of the same sort was noticed in the only fish I succeeded in bringing home alive. It could scarcely move after an early September night in Indiana. A still greater reaction was noticed in several I succeeded in bringing alive to Louisville and which succumbed to the frosty weather on the way from the depot to the hotel.

### TRANSPARENCY OF WATER.

In all caves in which collections were made the water is clear as crystal. It will easily rank with the water of Lake Tahoe and of the limestone springs of Florida, as among the most transparent natural water in the world. Fishes can readily be seen at depths of 15 and 20 feet or more, with the aid of a bicycle acetylene lamp. The water at the Vento Spring is of the same nature, but I was informed that it becomes slightly roiled after heavy rains.

### CHEMICAL COMPOSITION OF WATER.

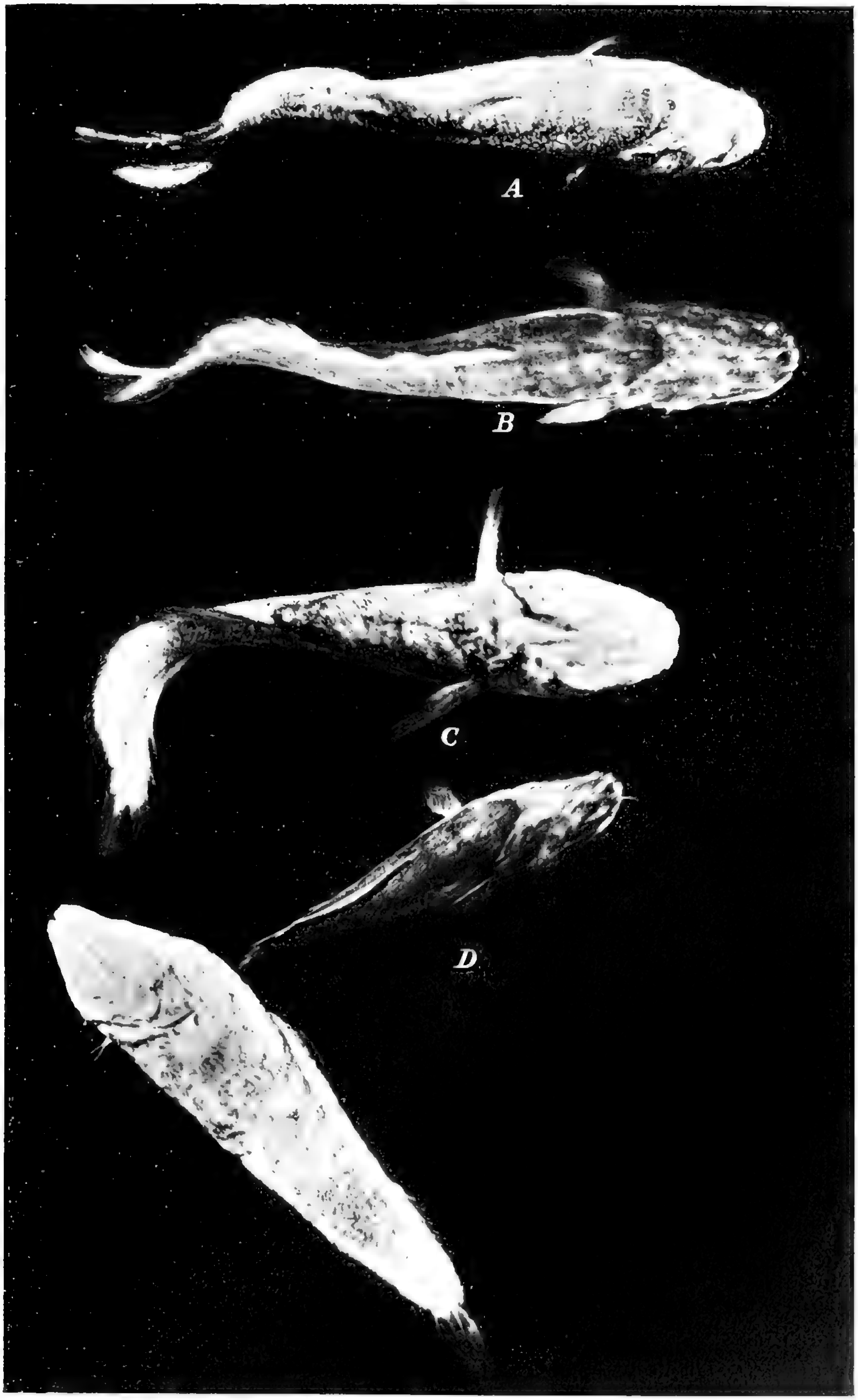
The water is everywhere highly charged with salts of lime and magnesium In all cases where the surface of the water is not disturbed by breezes, a crust of these salts forms like a thin ice over the surface of the water. When one disturbs the water, the crust breaks up into small fragments which fall through the water like snow through the air. Occasionally a larger flake, a foot square, may fall to the bottom; sooner or later they are dissolved again. With falling of the level of the water some of the crust is left on shore and gives an index of the amount of rise and fall in the water during a year.

### FLUCTUATION IN AMOUNT OF WATER.

The ordinary fluctuation in the amount of water in the caves is very small — about one foot during a year — judging by the flakes of lime left on the banks. I have mentioned elsewhere that, after long-continued rains, water flooded the entire region about Modesta, the cave was full to the top, and the water stood several feet over the ground. All of this retreated in a few days. Such fluctuations are very rare.

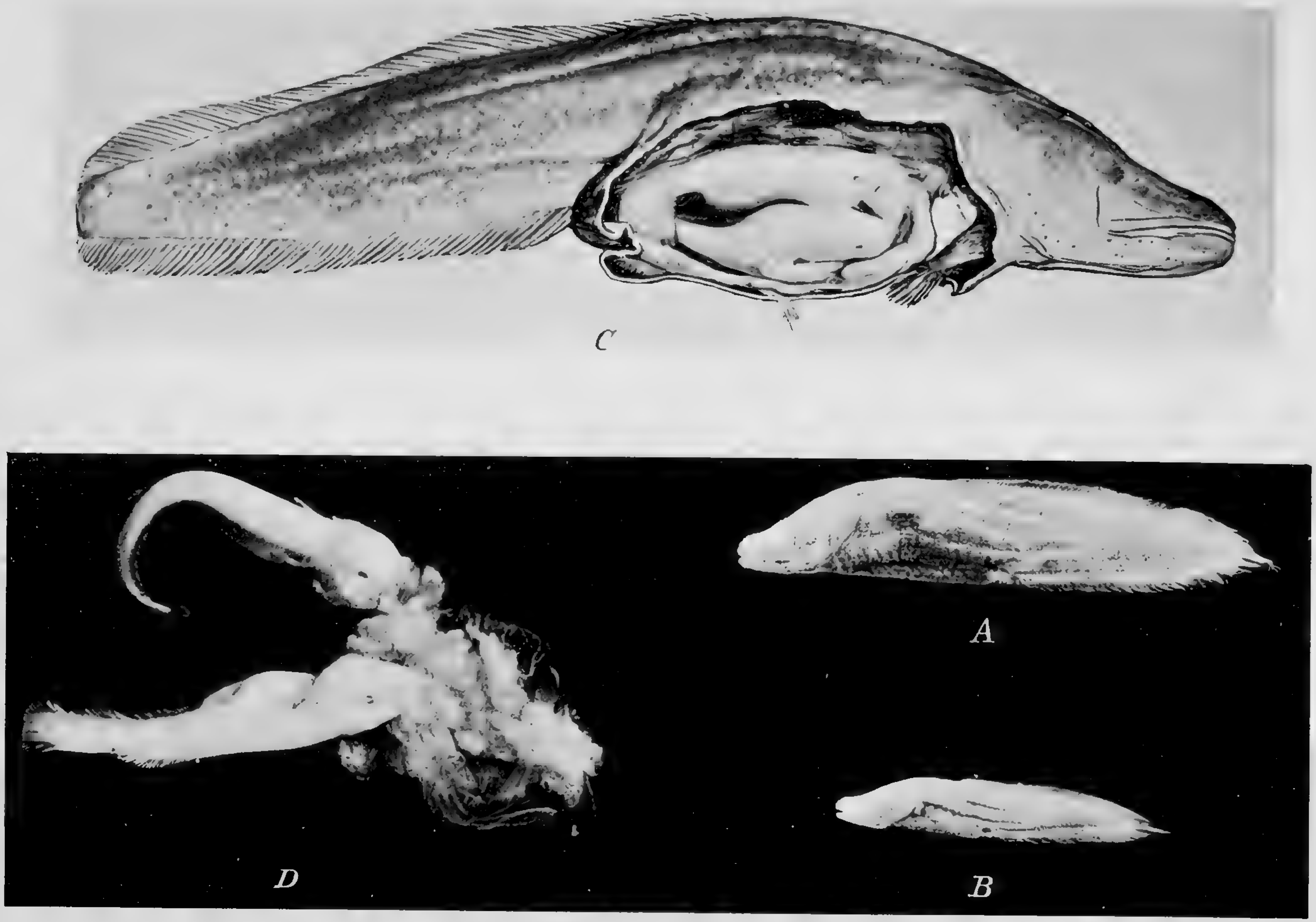
### SIZE OF ENVIRONMENT.

Concerning the size of the environment little can be said. The pools accessible are easily measured, none of them exceeding a few square meters in surface, but the size of the underground connections is naturally unknown. The rapid disappearance of the water after heavy rains indicates extensive underground channels.



Living Stygicolas.

Position of body and fins in swimming and differences in color of different individuals.



Views of Lucifuga.

A and B. From photographs of preserved specimens.
C. (No. 76) containing 4 young, (76a, 76b, 76c, 76d,) nearly fully grown. For the eyes of the adult see plate 27. For the eyes of the young see plates 16, 17, and 18, 24 F and E.
D. Two young, each fastened by mouth to lobe of ovary. Nearly fully grown to be born.

## BIOLOGICAL ENVIRONMENT OF STYGICOLA AND LUCIFUGA.

### ASSOCIATES.

During March small frogs are found abundantly at the margins of the pools in some of the caves. I do not know that these affect the lives of the blind fishes in any way. Tadpoles were found in the Carboneria well. It is possible that these may form some part of the food of the fishes during some seasons of the year. They are but casual associates of the blind fishes in some of the caves.

Fishes other than the blind ones were found in Ashton and some of the small open caves about Modesta. They were all *Girardinus metallicus* Poey, a species very abundant all the way to Pinar del Rio. The female reaches a maximum length of 79 mm., but is usually much smaller; the maximum length of the male is 45 mm. The largest specimens taken in Ashton are 41 mm. and 38 mm. These fishes are active swimmers, living near the banks, and while a few may be captured by the blind fishes, they are themselves too small to attack even the young of the blind fish.

### FOOD OF STYGICOLA AND LUCIFUGA.

The blind fishes are carnivorous, securing living prey. Their food consists largely of 4 species of crustaceans, 3 of which are blind cave forms. Probably every living animal of the proper size is used by the blind fishes for food.

### Cirolana cubensis Hay.

This species was described by Hay in Proceedings of the National Museum, VI, page 430, as follows:

> Body oval, a little more than twice as long as broad, widest a little behind the middle, rather strongly convex, and perfectly smooth. Head a little broader than long, slightly produced in front. Mesosome broader, with its greatest width at the fifth segment; coxal plates of the second, third, fourth, fifth, and sixth segments successively more enlarged and more strongly produced backward at an acute angle. The plate of the seventh segment is about the same size as the one preceding it. Metasome narrower than mesosome, of five segments, each of which, except the last, has the lateral angles strongly produced posteriorly; telson as long as the metasome, its margins gently curved and convergent for about two-thirds of its length, and then rather abruptly strongly convergent to form a short, obtuse tip. The eyes are altogether wanting. First antenna with three basal segments and a short flagellum which, when extended backward, reaches slightly beyond the posterior margin of the first thoracic segment. Second antenna with five basal segments, and a long, slender flagellum which may extend slightly beyond the middle of the body and is composed of about twenty-nine segments. The mandible, maxillæ, and maxillipeds do not present specific characters of importance, being of the type usual in the genus. The appendages of the mesosome are of moderate strength, and are armed with a few rather stout spines and stiff setæ. The branchial appendages of the metasome are membranaceous and small; the uropoda are well developed, the outer branch lanceolate in outline, the inner much broader and very slightly longer, and with the tip somewhat accuminate; both branches and the margins of the telson as well bear a rather dense fringe of hairs. Color in alcohol, white, with no markings of any kind. Length, 5 mm.
>
> Of the species of *Cirolana* known to inhabit American waters, *C. mayana*, which occurs on the coast of Yucatan and Colombia, is the nearest relative of the present species. Between the two, however, there are several important structural differences. The physiological differences between this species and all the others of the genus must be very great to admit of its living in the subterranean streams of fresh water. It may be added that *Cirolana cubensis* is very distinct from *Cirolanides texensis* Benedict, which occurs in the waters which flow from the large artesian well at San Marcos, Texas.

This species is everywhere abundant and may attack the fishes if it succeed in attaching itself to them. I have not caught any fish with them attached, but in small aquaria in which many of them were placed as food for the fishes they soon turned the tables and fastened themselves upon the fishes. In some of the caves cirolanas exist in vast numbers. At the base of the shaft of Tranquilidad they were so numerous and voracious that it was impossible to stand in water long enough to light our lamp. They fastened themselves in numbers on the feet and went to work with such a will that it was impossible to stand still.

### **Palæmonetes eigenmanni Hay.**

This extremely slender and graceful shrimp is abundant in all the caves. It is essentially pelagic in habit, though it is frequently seen resting on various objects on the bottom. Its eyes have been described by Pike. The species was described as follows in the Proceedings of the U. S. National Museum:

> Carapace thin, very delicate and transparent, in form slightly compressed near the middle of the body but rather broad anteriorly; the anterior border, below the eye, is produced as a broad, obtuse angle, which bears, near its lower margin, an acute, forwardly directed spine; this spine is the anterior end of an obscurely marked ridge, which extends obliquely downward and backward along the sides of the carapace. The rostrum is long, slender, compressed, and rather markedly upcurved; on its superior margin it bears a row of 6 or 8 slender, acute teeth, which begins well back on the carapace and extends forward to the rostrum; these teeth are directed obliquely forward; the inferior margin is unarmed; the tip of the rostrum is acute and reaches forward to a point opposite the distal extremities of the antennal scales. The eyes are much reduced in size, are without pigment, and the corneal surface comes to an obtuse point in front. The first antenna has the basal segment well excavated above and provided with a small, acute spine at the outer distal angle; there are two long and one short flagella, the short one slightly exceeding the rostrum, the long ones somewhat longer than the body. The second antenna has the basal segment provided with a small spine near the distal end; the antennal scale is broad and with subparallel margins; the tip is slightly rounded, and there is a small, obtuse spine at the outer distal angle; the flagellum is slender and about twice as long as the body. The mandible has an incisor portion with three or four sharp teeth, a small molar surface with several obtuse teeth, but is without a palpus. The third maxilliped is not strongly developed and presents no characters of importance. The first pair of pereiopods is chelate, and except for its much smaller size is exactly like the second; the chela is slender and weak; the carpal segment is long and slender; the meros is of about the same length, but stouter; the remaining segments short and rather thick. The remaining pereiopods are very long and slender. The abdomen is of the form usual in this genus, but the sixth segment is neither elongate nor compressed; the telson narrows gradually from the base to the obtusely angulate tip; on the upper surface there is on each side at about the middle and again about one-fourth the distance from the tip a small, appressed spine; at the tip there is on each side one minute and one long, slender spine, and in the middle a fringe of setæ. Color in alcohol, white. Length, 23 mm.
>
> They differ very markedly from *Palæmonetes antrorum* Benedict, hitherto our only known blind *Palæmonetes*, in the shape of the rostrum and the character of the chelæ. The shape of the eye is rather remarkable, even in a group, where through atrophy the eye tends toward the conical form. I know of no other in which it is produced into a blunt point. So far as I have been able to ascertain, this is the first record for this genus in Cuba. In the material from San Isidro there is one specimen which agrees in every way with the types, but the other two differ in such a manner as to lead me to believe that a second species may be found to inhabit the subterranean waters of Cuba. The two specimens just mentioned have the sixth segment of the abdomen 2.5 times as long as deep, and the antennal scale is more slender and acute. Unfortunately, the rostrum of one is entirely gone, while of the other only the abdomen remains.

### Epilobocera cubensis Stimpson.

This crab, which reaches a width of several inches, was observed in many of the caves. It is probably found in all of them though not in great abundance. If the adult affects the blind fishes at all, it is to feed on them. I have found the young of this species in the stomach of *Stygicola*.

### Gammarus cæcus Weckel.

The following technical description will be found in Proc. U. S. Nat. Mus., XXXII, page 47.

> Eyes absent. First antennæ more than half as long as the body; second segment of the peduncle slightly longer than the first and about three times as long as the third; flagellum composed of twenty to thirty elongated segments, each bearing a few short hairs at the distal end; secondary flagellum reaching slightly beyond the third segment of the primary flagellum, composed of four segments, the distal one short and furnished with long hairs. Second antennæ are about two-thirds as long as the first pair with the peduncle extending far beyond that of the first pair; ultimate segment of the peduncle only slightly longer than penultimate which is greatly elongated and about equal in length to the antepenultimate; flagellum composed of about twelve segments, which are shorter than those of the first antennæ and furnished with more hairs.
>
> The carpus of the first gnathopods of the male is triangular and elongated, with the anterior margin furnished with a few long hairs and numerous short ones; propodus narrower than the carpus, twice as long as broad, with the anterior margin concave, armed sometimes with a fascicle of hairs, the posterior margin convex, and the palm almost straight, slightly convex, and armed with four or five spines and a few short hairs; dactyl as long as the palm and fitting it closely. Second gnathopods with a carpus broader than in the first pair but similarly armed; propodus not so broad as the carpus, about twice as long as broad and larger than in the first gnathopods; posterior margin almost straight; anterior margin slightly convex and usually furnished with one or two fascicles of hairs; palm very oblique, slightly concave at the center, armed with five or six spines at the tip of the closed dactyl, and one or two spines and a few short hairs on the margin; dactyl strongly curved, as long as the palm.
>
> Both margins of the coxal plates of the third, fourth, and fifth peræopods are serrate and furnished with spines, those on the anterior margin being smaller than those on the posterior. Posterolateral angles of the third and fourth abdominal segments are produced backward and end in a blunt tooth. The last two or three abdominal segments are furnished dorsally with a few short spines. The first uropods project slightly beyond the second pair. In both pairs the rami are about equal in length and slightly longer than the peduncle. The third uropods were broken off in the few specimens which I had for examination. Telson cleft to the base, armed distally with a few short spines.

I found this blind amphipod in Modesta in the roots of trees. It was not abundant and was not observed in any of the other caves in which no special search was made for it. It was hidden among the rootlets of Ficus in a way in which it would not be noticed unless special care was taken to look for it. It is quite probable that it may be found in many of the caves.

In addition to the above mentioned species dragon-fly larvæ were found in the stomachs of some of the fishes.

### PLANTS.

In parts of Ashton a green alga forms a dense mass over many square feet of bottom. Young lucifugas are abundant in the alga, but this is the only instance of its occurrence in association with blind fishes and it scarcely deserves consideration as part of their normal environment.

The only plant worth considering as forming part of the biological environment of the blind fishes is the tree sending roots to the water. The roots break up into innumerable rootlets harboring numberless cirolanas and many young and small lucifugas. These trees are found in all the caves of the Cañas region. The roots sometimes extend vertically as much as 40 feet before striking water. At other times roots run along the ground down the slope of the cave as in Ashton, finally breaking up into rootlets (fig. 70 and plate 12).

### GENERAL HABITS OF LUCIFUGA AND STYGICOLA.

The position in the water and action of body and fins in swimming of *Stygicola* are amply indicated in plate 14, which is from instantaneous exposures on fishes confined in a 5-gallon aquarium. It is seen that the posterior part of the body moves from side to side, eel-fashion. The long dorsal and anal fins move in the same way, waves of motion passing from in front back. These fins, on account of this motion, are not well shown in the photographs. The pectorals move independently of each other. One may be forward, the other back. They are used in guiding largely. When the fish is swimming very slowly, the wave-movements passing along the dorsal and anal fins are the chief means of locomotion. In swimming rapidly the motion of the body comes chiefly into play. The fishes swim indifferently up or down, with the back up or lying on their sides. The actions of *Lucifuga* are essentially like those of *Stygicola.*

These fishes are much more readily disturbed than *Amblyopsis* of the Indiana caves, and when disturbed they swim swiftly in a less distracted way. On the whole they are much harder to catch than the *Amblyopsis.*

The action of the stygicolas in the Carboneria well in daytime and at night has been detailed. Two instances that seem to indicate that fishes "remember" localities must be put on record for what they are worth. One of these is of a fish at the right end of the "M" Cave, and the other in the left, dark part of the Donkey. In the "M" Cave the same fish, three times within an hour and a half, apparently made straight for an opening under the wall of the cave and escaped. In the Donkey Cave the same thing happened about a big stalagmite that rises out of the water. Several times within half an hour the fish came out, but each time it darted back among the nooks in the stalagmites with apparently as much decision as a mouse in seeking its hole. Perhaps in both cases the action was a reaction merely to the vibrations set up by my net. Perhaps the location of the solid stalagmite and the wall were perceived by the approaching fish and the escapes into nooks below the wall were simply necessary sequences in following along the solid wall until an opening was reached. Whatever it was, the repeated escape of the two fishes was as interesting as it was aggravating. Very frequently when disturbed they descend in the water and escape into depths beyond the reach of the net.

The character of food has been detailed under the head of Biological Environment. I am unable to give any direct observations on the securing of this food.

### BREEDING HABITS OF STYGICOLA AND LUCIFUGA.

In March of 1902, on my first trip, Mr. Riddle secured a female lucifuga containing 4 young, lacking but 3 or 4 mm. of being as long as the smallest lucifugas caught in the caves (plate 15, fig. c). This was the first intimation we had that these fishes are viviparous. No other embryos were obtained at that time. An examination of the ovaries of all the females caught and the size of the young led me to suppose that March was the close of the breeding season. With the grant from the Carnegie Institution I expected to remain in Cuba during the entire breeding season to secure a full series of embryos and to rear young in the light. Unfortunately for this plan the fish seem to have no general breeding season, and the appropriation was exhausted in determining that fact. I visited Cuba late in October, which was supposed to be the beginning of the breeding season if March was the end, but there was no indication that this time was near the breeding season. I had collections made early in December and again visited Cuba late in that month. But while, as before, there were indications that some individuals were ready to breed, there was no indication of the approach of a general breeding season. I next had collections made the first week in May without results. I revisited the caves late in August and early in September and finally, near the end of June, sent two of my students, Mr. John Haseman and Norman McIndoo, to the caves. The former had accompanied me on one of the trips, and both were in every way thoroughly competent to get everything possible.

To summarize: The caves were examined by myself and Mr. Riddle early and late in March, 1902; by Mr. Martinez early in May, 1903; by Mr. Haseman and Mr. McIndoo late in June, 1905; by myself and Mr. Hankinson late in August, 1904; by myself and Dr. Beede late in October, 1904; by Mr. Martinez in December 1, 1903; and by myself and Mr. Haseman late in December, 1903.

The net results of these numerous trips for *Lucifuga* are: Late in March I secured one female with young about 20 mm. long, or nearly ready to be born; the ovaries in most of the other females were minute, the largest eggs measuring 356 μ; in two ovaries there were eggs 560 μ and 850 μ in diameter, both of these containing spermatozoa. Late in June a female with 15 young, 12 mm. long, was obtained; the ovaries of the remaining fishes were small. On August 23 a female with 10 nearly grown young was obtained. The ovaries of all the others were minute. Late in October and December the ovaries of all females secured were minute.

The young from the female in March were at least 3 months old. This would give a breeding period whose outside limits would extend from December to the end of August. The examination of numerous ovaries does not indicate a general breeding season, though a larger per cent contained large eggs in March than in other seasons. The best season to get material is probably March to May.

The net results for *Stygicola* are:

In March the ovaries of *Stygicola* are mostly small, with eggs not exceeding 200 μ. One female taken at this time contained eggs 600 to 700 μ in diameter and her ovary was abundantly supplied with spermatozoa. In May no mating females were secured. In June the ovaries were mostly minute. Two of those secured contained turgid ovaries in which the structures were distinctly lobulated.

On September 1, I obtained a female with one young from the Carboneria. Other females had large ovaries, probably recently freed from young. Most females had small ovaries. One contained large eggs. The rest contained small eggs.

On October 30, I obtained a *Stygicola* from Alacranes containing two young. The mother was 92 mm. long and *her ovary contained eggs* 880 μ *long, which were evidently mature.*[1] At the same time I obtained 47 other females from 77 to

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[1] In an ovary containing spermatozoa in abundance, days if not months before the ripening of the eggs, an occasional early ripening should naturally result in the development of the embryo. The present case is probably one of this sort. Two eggs evidently started to develop long before the others were mature. The ripening of the eggs at different times may lead to different sized larvæ in the same ovary unless the earlier larvæ digest the spermatozoa present before the other eggs become ripe.

115 mm. from Alacranes and Cañas, in all of which the ovary was empty and in most cases at its minimum.

In December all the ovaries but two were minute. In one ovary a single large egg 720 μ was found, in the other the ovary was large and the eggs reached a maximum of 640 μ. Thus, nearly mature eggs were found in December and March, and young in September and October.

If the species breed annually and irregularly throughout the year and the young are carried but 3 months, at least one-fourth of all the females caught at any season of the year should be with young. If the young are carried but 2 months, one-sixth of all the females should be with young. If the species breed at some definite season of the year and this period is not more than 3 months long, all of the females should be with young near the middle of the breeding season.

The results are wide of any of these marks; and the only conclusion possible is that either there is no definite breeding season, but individuals breed at any time during the year, or the fishes breed only at longer intervals than a year, and in either case while breeding they migrate to undetermined regions. That these regions are not far away is shown by the fact that occasionally breeding females reach the upper accessible parts of the cave. Between breeding times they are found in the upper, readily accessible parts of the cave.

I found that while *Amblyopsis* probably breeds throughout the year a larger per cent breed in March than in other seasons. A similar condition may exist in the Cuban blind fishes.

### THE OVARIES OF STYGICOLA AND LUCIFUGA.

The minute structure of the ovary of *Lucifuga* is elsewhere described. The ovary consists of a pair of delicate walled sacks united behind and with the oviferous tissues attached along the middle of its dorsal and ventral wall except for a short distance behind. It is placed in the mesentery between the dorsal wall of the body cavity and the rectum and stomach. In enlarged ovaries the oviferous tissue is seen to be lobulated, the lobules being attached anteriorly and free posteriorly. These lobules are arranged like shingles, the anterior ones overlapping the posterior ones. When the ovaries contain no larvæ or ripe eggs, they extend far forward, the posterior oviferous tissues reaching but little behind the stomach. When eggs mature, the ovary becomes turgid and the oviduct apparently shortens, so that the posterior part of the stomach comes to lie in the fork near the anterior end of the ovary.

The spermatozoa are evidently, as in *Cymatogaster*, which is another viviparous fish, transferred to the female long before the eggs are mature. When mature the eggs are probably 850 μ in diameter, or even larger. Spermatozoa were found in an ovary containing eggs but 560 μ in diameter.

The number of young found in *Lucifuga* were 4, 15, and 10 respectively. The young were nearly all turned with their heads toward the front of the ovary, a condition duplicated in the ovary of *Cymatogaster* with *nearly* mature young. The condition of the young in the ovary with 4 young is well shown by the photograph (plate 15, fig. c). There were 2 young on each side. The largest eggs in this ovary were 200 μ in diameter.

The condition in a female 90 mm. long containing 15 young, about 12 mm. long, was as follows: there were 11 on the left side, one of which had an ovarian lobe in its mouth, and several had the gill covers hooked over ovarial lobes, the rest being free in the cavity (plate 15, fig. D). There were 4 on the right side, one of which had the head turned to the rear, and one was so firmly attached to the ovarian lobe by the gills that it was practically impossible to get it loose without damage.

One ovary of *Stygicola* containing 11 large eggs, at least one of which is free in the ovary, is distended much more than the few eggs would warrant, being 16 mm. long and 12 mm. wide. The outer tunic is quite thin. The eggs are nearly of the same size and measure 848 $\mu$ in diameter. The general features of the ovary of this species are given in plate 27, fig. A. The details of the structure are given in another chapter by Lane.

## THE EYES OF LUCIFUGA.

The snout of *Lucifuga* is broad and depressed to the posterior edge of the maxillaries — duck-bill shaped. The eye is distinguished without difficulty in the translucent living individuals, and even in specimens preserved in formalin or alcohol it is readily distinguished up to very old individuals.

In the older specimens the skin over the eye readily discloses the location of the organ. There is over the eye in these specimens a hemiovate elevation separated from the rest of the skin of the head by a distinct groove. The skin in this ovate arch is not any less abundantly supplied with pigment than any other part of the head, and there are no other distinguishing features to indicate that it is better adapted to admit light than any other part of the skin of the head. In some cases it is even more densely pigmented than neighboring regions. The region is proportionately larger in young individuals than in old, but is more conspicuously demarked in the older than in the young.

Removing the skin shows that beneath the ovate arch lies a mass of orbital fat, approximately in the center of which the eye lies embedded. The orbital fat-mass seen from above has an oval shape, considerably longer in the axis of the head than transversely. Behind, the mass touches the orbital process of the frontal bone. The eye is placed approximately over the middle of the maxillary.

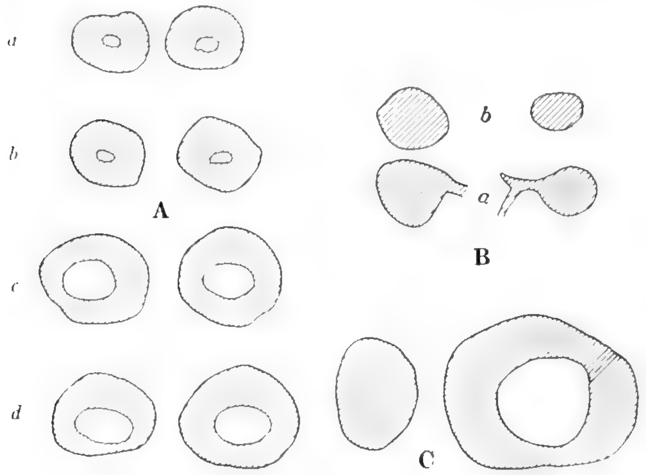
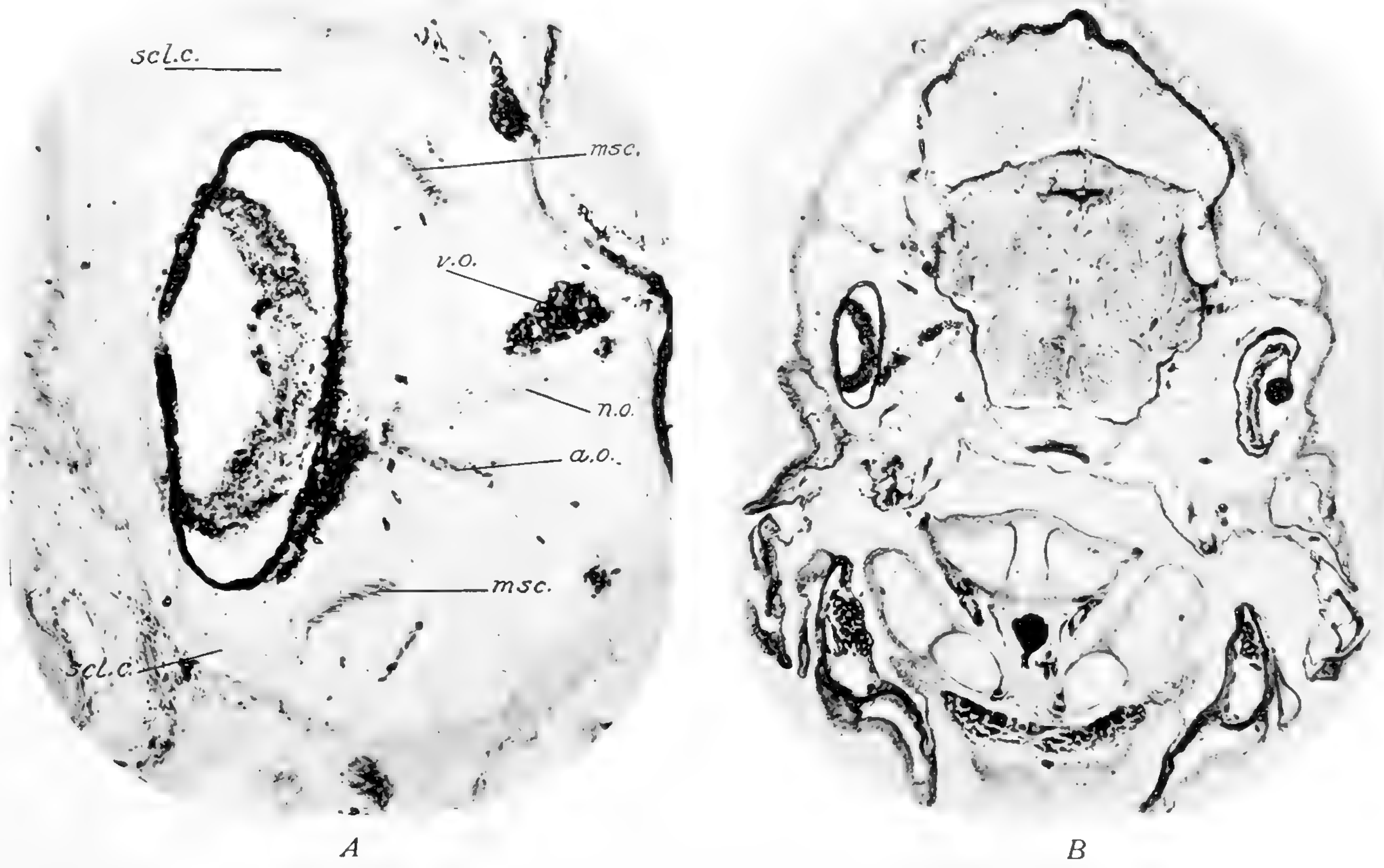


Fig. 72.

(A) Outline Camera Drawings of Eye of 4 Young of Female shown in plate 15 *C*, from Sides, Left Eye on Left, Right Eye on Right, so that Middle of Pairs is Anterior. *a*, Fish 18 mm. long; *b*, 18.5 mm.; *c*, 19 mm.; *d*, 20 mm. For details of these Eyes see figs. plates 16 to 18, 16 mm. and 4.
(B) Eyes of Mother of 4 Young, shown in A, drawn to Same Scale: *a*, from above; *b*, from sides. For sections, see plate 21.
(C) Outlines of Eyes of No. 95, a Fish 53 mm. For sections see plate 20 C and plate 24 A, 16 mm. and 6.

The proportion of the orbital space or socket occupied by the eye differs greatly in individuals of different sizes. In younger individuals, just about to be born, the eye fills a large part of the socket (plate 16, fig. B), while in the old it forms an insignificant dot in a mass of fat and connective tissue, hundreds of times larger than the eye (plate 21). The relation of the eye to the surface is similarly conditioned with age. In the young it lies near the surface, while with age it becomes farther and farther removed, retaining however its relative position in the orbital fat-mass until old age, when possibly it may move nearer to the skull.

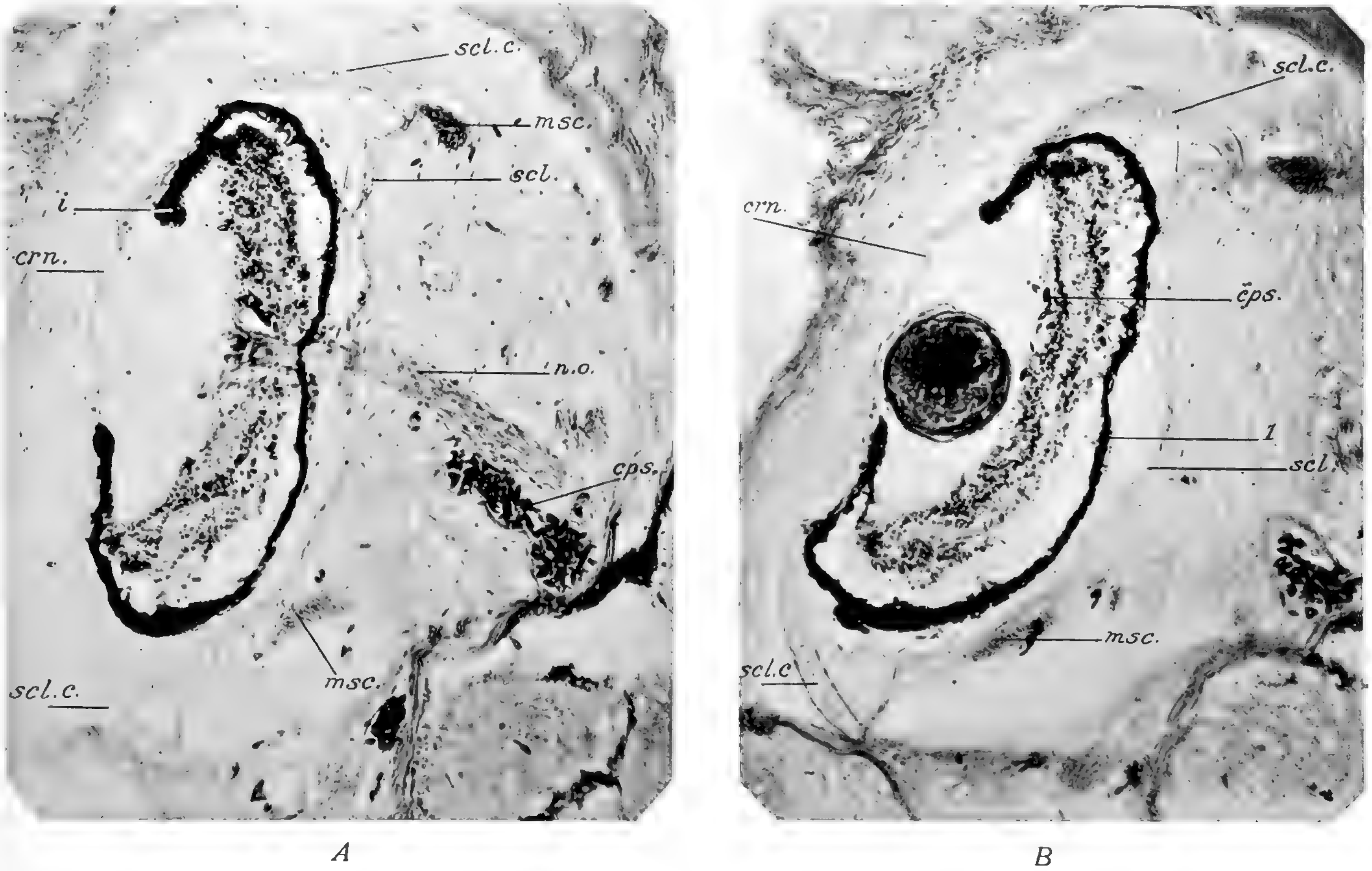
Seen from the surface, that is without sectioning, the eye presents great fluctuations in size. These are in part conditioned by the size of the individual, but in part are independent of size. Other things being equal, the eye decreases in size progressively from birth to its disappearance in extreme old age. This process is accompanied by, if it is not responsible for, the appearance of pigment masses. These are either intimately associated with the eye, as in the development of great



Sections of Lucifuga.

A. Section through head of one of the young, shown in plate 15 C, specimen (76*a*), showing relative size of eye to eye-socket and head. × about 60.

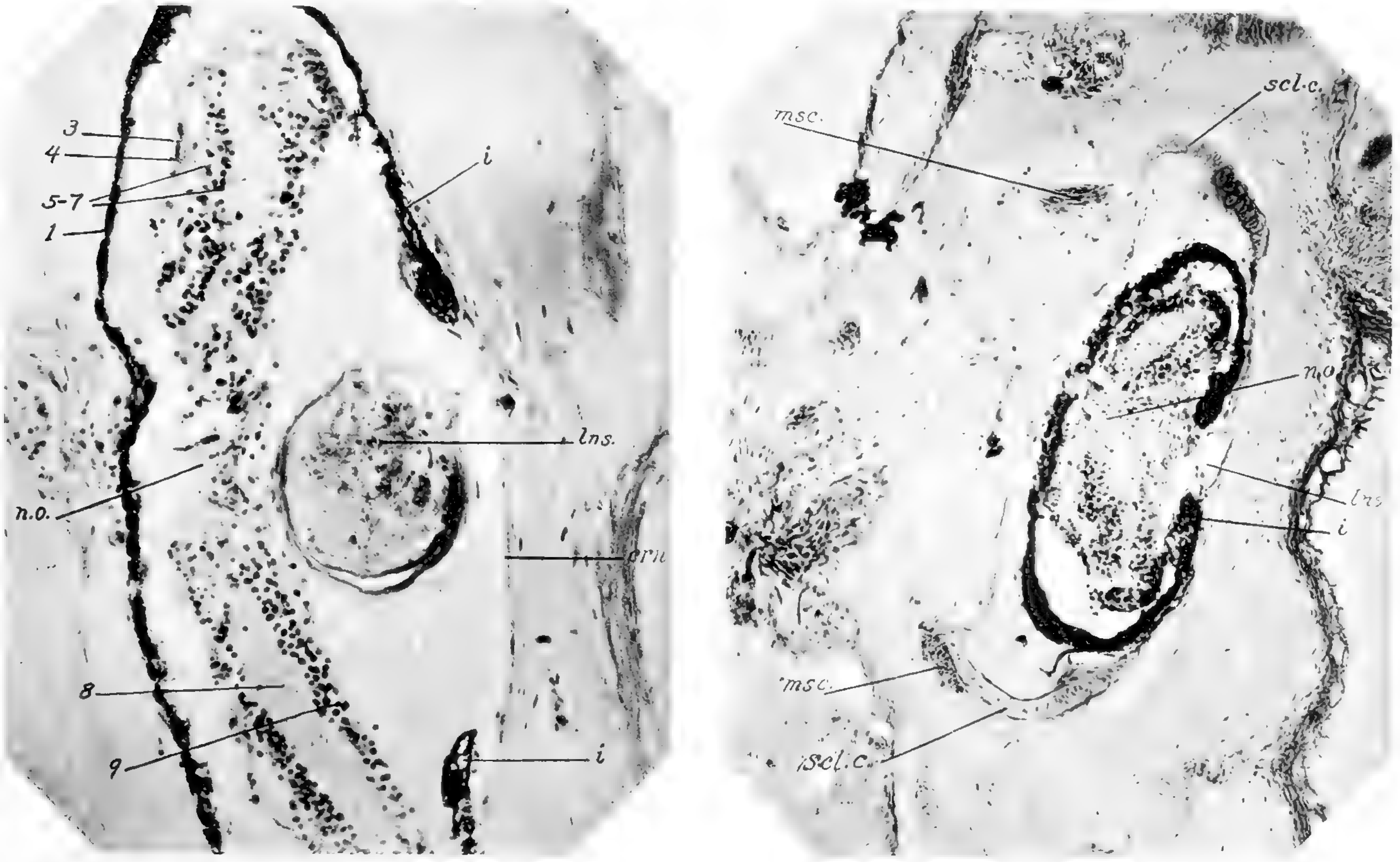
B. Section through left eye of specimen (76*a*), showing blood-vessels, optic nerve, scleral cartilages, eye-muscles, etc. × 200.



Two sections through right eye of Lucifuga, No. 76*a*.

A. Through entrance of optic nerve shows scleral cartilages (*scl. c.*), eye-muscles (*msc.*), blood-vessels (*cps.*), etc.

B. Passes through middle of lens. × 200.



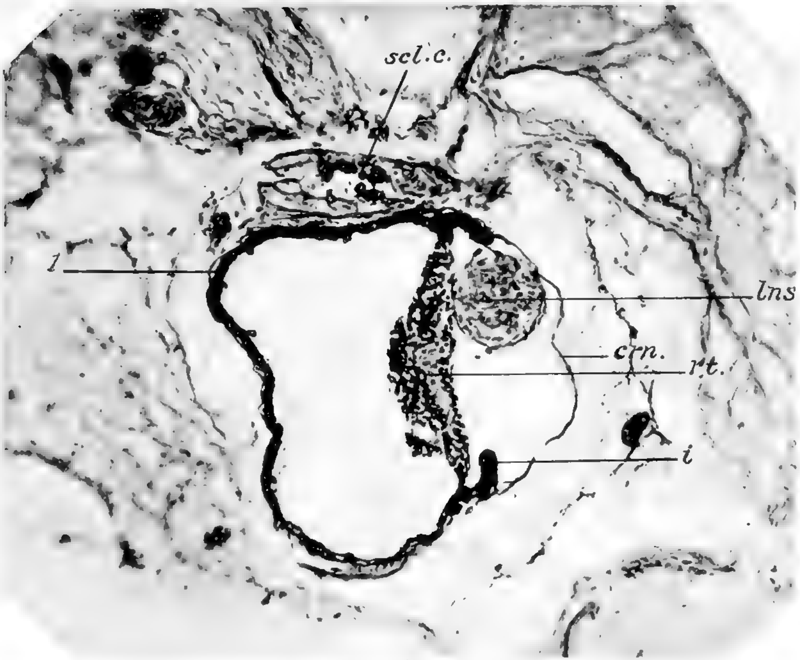
A

B

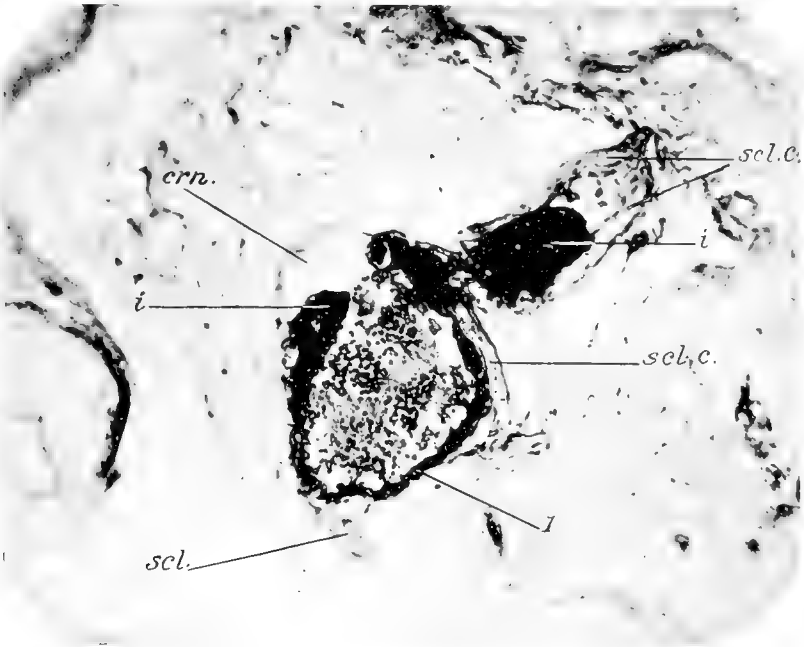
Sections of Eyes of Lucifuga.

A. Horizontal section through left eye of No. 76*d*, showing contents of lens capsule and layers of retina, seen from above. × 375.

B. Transverse section of No. 76*b*, eye of brother or sister whose eye is represented in fig. A. × 200.



*A*



*B*

Eyes of Lucifuga, 25 mm. long.

A. Right eye, showing vesicular arrangement of pigment layer and retina and folding of sclera. From above. × 100.

B. Left eye, shriveled and sclera similarly folded.

pigment cushions on the eye, or in extra ocular regions at times in contact with the sclera, at other times in the orbital fat some distance removed from the eye.

While, other things being equal, we find a progressive decrease in the size of the eye with age, we do not find that individuals of the same size have eyes of the same size. On the contrary, the eyes of individuals of approximately the same length may be very different in size and, as we shall see later, in structure also. For instance, of 4 young taken from the ovary of one mother and differing from each other by not more than 2 mm. in total length, we have the eyes of two individuals without a lens and the eyes of the other two with large lenses. The eyes measure 272, 320, 384, and 416 $\mu$ respectively, or, after clearing in xylol, which permitted a more minute measurement, 260, 280, 375, and 425 (fig. 72, A). Detailed measurements of these eyes will be found in the following table:

*Measurements in $\mu$ of Eye of Female Lucifuga and of Four Young contained in her Ovary.*

[$x$, as they were taken from the ovary; $y$, cleared in xylol; $z$, sectioned.]

| No. | Length in mm. | Condition. | LEFT EYE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Longitudinal. | Vertical. | Medio-distal. | Between cartilages. | Pupil longitudinal. | Pupil vertical. | Lens. | Optic nerve in eye. | Gptic nerve outside. |
| 76 | 65 | $x$ | 170 | 160 | 152 | ........ | 00 | 00 | 00 | 20 | ........ |
| 76[1] | 18 | $x$ | 416 | 368 | ........ | ........ | 200 | 144 | ........ | ........ | ........ |
| | | $y$ | 425 | 365 | ........ | ........ | 200 | 140 | ........ | ........ | ........ |
| | | $z$ | ........ | 340 | 120 | 412[5] | ........ | 120 | 80 | 12 | ........ |
| 76[2] | 18½ | $x$ | 272 | 256 | ........ | ........ | 55 | 40 | ........ | ........ | ........ |
| | | $y$ | 260 | 250 | ........ | ........ | 50 | 40 | ........ | ........ | ........ |
| | | $z$ | | 240 | 88 | 320[5] | | 36 | 00 | 12 | |
| 76[3] | 19 | $x$ | 320 | 256 | ........ | ........ | 80 | 80 | ........ | ........ | ........ |
| | | $y$ | 280 | 230 | ........ | ........ | 75 | 50 | ........ | ........ | ........ |
| | | $z$ | ........ | ........ | ........ | ........ | ........ | ........ | ........ | ........ | ........ |
| 76[4] | 20 | $x$ | 384 | 352 | ........ | ........ | 192 | 144 | ........ | ........ | ........ |
| | | $y$ | 375 | 350 | ........ | ........ | 175 | 130 | ........ | ........ | ........ |
| | | | 300 | ........ | 148 | 455 | 144 | ........ | 68 | 20 | 24 |

| No. | Length in mm. | Condition. | RIGHT EYE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Longitudinal. | Vertical. | Medio-distal. | Between cartilages. | Pupil longitudinal. | Pupil vertical. | Lens. | Optic nerve in eye. | Optic nerve outside. |
| 76 | 65 | $x$ | 225 | 208 | 200 | ........ | ........ | 12 | 00 | 20 | ........ |
| 76[1] | 18 | $x$ | 362 | 356 | ........ | ........ | 208 | 152 | ........ | ........ | ........ |
| | | $y$ | 360 | 250 | ........ | ........ | 200 | 135 | ........ | ........ | ........ |
| | | $z$ | | 212 | 168 | 376[5] | | 128 | 80 | ........ | ........ |
| 75[2] | 18.5 | $x$ | ? | ? | ........ | ........ | ? | ? | ........ | ........ | ........ |
| | | $y$ | 250 | 225 | ........ | ........ | 50 | 25 | ........ | ........ | ........ |
| | | $z$ | | 248 | 88 | 300[5] | ? | 36 | 00 | 8 | ........ |
| 76[3] | 19 | $x$ | 320 | 288 | ........ | ........ | 64 | 56 | ........ | ........ | ........ |
| | | $y$ | 275 | 210 | ........ | ........ | 60 | 40 | ........ | ........ | ........ |
| | | $z$ | | | | | | | | | |
| 76[4] | 20 | $x$ | 400 | 368 | ........ | ........ | 224 | 160 | ........ | ........ | ........ |
| | | $y$ | 375 | 315 | ........ | ........ | 185 | 140 | ........ | ........ | ........ |
| | | | 340 | ........ | 136 | 420 | 176 | | 212 | 20 | 20 |

[1] Lower embryo of right ovary.

[2] Upper embryo of left ovary.

[3] Lower embryo of left ovary.

[4] Upper embryo of right ovary.

[5] Vertical distance between inner margins of scleral cartilage.

Still more striking is the variability in the size of opposite eyes in the same individual whatever its length. There are minute differences in the size of the eyes of the two sides at all times, the individual with two eyes exactly alike is probably not to be found, but the differences in mind are of a much larger order. For instance, in the mother of the 4 young mentioned above, the left eye had a longitudinal diameter of 170, the right eye 225; that is, the right eye was a third longer than the left. Instances of this sort are by no means rare, there being a marked difference in a number of the individuals secured. In one of the oldest secured, the eye of one side is all but gone, that of the other still well defined (plate 23 and plate 24, D). In a much younger one, 43 mm. long, I have found no eye in one side. In another the left eye bears the ratio of 1 to 3 to the right eye, which is therefore almost nine times as large as the left (plate 24, figs. A and B).

Such big differences between the eyes of the two sides, fluctuating in amount in different individuals, but readily seen in living specimens, are found in about 10 per cent of individuals. Sections usually showed that such differences whenever they existed were largely to be found in the pigment layer which in the large eyes was vesicular and the retina shriveled and retracted to the pupil, leaving a large space between the pigment epithelium and the rest of the retina (plate 21, fig. B; plate 22, fig. A; plate 24, figs. A and C).

NOTE. — One element of error is present in the exposition of the eye of *Lucifuga*. *Lucifuga* and *Stygicola* live together in the same caves. There is no difficulty in distinguishing these after they reach a certain size. What that size is I can not say, but at 60 mm. they are conspicuously different. The smallest specimen of *Stygicola* unquestionably determined is 60 mm. in length. Possibly the two species are superficially indistinguishable when young, and some of the young specimens mentioned below 60 mm. and used in preparing the following account may in reality be stygicolas. All specimens below 60 mm. secured had the characters of *Lucifuga*. The probability of this possible error is not as great as it may appear at first sight, as an analysis of the origin of the specimens less than 60 mm. will show. Seven of the specimens less than 60 mm. sectioned are from the cave of Jaiguan. From this cave 23 fishes were taken, 5 of which were stygicolas. The smallest of the stygicolas was 81 mm. and considerably larger than the smallest undoubtedly distinguishable specimens. If there were no specimens of *Stygicola* between 60 mm. and 81 mm. long when they could have been readily distinguished, it is probable that there were none smaller. The 3 smallest specimens of *Stygicola* measured 81, 90, and 97 mm. respectively. From Hawey I secured only *Lucifuga*, at least 3 of them being larger than the smallest specimens, permitting an unquestioned determination. From La Fria the only 2 over 60 mm. long were *Stygicola*, while those below 57 mm. were apparently all lucifugas. Two of those sectioned, 54 and 57 mm. long, may be considered lucifugas without a doubt. This leaves one 27 mm. and one 28 mm. in doubt. In Los Baños we secured no large specimens; all the small ones were referred to *Lucifuga*. In Ashton large and small were all referred to *Lucifuga*, the smallest one sectioned from this place being 53 mm.; it is undoubtedly a *Lucifuga*.

The proportion of stygicolas to lucifugas among individuals over 60 mm. is: stygicolas, 43; lucifugas, 36. *Lucifuga* does not reach a size over 104 mm., and comparing the ratios of lucifugas to stygicolas, between the smallest determined *Stygicola* 60 mm. and the largest *Lucifuga* 104 mm., we get *Stygicola* 32 mm., *Lucifuga* 36 mm., or a ratio of 1 to 1.25. But of the 32 stygicolas between 60 mm. and 94 mm., 10 came from the "M" Cave which is remote from the region where lucifugas were found. Eliminating these, we would get a ratio of 22 to 36, or 1 to $1\frac{7}{11}$, for the region where both are found. This, other things being equal, would give us the probability that any of the younger specimens found in the region where both species were found was a *Stygicola* or a *Lucifuga*. More than this, in the "M" Cave, about 60 miles removed from any cave in which *Lucifuga* was found, *Stygicola* is very abundant, but we secured no specimens less than 60 mm. long in five trips, nor were any small ones found in the Donkey and Carboneria, where only *Stygicola* occurs and where it is abundant. This makes it seem probable that the young of *Stygicola* live in deep water and are not found in the open sink holes or that their habits otherwise prevent them from being found.

One more element tends to show that all the young are *Lucifuga*. *Lucifuga* differs from *Stygicola* in the shape of the nape, the scales of the head, the teeth, and the number of fin-rays. These characters in the young were always those of *Lucifuga* as far as could be made out.

With these preliminary remarks the details of the structure of the eyes of different individuals may be given. The different parts of this account may be begun with a description of the conditions obtaining in the 4 young and their mother (referred to as 76) since there can never be any question concerning the genetic relationship of the eyes.

### THE EYE MUSCLES.

The six normal eye muscles are all present in the young of 76, both in those with a large eye and those with a small eye. The muscles in one of the large-eyed specimens and one of the small-eyed specimens have the following maximum diameter:

| | *d*[1] | *c* | | *d* | *c* |
|---|---|---|---|---|---|
| Dorsal oblique... | 28 | 36 | Anterior rectus... | 24 | 16 |
| Ventral oblique.. | 40 | 40 | Ventral rectus.... | 40 | 20 |
| Dorsal rectus.... | 16 | 20 | Posterior rectus.. | 16 | 12 |

These muscles can all be traced quite readily from their origins to their insertion (plates 16 to 18, *msc.*) and are apparently quite normal.

In the mother of these young the oblique muscles can be very readily traced in the socket in front of the eye, but their insertion in the eye is by fibers bent nearly at right angles.

The dorsal, ventral, and posterior rectus of the left eye can be traced from their origin to their insertion. The posterior rectus is an exceedingly slender thread, and with the ventral rectus diverges from their origin, they converge again at their insertion. The dorsal and ventral recti are merged with the oblique muscles so that they appear as continuous strands, with the fibers mentioned above diverging from their union. (See also plate 22, fig. C.)

In the right eye the posterior rectus is attached to the eye independently, the ventral rectus and oblique are much more remote from the eye than in the left eye at the point where the connecting fibers are given off to the eye.

In one of the largest individuals, 93 mm., the oblique muscles can be seen in the socket, but I have not been able to connect them with the eyes. The dorsal and ventral recti are present and possibly the posterior rectus. The muscles are, in other words, not so very different from those in 76.

### THE SCLERA.

The sclera is most highly developed in the eyes of unborn young about 20 mm. long. It is well developed, with its cartilages, in 12 mm. young. Its most striking feature is the large scleral cartilage. This in the young 20 mm. long is a segment of a hollow sphere with a large opening for the iris. The edges of the proximal opening are at times curved in. It resembles a convex shield with an opening in

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[1] The four young specimens bear the serial numbers 76, a, b, c, and d.

the center. In these early stages the cartilage is usually in contact with the iris in front, but diverges widely from the eye proximally and not infrequently extends beyond the eye. The inevitable conclusion is reached by an examination of such figures as scl. c. of plate 16, figure A; plate 17, figures A, B; plate 18, figure B, that the sclera was built for an ontogenetically or phylogenetically much larger eye than the largest found, and that the sclera has not been reduced at the same ratio as the eye itself. There is here no possibility of an artificial shrinking causing the space between the sclera and the eye, because this space is filled with undisturbed tissue, and the only indication of a shrinking is sometimes noticeable proximal of the eye, between it and the fibrous part of the sclera.

The ratio of the largest eye found in the young (76 *a*) to the eye suggested by the sclera is about as 45 to 85; in the smallest eye among the young of 76 (*i. e.* 76 *b*), it is about as 20 to 49. The eye, however, even if as large as suggested by the scleral cartilage, would still be a very small eye, unless the scleral cartilage formed but a rim over the front of the eye.

The cartilage is only about one cell deep, except near the outer rim where it is occasionally thickened. Over the back of the eye stretching from the proximal edge of the scleral cartilage there extends a slack membrane very much thinner than the cartilage and apparently continuous over the surface of the cartilage as an exceedingly thin membrane. Near the scleral cartilage this proximal membrane has a definite outline which is at times lost toward the optic nerve, the membrane becoming flocculent and its substance less readily distinguishable from the connective tissue filling the socket. A similar membrane more uniform in outline and consistency over the front of the eye represents the cornea.

*The scleral cartilages degenerate shortly after birth.* In the eyes of recently born individuals they differ from those in the eyes of the unborn by fitting close to the bulb. They have apparently been drawn to the bulb and in this process lost their symmetrical shield shape and are at times bent in acute angles, at other times their free margins project considerably beyond the eye. In one case, an individual (No. 203) 25 mm. long (scl.c. plate 19), the cartilage in shrinking to the eye was thrown into a fold extending some distance from the eye. The pockets formed between the layers of cartilage in this fold are filled with pigment apparently belonging to the retina. This peculiarity is found in both eyes of this individual. The cartilages in free living individuals are much more variable than in the unborn young, and even in one individual only 28 mm. the cartilage of one eye has entirely disappeared, while that in the other is a minute bar folded upon itself. In only a single case, to be described shortly, were there any traces of cartilage in specimens over 40 mm. long. The fibrous part of the sclera differs greatly in thickness in different eyes of older fishes or even in the same eye.

The greatest amount of difference between the sclera of the mother and the unborn young described above (76) is undoubtedly found in the cartilage. In the right eye of the mother there is no definite cartilage at all; there is a nodule of substance at the lower margin of the iris that may be the remnant of the cartilage, but otherwise there is nothing in this eye to indicate that there ever was any cartilage associated with it at any time. In the left eye of the same individual are two nodules of cartilage, one tangent to the dorsal surface of the eye (plate 21, fig. A), the other in a vertical section through the middle of the eye somewhat below the level of the optic nerve. The former retains its distinct cartilaginous nature while the latter has lost it to such an extent that it is only by inference that it can be considered of cartilaginous origin. These are the only cartilages seen in eyes of individuals over 40 mm. long. The fibrous part of the sclera is as well developed as in the younger eyes, and indeed near the nodules of cartilage in the left eye it is distinctly thicker than in the younger stages. The sclera as a whole no longer forms a capsule much larger than the eye; it fits snugly against the eyeball, except in the cornea of the right eye, where it forms an arch over the iris and pupil in the normal way. Where the cornea joins the sclera proper in the right eye, there is again a material thickening of tissues.

The cornea in older individuals undergoes many modifications. It retains its shape for but a short time after birth. In 16 individuals over 24 mm. long it retained its original outline in only 4 eyes in 4 different individuals, one 28 mm. (plate 20, fig. A), one 38 mm., one 53 mm., and one 65 mm. long (plate 21, fig. A), the mother mentioned above. In the other eyes the aqueous space is obliterated, and the cornea more or less disintegrated. In cases where the vitreous cavity had disappeared, and the pupil had become closed, the cornea was at times replaced by a lenticular mass, cellular rather than fibrous (plate 20, fig. C).

The points of interest are that the sclera develops early and on a scale much beyond the present needs of the eye, *i.e.*, it preserves a past phylogenetic stage far better than the other parts of the eye, and yet ontogenetically it degenerates much more rapidly than any other part, with the possible exception of the lens.

### THE CHOROID AND RETINAL BLOOD-VESSELS.

In unborn young about 20 mm. long there is considerable space between the sclera and choroid. At first sight this may be taken as the result of shrinkage on the application of reagents, but a closer inspection shows the space to be filled with an undisturbed gelatinous substance interspersed with nuclei. It represents the suprachoroidal lymph space. Immediately in contact with the eye, the gelatinous matrix is replaced by fibers. The normal condition of the gelatinous layer is further testified to by the dendritic choroidal pigment cells that are scattered through it and occasionally are arranged into a thin layer, dividing the mass into approximately two equal layers. Still further evidence is given by the occasional blood-vessels passing through it.

In 76 *a* there is a fine capillary meshwork in the choroid. In the meridian of the optic nerve an artery approaches the entrance of the optic nerve from below and a vein much thicker leaves it above. The vein is made up of two branches in the choroid near the entrance of the optic nerve, one branch coming from above, the other from below. The artery enters the retina along the lower edge of the optic nerve. The vein leaves the retina in this eye over the lower margin of the iris. The meshwork of blood-vessels over the inner surface of the retina contains many far beyond capillary size, closely approaching in thickness the retina itself. There is a median vessel extending from the lower edge of the pupil along the surface of the retina up to a level with the upper surface of the lens.

In the left eye of 76 the ophthalmic vein measures 50 $\mu$ in diameter, while the eye itself measures but 170 $\mu$. In the eyes of this individual I have not been able to make out any blood corpuscles, nor have I been able to identify the ophthalmic artery.

In a specimen 94 mm. long the ophthalmic vein can readily be traced. In a specimen 93 mm. the ophthalmic vein of the right eye is seen to measure 40 μ as compared with a diameter of the eye of about 100 μ. A few blood cells are seen in this eye. A considerable mass of pigment is developed in the choroid, in places 15 μ thick. It is not possible to make out any vascular network either in the choroid or in the eye. Very few blood-vessels are seen about the eye itself, although the vessels leading to and from the eye are very large and filled with blood corpuscles.

In the eyes of older individuals there is a great diminution in blood in and about the eye. The capillary meshwork in the choroid and the vitreous vessels are no longer readily distinguishable, their reduced size being further indicated by the absence or inconspicuousness of the large choroidal veins seen in 76 *a*. The ophthalmic vein is, however, very large and well filled with corpuscles in even the oldest individuals. It has here the appearance of a sinus rather than a vessel. Certainly the necessity of the eye does not require a vessel equal to nearly half of the total diameter of the eye as in the case of 42.

The entire vascular arrangement gives the impression of being abnormal. A key to the large blood-vessels or sinuses is probably found in several of the eyes of *Stygicola* to be described later. In them it was definitely determined that blood lakes had formed in and about the eye that were entirely cut off from the circulation.

### PIGMENT MASSES NEAR THE EYE AND THEIR SIGNIFICANCE.

Near the eyes of all specimens above a certain size there are found masses of pigment. They are probably cells gorged with pigment which are aggregated in one or several masses. For instance, near the left eye of the largest fish examined, there is a large (80 × 128 μ in section) pigment mass 144 μ from the eye. It is oval in its proximal end; truncate in its distal. Some of the denser fibers of the capsule surrounding the eye extend out to it. Another less distinct pigment mass is found in contact with the eye in a manner to make it difficult to determine its relation to the eye. It may be part of the retinal pigment (plate 23, fig. B). On the right side there are several pigment masses located in the orbital fat near the eye: one, 80 × 96 μ; another circular mass, 32 μ; another, 80 μ in diameter near the eye; and still another, 32 × 48 μ. Some of these are evidently composed of lobes or distinct subsidiary masses. In very thin sections it can be seen that the cells composing the masses are filled to distention with granules about 0.7 μ in diameter, just such as are found in the pigment of the retina and in the subepithelial pigment of the skin. The cells measure 9 to 14 μ in diameter. They are rounded, sometimes flattened where they are in contact. When fully pigmented their well-defined outlines and the occasional undoubted relation of nuclei to them are the only indications that they are cells.

Remote from the densely filled cells, a number of cells can be made out in one individual in which the nucleus is located at one margin and the cytoplasm contains a few, or even but one, granule, while in others no granules are found. The nucleus is always kidney-shaped with the concave side toward the cytoplasm. There is, for instance, one nucleus near one of the large masses, similar to the nuclei in the mass flattened on one side and associated on that side with a hyaline bag of definite outline and containing a number of the pigment granules; near it is another with more pigment in a more elongate mass. In the youngest individual (38 mm.) with whose eyes pigment was found associated, it is close to the optic nerve on one side of the body and along a fibrous strand on the other.

The cells are fully charged with pigment, and no cells could be found with but a few granules. In the next largest (43 mm.) there is a large pigment cushion on the posterior face of the left eye. There are also a few fully pigmented cells scattered distad from the eye.

In individuals 44 mm. and 54 mm. long the pigment is also associated directly with the eye, but the parts can not be readily distinguished.

In an individual 53 mm. long there is a mass distad from the right eye over the pupil, and another proximal to the left eye. These are the beginning of the masses seen near the eye in older individuals.

In an individual 57 mm. long there are small masses of pigment cells some distance removed from the eye. On the left side the mass exceeds the size of the eye.

In the left eye of an individual 63 mm. long there is a large amount of pigment immediately around the eye and also masses removed some distance from the eye. The same is true of the right eye, which is large and vesicular.

In an individual 65 mm. long there is a small pigment mass remote from the eye and a larger amount directly associated with it.

In an individual 69 mm. long (plate 22, fig. A) there are masses of pigment near the eye which is vesicular. In an individual 80 mm. long small masses are found near the eye and there is much pigment in the eye.

In the right eye of an individual 84 mm. long there is a very thick (30 $\mu$) mass of quadrate pigment cells in the choroid along the lower surface of the eye. The pigment layer of the retina is but 4 $\mu$ thick and there is a lenticular mass of pigment cells, 46 × 34 $\mu$ in section, in the pupil. The vitreous cavity is obliterated.

In one of the largest fishes, 93 mm., there are large masses near the eye as well as a cushion of pigment affixed to the eye (plate 22, fig. B, *pi. s.*).

From the above it is seen that the pigment masses make their appearance at about the time the eye begins to actively degenerate, a short time after birth, and that they reach their maximum development when the eye has reached the vanishing point. The masses are first seen in a fish 38 mm. long in association with the optic nerve and the muscles near the eye. In slightly older individuals the pigment masses appear as lenticular cushions applied to the sclera, and in still older, when the fish has reached 50 mm., other masses are seen more or less remote from the eye, although pigment cushions may still be seen in some of the larger specimens. In the very largest there are several masses in the neighborhood of the eye or where it has disappeared.

While it is practically impossible to make out the structure of the pigment masses in their most intense development, it is evident that they are made up of rounded bodies densely pigmented, several of which are bound by fibrous tissues into subsidiary masses many of which together form the larger masses described.

No doubt the smaller rounded bodies are cells. In their most intensely pigmented condition it is impossible to demonstrate this. In certain favorable cases the individual pigment granules can be made out, as well as their arrangement in the cell. In the very largest individuals some cells were found that contained but one or very few pigment granules.

The appearance and gradual increase of these pigment cells and masses with the beginning and progressive degeneration of the eye makes an intimate dependence of the one phenomenon on the other very plausible. That pigment cells may sometimes appear and become pigmented at some distance from the degenerating eye is seen in the optic cavity of the largest individuals, where cells with but few pigment granules were seen remote from the eye. Furthermore no phagocytes or pigment cells in the process of gorging were seen in the eye. But in one case at least there were found a number of fully pigmented cells between the pigment layer and the rest of the retina. There seems to be little doubt, therefore, that there is direct association of at least some pigmented cells with the degenerating eye. Other indications as to the possible origin of the pigment masses are given under the head of the lens. In some of the degenerating lenses cells containing pigment granules were found. These cells are 6 $\mu$ to 9 $\mu$ in diameter. They are most numerous in the lens of an individual 25 mm. long before accumulation of pigment cells into masses has taken place.

I have noticed similar pigment accumulations in the eye of *Amblyopsis*.

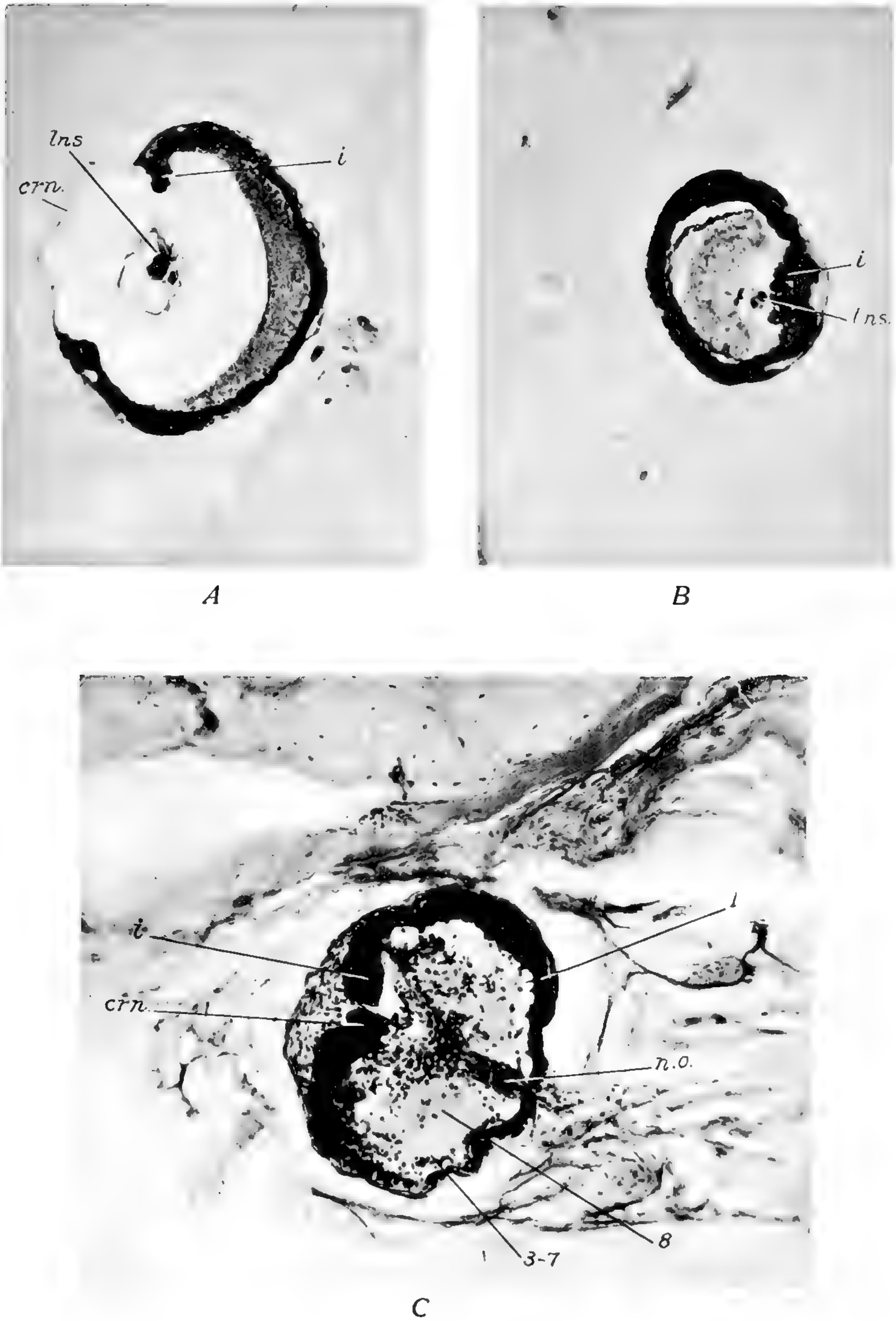
Pigment is found in very variable quantity and variously scattered in the connective tissue surrounding the eye. The amount of this pigment seems to vary inversely with the amount of pigment in the eye itself and to increase with age.

### THE LENS.

The variation in the lens is not equaled even by the variation in the sclera. Here, as in the sclera, we may begin the account with a description of the conditions in the 4 unborn young taken from the ovary of a single mother. *In 2 of these, a and d, the lens is still present; in the other 2 there is no indication of it.* In *a* and *d* it consists of a sphere (plate 17, fig. B, plate 18, fig. A) incased in a fibrous membrane of varying thickness, flocculent peripherally, becoming dense and firm and containing nuclei proximally. The contents of this membrane are evidently undergoing histolysis. It is an amorphous, granular substance with partially dissolved masses, some of them still showing nuclei. At other places the nuclei have degenerated into black chromatin lumps. There is absolutely no indication of lens fibers. The cortical layer of the mass is at times compact over the distal surface and this is the only indication of an epithelium covering this part. In the lens of *a* stained with iron hæmatoxylon, the center which chiefly contains the masses mentioned above is in part quite black. In *b* of these young the only indication of the lens is a small vacuity in the connective tissue between the edges of the iris (plate 18, fig. B). There is nothing about this space except its position to indicate that it was ever in the remotest way connected with a lens or its capsule. The lens in still younger ones (12 mm.) is much as described in *a* and *d*. It consists of a fibrous capsule filled with a mass of undifferentiated cells.

In small individuals, ranging from birth with a length of about 24 mm. to 38 mm. in length, the lens is usually present in a more or less advanced degree of degeneration. In the degeneration the solid contents of the lens capsule largely disappears, the capsule collapsing or not.

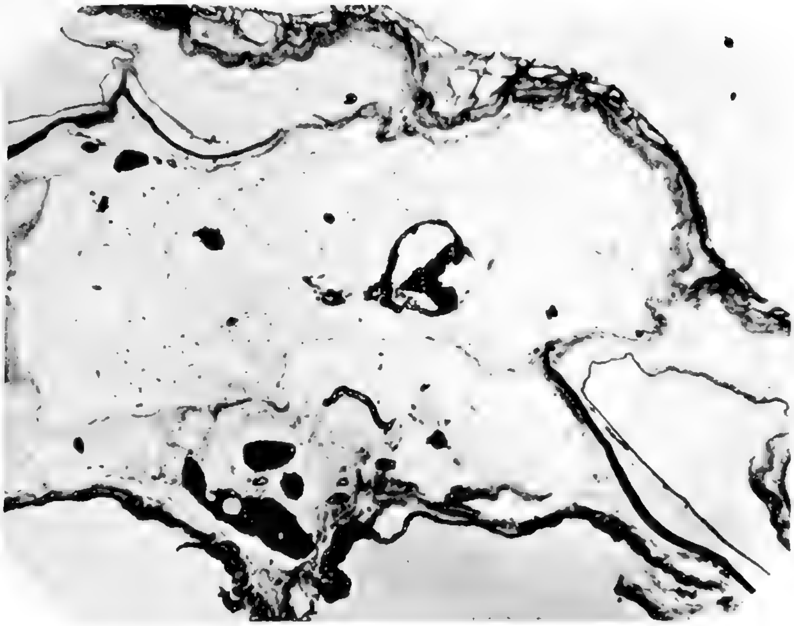
In a young 25 mm. long the lens capsule of the right is very much shriveled, like a collapsed balloon and contains only about a dozen small cells, some of them nucleated, others in part filled with dark brown pigment granules. These look not



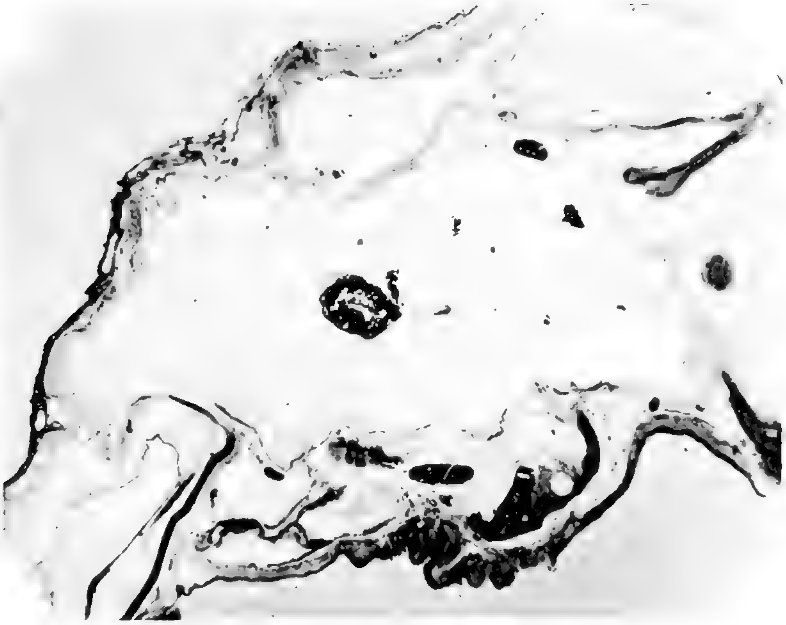
Eyes of Lucifuga.

A and B. Eyes of opposite sides of young, 28 mm. long, showing great differences in size of eyes and also of general structure. In A lens capsule shows well.

C. Eye of individual 53 mm. long. x 200. Eye of opposite side very large and vesicular (represented in plate 24A.)



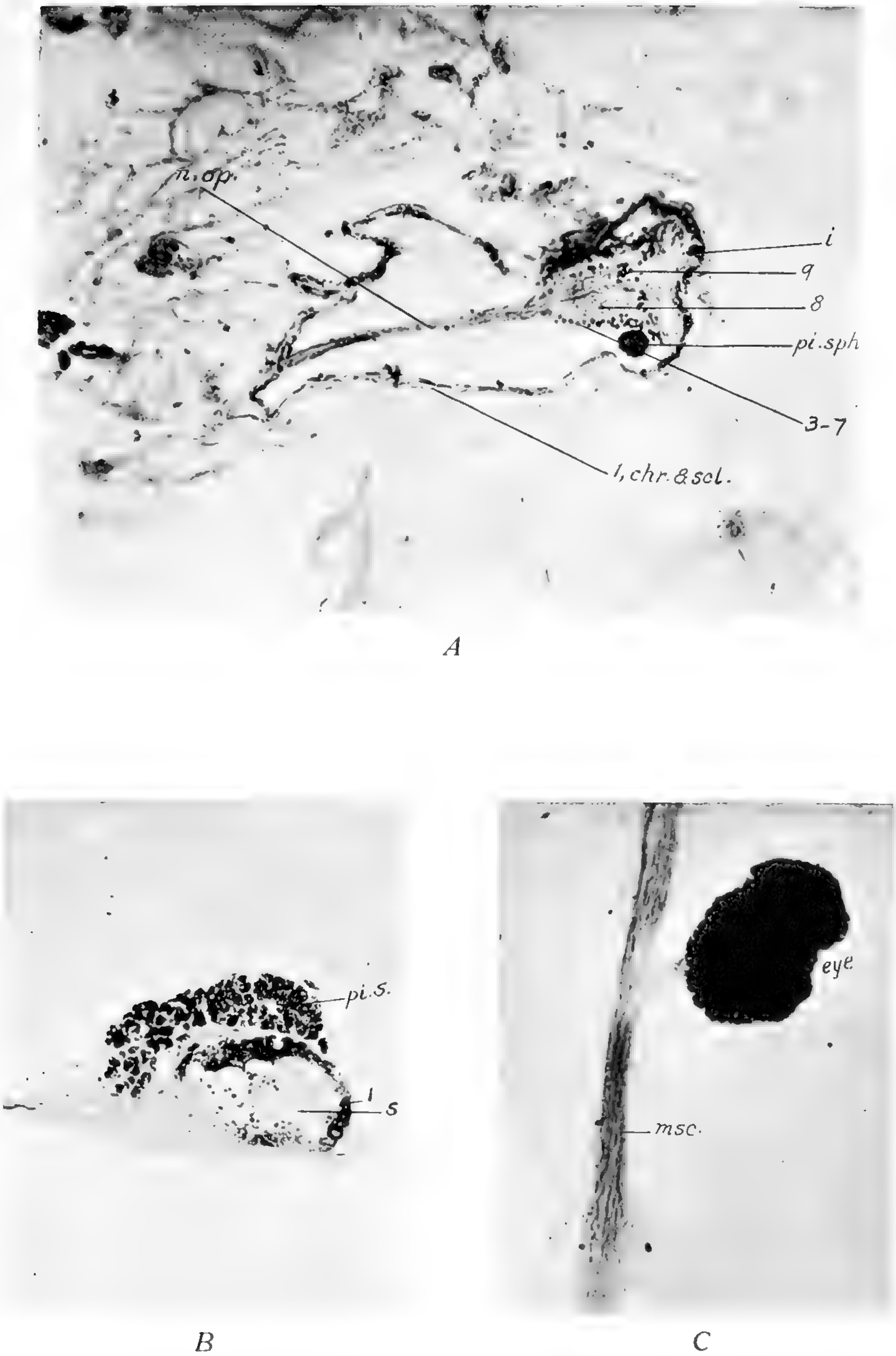
B



A

Sections through left and right eye-cavities of Lucifuga, specimen No. 76 (see plate 15C).

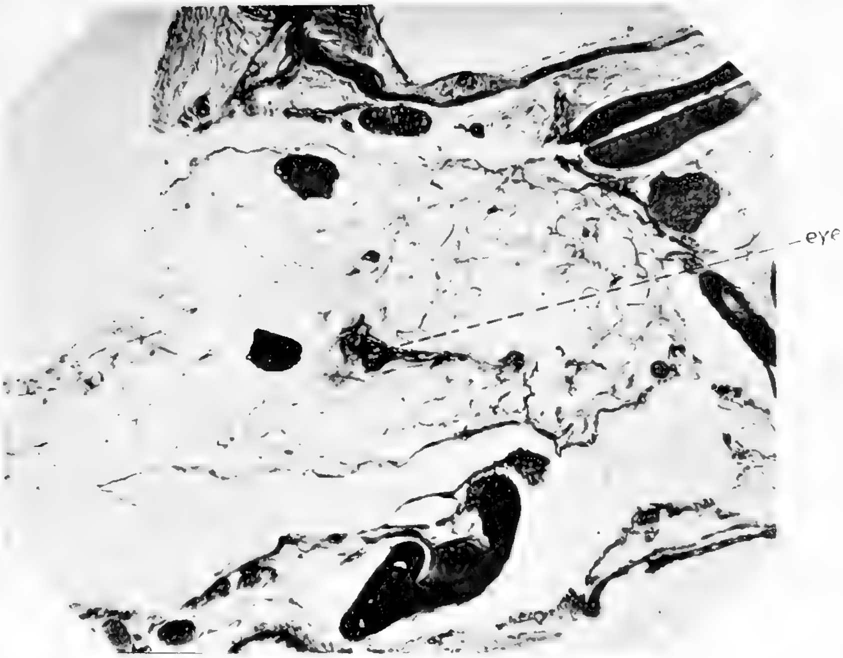
These figures have same magnification as plate 16B (of head of one of her young) with which they should be specially compared. Eye-cavities very large as compared with those of the larva (plate 16B), while eyes are much smaller. × 60.



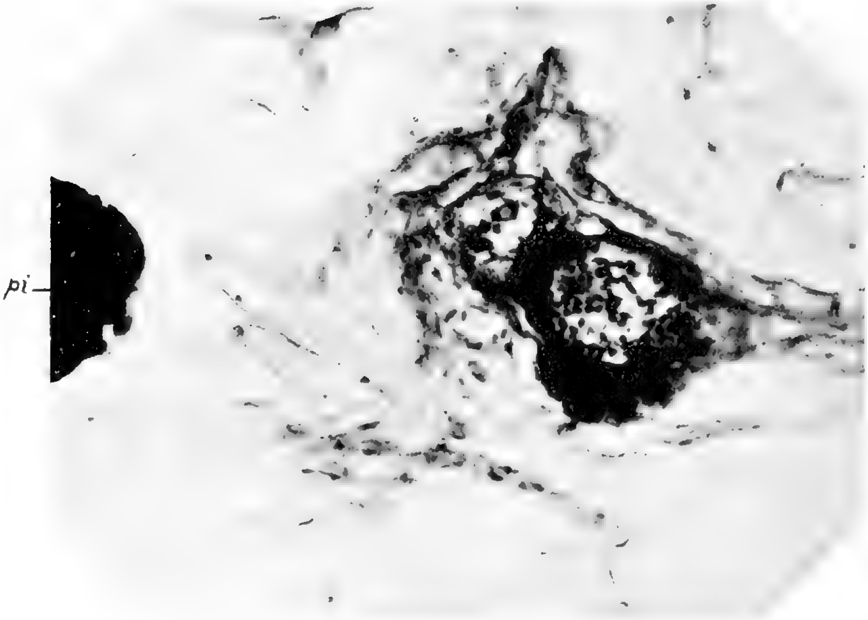
A. Right eye of a Lucifuga, 69 mm. long. The pigment layer with choroid and sclera (*1, chr.* & *scl*) is vesicular, walls of vesicle having shriveled somewhat. Section is along optic nerve (*n. op.*) and shows large, round pigment mass (*pi. sph.*) between pigment layer and lower part of retina. X 60.

B. Left eye of adult, 93 mm. long. Eye has become very small, pigment layer incomplete. Very large mass of pigmented cells has accumulated over and in front of eye; strand of connective tissue (with nerve fibers) extends from front of eye out toward surface. X 60.

C. Oblique (lower) and rectus muscles sending common tendon to eye. From fish 44 mm. long.



A



B

Eye of an old Lucifuga, 94 mm. long.

A. Left eye-socket with contained eye and pigment-mass at its left. × 60.

B. Part of same section, × 375, showing fibrillar network about eye. Eye a nodule of cells in which distinction can only be made between pigment and retina. Part of pigment mass at extreme left. (For right eye see plate 24 D).

unlike white corpuscles that have been abundantly fed with pigment granules. Whether they carried these in with them or whether the remnant of the lens had undergone a pigment degeneration, I am not able to say.

The eye of the other side is much damaged in sectioning, but is essentially the same.

In a young 24 mm. long, evidently just born, the lens capsule of both eyes is a large balloon, little wrinkled, and containing but little stainable material, all of it of the same nature as that described above.

In a young 27 mm. there is no remnant of a lens in the left eye, while in the right there is the filmiest suggestion of the lens capsule, but nothing more.

In an individual 28 mm. long the lens of the right eye is represented by a nearly empty capsule, that of the left is shriveled, contains pigment, and is entirely in the vitreous space, the pupil having closed.

In an individual 38 mm. long the left lens is represented by a large empty collapsed capsule, that of the right being small and collapsed.

The lens capsule is the last part of the lens to disappear. In specimens over 40 mm. long, it was observed in only two doubtful cases; in all others there was no trace of it left.

It is quite evident from the structure of the lens displayed in the unborn young 18 mm. long that it had passed its point of highest organization and was obviously far along on the route of degeneration. Indeed the lenses of the young (12 mm.) show no signs of fiber formation and also show indications that they have begun to degenerate.

Conspicuous and remarkable are the fibrous lens capsule which persists after its contents have disappeared, the irregularity of the contained cells in their highest development and their irregular distribution, and finally the pigment-fed phagocytes in the capsule.

## THE RETINA.

On account of the fluctuation in the size of the eye it is difficult to determine whether the end of its development is reached with a length of 12 mm. or not until a length of 20 mm. In the 4 embryos, 76 *a*, *b*, *c*, and *d*, about 20 mm. long, the eyes fluctuate from a maximum 425 $\mu$ in longitudinal diameter in the longest, to 260 $\mu$ in the shortest. If the embryo with the smaller eye had been of smaller size, it would have been but natural to come to the erroneous conclusion that the eye increases with age till the fish reaches a length of 20 mm. The same is true in respect to the differentiation of the retina. One can not say in general that the retina progresses in any respect between the length of 12 mm. and 20 mm. I can only say that the most highly developed retina was found in an unborn individual 20 mm. long (plate 18, fig. A, and plate 24, fig. E).

In the retina of the youngest individuals (12 mm.) there is a distinct differentiation into a ganglionic layer occupying 0.24 of the total thickness, an inner fibrous layer of the same thickness, a nucleolar layer 0.32 of the total, and a pigment layer occupying 0.20 of the entire retina. The boundaries of the different layers are not equally regular at all places, and the nuclear or ganglionic layer sends a connecting series of nuclei in an irregular manner through the reticular layer in different places. The pigment layer is well pigmented. The inner cell layer of the uvea is not pigmented and forms a distinct ciliary process. Between the latter and the rest of the retina there is an accumulation of elongate nuclei.

This retina has reached a stage in an irregular process of histogenesis, or it has earlier stopped at such a stage of differentiation, or finally, it has reached its present condition as a degeneration from an earlier, more highly differentiated stage. From the material at hand it is impossible to determine when the retina reaches its highest stage of development and when it begins to degenerate.

A slightly higher stage of differentiation is found in one of the eyes of one of the unborn young of 76. In this eye, the retina has about the same total thickness. There is found in places a very distinct separation of the outer layer of nuclei into an inner layer, a reticular layer, and an epithelial layer. To one of the epithelial nuclei a cone is found attached (plate 24, fig. E). A ciliary process is not seen in this eye nor in the group of elongate nuclei so conspicuous in the younger stage. The inner layer of the uvea, as well as the outer, is pigmented.

Beyond birth only general processes can be described without entering into a minute description of each eye. The retina degenerates progressively and it seems to do this accompanied by one of two modifications in the general structure of the eye. The eye may shrivel (plate 20, figs. B, C; plate 21, fig. A), the pigment layer lying close against the rest of the retina; or the pigment layer may separate itself from the rest of the retina and become very greatly distended, the retina itself forming but a small segment of the eye vesicle (plate 22, fig. A; plate 24, figs. A, C). Plate 22, figure A, represents such an eye, in which the retina is well contracted and the pigment layer shriveled. The optic nerve passes through the vesicle. The beginning of such a modification is probably to be seen in plate 21, figure B. In other cases the retina is drawn out laterally (plate 24, figs. A, C). Such vesicular eyes were also found in old individuals of *Amblyopsis*. There does not seem to be any increase in the amount of pigment, and, since it is scattered over a larger area, the pigmented layer of these vesicular eyes is less densely pigmented than that of the shriveled eyes. In one eye conditions normal to a fish eye are more nearly retained.

I am not able to say that one part of the retina undergoes a more rapid degeneration than another. They all reach the vanishing point with extreme old age.

In an old individual (94 mm.) the eye of one side consisted of a few vacuoles surrounded by nucleated fibrous tissue (plate 24, fig. D). It is impossible to determine to what these parts of the eye belonged. There are also scattered pigment granules and cells, while near this eye are a few pigment masses. The eye of the other side is better preserved and represented in plate 23. In one eye, which is shriveled to very small dimensions, a peculiar lenslike structure occupies most of the interior. Such lenslike structures I found in *Amblyopsis* and erroneously considered them the lens. In *Rhineura* it is distinctly seen that the structure fills an invaginated pocket of the pigment layer.

A census of a series of eyes of individuals from the time of birth to old age gives us the following statistics concerning the lens, the vitreous space (that is, between retina and iris), and the aqueous space (between iris and cornea):

*Statistics of Lens, showing Vitreous Space (between Retina and Iris) and Aqueous Space (between Iris and Cornea).*

| | | LENS. | | VITREOUS SPACE. | | AQUEOUS SPACE. | |
|---|---|---|---|---|---|---|---|
| mm. | | Left eye. | Right eye. | Left eye. | Right eye. | Left eye. | Right eye. |
| 25 | (66) | Empty capsule | Collapsed empty capsule | Large | Very large | Large | Large |
| 25 | (103) | Large, entirely filling vitreous cavity | Large | Small, filled by lens | Large | Large | Large |
| 24 | 67 | Large capsule | Large, empty capsule | Large | Large | Large | |
| 25 | 105 | * | Large | Large | Large | Moderate | Large |
| 27 | 110 | 0 | Filmy capsule | 0 | Large | 0 | |
| 28 | 64 | Capsule with pigment | Nearly empty capsule | Collapsing | Large | 0 | Moderate |
| 28 | 111 | ? | Capsule with pigment | Moderate | Very small | 0 | 0 |
| 38 | 62 | Large, empty | Small, collapsed | Left very large | Right? | Large | ? |
| 43 | 61 | 0 | ? | Large | ? | 0 | ? |
| 44 | 104 | 0 | 0 | 0 | Very small | 0 | 0 |
| 53 | 82 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | 95 | 0 | 0 | ? | Very small | Very large | 0 |
| 54 | 109 | 0 | 0 | 0 | 0 | 0 | 0 |
| 57 | 75 | 0 | 0 | 0 | 0 | 0 | 0 |
| 65 | 76 | 0 | 0 | 0 | Large | 0 | Large |
| 69 | 56 | ? | Empty vesicle | ? | Small | ? | 0 |
| 80 | 53 | 0 | 0 | 0 | 0 | 0 | 0 |
| 84 | 51 | 0 | 0 | 0 | 0 | 0 | 0 |
| 93 | 42 | 0 | 0 | 0 | 0 | 0 | 0 |
| 94 | 29 | 0 | 0 | 0 | 0 | 0 | 0 |

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* In the left eye the lens is not distinguishable, but is probably represented by a collapsed capsule in part filling the vitreous cavity.

## THE EYES OF STYGICOLA.

The account of the eyes of *Stygicola* is based (1) on two young born October 20, each about 20 mm. long; (2) on the mother of the above, 92 mm. long; (3) on various other older fishes, from 60 to 135 mm. long.[1] The early stages of the development and the history of the eye between 20 and 60 mm. is not known.

On October 30 I obtained a *Stygicola* at Alacranes. She gave birth to two young on the evening of October 31, at Cañas. They were born tail foremost. The ovary of this specimen contained eggs 0.88 mm. in diameter, or nearly ripe. The 2 young are referred to as 125 *a* and 125 *b*.

The head of 125 *b*, seen from above, is represented in plate 25, figure B, and the eyes are represented by plate 25, figure C. The eyes of the one born at Cañas (125 *b*) were symmetrical, nearly of the same size. The eyes moved, and as far as I could judge were as readily movable as the eyes of other young fishes.

The eyes were silvery, the argentea being apparently well developed. The iris was well distinguished, the pupil too large for the lens, having a downward directed notch continuous with the choroid fissure which is still visible as a pigmentless streak. While small, there was nothing in the general appearance of the eye that would lead one to conclude it might not be functional.

The eyes are so placed in relation to the brain that a line tangent to their posterior faces would be tangent to the anterior face of the optic lobes. This condition corresponds very well to the position in *Lucifuga* of equal size.

*Table of Measurements.*

| Current No. | 125 *a* | | 125 *b* | | 1 | | 126 | | 125 | | 117 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Size. | 20 mm. | | 20 mm. | | 60 mm. | | 88 mm. | | 92 mm. | | 135 mm. | |
| Side. | *l* | *r* | *r* | *l* | *r* | *l* | *r* | *l* | *r* | *l* | *r* | *l* |
| | μ | μ | μ | μ | μ | μ | μ | μ | μ | μ | μ | μ |
| Vertical diameter... | 413 [1] | 312 | 566 [2] | 560 | 95 | 170 | 320 | 250 | ... | 198 | 113 | 130 |
| Medio-distad....... | 284 | 251 | 191 | 198 | 160 | 170 | 312 [4] | 210 [4] | ... | 256 [5] | 140 | 110 |
| Pupil.............. | 130 | 67 | 128 | 122 | ... | ... | 153 | 30 | ... | ... | ... | ... |
| Lens............... | 92 | 74 | 78 | 73 | ... | ... | 45 | 50 | ... | ... | ... | ... |
| Pigment........... | 6.7 | 4.5 | 11 | 11 | ... | ... | 11 | 11 | ... | ... | ... | ... |
| Outer nuclear...... | 15.8 | 18 | 13 | 18 | ... | ... | ... | ... | ... | ... | ... | ... |
| Granular.......... | 18 | 24 | 26 | 20 | ... | ... | 58 | 56 | ... | ... | ... | ... |
| Ganglionic......... | 9 | 12.6 | 11 | 9 | ... | ... | ... | ... | ... | ... | ... | ... |
| Hyaloid........... | 6.7 | .... | 9 | 9 [3] | ... | ... | ... | ... | ... | ... | ... | ... |
| Blood-vessel in eye.. | 45 | 24 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Optic nerve........ | .... | 20 | 31 | ... | ... | ... | ... | ... | ... | ... | ... | ... |

[1] From outer margin of scleral cartilage, unless otherwise stated.
[2] From outside of pigment to outside of pigment.
[3] Total thickness of retina 67 μ, as compared with 237 μ in *Zygonectes*.
[4] These eyes lie 0.5 mm. below the surface.
[5] This eye lies 0.3 mm. below the surface.

About the left eye of the second young (125 *a*) there was a large accumulation of blood, which in section is seen to be in the choroid layer and mixed with the orbital fat. Measurements of the eyes of the young, as well as of the mother, are shown in the above table, and see also plate 25, G.

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[1] A single larva obtained on September 1, between 10 and 12 mm. long, is not well enough preserved to be considered.

In these young the eyes are in contact with the skin and fill a large part of the fibrous orbit. With age the eyes come to be farther and farther removed from the skin, and lie in the orbital fat, which may be many times the size of the eye. For instance, in the mother of the young (125 *a* and *b*) the eyes are approximately in the middle of the large eye cavity, which is over a thousand times as large as the eye, having on the left side a vertical diameter of 1.8 mm. and a lateral diameter of 3 mm., whereas the eye has an average diameter of but 0.2 mm. The eyes are about 0.13 mm. removed from the surface. The eye cavity is filled with cavernous connective tissue meshwork holding fat. About the eye the meshes are stronger and very rich in blood-vessels. About the eye in this individual, as in all old ones, there are also large accumulations of pigment.

Parts of the eye have certainly begun to degenerate before birth. The lens leads in this respect. After birth there is a rapid general degeneration of the eye. This is not directly proportional to the increase in size of the fish. For instance (see table), in a specimen 60 mm. long the eyes are distinctly farther reduced than in one of 88 mm. The left eye (plate 25, fig. G) in life was surrounded by stagnant blood. The choroidal blood-vessels were distended and the vessels of the vitreous body were also abnormally large. The entire eye was compact, and the retina, slightly withdrawn from pigment layer by reagents, shows a drawn-out process indicating an intimate relation between two layers. Figure F (right eye) shows eye normal to this stage. The retina has shrunken away from the pigment layer somewhat and an artificial space has also been formed in places between sclera and choroid.

As in *Lucifuga*, the eyes of opposite sides have at times undergone different modifications; the eye on one side may be contracted into a compact ball, while on the other it is distended into a hollow sphere, eight or ten times as great in cubic contents. The left eye of 125 *a* and the left of 126 (plate 25, fig. G, and plate 26, fig. A) indicate that in these two eyes at least, the compression is associated with an accumulation of blood in the choroid vessels and in the orbital fat. While this blood does not have the appearance of a clot, the corpuscles have a very different staining reaction from those in the vessels. In 126 *l* there is a small vessel in front of the iris which contains normal blood (plate 26, fig. A, *cps.*), otherwise this eye is shut off from the circulation. The left eye of 125 *a* was certainly cut off from the circulation by the formation of a large blood lake about the eye. There is evidence in the right eye of 125 that extra limital blood has accumulated about this eye also. It would seem from these examples that one of the principal causes of degeneration is a disturbance in the circulation.

Figure A of plate 26 shows the left eye of No. 126, 88 mm. long. The choroid blood-vessels are distended with blood corpuscles which stain differently from those in one of the choroid vessels. Other spaces or vessels filled with blood were found in tracts passing through the orbital fat-mass, past the eye. The iris was infolded and the pupil closed with a fibrous tissue containing blood-vessels. The lens was a flaccid membranous bag containing pigment granules and a few nuclear remains. The pigment layer variously pigmented (1) appears in two layers in places, and within it are found large rounded masses of pigment. The retina consists of ganglionic cells, and an outer layer of cells and a reticular layer, approximately divided in the middle by an irregular cellular layer. The optic nerve in the figure is supplied from neighboring sections.

### THE EYE MUSCLES.

The eye muscles of 125 *b* are well developed, with some anomalies in one of the recti of each side.

The two oblique muscles arise just below the point of exit of the olfactory nerve from the brain cavity, downward and medial of the middle of the olfactory pit. They are attached to the membrane connecting the ethmoid with the vomerine cartilage. They extend backward in a canal bounded above by the ethmoid, below by the vomer, and laterally by another cartilage. The upper oblique is regularly horizontal-oval, measuring 34 μ by 48 μ. The lower oblique is slightly crescent-shaped in section with a diameter of 25 μ by 83 μ. These muscles are attached on the sclera so that the tips of their insertion are just in contact with the posterior rim of the scleral cartilage. The superior and inferior recti have their points of insertion on the cartilage just outside the insertions of the oblique.

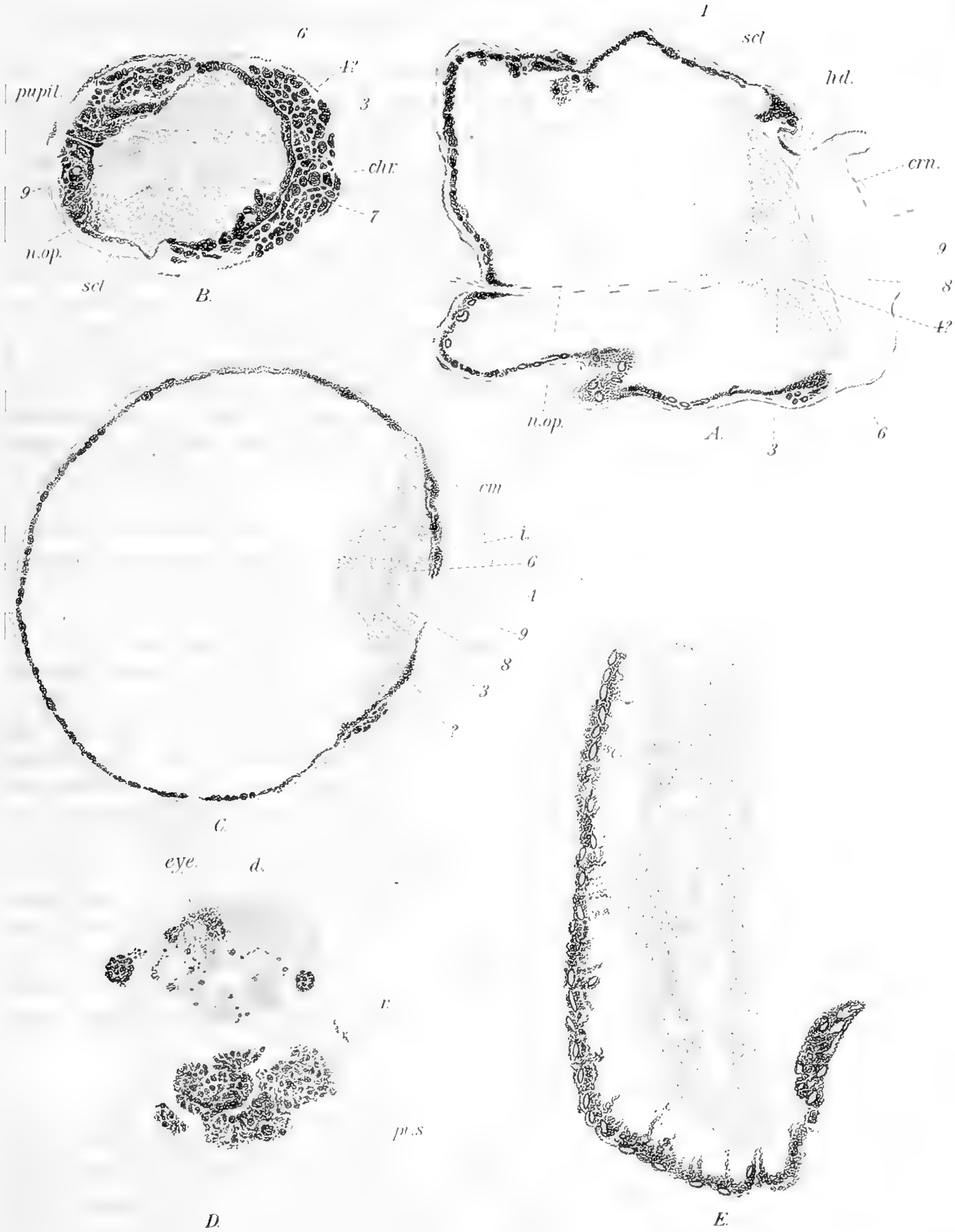
The anterior rectus of the left side is inserted on the anterior face of the scleral cartilage. It has a diameter of 20 μ near its insertion. It has its origin just *in front* of the exit of the optic nerve. On the right side the muscle arises just *below* the exit of the optic nerve, extends out and then curves down and joins the fibers of the inferior rectus, following the fibers of this muscle and becoming indistinguishable from them.

The posterior rectus arises far back, just below the origin of the ear capsule. It extends out and forward, with a diameter of about 30 μ and attaches to the posterior face of the eye.

The superior and inferior rectus muscles are much stronger than the others; they arise much farther forward than the posterior rectus, about on a plane connecting the posterior faces of the eyes. The upper rectus has a broad point of origin, the inferior rectus a narrower one below it. The upper rectus curves upward, forward, and out; the lower runs in a nearly straight line obliquely down, out, and forward. On both sides the upper rectus gives off fibers to the lower. The method of the two sides is different; on the right a compact bundle of fibers branches off from the root of the muscle, passes toward the lower rectus to whose inner face they become joined. The fibers pass from the origin of the superior to the insertion of the inferior rectus. While some fibers seem to have a similar course on the left, the conspicuous thing here is that fibers form an arch between the upper and lower recti, their origin and insertion being both on the *eye*. The important point is that in the eyes of the young the muscles, while varying to a degree on the two sides, are all well developed. The muscles are still conspicuous in a specimen 97 mm. long, but in the mother of the young described and in older fishes, I have not been able to find any muscles (plate 25, figs. E, F, G, *msc.*).

### THE SCLERA.

The scleral cartilage is well developed at birth. Whereas, in *Lucifuga*, it formed a partial shield over the distal face of the eye, its pupilary diameter being much less than the diameter of its proximal rim, it here forms a ring about the equator of the eye the diameter of whose proximal rim is less than that of the distal opening. The walls of the ring are thickest in front, where they reach 30 μ, tapering backward. The ring in some cases fits the eye and does not, as in *Lucifuga*, suggest



## Eye of Lucifuga.

A. Right eye of Lucifuga, 53 mm. long. Left eye compact; shown in plate 23 A. Retina drawn from one section; optic supplied from several sections. × 440.

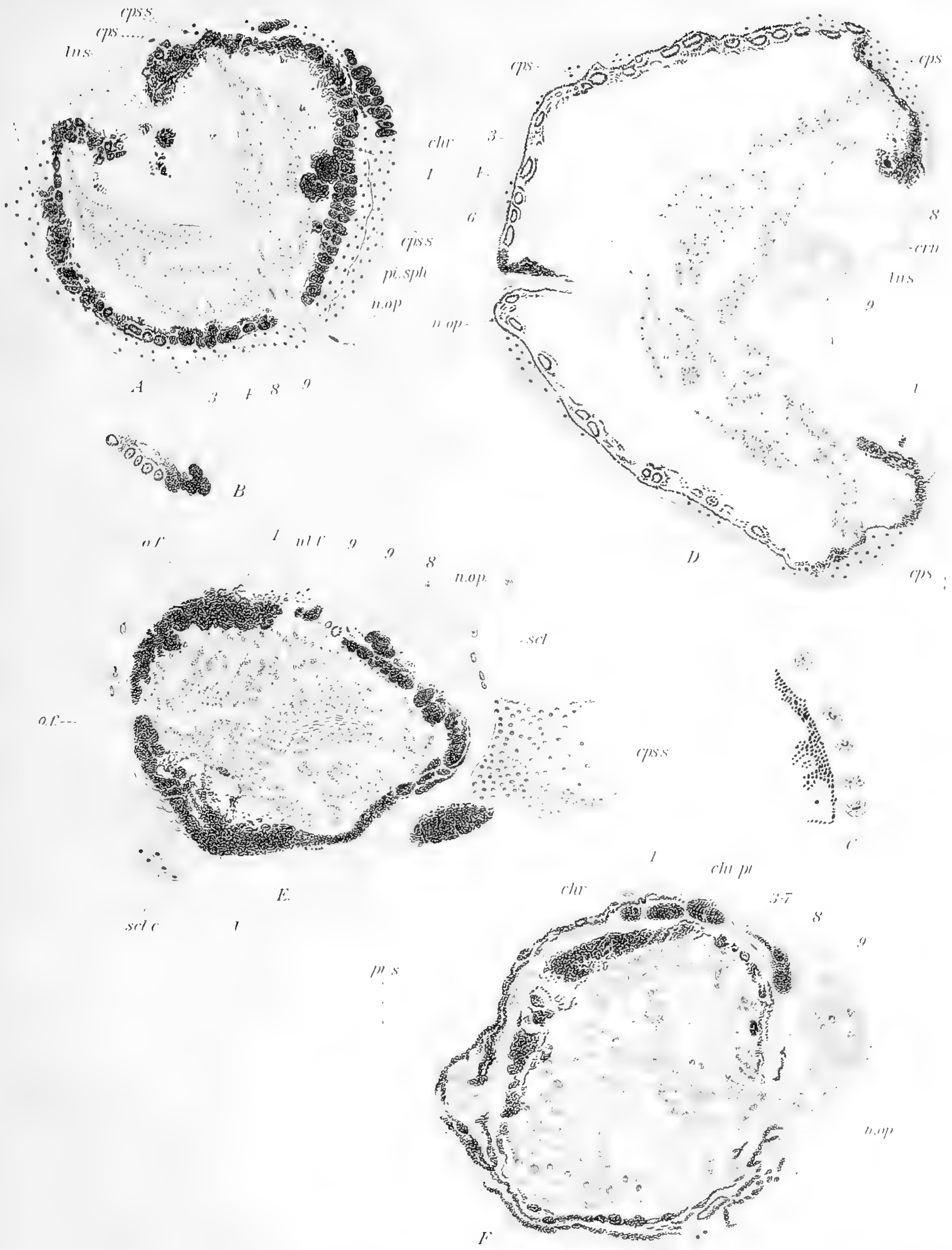
B and C. Left and right eye of Lucifuga. × 270.

D. Right eye of Lucifuga, 94 mm. long. (For left eye see plate 23.)

E. Part of retina of eye of Lucifuga shown in plate 18 A.

Eyes of Stygicola and Lucifuga.

A. Lucifugas 20 mm. long and ready to be liberated. See plate 15 D for others from the same ovary.
B. Dorsal view of head of a young Stygicola, about 20 mm. long.
C. (*a*) right and (*b*) left eyes of a young Stygicola 20 mm. long (No. 125*b*). In *b* details of marking of iris and ball are shown.
D. (*a*) and (*b*) right and left eyes of the only other individual from the same ovary (No. 125*a*).
E. Section of the left eye shown in C *b*.
F and G. Vertical sections of right and left eyes shown in D *a* and *b*. Seen from in front. × 390.
H. Section through middle of lens of left eye.



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Eye of Stygicola.

A. Left eye of Stygicola, 88 mm. long. From behind. × 390.
B. Fragment of pigment-layer, poor in pigment. From a neighboring section.
C. Pigment-cell from choroid of same eye; few sections removed.
D. Right eye of same fish, with same magnification.
E. Left eye of Stygicola, No. 125 (mother of 125 *a* and *b*). 92 mm. long. From in front. × 390. Section obliquely through optic nerve.
F. Left eye of Stygicola, 135 mm. long. From behind. In left of figure outline of mass of pigment lying distad from eye is drawn. × 700.

a larger eye, but in others it is considerably larger. The cartilage degenerates rapidly. In the eye of the mother of 125 *a* and *b* (92 mm.) only a few cells are left (plate 26, fig. E, *scl.c.*).

This history of the scleral cartilage in *Lucifuga* and *Stygicola* is in distinct contrast to its history in *Amblyopsis*. In the latter it appears as the last of the eye structures and remains after everything else has disappeared. The early history in *Lucifuga* and *Stygicola* is not known, but it disappears even more rapidly than the lens, only a few cells sometimes remaining longer. Aside from the cartilage the sclera consists of a thin, fibrous, nucleated membrane over the proximal face of the eye and a similar membrane, and the cornea over the distal face. The cornea may remain for a long time after birth or it may, especially if the eye becomes compact, disappear and be replaced by an accumulation of cells such as have been seen in *Amblyopsis* and *Lucifuga*. With age the sclera becomes a fibrous capsule of varying thickness (plate 26, figs. E and F).

### THE CHOROID AND THE ORBITAL PIGMENT.

The choroid in the eyes of the young consists of a thin membrane containing blood-vessels and pigment cells. Its structure can best be seen where it has accidentally become removed from the pigment layer by reagents. The blood-vessels may become so distended with blood that the thickness of this layer becomes several times its normal thickness. Between the choroid and the sclera in the young is a well-developed suprachoroidal lymph space. In contrast with *Lucifuga*, where this space is largest between the choroid and scleral cartilage, it is usually thin or absent in this region but comparatively well developed on the proximal face of the eye. In the old it is not evident (plate 25, figs. E and F, *chr. l.*). As in *Lucifuga* there appears, concomitant with the degeneration of the eye, an accumulation of pigment in the orbital fat or in the choroid. The outlines of such a mass in contact with the eye are shown in plate 26, figure F, representing the eye of a fish 135 mm. long. The accumulations of pigment in both eyes are very large — larger than the eye.

### THE LENS.

As in the eyes of *Lucifuga*, the lens degenerates and disappears more rapidly than other parts. The methods of degeneration are seen in the lenses of 125 *a* (plate 25, fig. H). The nuclei become distended, the chromatin accumulating in a few nucleoli-like granules. The membranes of the nuclei next dissolve and there results a mushy mass containing lumps of chromatin. The contents of the lens capsules are next removed in a manner not clear. Toward the end of this process the lens may be found to consist of a shrunken, fibrous membrane containing pigment granules and accumulations of pigment — possibly cells. There is not a vestige of the lens left in old individuals.

### THE IRIS.

The iris in the young appears much darker from the surface than the rest of the eye. In sections it is found to be not more densely pigmented than the pigment layer. The epithelial part may be entirely pigmented, or the inner cells may be partially free from pigment. At birth the pupil is larger than the lens and it remains so, becoming even larger in those eyes which become distended. In the eyes which contract, on the other hand, it becomes closed and the opening finally is entirely obliterated. The irideal parts are then indicated by a layer of pigment much thicker than elsewhere about the eye (plate 26, figs. E and F). This pigmentation over the front of the eye is not unique in the Cuban blind fishes. It is well marked in *Typhlomolge* and so striking in *Troglichthys* that Kohl in this species ruled the irideal pigment entirely out of the eye. Where the iris joins the retina, in the ciliary region, there are cells with elongated nuclei as usual.

### THE VITREOUS BODY.

The vitreous space and its history differ greatly in different eyes. For instance, in 125 *a*, right, it is very large, while on the other side it is almost entirely filled with the enlarged hyaloid blood-vessels (plate 25, figs. F and G). The factors that condition its structure in these two eyes also control its later history. In the eyes that contract the space becomes rapidly reduced and finally becomes obliterated (plate 26, figs. A and F). In the eyes that become vesicular it remains, unless the vesiculation is so pronounced that the eye becomes a hollow sphere with the pigment layer forming the larger part of the circumference, and the retina is literally turned out to form the front part of the sphere. In the latter case the vitreous space, or body, naturally is turned entirely out of the eye.

Between the vitreous body and the hyaloid membrane, or in the latter, pigment cells are sometimes found in old individuals.

### THE RETINA.

The pigment layer in the young 20 mm. long is a thin epithelial layer, well pigmented. In places where it is artificially separated from the rest of the retina processes extend from the pigment layer down, and from the nucleated layer up, indicating more than mechanical contact between the layers (plate 25, fig. G). Such conditions argue that there is a beginning, at least, of the differentiation of cones. The pigment layer never becomes more highly differentiated than in these young. In eyes that become distended the amount of pigment being scattered over a wider area is much less dense at any one point. (Compare right and left eyes of 126, plate 26, figs. A and D.)

Sometimes pigment cells, or simply accumulations of pigment, separate from the layer and come to lie between the layer and the rest of the retina. These are also found in *Lucifuga* (plate 26, fig. A). Whether this is a case of active migration of pigment cells or simply a result of mechanical crowding, I am unable to say.

The remaining layers of the retina may best be considered together. In young 20 mm. long (plate 25, figs. E, F, G) these consist of a ganglionic layer consisting of several series of cells. A nuclear layer, also consisting of several series of cells, lies immediately beneath the pigment layer. The nuclei are similar, there being little differentiation. Between the nuclear layer and the ganglionic layer is a sharply defined, broad, reticular layer. This is differentiated into a wider outer, a narrower inner, and a very narrow, more densely staining intermediate layer. In favorable sections stratifications can be made out in this entire layer.

Müllerian fibers are seen, but I have not identified the nuclei belonging to them.

I am not sure whether the conditions seen in older eyes are to be taken as the result of retrogressive changes, or of an abortive differentiation of an outer reticular layer with a separation of the nuclear into an outer and inner nuclear layer.

The definiteness of the reticular and outer nuclear layers is no longer found in older fishes. Instead, there is found an irregular series of cells bounding the epithelial face of the retina (plate 26, fig. A). The other nuclei of the outer layer, seen in younger fishes, are scattered irregularly through the retina, leaving, however, a distinct, inner reticular layer. The outer reticular layer (4) so formed is much thicker than in normal retinas, and it is otherwise so irregular that it can scarcely be considered the homologue of the outer reticular layer of normal retinas.

In the oldest eye examined even this degree of regularity is gone (plate 26, fig. F). There is an undoubted reduction in the number of nuclei, remains of which are seen as dark granules among the nuclei. The character of the nuclei in the older individuals differs; some still show granules, while others stain uniformly.

The optic nerve is very well developed in the young 22 mm. long, and can be as readily followed to the brain as in normal eyes. It becomes proportionately more slender with age. It is observable even in the oldest eyes, but in them it has been impossible to trace the optic nerves outside the bulb.

## ON THE OVARY AND OVA IN LUCIFUGA AND STYGICOLA.

(By Henry H. Lane.)

In the Biological Bulletin, vol. 6, No. 1, December, 1903, the ovarian structures of Cuban cave fishes, *Lucifuga* and *Stygicola*, were described as minutely as the few specimens then at hand would allow. A much larger series of the ovaries of these fishes has since been put at my disposal. A study of these 44 ovaries (21 of *Lucifuga* and 23 of *Stygicola*) enables me to correct some minor errors and to make some observations additional to those already recorded, and these are submitted as follows:

A few terms may be defined for the sake of clearness. These are:

*oviduct:* the unpaired duct leading from the ovary to the urogenital pore. It is not in Teleosts generally the homologue of the Müllerian duct of other vertebrates.

*ovisac:* the anterior enlargement and continuation of the oviduct, covering the ovary proper.

*ovary:* the organ containing the ova. It is, however, sometimes convenient to speak of the ovisac and ovary proper together simply as the "ovary." In such cases the context prevents ambiguity.

*stroma:* the tissues of the ovary proper other than the ova and their follicular membranes.

### GROSS STRUCTURE.

Externally the ovary is a Y-shaped, subcylindrical organ (plate 27) with a bilateral arrangement of the stroma. Its greatest diameter is usually immediately posterior to the point where the two horns begin. These horns of the ovary are right and left in position and may be long enough to inclose between them the posterior part of the stomach, though there is much variation in their length (plate 27). Within the ovisac the stem of the Y is divided by a median partition with which the ovarian structures proper are associated, in some ovaries more distinct than in others. This median sagittal partition extends posteriorly to the region I have chosen to consider the beginning of the oviduct, where in most cases only the part attached to the ventral wall persists; in others the part attached to the dorsal wall is also present, though separated from the ventral part by a fissure. From the tips of the ovarian horns slender but comparatively strong threads of connective tissue, inclosing blood-vessels, run cephalad and fasten to the peritoneal walls, thus assisting in securely holding the ovary in position. Dorsally, a *mesovarium* suspends the organ from the peritoneal lining of the body cavity, while ventrally there is a corresponding attachment, the *mesorectum*. Each horn of the ovary is supported by its own fold of peritoneum and these two become united at or near the point of division of the horns and are continued posteriorly as the single, thicker mesovarium supporting the body of the ovary and the oviduct. The mesorectum is not always complete in the region of the ovarian horns.

The *oviduct*, which has its external orifice at the urogenital pore, increases gradually in size as it extends forward toward the ovary and finally becomes the *ovisac* surrounding the ovary proper.

The size of the ovary varies with the age and size of the females as well as the state of development of the embryo or ova within it. One specimen, a *Lucifuga* 65 mm. long, had an ovary only 16 mm. long and 8 by 9 mm. in largest diameters, altho it contained 4 nearly ripe young, each 18 to 20 mm. in length. One non-pregnant *Lucifuga*, 83 mm. long, had an ovary but 12 mm. in length. These measurements were made on preserved specimens.

The point of division into the two horns is usually a little less than halfway from the anterior tip of the ovary to its posterior end. The two horns themselves are rarely equal in size, though there is no evidence of any tendency toward an unpaired condition through the "phylogenetic resorption" of one side such as Ryder found in *Gambusia patruelis*.

The space between the ovisac and the inclosed ovary varies in size and is continuous with the lumen of the oviduct. The growth of the young results in a gradual stretching of the ovisac, and to a certain extent of the oviduct also, so that near the close of gestation these structures are so extremely thin that their cellular nature can not be satisfactorily made out (plate 29, figs. A, B, C). Apparently within a short time after the birth of the young, the ovisac and oviduct contract and reassume the form and appearance found in the ovaries of mature non-pregnant females. The wall of the ovisac is then quite thick (*ov.s.*, plate 29, fig. D) and the lumen very small.

The stroma of the non-pregnant ovaries is a bilateral mass which occupies most of the space within the ovisac. Its general shape resembles that of the ovisac, being fusiform in its main part, with its greatest diameter just posterior to the division into the two horns. The horns of the stroma are attached to the horns of the ovisac along their median surfaces (plate 29, fig. A), the whole stroma forming a V. In the stem of the V the stroma forms a median dorso-ventral partition within the ovisac, which is to be looked upon as representing the area of fusion of the originally distinct right and left ovisacs (fig. B). Near its posterior end this partition is cut across laterally by a fissure and the two prongs thus formed, one dorsal, the other ventral, gradually disappear toward the oviduct (figs. C and D). Sometimes the dorsal one disappears first, sometimes both are equally extensive.

The stroma has many somewhat pointed and comparatively large lobes, which are usually connected with the main mass by a slender "neck" of tissue. Dr. Eigenmann observed that these lobes are sometimes held in the mouth of the young fish during a part at least of its later development. Whether the young derives any nourishment from the lobes can not be stated with certainty. The whole stroma in the non-pregnant ovaries is distended by a large amount of lymph and adipose tissue contained in the sinuses described below, especially when approaching the reproductive period as shown by the maturity of the ova.

The largest ova can be seen through the ovisac by the unaided eye as opaque white dots in the preserved specimens (plate 27). The follicles surrounding these ova usually lie some distance beneath the surface of the stroma and a tubular indentation of the epithelial covering of the latter extends down to the follicle (plate 29, fig. E). The blind end of this pit is so closely applied to the follicular membrane that it usually requires a very close inspection to discover its independence. It is then found that the follicular membrane at this place is only a single cell layer in thickness. Stuhlmann describes a similar indentation of the epithelium over the ova in the ovary of the viviparous blenny, *Zoarces viviparus* Cuvier.

By the time the young are well advanced, *i.e.*, 18 to 20 mm. long, the lymph sinuses of the stroma have mostly lost their contents and the stroma itself has become very greatly reduced and compressed into a narrow median wall (plate 29, fig. B). There are no "pockets" in which the young are carried as in *Cymatogaster* and other *Embiotocidæ* (Eigenmann), or as in *Anableps* (Wyman), but instead the young attach themselves by the mouth to the ovarian lobes, or lie free within the lumen of the ovisac.

The single oviduct, as well as the ovisac, is widely distended in pregnant females when the young are well advanced. In the non-pregnant females the duct is a cylindrical, thick-walled, muscular tube with numerous folds on its inner surface, which is covered with a layer of columnar epithelium similar in all respects to that of the ovisac.

### MINUTE STRUCTURE.

*The Ovisac.*—The ovisac, as noted, varies greatly in appearance, depending on the length of the pregnancy or the time since the close of that period. In normal, non-pregnant ovaries it varies from 100 to 150 $\mu$ in thickness. Structurally it is composed of at least 4 cell layers. The outermost is the ordinary peritoneal layer continuous with the lining of the body cavity; second, a thicker layer of longitudinal muscle fibers which lie immediately below the peritoneal covering; third, a somewhat thicker band of transverse muscle fibers; fourth, the inner lining of epithelium, which, in some instances at least, contains numerous blood capillaries. In the case of pregnant females, the ovisac is more or less thinned through stretching, until, when the young are well advanced, the cell layers can scarcely be distinguished. (See plate 29, figs. A, B, C.)

*The Ovary.*—The ovarian structures proper are highly vascular and much lobed. In some instances the egg follicles are surrounded by a network of blood capillaries. The greater part of the stroma is split up into numerous sinuses (*st.*, plate 28), many of which are larger than any of its blood-vessels. The epithelial layer covering the stroma frequently contains numerous capillaries each with a diameter of 5 to 8 $\mu$ (plate 29, fig. F). In some instances these capillaries are very numerous; in others, they are scarcely, if at all, perceptible. This difference is due to the different degrees of distention of the stroma by the lymph in its sinuses. The stroma itself consists of a mass of connective tissue and non-striated muscle fibers in which are embedded the ova in various stages of development.

*The Follicle.*—The smallest ova (5 to 10 $\mu$ in diameter) have no trace of a follicular membrane around them individually. Somewhat larger ova (100 to nearly 400 $\mu$ in diameter) are surrounded by a single layer of elongate cells, quite similar to the stroma cells. In the case of more mature ova (over 400 $\mu$ in diameter) there is a distinct follicle consisting of a single layer of appressed quadrangular cells about 6 $\mu$ in depth; outside of this is a layer of somewhat irregular cells, in many cases surrounding blood capillaries 6 to 10 $\mu$ in diameter. The thecal wall outside the capillary layer consists of from 1 to 3 cell layers of long, spindle-shaped cells resembling those composing the stroma itself. The medium-sized ova (about 400 $\mu$ in diameter) lie close beneath the surface of the ovary (*o.*, plate 29, fig. D), but the largest ova (600 $\mu$ and over in diameter) are usually found rather deeply embedded within the stroma, except for the tubular indentation from the surface of the latter which reaches down to the follicle and possibly affords later a means of entrance of the spermatozoa to the mature egg.

*The Ova.*—In the smaller ova (under 400 $\mu$ in diameter), the nucleus is usually quite distinct and has approximately one-third the diameter of the whole ovum. The cytoplasm, not yet deeply laden with deutoplasm, has usually a reticulated or alveolar appearance. In the larger ova (over 400 $\mu$ in diameter), the cytoplasm becomes more and more heavily laden with deutoplasm, until in the largest it is almost wholly obscured by the latter. The nucleus at the same time becomes correspondingly more difficult to find, not increasing much in size as the ovum develops. No traces of maturation were detected in even the largest ova found (750 $\mu$ and over in diameter).

More or less deeply within the stroma the ova arise in masses of several hundred ova each. In size the smallest discernible ova measure from 5 to 10 $\mu$ in diameter and have well-defined nuclei (*s.o.*, plate 28, fig. A). As development proceeds a number of ova in each "nest" may increase more than the others; at a later stage it can be seen that a few of these are gaining on their fellows; and still later *one* is seen to be outstripping all the others in that "nest." Sometimes one (*l.o.*, plate 28, fig. A) gains the ascendency so early that the remainder (*s. o.*, plate 28, fig. A) never show any marked increase or difference in size among themselves. In any case, in the final stage of development, *a single ovum is left in the "nest,"* and this now seems to migrate till it rests just beneath the surface of the ovary itself (*o.*, plate 28, fig. C). Where several large ova seem to have been developed in one "nest," close scrutiny, at least in the case of the less fully developed ova, invariably reveals a separating layer of very thin, elongated stromal cells (*st.c.*, plate 28, fig. F) such as originally surrounded the whole "nest" (compare with *st.c.*, plate 28, figs. A or D), thus showing that these larger, closely adjacent ova are derivatives each from an originally distinct "nest."

The fate of the other ova which at first lay in the same "nest" with the larger ovum is a question of interest. Two possibilities suggest themselves: either the growing ovum absorbs the neighboring ova into its own substance, or they disintegrate *in situ* without becoming a part of the larger ovum.

Certain of the larger ova very strongly suggest the first possibility. In these the smaller ova are grouped together at the side of the much larger one, or may even surround it, and are apparently undergoing a greater or less amount of disintegration. In the case shown (*a. o.*, plate 28, fig. B) this disintegration has gone so far that the outlines of the small ova are quite indistinct and in some cases apparently only the nucleus remains, and this, too, is no more than an irregular mass of chromatin.

*The atrophy of the small ova is evidently a rapid one, for there is no sign of any pigmentation or other mark of a gradual degeneration of the cells.* Moreover, wherever a "nest" contains a larger ovum and smaller atrophying ova around it, the cytoplasm of the ova is confluent, at least in the case of those most advanced in disintegration. This in itself is good evidence of the assimilation of the degenerating ova by the larger one. In short, *there is here a struggle for existence among the ova of each "nest."* The successful ovum either *produces a rapid degeneration* of the surrounding ova, or taking advantage of such a condition *produced in them by some unknown factor, assimilates them* into its own substance. It is hard to determine what is the all-important cause of the initiation of the more rapid growth of the superior ovum, but one possibility is that of a more fortunate situation in regard to the source of nutrition. In most cases, if not in all, *the ova which gain in size over the others lie in such a position in the "nest" as to be more nearly in contact with the stromal blood capillaries than the others*, and this very likely furnishes the explanation of the phenomenon noted.

In case, for any reason, *no ovum in a "nest" develops, all the ova in that "nest" undergo a slow, pigmented degeneration, or atrophy.* The evidence for this lies in the presence within the stroma of masses of yellowish-brown cells which do not stain with hæmatoxylon. For convenience I shall speak of them as the "yellow cells." Their nuclei may be evident only as small, deeply staining masses of chromatin; or the chromatin may have the form of a more or less definite spireme; but in many cases the nuclear material shows signs of karyorrhexis, being decidedly broken up and apparently migrating into the cytoplasm of the cell. A reference to plate 28, D and E, will show that these cells can not be red blood corpuscles, for, not only do they not lie within a blood-vessel, but they are also more than twice as large as undoubted red blood corpuscles in the same section. They have none of the characters of leucocytes or phagocytes, but *they do exhibit the typical brown or yellowish pigmentation of degenerating epithelial cells.*

As shown (*st.c.*, plate 28, fig. D), the yellow cells lie within a space surrounded by exactly the same sort and arrangement of stromal cells as those which inclose undoubted "nests" of ova. Hence they must be regarded as either degenerating ova or cells which have taken the place of ova. As already stated, they have none of the characters of phagocytes. One other possibility is suggested by the fact of the viviparity of these fishes; that is, the possibility that these yellow cells may represent a sort of *corpus luteum.* Aside from the structure of these cells, which do not have more than a very faint resemblance to the lutein cells of mammalian *corpora lutea*, one consideration very effectually disposes of this possibility; namely, the fact that *these yellow cells do not occur in the larger ovaries, i.e.*, in those of the more mature females, *but on the contrary they occur in the smaller and even the smallest ovaries at hand.* They certainly can not therefore be of the nature of *corpora lutea* cells. That *they are degenerating ova* seems to me the most probable conclusion, for the following reasons:

- (*a*) The "yellow cells" occur only in masses, exactly similar, in point of number and size of cells as well as in position in the stroma, to the "nests" of young ova.
- (*b*) The masses of "yellow cells" are surrounded by the same sort and arrangement of stromal cells as surround the "nests" of ova.
- (*c*) The "yellow cells" exhibit the typical brown pigmentation of slowly atrophying epithelial cells.
- (*d*) There are no cells of sufficient size in these ovaries which could have these characters except degenerating ova in "nests."

These fishes are undoubtedly descended from oviparous forms, and viviparity is probably a comparatively recent acquirement, though most probably attained before the change of habitat from the sea to the underground streams of Cuba. Some at least of the marine members of the Brotulidæ are also viviparous. *The production of the many "nests," each with its hundreds or even thousands of young ova, is a reminiscence of the oviparous condition*, when it was necessary for the preservation of the species that a multitude of young be produced, as in the case of the oviparous fishes. The condition of viviparity, providing as it does for the greater safety of the young during the most critical period of their development, and their habitat in caves where the number of enemies is probably greatly less than in the sea enable these species to maintain themselves by the production of fewer offspring.

### SUMMARY

1. The *ovary* in *Lucifuga* and *Stygicola* consists of a mass of *stroma* containing the *ova* and covered with epithelium; the whole structure is V-shaped and is contained within the *ovisac;* the latter is continued to the urogenital pore as the *oviduct.*
2. The *epithelium*, lining the ovisac and covering the ovary proper, is unique in that it frequently contains numerous blood capillaries.
3. The sinuses within the stroma are filled with lymph and adipose tissue.
4. *Lucifuga* and *Stygicola* are viviparous blind fishes which give birth to but few young, 2 to 15 so far as yet observed.
5. The young are not developed in separate sacs, but lie within the lumen of the ovisac, gradually compressing the ovarian stroma as they develop.
6. The ova arise in "nests" or masses of several hundred each. The smallest observed have a diameter of 5 to 10 $\mu$.
7. *One ovum from each "nest" is developed to maturity; the other ova of the "nest" undergo rapid degeneration and are ultimately absorbed into the substance of the large ovum.*
8. *In those "nests" in which none attains maturity, all the ova undergo a slow, pigmented degeneration in situ.*
9. *The destruction of so many ova at an early stage is an adaptation to the viviparous habit.*
10. *Viviparity is probably a comparatively recent acquirement of these fishes,* though attained before these genera left the sea for the fresh-water cave streams.

## CONCLUSIONS IN REGARD TO LUCIFUGA AND STYGICOLA.

1. *Lucifuga* and *Stygicola* are two marine fishes that have remained in the cracks and caves of the coral beaches which they inhabited, as these caves were elevated and became filled with and enlarged by fresh water. They have become entirely adjusted to a fresh-water environment.

2. *Stygicola* is known from both the north and south slopes from Alacranes to Matanzas and Alfonso XII. *Lucifuga* is known only from the south slope west of Havana.

3. The caves in which the fishes were found are all well lighted, but are always connected with dark underground channels. Each cave has only a limited supply of fishes that may be replenished from the underground channels.

4. *Lucifuga* and *Stygicola* are negatively heliotropic. They are adjusted to withstand but slight temperature changes. They feed on crustaceans and odonata larvæ.

5. Both species are viviparous, giving birth to 2 to 15 young about 25 mm. long. Both probably breed throughout the year. Spermatozoa are transferred long before the ripening of the eggs. *Lucifuga* breeds probably most abundantly through March and May in shallow places. Its young are abundant near the surface. *Stygicola* breeds in unknown places and its young are not seen near the surface.

6. The eye decreases in size progressively from birth to extreme old age concomitantly with the appearance of masses of pigment cells in the orbital fat.

7. The eye varies greatly in different individuals of the same size — from 260 to 425 $\mu$ in length, in brothers and sisters in the same ovary.

8. The ontogenetic degeneration results either in the shriveling of the entire structure or the great distention of the pigmented layer. One process may be found on one side, the other on the other side of the same individual.

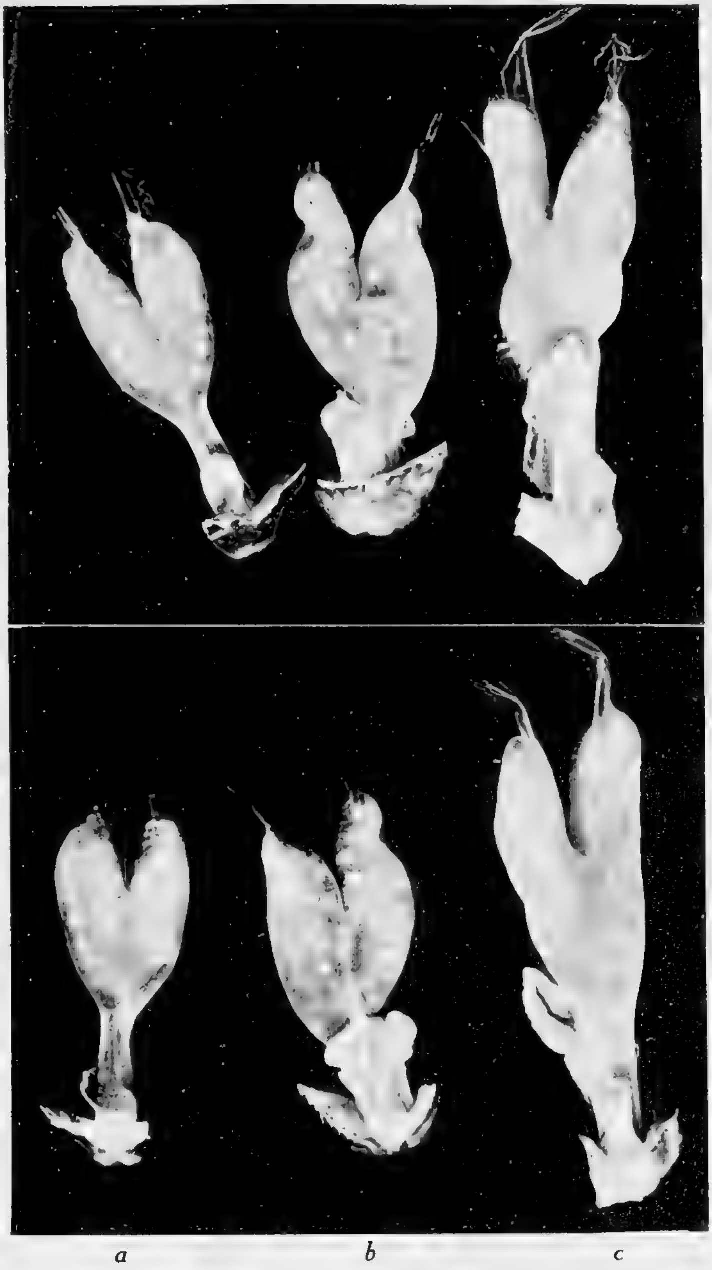
9. The eye muscles are all present in the young, but undergo a variable amount of degeneration with age, disappearing entirely in very old of *Stygicola*.

10. The sclera is self-determining in both *Lucifuga* and *Stygicola*. In *Lucifuga* the cartilages at the time of birth are too large for the eye, forming a shield over the face of the eye. In *Stygicola* it forms a ring about the middle of the eye. After birth they very rapidly degenerate and disappear entirely by the time *Lucifuga* has reached less than half its maximum length. In *Stygicola* it remains longer.

11. There is evidence that there is an early disturbance of the vascular system of the eye resulting in the formation of large blood lakes about the eye.

12. The lens has begun to degenerate before birth. Its contents liquefy, the capsule shrivels, and finally disappears at a length of about 40 mm.

13. It has not been determined when the histogenesis of the retina ends and its degeneration begins. The most highly developed retina was found in an unborn young of *Lucifuga* 20 mm. long. In this retina the outer nuclear, outer reticular, inner nuclear, inner reticular, and ganglionic layers are more or less distinctly represented.



Ovaries of Lucifuga and Stygicola.

A. Dorsal aspect. Round, opaque, white dots are larger ova seen through stroma and ovisac.

B. Ventral aspect. × 2 diameters.

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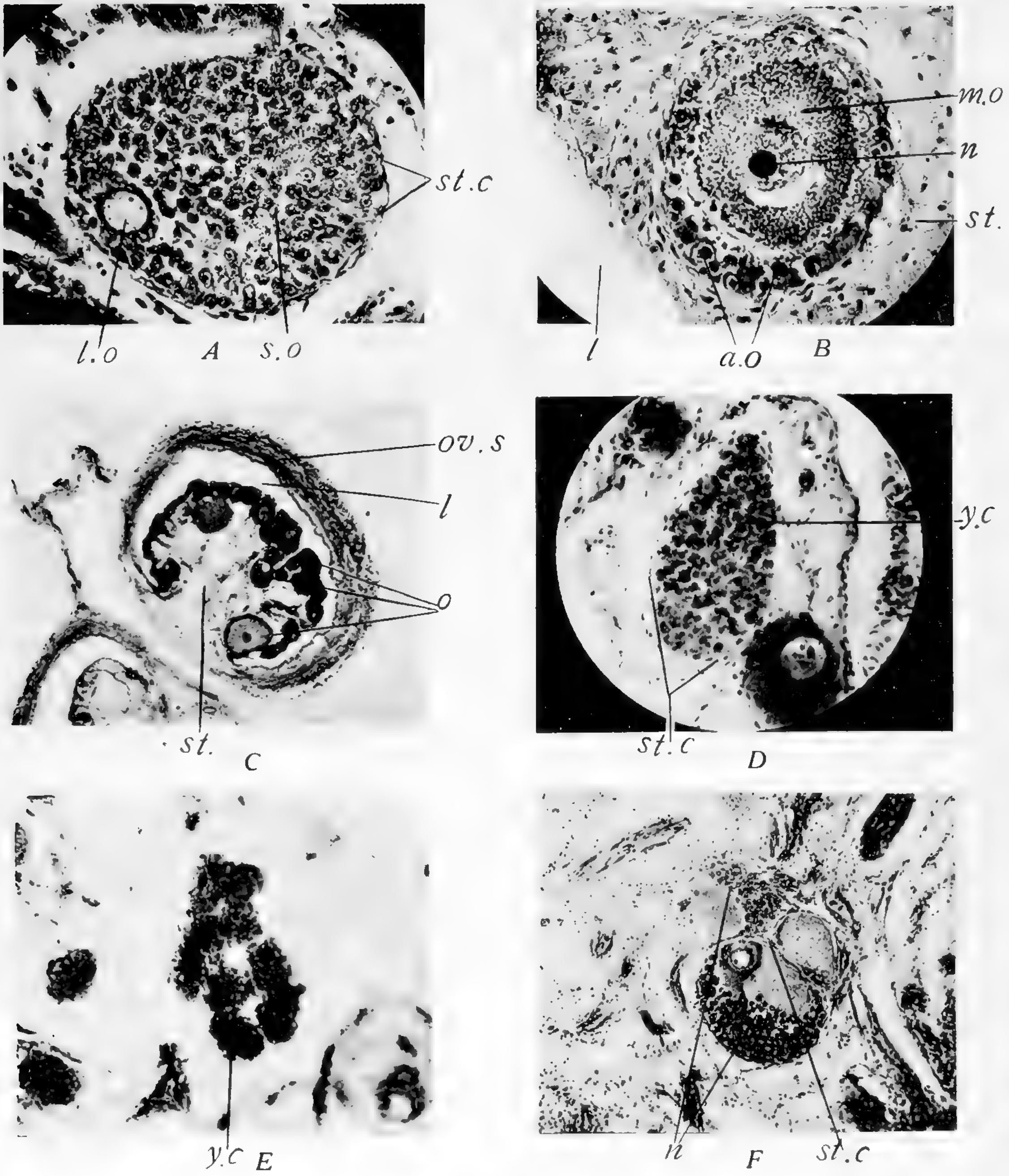

A. "Nest" of small ova (*s. c.*), each about 10 $\mu$ in diameter, one larger ovum (*l.o.*) 50 $\mu$ in diameter. Whole nest contained within special arrangement of stromal cells (*st. c.*). × about 300 diameters.

B. Developing ovum (*m. o.*), surrounded by rapidly atrophying small ova (*a. o.*); *l.*, lumen of ovisac; *n.*, "germinative spot"; *st.*, stroma. Diameter of large ovum, 120 $\mu$. × 500 diameters.

C. Cross-section of one horn of ovary. *l.*, lumen of ovisac; *o.*, ova; *ov. s.*, ovisac; *st.*, stroma. Guide line to *st.* crosses place of attachment of stroma to median wall of horn of ovisac. × about 50 diameters.

D. "Nest of yellow cells." Diameter of individual "yellow cells" (*y. c.*) about 15 $\mu$ *st. c.*, arrangement of stromal cells around yellow cells, as around "nest" of small ova (*st. c.*, in fig. A.) × 200 diameters.

E. Few "yellow cells" more highly magnified to show pigment-granules and general appearance of slow degeneration. Nuclei can not be distinguished in photograph, though distinct enough in section. × about 800 diameters.

F. 3 adjacent "nests" of ova (*n.*), each with developing ovum; *st. c.*, stromal cells which separate "nests" and likewise developing ova from one another. × 210 diameters.

Sections of Ovaries.

A. Cross-section through horns of pregnant ovary.
B. Cross-section through middle part of pregnant ovary. Ovisac collapsed when fetuses were removed.
C. Cross-section through posterior part of pregnant ovary.
D. Cross-section of non-pregnant ovary with stroma in two lobes, one dorsal, other ventral.
E. Part of cross-section of non-pregnant ovary.
F. Part of epithelial covering of non-pregnant ovary showing capillaries (*cps*).

# THE
# CAUSES OF INDIVIDUAL AND PHYLETIC DEGENERATION

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It may now be profitable to take up the causes leading to the small degree of degeneration found in *Chologaster*, the degeneration of the eye in *Amblyopsis*, *Typhlichthys*, and *Troglichthys* to a mere vestige, together with the total disappearance of some of the accessory structures of the eye, as the muscles, in some of the species. In the outset of this consideration we must guard against the almost universal supposition that animals depending on their eyes for food are or have been colonizing caves, or that the blind forms are the results of catastrophes that have happened to eyed forms depending on their eyesight for their existence. This idea, so prevalent, vitiates nearly everything that has been written on the degeneration of the eyes of cave animals.

The degeneration of organs ontogenetically and phylogenetically has received a variety of explanations.

(1) The organ diminishes with disuse (ontogenetic degeneration, Lamarck, Roux, Packard) and the effect of this disuse appears to some extent in the next generation (phylogenetic degeneration, Lamarck, Roux, Packard).

(2) Through a condition of panmixia the general average maintained by selection is reduced to the birth mean in one generation (ontogenetic, Romanes, Lankester, Lloyd Morgan, Weismann) to the greatest possible degeneration in succeeding generations (phylogenetic, Weismann), or but little below the birth average of the first generation (Weismann's later view, Romanes, Morgan, Lankester).

(3) Through natural selection (reversed) (the struggle of persons) the organ may be caused to degenerate either (*a*) by the migration of persons with highly developed eyes from the colony living in the dark (Lankester), or (*b*) through economy of weight and nutriment or liability to injury (phylogenetic purely, Darwin, Romanes).

(4) Through the struggle of parts (*a*) for room, an unused organ in the individual may be crowded (ontogenetic, Roux), (*b*) for food, this may lead to the development of the used organ as against the disused through a compensation of growth (Goethe, St. Hilair, Roux); this ontogenetic result becomes phylogenetic through transmission of the acquired character (Roux), or is in its very nature phyloblastic (Kohl).

(5) Through the struggle between soma and germ to produce the maximum efficiency of the former SOMA with the minimum expenditure of the latter GERM (ontogenetic and phylogenetic, Lendenfeld).

(6) Through germinal selection, the struggle of the representatives of organs in the germ (ontogenetic and phylogenetic, Weismann).

(7) To these special considerations should be added the recently suggested general process of mutation.

The idea of ontogenetic degeneration is intimately bound up with the idea of phylogenetic degeneration. Logically we ought to consider first the causes of individual degeneration and then the processes or causes that led to the transmission of this. Practically it is impossible to do so, because many of the explanations are general. Only number (4) of the above may be taken in the ontogenetic sense purely, though it was certainly also meant to explain phylogenetic degeneration. In many of the explanations of particular cases of degeneration more than one of the above principles are invoked, though only one was meant to be used. In most cases, however, the discussions of degeneration have been in general terms, without direct bearing on any specific instance of degeneration in all its details. It must be evident that such discussions can only by accident lead to right results.

By the Lamarckian ontogenetic degeneration is considered the result of lack of use and consequent diminished blood supply. The results of the diminution caused by the lack of use during one generation are transmitted in some degree to the next generation, which thus starts at a lower level. A continuation of the same conditions leads finally to the great reduction and ultimate disappearance of an organ.

No one, so far as the author knows, has attempted, or, perhaps better, succeeded, in accounting with this factor in detail for the degeneration of the eye. Packard's explanations are evidently a mixture of Lamarckism and Darwinism.

Packard says, "When a number, few or many, of normal seeing animals enter a totally dark cave or stream, some may become blind sooner than others, some having the eyes slightly modified by disuse, while others" may have in addition physical or functional defects, especially in the optic nerves and ganglia. "The result of the union of such individuals and of adaptation to their stygian life would be broods of young, some with vision unimpaired, others with a tendency to blindness, while in others there would be noticed the first steps in degeneration of nervous power and nervous tissue." Packard evidently had invertebrates in mind. He clearly admits the cessation of selection or panmixia which is implied by his supposition that those born with defects may breed with the others. He supposes that the blind fauna may have arisen in but few or several generations, a supposition that may be applicable to invertebrates, but certainly may not be applied to the vertebrates. At first those becoming so modified that they can do without the use of their eyes would greatly preponderate over those "congenitally blind." "So all the while, the process of adaptation going on, the antennæ and other tactile organs increasing in length and in the delicacy of structures, while the eyes were meanwhile diminishing in strength of vision and their nervous force giving out, after a few generations, perhaps only two or three, the number of congenitally blind would increase, and eventually they would, in their turn, preponderate in numbers." Packard seems here to admit the principle of degeneration as the result of compensation of growth, the nervous force of the eye giving out with the increase of the tactile and olfactory organs. It is somewhat doubtful in what sense the term "congenitally blind" is used, but it probably means born blind as the result of transmitted disuse rather than blind as the result of fortuitous variation. The effects of disuse are thus supposed through their transmission to have given rise to generations of blind animals. The continued degeneration is not discussed.

In 1873, 1874, and 1890, Romanes, in a series of articles in "Nature" and later in "Darwin and after Darwin," II, page 291 *et seq.*, maintained that the beginning of degeneration is due to cessation of selection, and continued degeneration to the reversal of selection and final failing of the power of heredity. Selection he supposed to be reversed because the organ no longer of use "is absorbing nutriment, causing weight, occupying space, and so on, uselessly. Hence, even if it be not also a source of actual danger, economy of growth will determine a reversal of selection against an organ which is now not only useless, but deleterious." This process will continue until the organ has reached "so minute a size that its presence is no longer a source of detriment to the organism, the cessation of selection will carry the reduction a small degree further; and then the organ will remain as a 'rudiment.'" Since, however, we can not consider that the force of heredity is everlasting, it will eventually fail and the organ dwindle still further and disappear. This failure of heredity, Morgan ("Animal Life," page 793) is unable to distinguish from the effect of disuse without which "the reduction of organs is difficult to explain."

The principles involved in this explanation are panmixia natural selection, and, according to Morgan, disuse transmission.

Weismann ("Nature," 1886, and "Essays," vol. II, 1) contended that cessation alone, or panmixia as he terms it, is sufficient to account for all degeneration. He later gave up this view for his theory of germinal selection, of which more later.

Roux, starting with the then generally accepted view that acquired characters are transmitted, attempted chiefly to explain degeneration in the individual. Degeneration is looked upon as the result of a struggle among the parts for (*a*) room and (*b*) food. He emphasizes the fact that a reduced functional activity continued for a long period reduces the functional possibility of an organ (page 176). The diminished use not only brings about this simple atrophy, but also the reduction, by stronger neighbors, to such a volume as is still of advantage to the animal. Disused organs that are not in the struggle for room may maintain themselves a long time. The struggle among parts for food, which implies the principle of compensation of growth of Goethe, need not take place through the withdrawal of blood, but may take place through the more active osmotic selection by the stronger organ of food that would otherwise go to the weaker.

Without doubting that both these principles are active agents in degeneration, it may be seriously doubted whether they were effective in the degeneration of the eyes in question. Certainly there can be no question of a struggle for room, for the position and room formerly occupied by the eye is now filled with fat which can not have been operative against the eye. The presence of this large fat-mass in the former location of the eye, the large reserve fat-mass in the body, the uniformly good condition of the fish, and the low vitality which enables them to live for months without visible food, all argue against the possibility that the struggle for food between parts was an active agent in the degeneration of the eyes.

Kohl considers that "Der Grund, und direkter oder indirekter Anlass zum Eintreten der Entwickelungshemmung ist Lichtmangel." The method of the direct operation of the lack of light he conceived to be as follows: The ancestry of blind animals lived where the light was uninterrupted and they had developed eyes. They got into an environment where the light was shut off more or less. The first generations retained their fully developed eyes without, however, being able to put them to full use. In consequence during phylogeny other organs became highly developed to compensate for the disuse of the eye. (Through natural selection?) Thus touch organs (*Myxine*, *Siphonops*) or the auditory organs (*Talpa* and possibly *Typhlichthys*) became more highly developed. The eye was unnecessarily highly developed. A process of degeneration (Rückbildung) began, which was never very extensive. Much more potent in placing the eye in harmony with its environment was the fact that every succeeding generation developed its eye less. This process of Hemmung of the eye did not begin until the developmental force began to go to the development of the compensating organs. On account of the loss of this developmental force the eye was unable to reach, in successive generations, the former grade. The degeneration is thus explained as the result of a struggle of parts, although this term is nowhere used, acting through the principle of compensation. The same objections may be offered to this explanation of Kohl as to all his theoretical discussions; they are based on the assumption of conditions and processes that have no existence. The high development of "compensating" organs is not primarily the result of the loss of the eye, but the high development of the former organs permitted the disuse and later degeneration of the later. His whole process is a phylogenetic one without a preceding ontogenetic one, though on this point he does not seem to be very clear himself, for on one page we are told that degeneration leads to retardation, and on another that degeneration is a consequence of retardation.

Lendenfeld endeavors to apply Roux's Kampf der Theile with reversed selection to explain the conclusions reached by Kohl on the processes and causes of degeneration. The struggle is represented to take place between the germ and soma, the former endeavoring to keep the latter at the lowest efficient point as weapon for the germ. If a series of individuals gets into the dark, the organs of vision are of no advantage, and reversed selection will bring about their degeneration. The saving in ontogeny appears first as a retardation and then a cessation of development.

Weismann later accepted the view of Romanes, Morgan, and Lankester of the inadequacy of panmixia to explain the whole phenomena of degeneration, and in his "Germinal Selection" rejects the idea of reversed selection and suggests a new explanation for what Romanes attributed to the failure of heredity and the Lamarckians to disuse transmission. The struggle of the parts, of Roux, has been crowded back by him to the representatives of these parts in the germ.

"The phenomena observed in the stunting, or degeneration, of parts rendered useless show distinctly that ordinary selection, which operates by the removal of entire persons, personal selection, as I prefer to call it, can not be the only cause of degeneration; for in most cases of degeneration it can not be assumed that slight individual vacillations in the size of the organ in question has possessed selective value. On the contrary, we see such retrogressions affected apparently in the shape of a continuous evolutionary process determined by internal causes, in the case of which there can be no question whatever of selection of persons or of a survival of the fittest, that is of individuals with the smallest rudiments." The gradual diminution, continuing for thousands and thousands of years and culminating in its final and absolute effacement, can only be accomplished by germinal selection. Germinal selection as applied to degeneration is the formal explanation of Romanes' failure of the hereditary force and the establishment of disuse effects in the heredity through the struggle of parts for food. "Powerful determinants will absorb nutriment more rapidly than weaker determinants. The latter, accordingly, will grow more slowly and will produce weaker determinants than the former." If an organ is rendered useless, the size of this organ is no longer an element in personal selection. This alone would result in a slight degeneration. Minus variations are, however, supposed to rest "on the weaker determinants of the germ, such as absorb nutriment less powerfully than the rest. This will enable the stronger determinants to deprive them even of the full quantum of food corresponding to their weakened capacity of assimilation and their descendants will be weakened still more. Inasmuch now as no weeding out of the weaker determinants of the hind leg (eye) by personal selection takes place on our hypothesis, inevitably the average strength of this determinant must slowly but constantly diminish, that is, the hind leg (eye) must grow smaller and smaller until it finally disappears altogether." "Panmixia is the indispensable precondition of the whole process; for owing to the fact that persons with weak determinants are just as capable of life as those with strong, solely by this means is a further weakening effected in the following generations."

This theory presupposes the complex structure of the germplasm formulated by Weismann. But granting Weismann the necessary structure of the germplasm, can germinal selection accomplish what is claimed for it? I think not. Granting that variation occurs about a mean, would not all the effects claimed for minus variations be counteracted by positive variations? Eye determinants, that on account of their strength secure more than their fair share of food and thereby produce eyes that are as far above the mean as the others are below, may leave descendent determinants that are still stronger than their ancestry. It is evident that a large, really extravagant development of the eye in such a fish as *Chologaster* would not effect the removal of the individual by personal selection, still less so in *Amblyopsis*, which not only lives in comparative abundance, but has lived for 20 months in confinement without visible food. It seems that all the admitted objections to degeneration by panmixia apply with equal force to germinal selection. This, however, would be changed were the effect of disuse admitted to affect the determinants, and this it seems Weismann has unconsciously admitted. So far we have considered germinal selection in the abstract only. In the concrete we find that degeneration is not a horizontal process affecting all the parts of an organ alike as Weismann presupposes, not even a process in the reverse order of phyletic development, but the more vital, most worked parts degenerate first with disuse and panmixia, the passive structures remain longest. The rate of degeneration is proportional to the past activity of the parts and the statement that "passively functioning parts, that is, parts which are not alterable during the individual life by function, by the same laws also degenerate when they become useless" is not applicable to the eyes. As one example of the unequal degeneration we need only call attention to the scleral cartilages and the rest of the eye of *Troglichthys rosæ*.[1]

All are agreed that natural selection alone is insufficient to explain all, if any, of the processes of degeneration. All either consciously or not admit the principle of panmixia, and all are now agreed that this process alone can not produce extensive degeneration. All are agreed that the important point is degeneration beyond the point reached by panmixia, the establishment of the degenerating process, whatever it may be, in the germ, or in other words, breaking of the power of heredity. It is in the explanation of the latter that important differences of opinion exist.

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[1] I must again guard against cross-counter conclusions. In the Brotulidæ the passive cartilages are among the first things to go.

Weismann attempts to explain the degeneration beyond the point which panmixia can reach by a process which not only is insufficient, if all his premises are granted, to produce the desired result without the help of use transmission, but has as its result a horizontal degeneration which does not occur in the eyes.

Romanes supposed degeneration, beyond the point which may be reached by panmixia, to be the result of personal selection and the failure of the hereditary force. The former is not applicable to the species in question and is denied by such an ardent Darwinian as Weismann to be applicable at all in accounting for degeneration. Moreover the process as explained by Romanes would result in a horizontal degeneration which has no existence in fact. The second assumption, the failure of hereditary force, is not distinguishable, as Morgan has pointed out, from the effect of use transmission.

The struggle of parts in the organism has not affected the eye through the lack of room, since the space formerly occupied by the eye is now filled by fat and not by an actively functioning organ. It is not affected by the struggle for food, for stored food occupies the former eye space. It could only be affected by the more active selection of specific parts of food by some actively functioning organ. It is possible that this has in fact affected the degeneration of the eye. The theory explains degeneration in the individual and implies that the effect in the individual should be transmitted to the next generation. This second fact seems but the explanation of the working of the Lamarckian factor.

Mutation can produce definitely directed evolution such as we find in the degenerating eye only when each step, each successive mutation, has an advantage over the mother or sister lines. I do not think that any one after familiarizing himself with the variation of the eye and its insignificance will maintain that this minute organ is now or has been for many generations of selective value. If it is not of selective value, mutation is as powerless to account for its condition as is natural selection of favorable variations.

The eyes of the two sides vary so much, independent of each other, that we are forced to conclude that there has been no check on their variation for a long period.

The only answer to the objection that the eyes are not the result of personal selection is that they may be so correlated with another organ inversely proportionate to it, that the selection of individuals with this other organ in favorable condition carries with it the selection of individuals with the eye in decreasingly imperfect condition. No such organ is available.

The Lamarckian view, that through disuse the organ is diminished during the life of the individual, in part at least on account of the diminution of the amount of blood going to a resting organ, and that this effect is transmitted to succeeding generations, not only would theoretically account for unlimited progressive degeneration, but is the only view so far examined that does not on the face of it present serious objections. Is this theory applicable in detail to the conditions found in the Amblyopsidæ? Before going farther, objections may be raised against the universal assumption that the cessation of use and the consequent panmixia was a sudden process. This assumes that the caves were peopled by a catastrophe. But it is absolutely certain that the caves were not so peopled, that the cessation of use was gradual and the cessation of selection must also have been a gradual process. There must have been ever widening bounds within which the variation of the eye would not subject the possessor to elimination.

*Chologaster* is in a stage of panmixia as far as the eye is concerned. It is true the eye is still functional, but that the fish can do without its use is evident by its general habit and by the fact that it sometimes lives in caves.

The present conditions have apparently existed for many generations, as long as the present habits have existed, and yet the eye still maintains a higher degree of structure than reversed selection, if operative, would lead us to expect, and a lower degree than the birth mean of fishes depending on their eyes — the condition that the state of panmixia alone would lead us to expect. There is a staying quality about the eye with the degeneration, and this can only be explained by the degree of use to which the eye is subjected.

The results in *Chologaster* are due to panmixia and the limited degree of use to which the eye is put. *Chologaster agassizii* shows the rapid diminution of the eye with total disuse.

The difference in the conditions between *Chologaster* and *Amblyopsis*, *Typhlichthys* and *Troglichthys* is that in the former the eyes are still in use, except when living in caves; in the latter they have not been in a position to be used for hundreds of generations. The transition between conditions of possible use and absolute disuse may have been rapid with each individual after permanently entering a cave. Panmixia, as regards the minute eye, continued. Reversed selection was inoperative, for economy can not have affected the eye for reasons already stated. Simply the loss of the force of heredity, unless this was caused by disuse or the process of germinal selection, can not have brought about the conditions, because some parts have been affected more than others.

Considering the parts most affected and the parts least affected, the degree of use is the only cause capable of explaining the conditions. Those parts most active during use are the ones reduced most, viz., the muscles, the retina, optic nerve, and dioptric appliances, the lens and vitreous parts. Those organs occupying a more passive position, the scleral cartilages, have been much less affected and the bony orbit least. The lens is one of the latest organs affected, and not at all during use, possibly because during use it would continually be in use. It disappears most rapidly after the beginning of absolute disuse both ontogenetically and phylogenetically. All indications point to use and disuse as the effective agent in molding the eye. The process does not, however, give results with mathematical precision. In *Typhlichthys subterraneus* the pigmented layer is affected differently from that of *Amblyopsis*. The variable development of the eye muscles in different species would offer another objection if we did not know of the variable condition of these structures in different individuals. Chilton has objected to the application of the Lamarckian factor to explain degeneration on account of the variable effects of degeneration in various invertebrates. But such differences in the reaction are still less explicable by any of the other theories.