THE INFLUENCE OF LIGHT AND DARKNESS ON THYROID AND PITUITARY ACTIVITY OF THE CHARACIN *ASTYANAX MEXICANUS* AND ITS CAVE DERIVATIVES

PRISCILLA RASQUIN

BULLETIN
OF THE
AMERICAN MUSEUM OF NATURAL HISTORY
VOLUME 93 : ARTICLE 7 NEW YORK : 1949

THE INFLUENCE OF LIGHT AND DARKNESS ON THYROID AND PITUITARY ACTIVITY OF THE CHARACIN *ASTYANAX MEXICANUS* AND ITS CAVE DERIVATIVES

THE INFLUENCE OF LIGHT AND DARKNESS ON THYROID AND PITUITARY ACTIVITY OF THE CHARACIN *ASTYANAX MEXICANUS* AND ITS CAVE DERIVATIVES

PRISCILLA RASQUIN

Scientific Assistant

Department of Fishes and Aquatic Biology

BULLETIN

OF THE

AMERICAN MUSEUM OF NATURAL HISTORY

VOLUME 93 : ARTICLE 7 NEW YORK : 1949

BULLETIN OF THE AMERICAN MUSEUM OF NATURAL HISTORY
Volume 93, article 7, pages 497–532, text figures 1–9,
plates 20–25, tables 1–6
Issued July 18, 1949
Price: $.75 a copy

INTRODUCTION

DURING THE COURSE of studies on pigmentation and behavior in response to light of the Mexican characin *Astyanax mexicanus* Fillipi and its cave derivatives, variations were noted among the separate forms in behavior, locomotor activity, fat deposition, body shape, and guanin deposition. These variations suggested some fundamental difference in the physiology of the eyed river form living in the light and the blind cave forms living in total darkness.

Five caves containing populations of blind fish are known to exist in a valley which drains into the Rio Tampaon, San Luis Potosi, Mexico. This river is populated with the eyed, pigmented form. Breder and Gresser (1941a, 1941b), Breder (1942, 1943), Breder and Rasquin (1947a, 1947b), and Rasquin (1947) present more detailed reports on these populations. This report is concerned with the blind inhabitants of only two of these caves and the eyed river fish, namely, a form from La Cueva Chica, *Anoptichthys jordani* Hubbs and Innes, a form from Cueva de los Sabinos, *Anoptichthys hubbsi* Alvarez, and the ancestral river form, *Astyanax mexicanus* from the Rio Tampaon. The first of these is extremely variable, perhaps owing to contamination with the river fish that presumably can enter La Cueva Chica and interbreed with the blind form. The fish from Sabinos are less variable and show no evidence of interbreeding with any other type. The two blind types are quite distinct, the fish from La Cueva Chica having retained more of the remnant eye capsule and more pigmentation than the form from Sabinos. All are sufficiently related to permit interbreeding, and hybrids have been obtained from Chica and river fish parents, and from Chica and Sabinos fish parents.

Several factors point to a difference in physiology among these three forms. The river fish in the light is of an evidently "nervous" temperament, capable of extremely active reactions on slight stimulus, but is usually quiescent, keeping within the shade of aquarium plants and facing out into the lighted area. The blind fish, on the contrary, have no point of optical fixation and are constantly in motion. They consume more food than the river variety, as their continual wandering evidently calls for the expenditure of more energy. Oxygen consumption studies by Schlagel and Breder (1947) have shown that the river fish consumes more oxygen when kept in total darkness than when kept in light, the impossibility of optical fixation probably keeping the river fish moving in the dark. At the same time the Chica fish were shown to consume more oxygen in the light than in the dark, the light apparently stimulating them to greater activity.

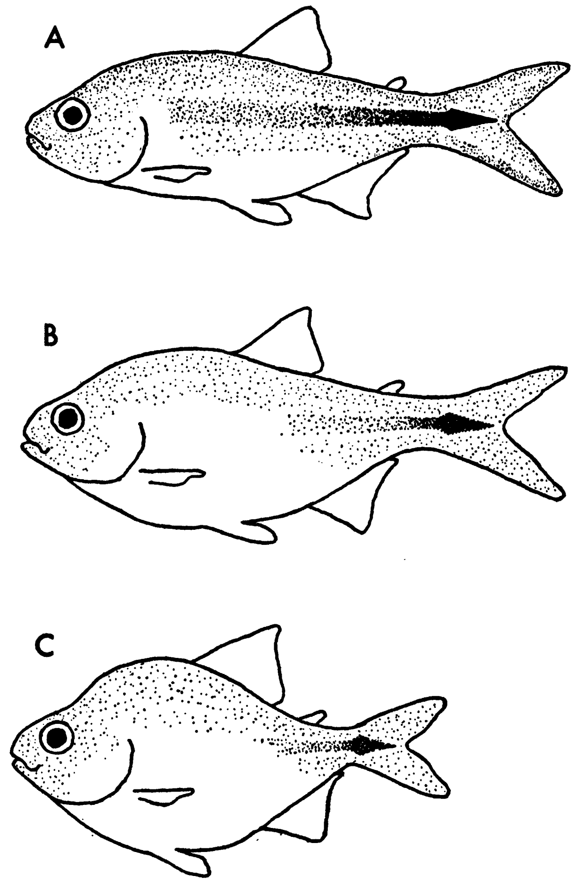


FIG. 1. Body contours of river fish. A. Normal river fish in the light. B. River fish three months in the dark. C. River fish two years in the dark.

Another factor bearing on this difference appears in the differential deposition of the metabolic waste product, guanin. The facts that additional guanin was laid down by

Chica fish when brought from the dark habitat into the light and that no such reaction took place in the Sabinos fish under similar conditions pointed to an assumption that some physiological activity was induced by the action of light present in one blind form and not in the other (Rasquin, 1947). Sections through the body regions of these fish showed that the Sabinos form had large quantities of adipose tissue, not only in the abdominal cavity but between the dermis and muscular layers of the head and body, which were absent in the other two forms in the light. In fact all three types become fatter when kept in the dark than when kept in the light. The river fish accumulates so much fat in the dark that the body shape is entirely changed. They are shorter than fish of a similar age kept in light, probably as a result of a slower rate of growth in the dark, but they are also deeper, and a definite hump appears behind the head in the region anterior to the dorsal fin. These changes in body shape are shown in figure 1.

Since Landgrebe (1941) indicated that the "silvering" or guanin deposition in the eel, salmon, and trout was independent of gonad development and since he was able to produce silvering in the salmon and brown trout by injections of thyroid extract, it seemed appropriate to consider the endocrine system of these fishes, particularly the thyroids. The results indicate that some accommodation for living in cave environments has been evolved in the endocrine systems of the cave varieties.

These experiments were carried out in a laboratory kindly provided by the Department of Birds of the American Museum of Natural History through the kindness of Dr. R. C. Murphy. I wish to thank Dr. Charles M. Breder, Jr., Dr. Ross F. Nigrelli, Dr. Harry A. Charipper, and Mr. James W. Atz for suggestions and criticism of the manuscript.

MATERIAL AND METHODS

THIS REPORT is divided into two sets of experiments. After considerable work had been done on the eyed and blind forms, it was found that the information available on the deriving river form was insufficient to make valid comparisons. Therefore a second set of experiments was undertaken on the eyed river form alone, using animals whose age and past history were known. These are called, respectively, the first and second experiments.

In the first experiment the river fish used were all aquarium-raised descendants of fish that were brought from the Rio Tampaon by an expedition sent out by the New York Aquarium in 1940. Of the Chica fish used, some were members of an eleventh generation raised in the light, and some were specimens newly brought from the cave by an expedition sent out from the Department of Fishes and Aquatic Biology of the American Museum of Natural History in 1946. The Sabinos fish used were all specimens newly brought from the cave by the latter expedition except for one specimen which had been living in the daylight of the laboratory for more than two years. The ages of all fishes brought from the caves were unknown. The laboratory-raised river fish were all approximately two years of age, and all the fish were sexually mature. Ten fish of each group used were killed and fixed at the time of collection in their natural habitats. The ages of these fish were unknown.

Darkness was provided by a small dark room built into the same room that housed the fish living in the light. For total darkness, the aquaria containing experimental fish were placed in black-painted plywood boxes which had light-tight covers, and these were kept inside the dark room. Temperature within the dark room never varied more than a degree from that of the outside laboratory and was kept between 68° and 80° F.

In the second experiment, the river fish used were all members of a single spawning. They were received in the laboratory two months after hatching, and those that were to be kept in total darkness were placed there at that time. Thus the dark-adapted fish had been accustomed to a light environment for the first two months of life only. Those fish that were to be kept in the light remained in tanks in the laboratory subject to ordinary conditions of daylight and darkness.

At monthly intervals one fish from the light and one from the dark were sacrificed, and studies were made on their thyroids and pituitaries. Thus a record was made on these glands during the period of most active growth.

Three river fish living in the light were blinded by severing the optic nerve when they were nine months of age. These fish remained in the light and were sacrificed at monthly intervals.

At nine and one-half months of age, five river fish living in the light were placed in separate small tanks, each containing 4 liters of .033 per cent solution of thiourea (thiocarbamide, c.p.). This concentration was used by Goldsmith, Nigrelli, Gordon, Charipper, and Gordon (1945). The solution was made up with conditioned water, that is, water in which fishes had been living, and the solution was changed weekly. It was necessary to add some floating plants to each tank to provide a cover for the fishes, as they exhibited too many fright reactions in the bare tanks. They were fed daily with their customary food, but their appetites were noticeably diminished. These animals were sacrificed at intervals of two weeks beginning one month after the start of treatment.

Simultaneously five river fish of the same age that had been living in total darkness were placed in similar individual tanks in the dark room. On alternate days these fish were fed their customary food and desiccated thyroid (Burroughs Wellcome tablets). One-quarter grain was the dosage employed until the fish had received a total of 3.75 grains, when the feedings were increased to one-half grain. The water in these tanks was not changed throughout the experiment. The animals were sacrificed at the same times as the thiourea-treated fish.

In the first experiment a total number of 62 fish was employed, 17 Sabinos fish, 27 Chica fish, and 18 river fish. The difficulty experienced in collecting these fish and that encountered in inducing them to reproduce in aquaria made it necessary to limit such use to as few as possible. Fish killed in Mexico at the time of collection were fixed in 10 per cent formalin. All the laboratory-raised fish were killed by decapitation. The lower jaw as far back as the operculum and gills was removed and fixed in Bouin's solution. The skin and upper part of the brain case were removed from the rest of the head so that proper quick fixation of the pituitary would be insured. This portion of the head was fixed in a freshly prepared Zenker-formol solution. The tissues were decalcified by the phoroglucin-nitric acid method and embedded in paraffin. For the study of the thyroids the tissue was sectioned serially at 7 microns and stained with Harris' hematoxylin and eosin, and in each case a slide was stained by Mallory's connective tissue method for a check on cytological details. For a study of pituitaries, sagittal serial sections were made at 5 microns and were stained with Scruggs' modification (1939) of the method recommended by Dawson and Friedgood (1938). Rasmussen's suggestion (1929) of a preliminary immersion in hematoxylin to stain the nuclei more prominently proved useless when followed by Dawson and Friedgood's technique. However, excellent differentiation was obtained by Scruggs' method and for quantitative work was found to be far superior to methylene blue and eosin. Dempsey and Wislocki (1945) reported that the cytoplasm of the basophilic cells of rat pituitaries showed a more marked affinity for methylene blue than for anilin blue, which stains acidophilic tissue. These authors consider that the anilin blue stains acidophilic substances in the basophiles and therefore is not a good indicator of true basophilia. Desclin (1940) also demonstrated basophilia in the pituitary glands of rats and guinea pigs which was not detected by anilin blue. However, the differentiation obtained with methylene blue and eosin is not nearly so sharp nor so reliable on this tissue, and since Scruggs described so many fish pituitaries using Dawson and Friedgood's technique, it was decided to use the same method for purposes of comparison.

In the second experiment the fish were killed by dropping them into Bouin's solution. As soon as they had ceased struggling they were decapitated and the lenses were removed from the eyes. After the usual decalcification and embedding, the whole head was sectioned transversely at 5 microns through the thyroid and pituitary regions. These sections were stained with a modification of Masson's connective tissue stain recommended by Lillie (1940), using Biebrich Scarlet and Fast Green. In this way a good differential stain was obtained on both glands, and the measuring of thyroid epithelial heights and the differential counting of pituitary cells were greatly facilitated.

Rawson and Starr's method (1938) for measuring the height of the epithelial cells of the thyroid follicles was applied, since Gorbman (1940) had called attention to the suitability of this technique as applied to goldfish. The original technique was followed except that one cell from each of 100, instead of 200, different follicles was counted, because these fish were small and some did not appear to have as many as 200 follicles. Furthermore, some of the follicles were so large that it was deemed advantageous to have at least 100 microns between sections used for counting in order that no follicle would be measured more than once.

Rasmussen's technique (1929) for making differential counts of the cells of the anterior lobe of the pituitary was found to be inapplicable to the fish pituitary in this case. The pituitaries of these fishes are too small and contain too few cells for statistically significant figures to be obtained. Levenstein's technique (1939) was also abandoned because a proper representation of the cells could not be made to fall within the counting area that he recommends. In the first experiment this latter method was modified as follows: A Leitz ocular was used which was marked in 100 equal squares, and all the cells that fell within the hundred squares were counted. The counting area was set in the center of the transitional lobe in the section, and in the beginning and ending sections this included all of the gland which was to be seen in the section. Beginning with the first section that contained pituitary tissue, every third section was counted. To check the results, every third section was again counted, beginning with the second section that contained pituitary tissue. This technique was not altogether satisfactory, mainly because fully satisfactory serial sections were almost impossible to obtain by the method used.

A more accurate technique was evolved in the second experiment where the changed histological method provided good serial sections. Here, all the cells that exhibited nuclei in the transitional lobe were counted in every third section. With the use of the same ruled ocular it was possible to count all the cells within the squares and, by moving the slide carefully, to count other fields in the same section without duplicating any field already counted. By this method no part of the transitional lobe was unrepresented in the final count.

The statistical methods followed can be found in Simpson and Roe (1939). The samples were compared for statistical significance by the use of the following formula for the derivation of the value of d/σ_d:

$$\sigma_d = \sqrt{\sigma_{M_1} + \sigma_{M_2}}$$

If d/σ_d is greater than 3 the difference is definitely significant, when it is greater than 2.5 it is usually significant, but when it is less than 2 it is not significant.

EXPERIMENTAL RESULTS

THE THYROID GLANDS of all these fishes consist of scattered follicles found in the region below the floor of the mouth and pharynx, reaching anteriorly from between the mandibular bones back through the gill arches to the anterior portion of the heart.

All the fishes that were killed and fixed in Mexico, whether in darkness or in light—that is, cave fish that were killed in the cave without being exposed to light (except that of the lamps necessary for collecting) and river fish that were taken from the surface waters of the river—show fairly inactive thyroids. The epithelium is squamous, the follicles are large and filled with colloid, and few vacuoles are seen in either cells or colloid. Gaylord and Marsh (1912) have already noted that the thyroids of trout kept in captivity in hatcheries show greater activity than those of truly wild specimens, and it may well be that some of the atypical results found in this study are owing to the confinement of the fish in relatively small spaces. That all were similarly confined does not necessarily imply that this factor was controlled, since it is possible that individual fish may react differently to the same confining conditions. Table 1 gives the mean thyroid epithelial heights of the fish that were killed when collected. This then represents the state of the thyroid of the fish in their natural habitat. It is interesting to note that the Chica fish that come from a cave where the population contains both eyed and blind specimens show a thyroid epithelial height that is statistically significantly different from that of either the Sabinos fish or the river fish.

If the typical condition of the gland of captive fish is assumed to be that found in the river fish reared under the normally recurring conditions of daylight and darkness of the laboratory, the follicles are large, and the epithelium ranges from squamous to low cuboidal in character. Occasional vacuoles are seen in the colloid and in the cells, mostly in the smaller follicles. The colloid has a smooth, homogeneous appearance. The tissue between the follicles consists of areolar connective tissue in which pigment cells are often found, and of adipose tissue and blood sinuses. The mean epithelial height of the thyroid cells of the river fish raised in the light, taken from table 3, is 4.480 microns. This is considerably higher than that shown in table 1 by the river fish killed at the time of collecting. The mean epithelial height of the latter is

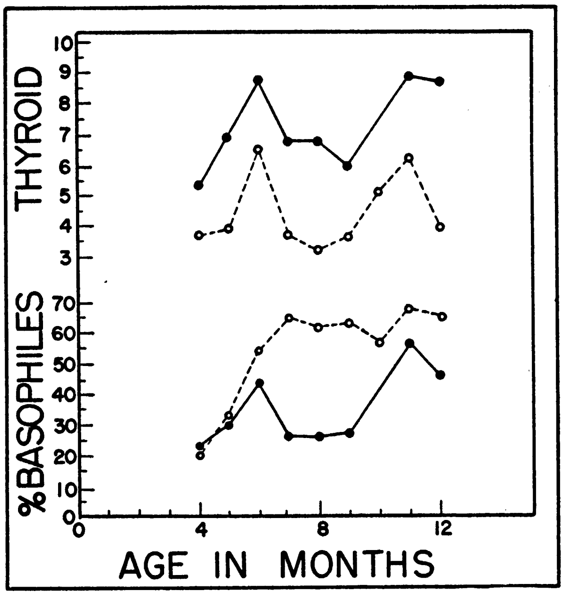


FIG. 2. Thyroid epithelial heights and percentages of basophiles in the transitional lobes of the pituitaries of river fish in light and dark, from four to 12 months of age. Solid line indicates fish in the dark, broken line indicates fish in the light.

2.539. This may not be a valid comparison, for the laboratory-raised fish were in the period of their most active growth, when the thyroid would be presumed to be more active than after full maturity had been reached. One laboratory-raised fish which was over two years old showed a thyroid epithelial height of 3.22 microns. The only captive fish that have shown the low epithelium comparable to that found in the fish taken from their natural habitats are some of the blind forms kept in darkness, one fish from Cueva de los Sabinos kept in light, and one river fish placed for one day in darkness. These values may be found in table 2.

Table 3 and figures 2 and 3 show the heights of the thyroid epithelium found in a series

TABLE 1

STATISTICAL EVALUATION OF THYROID CELL HEIGHTS OF FISHES FIXED IN THEIR NATURAL HABITATS

Fish	Sex	Standard Length (in mm.)	Mean Cell Height of Individuals (in Microns)	Standard Error	Mean Cell Height of Each Group (in Microns)	Standard Error
Cueva de los Sabinos						
1	♂	46	*2.540*	.0735		
2	♂	43	*2.570*	.0721		
3	♂	47	*2.580*	.0690		
4	♂	43	*2.500*	.0721		
5	♀	56	*2.445*	.0697	*2.603*	.002515
6	♀	60	*2.330*	.0653		
7	♀	61	*3.125*	.1218		
8	♀	50	*2.560*	.0727		
9	♀	45	*2.600*	.0744		
10	♂	—	*2.780*	.0825		
La Cueva Chica						
1 (eyed)	♀	51	*2.750*	.0783		
2 (eyed)	♀	60	*2.810*	.1006		
3 (eyed)	♂	45	*2.710*	.0634		
4 (eyed)	♀	47	*2.475*	.0757		
5 (eyed)	—	—	*2.780*	.0752	*2.805*	.002506
6 (eyeless)	♀	45	*2.730*	.0885		
7 (eyeless)	♂	49	*3.815*	.1018		
8 (eyeless)	♂	46	*3.030*	.0854		
9 (eyeless)	♂	52	*2.855*	.1022		
10 (eyeless)	—	—	*2.090*	.0613		
Rio Tampaon						
1	♀	52	*2.690*	.0835		
2	♀	56	*2.340*	.0636		
3	♀	49	*2.605*	.0888		
4	♂	48	*2.620*	.0711		
5	♂	46	*2.650*	.0847	*2.539*	.002451
6	♂	44	*2.585*	.0728		
7	♀	54	*2.480*	.0653		
8	♀	48	*2.425*	.0548		
9	♂	42	*2.420*	.0752		
10	—	—	*2.570*	.1035		

of laboratory-reared river fish from the ages of four to 12 months. This information indicates that there may be a cycle of thyroid activity in this species. The epithelial heights varied from a low of 3.29 microns to a high of 6.56 microns. The next highest value, 6.25 microns, was reached after an interval of five months. The first high point which occurred at six months of age was simultaneous with the appearance of mature gametes in the gonads. At four and five months of age the gonads were immature although well differentiated. In the fish that was six months old and dark adapted for four months, one ovocyte was seen embedded in otherwise normal, mature, testicular tissue. This was the only sexual anomaly found in all the fishes examined.

The data concerning the mean thyroid epithelial heights in light and dark in table 3 were examined statistically. The ten-month-old animal was omitted because there was

TABLE 2

EXPERIMENTAL CONDITION OF THE FISH AND THE STATISTICAL EVALUATION OF THYROID CELL HEIGHTS

Fish	Mean Cell Height in Light (in Microns)	Mean Cell Height in Dark (in Microns)	Standard Error[a]
Sabinos, kept over 2 years in light	*2.37*		.0687
Sabinos, brought from cave in darkness, kept 2 months in dark in laboratory		*2.39*	.0655
Sabinos, brought from cave plus 1 year in dark in laboratory		*5.99*	
Sabinos, as above, 1 day in light	*5.50*		
Sabinos, 2 days in light	*4.02*		
Sabinos, 3 days in light	*3.37*		
Sabinos, 6 days in light	*4.07*		
Chica, raised in light	*3.24*		.1090
Chica, raised in light	*3.14*		.1022
Chica, 8 months in darkness with controlled ecological conditions		*2.52*	.0650
Chica, 8 months in darkness with controlled ecological conditions		*2.69*	.0762
Chica, 3 months in dark		*4.02*	.1108
Chica, 3 months in dark		*4.56*	.1550
Chica, 13 months in dark		*4.41*	.1540
Chica, 13 months in dark, 1 day in light	*3.41*		.1190
Chica, 13 months in dark, 2 days in light	*5.33*		.1910
Chica, 13 months in dark, 3 days in light	*3.64*		.1110
Chica, 13 months in dark, 6 days in light	*3.30*		.1110
Chica, 13 months in dark, 8 days in light	*3.64*		.1320
Chica, 13 months in dark, 10 days in light	*4.02*		.1360
Chica, 13 months in dark, 13 days in light	*3.15*		.1100
Chica, 13 months in dark, 16 days in light	*3.18*		.0890
Chica, 13 months in dark, 20 days in light	*3.05*		.0696
Chica, 13 months in dark, 23 days in light	*3.10*		.0886
River fish raised in light	*3.22*		.0800
River fish, 2 years 3 months in total dark		*6.75*	.2086
River fish, 2 years 3 months in total dark		*5.92*	.1980
River fish, 2 years 6 months in total dark		*8.42*	.3090
River fish, 3 months in total dark		*8.94*	.3000
River fish, 3 months in dark, 1 day in light	*3.32*		.1430
River fish, 3 months in dark, 7 days in light	*4.45*		.1640
River fish, 1 day in dark		*2.73*	.0880
River fish, 7 days in dark		*4.66*	.1880

[a] Because in each case *N* equals 100, the standard deviation, not given, is 10 times the standard error.

none sacrificed from the dark at the same time. Although there is considerable variability among these thyroids, the difference between them in light and dark is clearly significant. The mean differences between the two fish of each group were averaged, and that value which is the mean difference between the thyroids in light and dark is 2.07. The standard deviation is 1.53 and the standard error is .54. The value for t is 3.8, indicating statistical significance.

Darkness was found to have a profound effect on the condition of the thyroid in the river fish. Placing such a fish for one day in total darkness seemed to have little or no effect, the mean thyroid epithelial height being 2.73 microns, as is shown in table 2. After one week in total darkness, the mean

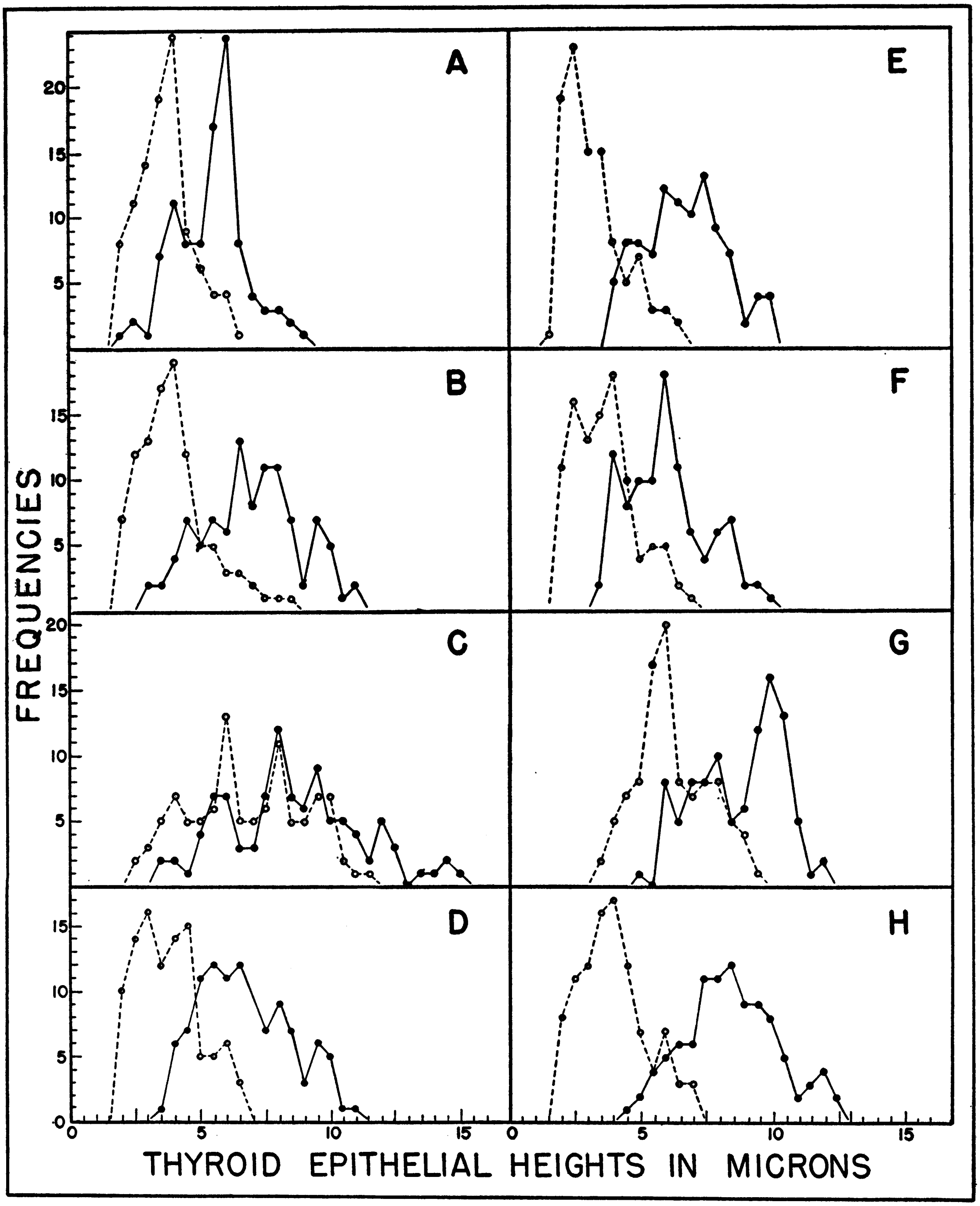


FIG. 3. Thyroid epithelial heights of pairs of river fish of the same age in light and dark plotted against the frequency of their occurrence. See text for explanation of frequency. Solid lines indicate fish in the dark, broken lines indicate fish in the light. A. Four months old. B. Five months old. C. Six months old. D. Seven months old. E. Eight months old. F. Nine months old. G. Eleven months old. H. Twelve months old.

height was raised to 4.66 microns. A few mitotic figures are seen in the tissue lying between the thyroid follicles. The nuclei are large and vesicular and have prominent nucleoli. Vacuoles can be seen in the cytoplasm, although no lumen of a follicle has yet appeared. Both large and small follicles are present and, in all but the extremely large follicles, vacuoles are seen in the colloid next to the epithelial cells. The colloid stains a deeper pink with eosin at the periphery, and in the Mallory-stained sections, no blue staining of colloid is apparent. No blood cells appear within the follicles nor does the gland appear particularly vascular.

The river fish kept for three months in total darkness shows a mean epithelial cell height of 8.94 microns. In addition to increased cell height the gland is extremely vascular. Many blood cells are seen within the large follicles, and the periphery of most of the follicles is surrounded by red blood cells. Gudernatsch (1911) has mentioned the presence of erythrocytes within the follicles of the teleost thyroid. Since these fish were killed by decapitation, it was at first thought that some pressure exerted by the act of cutting off the head might have ruptured some follicles and forced blood into them. However, a careful study of serial sections showed ruptured follicles to be rare, and the adjacent tissues did not exhibit any undue infiltration of erythrocytes. The erythrocytes may infiltrate through the wall of the follicle,

TABLE 3

Differential Counts of the Cells of the Transitional Lobes of River Fish Kept in Light and Darkness, with Heights of Thyroid Epithelium

Age of Fish (in Months)	Sex	Time in Light (in Months)	Time in Dark (in Months)	Total Number of Cells Counted	Per Cent of Acidophiles	Per Cent of Basophiles	Per Cent of Chromophobes	Mean Thyroid Epithelial Height
4	♂	4		2,100	79	20	1	*3.710*
4	♀		2	2,101	76	23	1	*5.400*
5	♂	5		4,764	66	33	1	*3.955*
5	♂		3	3,523	69	30	1	*6.975*
6	♀	6		5,283	41	54	5	*6.560*
6	♂		4	7,049	56	43	1	*8.635*
7	♀	7		5,713	31	65	4	*3.760*
7	♂		5	4,197	68	26	6	*6.705*
8	♀	8		9,889	36	61	3	*3.290*
8	♂		6	4,730	73	26	1	*6.730*
9	♂	9		11,779	30	62	8	*3.680*
9	♂		7	3,899	71	27	2	*6.060*
10	♂	10		13,722	35	57	8	*5.145*
10	♂		8	—	—	—	—	
11	♂	11		22,783	25	68	7	*6.250*
11	♀		9	11,165	40	56	4	*8.790*
12	♂	12		11,965	30	65	5	*3.965*
12	♀		10	9,129	52	46	2	*8.485*

as they can be seen between the epithelial cells. In the larger follicles the colloid is deeply stained in the center, but lightly stained near the cells. Vacuoles are seen both inside the cells and in the colloid. Mallory-stained sections show most of the colloid stained a pale blue. In some of the larger follicles the center colloid takes a deep orange stain, while the peripheral colloid is blue and less dense. Some follicles exhibit only pale blue colloid which is vesicular and granular in character. Only the very smallest follicles show a completely orange-stained colloid.

In the river fish kept for over two years in absolute darkness, the thyroid epithelial heights range from 5.92 to 8.42 microns. The follicles are fewer in number and range from a medium to a small size. Vacuoles are prominent in both colloid and cells. The colloid appears smooth and fairly dense, staining more intensely at the periphery than in the center. Some follicles are completely devoid of colloid. Nuclei are basal in position and vesicular with prominent nucleoli. No mitotic figures were observed. Adipose tissue has increased in the thyroid area, and there

TABLE 4

COMPARISONS MADE FOR STATISTICAL SIGNIFICANCE

Pairs of Fish	Mean Thyroid Height (in Microns)	Standard Error[a]	Significance
Sabinos in light Sabinos in dark	*2.37* *2.39*	.0687 .0655	.2 no sig.
Chica in light Chica in dark	*3.24* *2.52*	.1090 .0650	5.6 sig.
Sabinos in dark Chica in dark	*2.39* *2.69*	.0655 .0762	2.9 prob. sig.
River fish in light River fish in dark	*3.22* *6.75*	.0800 .2086	15 sig.
Chica 1 day in light Chica 2 days in light	*3.41* *5.33*	.1190 .1910	8.5 sig.
River fish 1 day in dark River fish 1 week in dark	*2.73* *4.66*	.0880 .1880	9.2 sig.
Catfish in light Catfish 2 months in dark	*3.58* *4.48*	.0890 .1086	6.3 sig.
Eyed river fish in light, 10 mos. old River fish blinded 1 mo., 10 mos. old	*5.14* *3.79*	.1616 .1084	6.9 sig.
River fish in light, 11 mos. old River fish blinded 2 mos., 11 mos. old	*6.25* *4.47*	.1308 .1306	11.4 sig.
River fish in light, 12 mos. old River fish blinded 3 mos., 12 mos. old	*3.96* *4.83*	.1289 .1397	4.5 sig.
River fish in light, 6 mos. old River fish in dark, 6 mos. old	*6.56* *8.63*	.1713 .2692	6.6 sig.

[a] Because in each case *N* equals 100, the standard deviation, not given, is 10 times the standard error.

is no indication of increased vascularity which was evident in the fish kept for only three months in the dark.

This reaction of the thyroid to total darkness is not exhibited consistently by any of the cave fishes. The blind fish from Cueva de los Sabinos show more variability in thyroid activity than those from La Cueva Chica. thyroid activity comparable to that shown in the light or one that shows a decrease in activity. The decrease in the height of the epithelium in the dark may be significantly different from that in the light as is shown in table 4. No river fish placed in the dark, with the exception of one placed there for only one day, has shown an inactive thyroid.

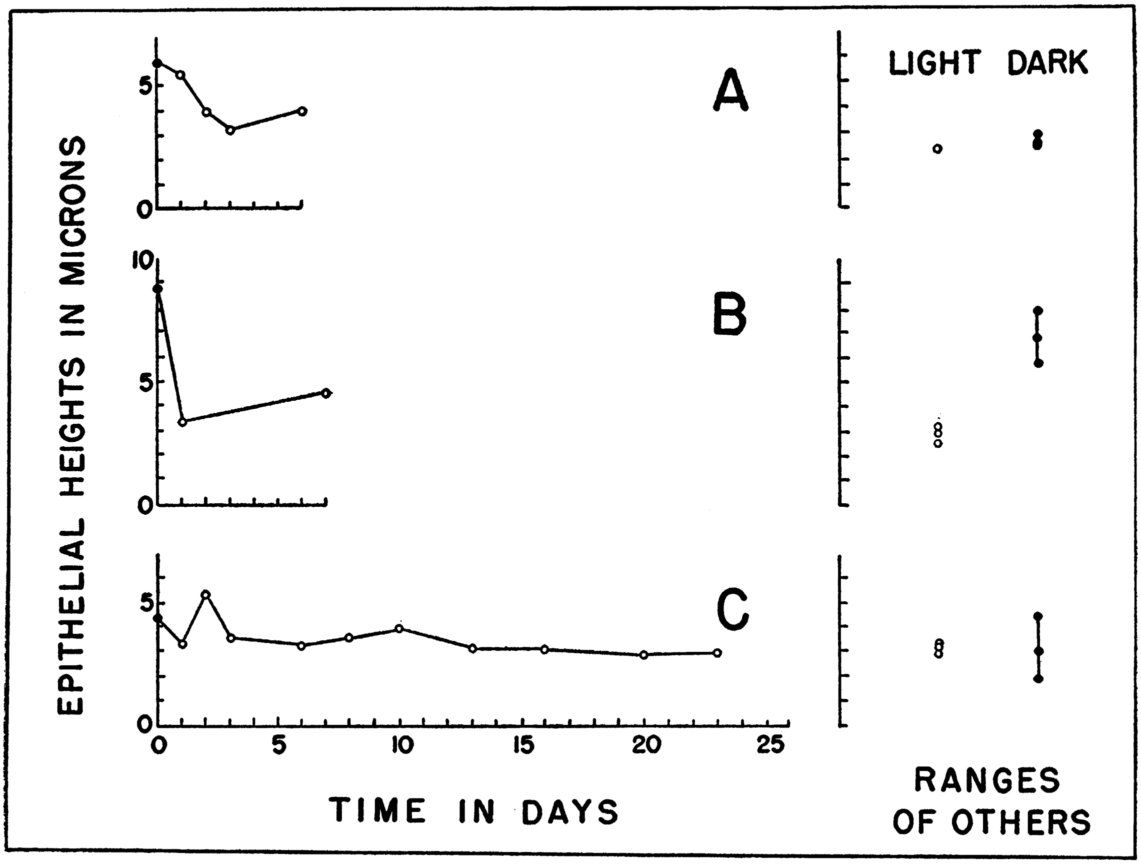


FIG. 4. Thyroid epithelial heights of fish brought from darkness into light. A. Sabinos fish. B. River fish. C. Chica fish.

As far as can be determined from the few specimens examined for this report, the effect produced by bringing them into light-living conditions is to reduce the height of the thyroid epithelium. However, one specimen kept two months in the dark exhibited a thyroid height no different from another kept two years in the light. The blind fish from La Cueva Chica when kept in the light show thyroid epithelial heights that are comparable to those of the ancestral river form in the light. In darkness, they may show a

Plates 20 and 21 show the various heights of the thyroid epithelium of the river, Chica, and Sabinos fishes in light and darkness. Table 3 gives the results gathered from the second experiment, and figure 2 shows these figures in graphic form with the mean epithelial height in microns plotted against the age of the fish in months. Two series of fishes from four to 12 months of age are compared, one series having been kept in total darkness from the age of two months. As the two fish, one from the light and one from the dark, were sacrificed at the end of each month, the thyroid epithelium of the fish from the dark was found to remain consistently and statistically significantly higher than the one from the light. Evidence of a cycle of thyroid activity was shown by both series of fishes. Figure 3 indicates for each pair the various heights of cells measured for each thyroid plotted against the frequency of their occurrence. The statistical significance for the pair at six months of age was calculated since these values were the closest. The value obtained for d/σ_d was 6.6, which is clearly significant. The values used in the calculation are given in table 3.

It has previously been observed (Rasquin, 1947) that the fish from La Cueva Chica reacted to exposure to light differently from the other cave varieties, by depositing an additional quantity of guanin beneath the skin. This deposition takes place during the three weeks subsequent to removal from the dark. A series of Chica fish were removed from the dark room into the ordinary conditions of the laboratory and sacrificed on successive days. Table 2 gives the number of days in the light and the corresponding thyroid epithelial heights during this three-week period, and the results are graphically shown in figure 4C. The fish killed in the dark on the day that the others were brought into the light showed a higher epithelium than normally found in such fish in either light or dark, that is, 4.41 microns. Further investigation revealed the fact that this fish was suffering from a disease of the kidneys not uncommon in this species,[1] and the more active thyroid might well be associated with a reaction of the body to the effects of the disease.

This series of fish had been subjected to enucleation, a complete removal of the blind optic capsule, before being placed in the dark 13 months previously. Thus they were rendered light insensitive as well as blind, so that the excessive deposition of guanin was not mediated through any optical connection with the pituitary.

A similar series of five Sabinos fish were sectioned. These were fish that had been brought from the cave in Mexico and subjected to only five days in the light before again being placed in darkness. They had been maintained in the laboratory for approximately a year before being brought out into the light and sacrificed on successive days. Another series of three river fish kept in the dark for three months was also studied. Figure 4 gives a graphic representation of the thyroid heights of the fishes in these three series. In all the fishes exposure to light seemed to bring about an initial drop in the activity of the thyroid with a subsequent secondary stimulation. In the Chica series a significant rise in thyroid level was noted on the second day, and after that the height of the thyroid epithelium returned to that normally found for the Chica fish in the light. Although this level is often higher than that usually found for these fish in the dark, it cannot be stated that the increased activity of the thyroid is responsible for the additional deposition of guanin, until further confirmation is established.

The Sabinos fish in this series, represented in figure 4A, exhibited a higher level of thyroid epithelium than any others studied. However, it is not comparable with the condition of hyperplastic thyroid found in the river fish in the dark; the gonads of the Sabinos fish showed no evidence of reduction, as did those of the river fish, and the shape of the body was unaltered.

The river fish series shown in figure 4B exhibits an extreme drop in the thyroid level on the first day of exposure to light, with a subsequent rise. Unfortunately the scarcity of animals did not permit a more nearly adequate representation of the immediate effects of continued light. However, figure 4 (above the caption "Ranges of others") shows the means and extremes of all other river fish studied after they had been in light for various periods of time, as well as the same values for other fishes kept in the dark. These extremes and means therefore represent the levels of the thyroid reached after considerable conditioning to living in the light.

Another indication of differing physiological function among these fishes is the deposition of fat underlying the dermis. The relative amount of fat increases from the river variety through the Chica to the Sabinos form. When kept in the light, the river fish

[1] Nigrelli (1943, fig. 16) has illustrated such a diseased specimen.

has very little subdermal fat. The Chica fish kept in the light have less than those kept in the dark, while the Sabinos fish in either light or dark have more than any of the others except the river fish kept in the dark. A remarkable increase in adipose tissue occurs in the river fish kept in total darkness. This accumulation reaches such a degree that the only to reduce the amount of adipose tissue but also to return the thyroid gland to a state of normal activity. Plates 22 and 23 show transverse sections through the bodies of these fish taken just anterior to the dorsal fin. In addition to the number of dark-adapted river fish used in the first experiment, 100 river fish were placed in the dark at the

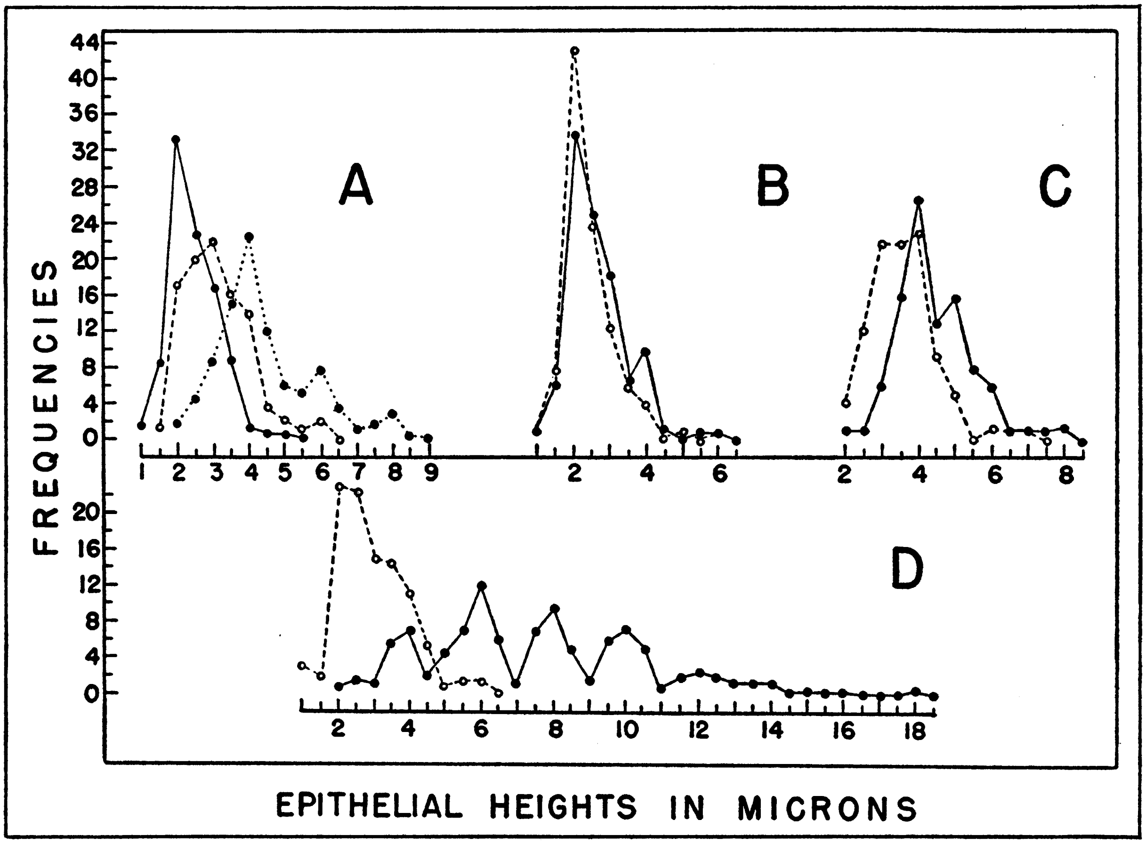
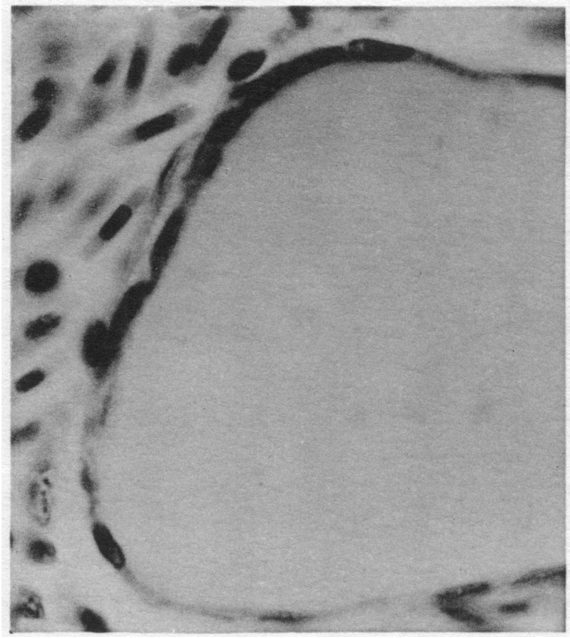


FIG. 5. Graphs of the thyroid epithelial heights and their frequencies of the four kinds of fish studied in light and dark. The solid lines indicate fish in the dark, broken lines indicate fish in the light, and the dotted line indicates fish three months in the dark. A. Chica fish. B. Sabinos fish. C. Catfish. D. River fish.

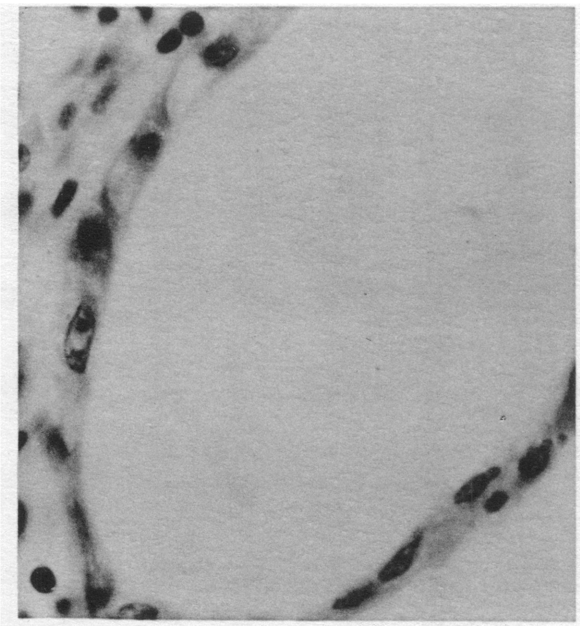
whole body contour of the fish is changed. (See fig. 1.) The change is evident after three months, but progresses much farther after two years. It is evident that important skeletal modifications are not involved, for when these fish are taken out of the darkness and returned to ordinary light conditions they return to their original shape after six to eight weeks. The reaction to light of these fishes brought out of the dark serves not beginning of the second experiment. All these fish showed the same alteration in body shape.

Figure 5 indicates graphically the thyroid heights of the four different kinds of fishes studied in light and dark, with the epithelial heights in microns plotted against the frequency of their occurrence. Solid lines indicate fishes in the dark; broken lines, fishes in the light. Figure 5A indicates the

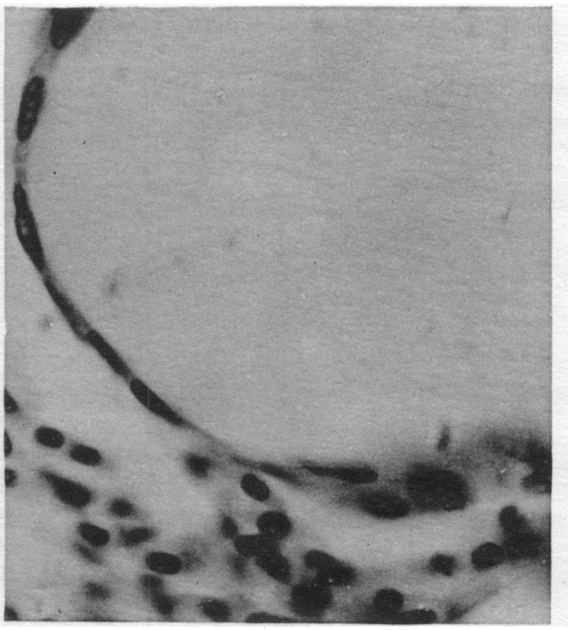


1

2

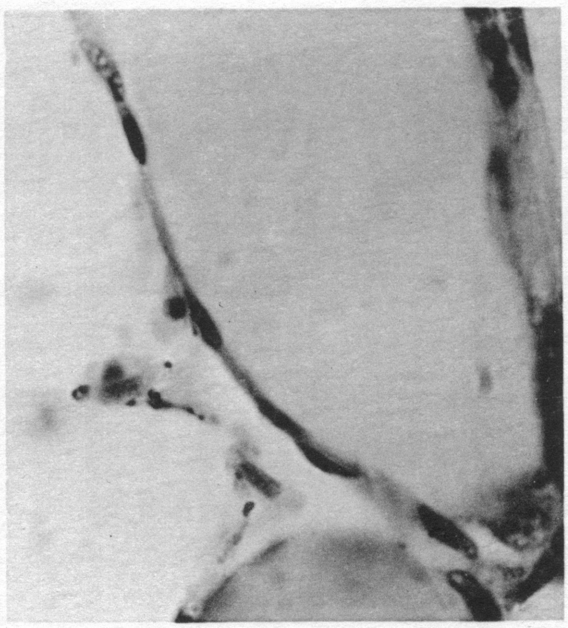


3

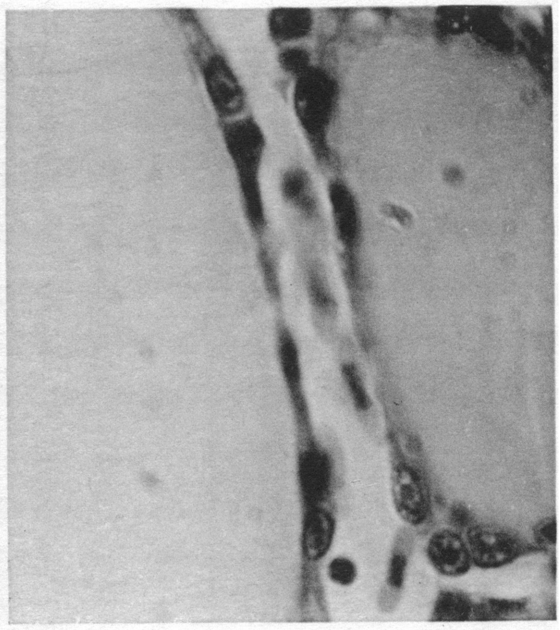


4

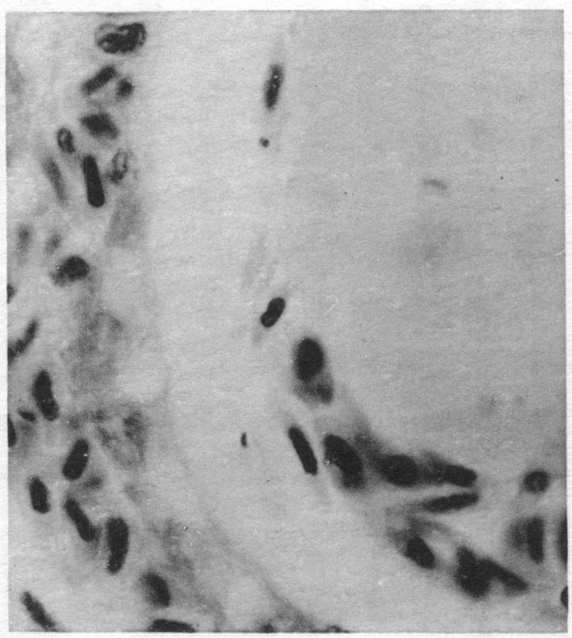
Thyroid epithelium. 1. River fish in the light. 2. River fish long in the dark. 3. Chica fish in the light. 4. Chica fish long in the dark. Magnification × 1500



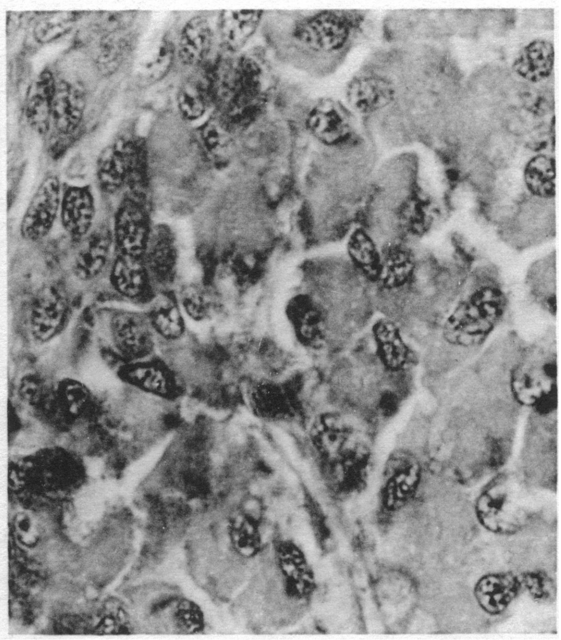
1



2

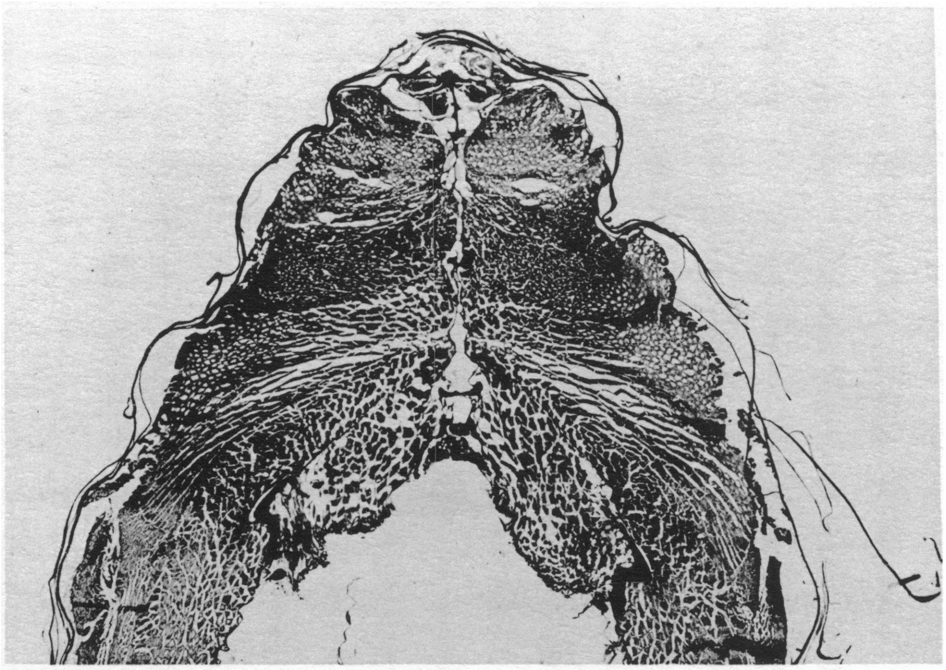


3

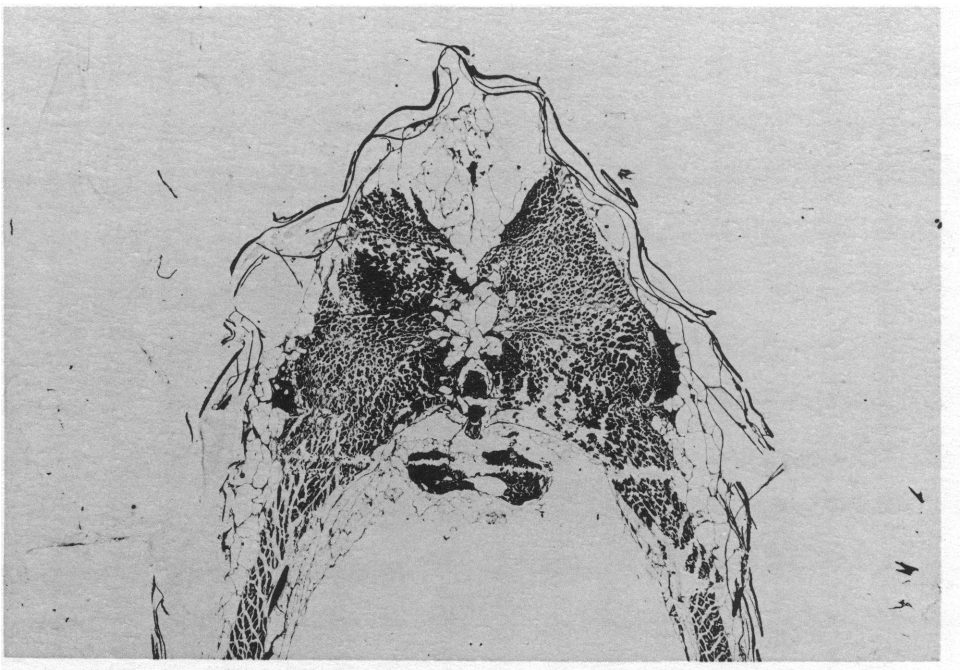


4

Thyroid epithelium and pituitary. 1. Sabinos fish in the light. 2. Sabinos fish long in the dark. 3. River fish three months in the dark, showing vascularization of the gland. 4. Transitional lobe of the pituitary of the river fish in the light. Cells with dark cytoplasm are acidophiles, others are basophiles. Magnification × 1500

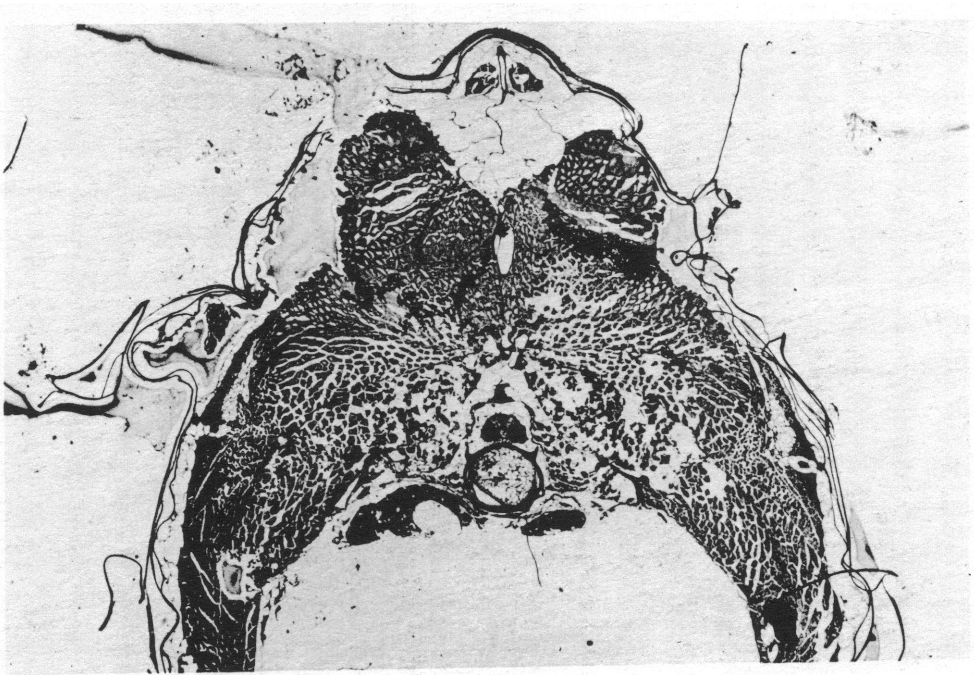


1

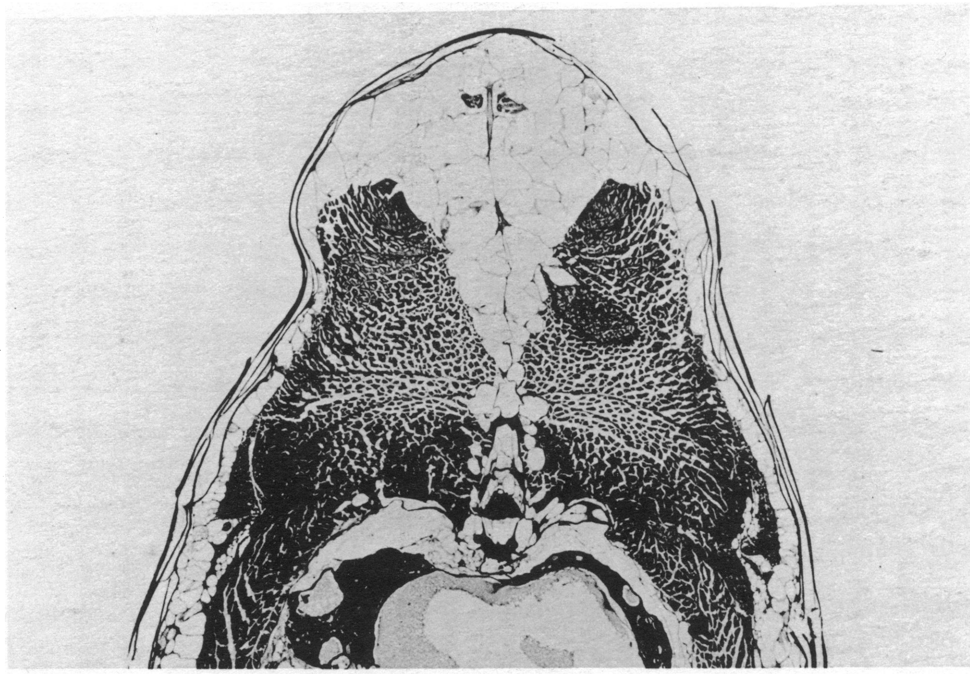


2

Transverse sections through dorsal body region anterior to the dorsal fin. 1. River fish in the light. 2. River fish long in the dark. Magnification × 14



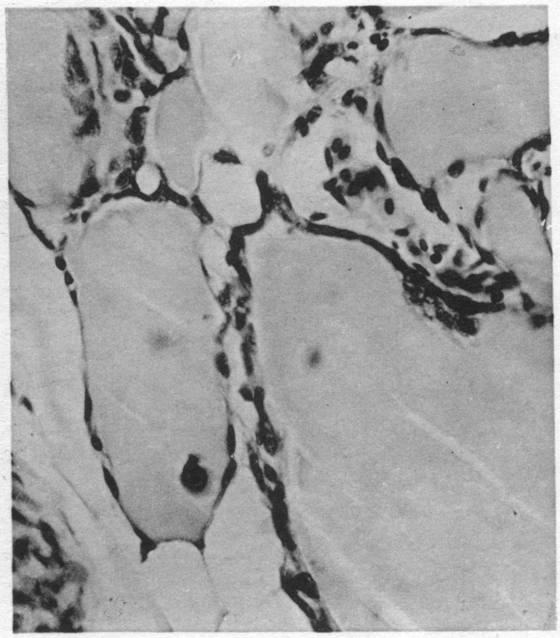
1



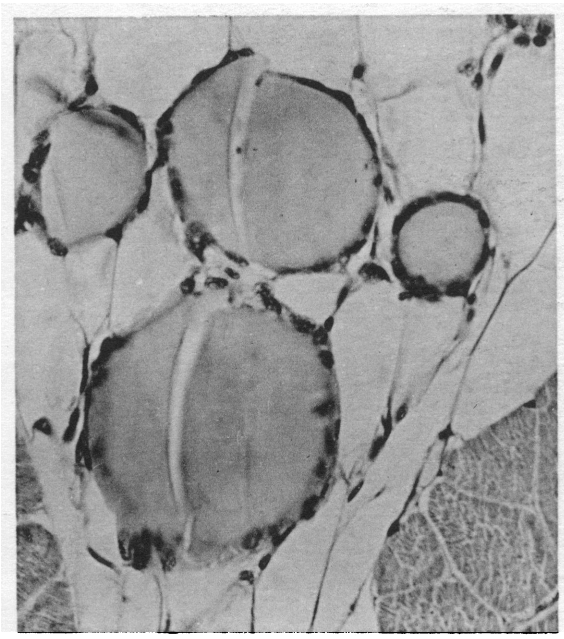
2

Transverse sections through dorsal body region anterior to the dorsal fin. 1. Chica fish in the light. 2. Chica fish long in the dark. Magnification × 14

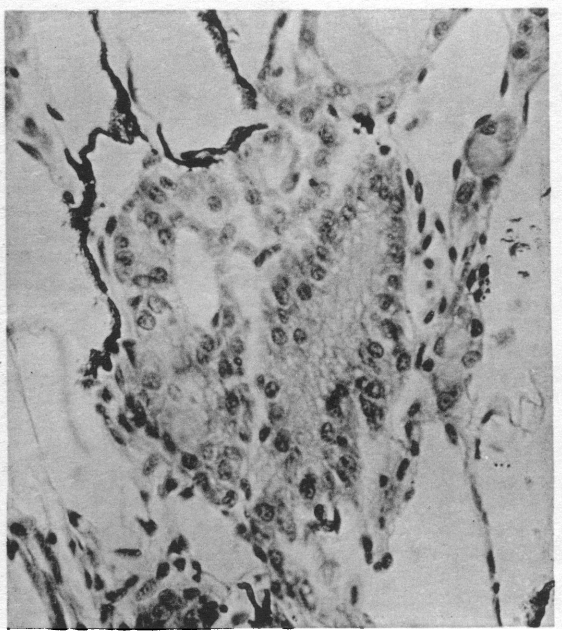
1



2

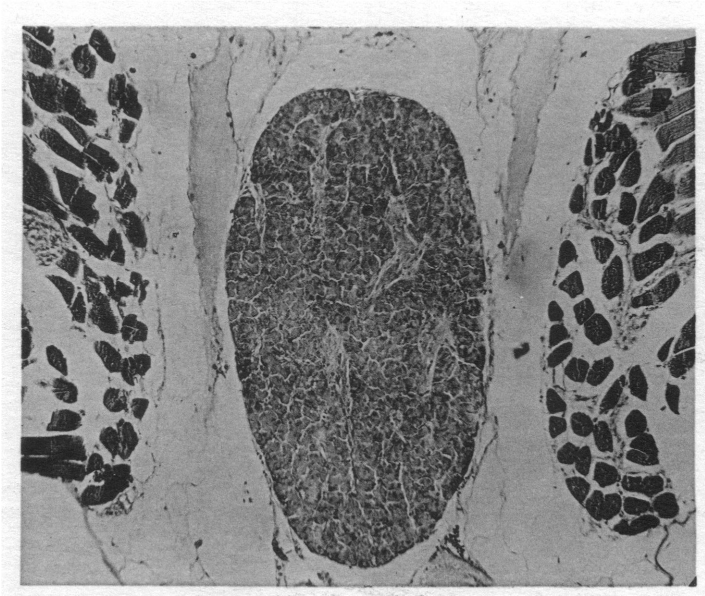


3

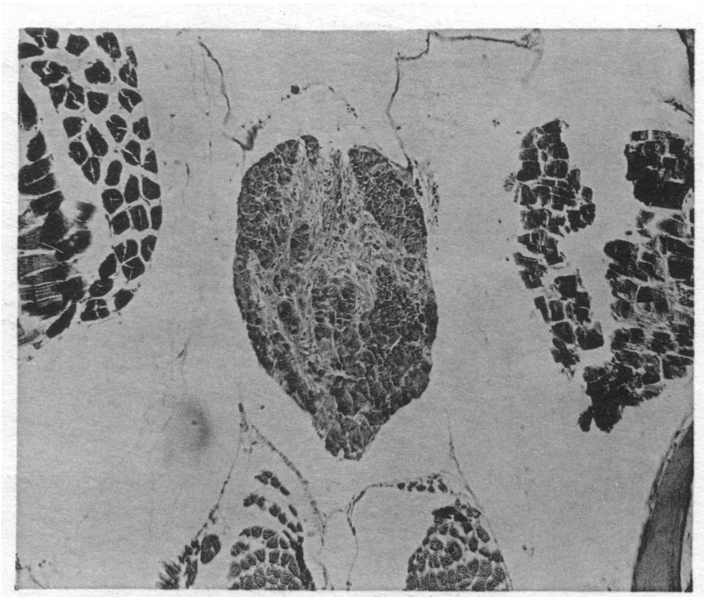


4

Thyroid epithelium of normal, thiourea-treated, and thyroid-fed river fish. 1. Normal river fish, 11 months old, nine months in the dark. 2. River fish of the same age in the dark, thyroid fed for six weeks. 3. Normal river fish in the light, 11 months old. 4. River fish in light of the same age, thiourea treated for six weeks. Magnification × 520



1



2

Transverse sections of the transitional lobes of river fish pituitaries. 1. River fish in the light, eight months old. 2. River fish eight months old, six months in the dark. Magnification × 110 mean of four Chica fish in light and four in dark, and the dotted line indicates the mean of two Chica fish that had been in the darkness only three months. The increased epithelial heights of these two Chica fish may possibly indicate that some considerable length of time is needed by the fish to accustom themselves to the different condition, or, again, it may be part of an activity cycle. This is somewhat similar to the river fish, the highest value of which (8.94) was attained by a fish that had been kept only three months in the dark. Figure 5B represents the mean of two Sabinos fish in the light and two in the dark. There is no statistical significance between these fish. However, the thyroid of the Sabinos fish taken as the first point in figure 4 shows a much more active gland than any of these. Of the two Sabinos fish from the dark represented in figure 5B, one was killed and fixed in the cave and the other had lived in darkness in the laboratory only two months. The fishes represented in figure 4 had lived in the laboratory in darkness for nearly a year. Again, the higher epithelium may be due to the confining conditions of the laboratory tanks, although the Chica fish have not been noted to show such a reaction, and the Sabinos fish kept more than two years in the light in the same sized tanks showed no such increase in thyroid epithelium. Figure 5D represents the means of two river

TABLE 5

MEASUREMENTS (STANDARD LENGTH BY GREATEST DEPTH IN MILLIMETERS) OF FISH RECEIVING THYROID AND THIOUREA TREATMENT
(The last measurement indicates the date on which sacrificed.)

Date (1947)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
			Thiourea treated in light		
9/ 8	53×18	52×18	46×12	53×19	53×22
9/15	53×18	52×17	46×11	53×19	53×20
9/22	53×17	52×18	46×15	53×19	53×21
9/29	53×17	52×17	47×15	53×19	53×21
10/ 6	53×17	53×17	47×15	54×19	54×21
10/13		52×16	47×15	53×19	54×20
10/20		52×17	47×15	54×18	54×20
10/27			47×15	54×19	54×20
11/ 3			47×14	54×18	54×19
11/10				54×17	54×19
11/17				54×17	54×19
11/24					54×19
12/ 1					54×19
			Thyroid fed in darkness		
9/ 8	36×16	32×14	36×16	37×17	37×17
9/15	36×16	32×14	36×16	37×17	37×16
9/22	36×16	32×14	36×16	37×16	37×15
9/29	37×16	33×14	38×16	38×16	37×16
10/ 6	37×17	34×14	38×16	38×16	38×15
10/13		33×14	38×16	38×16	38×15
10/20		32×13	38×16	37×17	37×16
10/27			38×15	37×16	37×15
11/ 3			37×16	38×16	38×16
11/10				38×16	38×16
11/17				38×16	38×16
11/24					38×16
12/ 1					38×16

fish in the light and four in the dark. Here the extreme difference in the activity of the thyroid in light and dark is at once apparent. No river fish taken from the light has ever shown the hyperplastic condition of those taken from the dark. The eyed, pigmented Chica form taken from the cave, which is superficially identical with the eyed river form, exhibits a normal thyroid comparable to the blind form from the same cave or the form from Cueva de los Sabinos. It is only the eyed form from the river that shows hyperplasia of the thyroid in darkness. Figure 5C represents two young catfish, *Ameiurus nebulosus*, one in the light and one in the dark. These show the same tendency towards a more active gland in the dark. The difference between the two catfish thyroids is not great but is statistically significant as is shown in table 3. These are nocturnal and cryptic in behavior, and the eyes are of relatively minor importance as compared with those of *Astyanax*. Therefore they may adapt themselves easier to confinement in the dark. It is noteworthy that there are more catfishes living in cave environments than representatives of any other group (Hubbs, 1938).

Table 5 gives the weekly measurements taken on river fish in the light that were treated with thiourea and on river fish in the dark that were fed desiccated thyroid. Although the thyroid epithelium responded to the treatment in both cases, the shape of the body remained unchanged. The administration of thyroid did not cause the fish in the dark to lose the excessive fat deposits, nor did the administration of thiourea to the normal animals in the light cause any such accumulation of fat. The measurements were taken as accurately as possible on live and struggling fish, but the inaccuracies inherent in such a technique were not great enough to conceal any change in form, had such a change resulted from the treatment. A slight indication of increasing slimness on the part of the thiourea-treated animals was probably owing to their decreased food intake.

Results of thyroid feeding and thiourea treatment are shown in table 5, figure 6, and plate 24. The first animals were sacrificed four weeks after treatment was initiated, and at this time the results were already apparent. The thyroids of the fish in the dark dropped to normal and in succeeding weeks

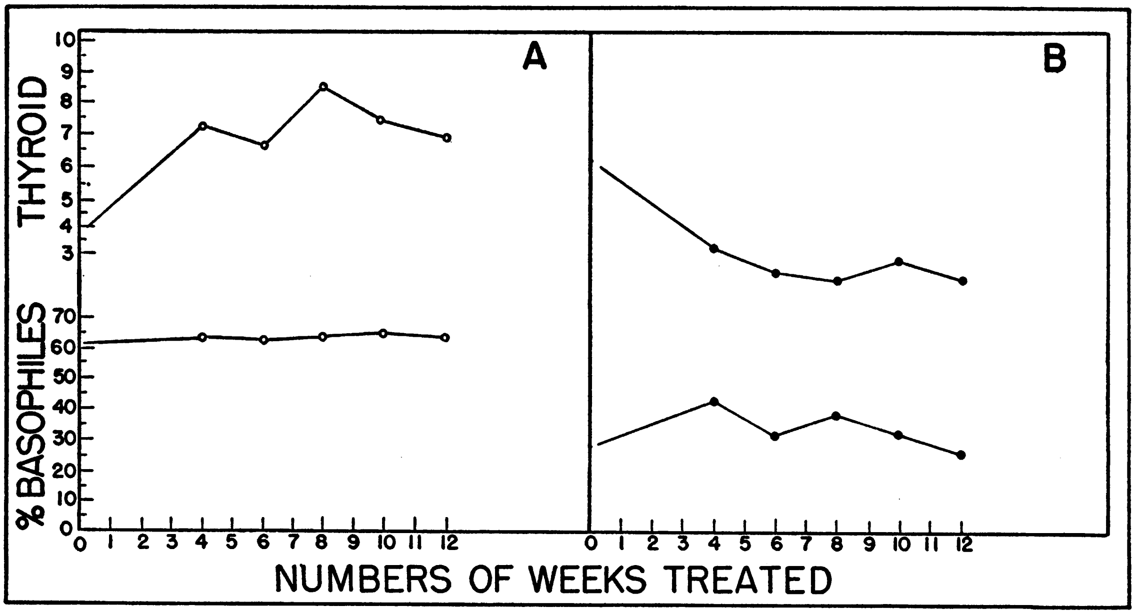


FIG. 6. Thyroid epithelial heights and percentages of basophiles in the transitional lobes of the pituitaries of river fish. A. Thiourea treated. B. Thyroid fed.

reached a low level seen only in fish taken in their natural habitat. Histologically, the follicles were large, filled with homogeneous stored colloid, the cells were squamous with very few vacuoles, and the whole gland presented a picture of the resting condition. The thyroids of those fish that received thiourea treatment exhibited epithelial heights of from 5.765 microns to 8.535 microns. No case was observed where the epithelial height was any greater than that shown by normal fish subjected only to darkness, nor did the histological picture presented by the thiourea-treated fish show any greater cellular activity than that presented by the fish that had been confined in total darkness. Photomicrographs of these glands are shown in plate 24.

Because of the increased oxygen consumption of the river fish when placed in the dark (Schlagel and Breder, 1947), it was thought appropriate to study the thyroids of normal river fish that were blinded and kept in the light. When deprived of optic stimuli these fish take up the constant wandering movements of the blind cave fish. The results of studies on three blinded fish from one to three months after operation are given in figure 7. From these it is apparent that the increased activity does not call forth increased activity on the part of the thyroid. Compared with normal fish of the same age, the blinded animals do not show as great variations in height of cells as the normal ones do. In all cases the blinded fish thyroids are statistically different from the normal, in two cases significantly below the normal, and in one cases significantly above. There are not sufficient data here to determine whether a thyroid activity cycle normal to the unoperated fish has been inhibited by removal of optic stimuli in the blinded animals or whether the thyroids of the blinded fish would go on to reach a similar peak, the two being simply in cycles that are out of phase. It is doubtful that some traumatic shock resulting from the operation had any bearing on these results, for a month was allowed to elapse after the operation before the first blinded fish was sacrificed, and the animals exhibited excellent health and hearty appetites at all times after the initial post-operative day.

A study of the literature concerned with the anatomy and histology of the teleost pituitary indicates considerable confusion. A comparison of the plates in articles by Bell (1938) and Matthews (1936) indicates that what Bell designates the pars intermedia is

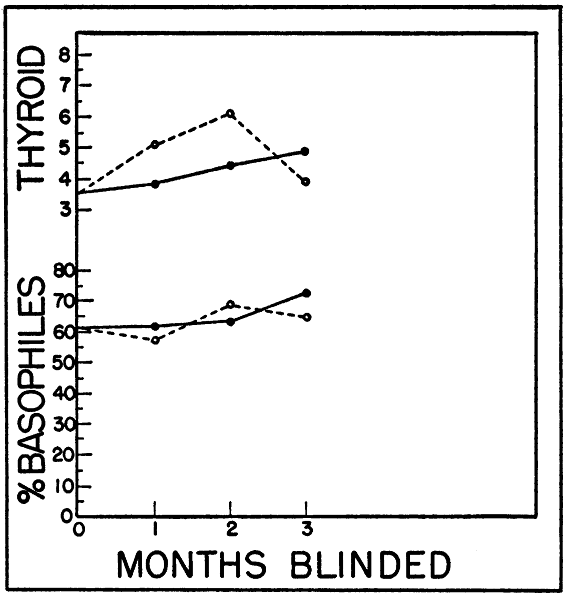


FIG. 7. Thyroid epithelial heights and percentages of basophiles in the transitional lobes of the pituitaries of normal and blind river fish in the light. Solid line indicates blind fish, broken line indicates eyed fish.

	I	II	III
Kerr	Anterior glandular portion	Middle glandular portion	Posterior glandular portion
Bell	Pars anterior	Übergangsteil	Pars intermedia
Matthews	—	Pars intermedia	Pars transitional
Bock	Anterior lobe	Übergangsteil	Intermediate lobe
Scruggs	Anterior lobe	Übergangsteil	Intermediate lobe
Bretschneider and De Wit	Pars anterior	Gonadotropic zone of pars anterior	Pars intermedia

labeled pars transitional by Matthews, and the "übergangsteil" of Bell is the pars intermedia of Matthews. Kerr's figures (1942) only add to this confusion. While it may make no difference what name is given to a particular part, so long as others can tell what is meant by the author, the names given to the various parts of the pituitary have come to carry a certain connotation of function. The names of the different parts as used by various investigators are listed in tabular form on page 517.

The changes that occurred in the histology of the pituitaries under discussion in this paper were confined to that portion under II on page 517, which will be called the transitional lobe. The three parts will be designated, respectively, as the anterior lobe, the transitional lobe, and the intermediate lobe, since most authorities have used these terms and there seems to be no necessity for using the German word.

The technique employed in the second experiment gave better statistical and histological results than that used in the first experiment, but the results obtained from a study of the pituitary glands of these fishes are not so clear-cut or reliable as those obtained from the thyroids. This results partly from the minute size of the gland and partly from the difficulty in obtaining good serial sections and well-stained preparations, since the entire head had to be decalcified and the gland sectioned *in situ*. Accurate differential counts are also difficult to obtain. Table 6 presents the analysis of the transitional lobes of the fish used in the second experiment.

TABLE 6

RESULTS OF THYROID FEEDING AND THIOUREA TREATMENT

Age of Fish (in Months)	Time in Dark (in Months)	Amount of Thyroid Fed (in Grains)	Length of Time Treated (in Weeks)	Mean Thyroid Epithelial Height (in Microns)	Total Cells Counted in Pituitary	Per Cent of Acidophiles	Per Cent of Basophiles	Per Cent of Chromophobes	Sex
Thyroid fed in darkness									
10½	8½	2.75	4	*3.225*	6,499	54	42	4	♂
11	9	5.25	6	*2.52*	5,182	66	32	2	♂
11½	9½	7.75	8	*2.415*	8,440	61	37	2	♂
12	10	10.25	10	*2.965*	6,043	63	33	4	♂
12½	10½	12.75	12	*2.21*	6,608	69	29	2	♀
Thiourea treated in light									
10½	None		4	*7.20*	11,827	30	65	5	♂
11	None		6	*6.40*	8,956	31	64	5	♂
11½	None		8	*8.535*	9,815	32	64	4	♂
12	None		10	*7.56*	15,743	28	63	9	♂
12½	None		12	*5.765*	11,958	31	62	7	♂
Normal untreated in light									
11				*6.25*	22,783	25	68	7	♂
12				*3.965*	11,965	30	65	5	♂
Normal untreated in darkness									
11	9			*8.79*	11,165	40	56	4	♀
12	10			*8.485*	9,129	52	46	2	♀

The transitional lobe in these fish contains secretory basophiles and acidophiles and a smaller number of chromophobes (see pl. 21, fig. 4). The anterior lobe contains only small chromophobe cells faintly staining with orange G and scattered deeper staining acidophile cells having an affinity for the same dye. The intermediate lobe shows the same condition, although the cells are somewhat larger and arranged in a more orderly fashion, giving the appearance of cords separated by blood sinuses. Contrary to Scruggs' (1939) description, based on several species of fishes, the cells of the intermediate lobe of the present material are chromophobic or very weakly acidophilic and show no affinity for the blue dye. Neither were any carmine cells found as described by Scruggs, but these differences may result from a different method of fixation. Only in the transitional lobe were basophiles found. This portion of the gland contains cells larger than in the other lobes, and much more brilliantly staining. Acidophiles are a brilliant orange, basophiles a brilliant blue, and the chromophobes colorless. When Masson's trichrome stain was used on Bouin-fixed material in the second experiment, the cells of both anterior and intermediate lobes take on an acidophilic appearance. In the anterior lobe, the cells all appear of one hue; in the intermediate lobe, acidophiles can be diffentiated from chromophobes by their more intense staining reaction. The transitional lobe shows intensely dark red acidophiles, bright green basophiles, and chromophobes that are either colorless or very weakly acidophilic. In the normal gland of the river fish, kept under ordinary conditions of daylight, chromophobes are not numerous, and the basophiles outnumber the acidophiles. Tables 3 and 6 give the differential counts on various fish from the second experiment. The acidophiles and basophiles appear to be scattered throughout the lobe fairly indiscriminately, except that the acidophiles are slightly more numerous next to the ramifications of the nervosa in the center of the gland and the basophiles are slightly more numerous at the periphery.

Ramifications of the infundibulum, or pars nervosa, also enter all the other parts of the gland. In each lobe they are bordered by cells characteristic of the respective part. In some glands, acidophiles appear clustered about these strands in the transitional lobe, particularly in the fish kept in the dark, but they are not seen outside this part, and therefore there seems to be no reason for identifying them as a part of the intermediate lobe. They are similar to the other granular acidophiles of the transitional part and in no way resemble the smaller, less granular, and less intensely stained acidophiles found in the intermediate lobe.

The only tissues that took the carmine stain were blood elements and the colloid

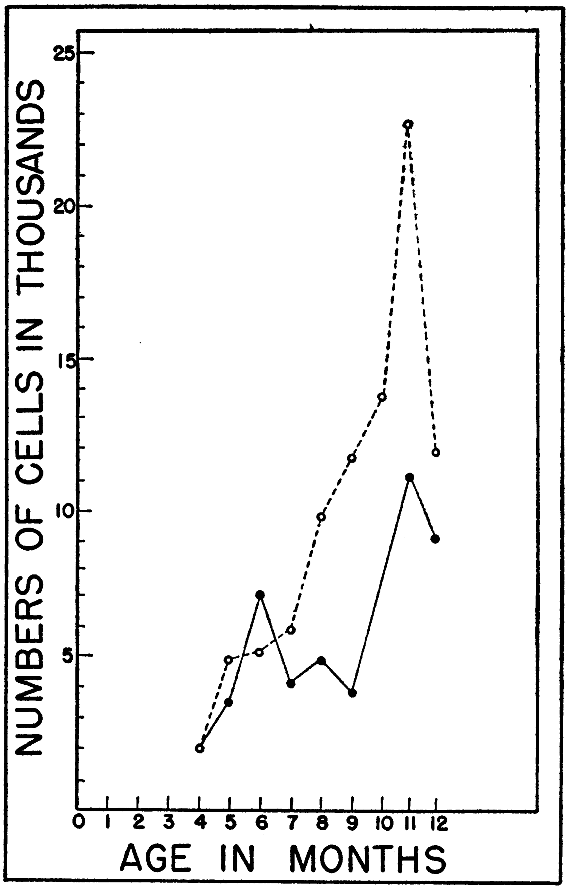


FIG. 8. Numbers of cells counted in the transitional lobes of the pituitaries of river fish in the light and dark from the ages of four to 12 months. Solid line indicates fish in the dark, broken line indicates fish in the light.

droplets that were seen in the intermediate lobe and in the nervosa.

A study of the differential counts made on the transitional lobes of the pituitaries reveals that in all the fishes kept in the dark the chromophobes have decreased in number and in some cases have all but disappeared. This is the only evident difference between the pituitaries of the river fish kept in the light and the one kept in the dark for three months. The pituitary from the river fish kept over two and one-half years in the dark was in too fragmented a condition for differential counting. The sections show, however, that the basophiles are fewer in number. The gland exhibits pathological tendencies in that there has been an infiltration of connective tissue, and cavities have appeared in the glandular portion which are either empty or filled with cellular debris. The volume of the gland has thus been considerably reduced.

It is unfortunate that the original technique employed in the first experiment made accurate counts of the blind fish pituitary cells impossible. A study of these sections gives the impression that all the fish kept in the light, whether eyed or blind, show a preponderance of basophiles over acidophiles in

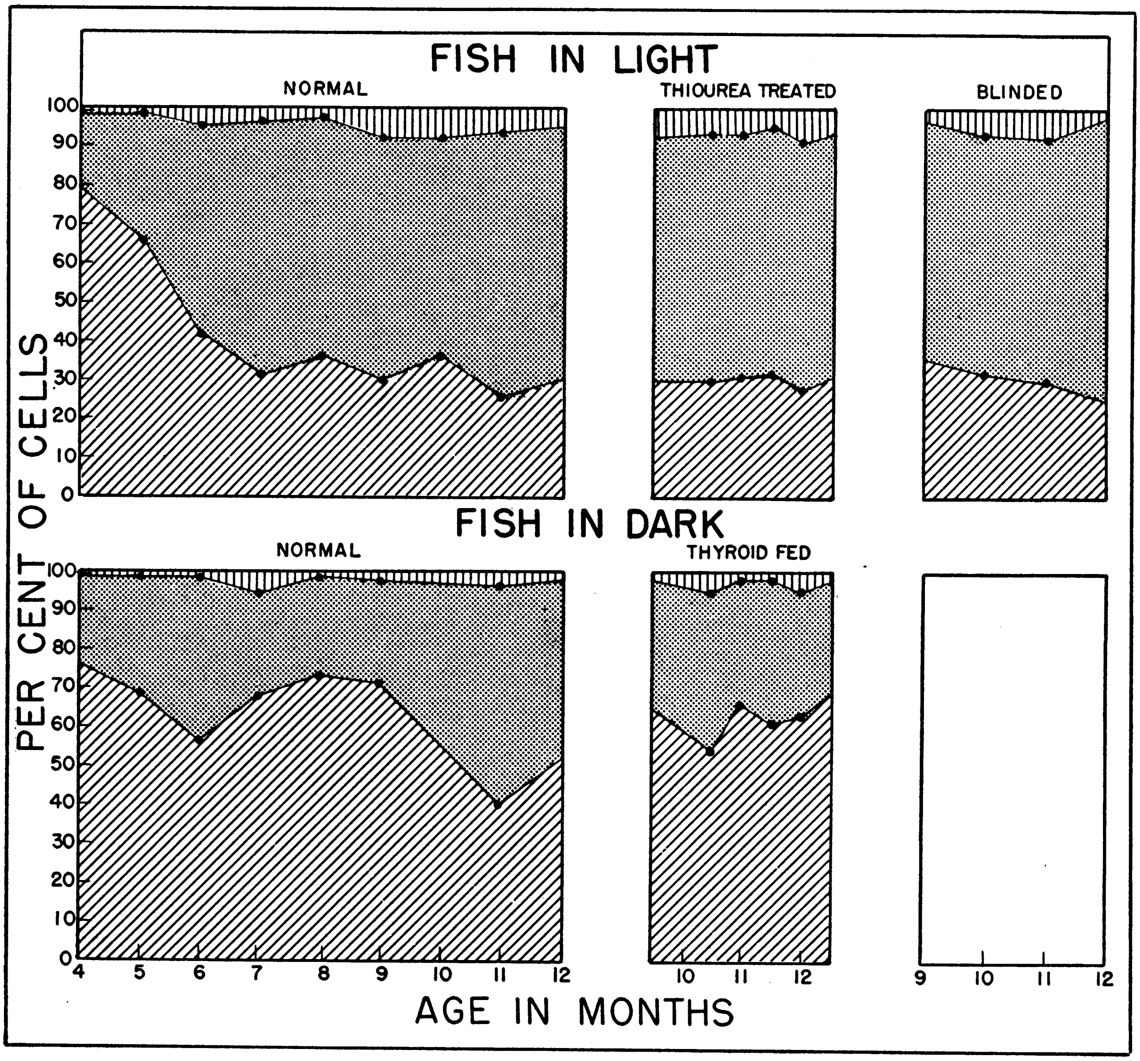


FIG. 9. Graphic representation of the percentages of cells in the pituitaries of river fish in the light and dark, both normal and experimentally treated. Vertical hatching indicates chromophobes, stippling indicates basophiles, and cross hatching indicates acidophiles.

the transitional lobes. In the pituitaries of the fish kept in the dark, there seems to be a reduction in the number of basophiles. Since these are purely subjective impressions they are clearly without much value.

The more accurate technique used in the second experiment confirmed the impressions of the first experiment on the pituitary. The animals raised in darkness exhibit a predominance of acidophiles in the transitional lobe, and those raised in the light show a predominance of basophiles. This ratio of acidophiles to basophiles takes place after sexual maturity. Before mature sex cells are seen in the gonads the transitional lobes of both sets of fish are predominantly acidophilic in character. Table 3 gives the differential counts obtained on fish kept in the light and dark from the ages of four to 12 months. At four and five months of age, before the onset of sexual maturity, the counts for the fish in light and dark appear approximately the same, that is, the percentages of acidophiles are considerably greater than those of the basophiles. From the age of six months to 12 months the transitional lobes of the pituitaries of the fish raised in the light show increasing percentages of basophiles. With one exception, the fish in the dark at 11 months of age, the acidophiles remain the most numerous cells in the transitional lobes of the pituitaries of the animals raised in total darkness. A smaller number of chromophobes is also seen in the dark-raised fish.

The total numbers of cells counted in each pituitary are shown graphically in figure 8. As the fish progress in age they show an increasing number of cells, but the fish raised in the light reach a consistently higher total. This is immediately evident when the sections are studied under the microscope. The transitional lobes of the animals raised in the dark retain a resemblance to the gland found in the immature fish, while the transitional lobes of the fish raised in the light show development to a state where the transitional part occupies more space than any other part in the pituitary, and the basophiles have increased in number to such an extent that the transitional lobe stands out very conspicuously from the rest of the gland owing to the differential staining. Plate 25 shows photomicrographs of sections taken through the transitional lobes of both the light- and dark-raised fish at eight months of age. No correlation was noted between the number of cells found and the sex of the fish.

Figure 2 pictures graphically the epithelial heights of the thyroid cells and the percentages of basophiles found in this second series of fish in light and dark. The apparent cycle of thyroid activity seems to be correlated with a similar cycle of basophilia in the pituitary, particularly in the dark-raised animals.

Table 6 and figure 9 give the results of differential counts on the transitional lobes of the pituitaries of thyroid-fed fish from the dark and thiourea-treated fish from the light, together with the results from normal fish of similar ages. It cannot be said that either form of treatment had any effect on the percentages of cells in the pituitary. Treatment with thiourea did not significantly alter the percentages of cells in the pituitaries of fish kept in the light. Feeding thyroid did not increase the percentages of basophiles in fish kept in the dark over those of the controls, nor was any effect seen on the pituitary counts of the fish that had been experimentally blinded and kept in the light. Results on the blinded fish are incorporated in figures 7 and 9.

DISCUSSION

BOTH VARIETIES of cave fishes have made some accommodation of the endocrine system to living in total darkness which the normal eyed river fish is apparently unable to make when transferred from light to darkness. Whatever factor is responsible for this accommodation has progressed further in the form from Cueva de los Sabinos than in the form from La Cueva Chica. The form from Cueva de los Sabinos shows either the same or greater activity of the thyroid in the dark than in the light, while the form from La Cueva Chica shows the same or greater activity in the light than in the dark. The latter also deposits additional quantities of guanin when exposed to the light, a reaction not shown by the Sabinos fishes. The thyroid of the Sabinos fish, as far as can be ascertained by the few specimens examined for this paper, has lost the ability to respond to the action of light with greater physiological activity.

Turner and Benedict (1932) have reported hypertrophy and colloid loss in the thyroids of chicks deprived of ultraviolet light. They found that the administration of potassium iodide would prevent these changes. Their experimental animals were not kept in the dark but under amber glass which, however, transmitted only 30 per cent of the visible rays. Bergfeld (1930) reported thyroid hypertrophy in rats deprived of ultraviolet light from four to six weeks. Bergfeld's results were not substantiated by Kenyon (1935) who obtained no hypertrophy or colloid loss in the thyroids of rats kept in the dark under controlled conditions of temperature and diet. Mayerson and Branch (1943) were also unable to demonstrate any thyroid hypertrophy in rats kept in the dark unless they were fed an iodine deficient diet. The river and cave fish used for this report received an identical diet which contained sufficient iodine to keep the thyroid at a normal level in the fish maintained in the light. The light to which these fish were subjected was deficient in ultraviolet, since the fish were kept in glass tanks that were covered with glass plates, and in addition the light was filtered into the laboratory through glass windows.

Histologically the thyroid of the dark-adapted river fish shows indisputable evidence of hyperactivity. The follicles are small and are often found completely devoid of colloid. Some glands appear engorged with blood, a condition which other investigators (Adams, 1933; Albert, 1945) have associated with the release of colloid. The height of the epithelial cells has increased tremendously. The nuclei are of the vesicular type with prominent nucleoli, and vacuolations appear in both colloid and cells, as is shown in plate 20, figure 2. Mallory-stained sections reveal that what colloid is present has been recently produced and not stored for any considerable length of time. With such evidence of thyroid activity the animals still manifest indications of low thyroid level.

The most remarkable of these manifestations is the accumulation of fat throughout the body. This increased deposition of fat is more marked in the subdermal layers of tissue than in the abdominal cavity. Well fed, captive fishes kept in the light regularly show an accumulation of fat within the abdominal cavity, but none exhibit the subdermal deposition which is so great in the river fish kept in the dark that the whole body contour is changed. MacKay and Sherrill (1942) determined that the plumpness of thyroidectomized rats was due to myxedematous changes in the tissues rather than to an increased fat content. While these fish may have a decreased thyroxin level it cannot be comparable to the loss resulting from thyroidectomy, and the changes involved in the river fish are not myxedematous. The fat is reduced by osmic acid and stained with Sudan IV. Relevant experimentation on fish is difficult to find in the literature, and it is necessary to bear in mind that conclusions drawn from a comparison with work on other vertebrates may not be valid.

Grobstein and Bellamy (1939) obtained altered body proportions by feeding thyroid to immature fish of the genus *Platypoecilus*. Administration of excess thyroid caused the fish to be longer in proportion to depth than the controls. There was no indication that the alteration was due to the presence or absence of fat. It may be that insufficient thyroid could cause the reverse proportions that were demonstrated by these river fish in the dark, where the fish are deeper in proportion to length than those reared under normal light conditions.

Another indication of deficient thyroid activity is a reduced growth rate in the river fish kept in the dark. These river fish from the dark are definitely shorter than fish of the same age living in the light as is shown in table 5. Breder and Rasquin (1947a) showed that when other ecological conditions were controlled there was no difference in the growth rate of young Chica fish kept in the light from those kept in the dark. Observations on the other varieties of cave fish indicate no differences in size or shape between those kept in the light and those in the dark, even when no precautions are taken otherwise to maintain identical ecological conditions. Of all these related characins, the river fish alone exhibits a reduction in the growth rate. Whether this is owing to an inhibition of proper development of the pituitary by darkness or to an insufficient supply of thyroid hormone alone cannot be determined from the results of these experiments. Further work on the evident imbalance of the endocrine system of these fish is in progress.

Schlagel and Breder (1947) have shown that the river fish consumes more oxygen in the dark than in the light. This probably results from the assumption of a continuous wandering movement characteristic of these fish when deprived of the possibility of optic fixation. The typical wandering movement of the cave varieties is assumed by the river fish after enucleation of the eyes (Rasquin, 1947). The tests for oxygen consumption on these fish were made on animals that were adapted to the dark chamber for only a few minutes. What would be the oxygen consumption of river fish, adapted to total darkness for more than two years, is unknown.

It may be that placing these river fish in a dark chamber where they cannot receive a retinal image is to force them into a state of constant activity, contrary to their normal behavior. Very few references can be found in the literature on the effects of enforced activity on the thyroid gland, and these are concerned with forms other than fish. Bast, Supernaw, Lieberman, and Munro (1928) studied the thyroid glands of rabbits exhausted from lack of sleep and found some evidence of increased activity in the thyroid in that the number of mitochondria was markedly decreased in the glands of the animals so exhausted. Lyne (1945) used rats fatigued by excessive swimming activity and found that their thyroid glands showed increased activity by higher epithelium and more numerous colloid vacuoles. These indications of increased activity are not nearly so great as those shown by the river fish kept in total darkness, but the rats were permitted recovery periods as they were exercised only once a day. The influence of darkness on the river fish is a constant one, allowing no periods of rest and consequent periods of recovery for the gland. Woitkewitsch (1946) exhausted the thyroid gland in carrier pigeons by repeatedly plucking the feathers that appeared after molting. Under normal conditions the appearance of the new feathers after molting is accompanied by increased activity of the thyroid. By this means he obtained extremely hyperplastic thyroids, the weight of which was double or triple those of the control birds. Fragments of these glands transplanted into tadpoles did not produce a specific rate increase in metamorphosis, and after the third plucking the smaller feathers did not develop again, indicating exhaustion of the gland, although the histological picture was that of great activity.

It was in this connection that three eyed river fish were experimentally blinded and kept in the light, and, as soon as the anesthesia wore off, the newly blinded animals assumed the constant wandering movements typical of the cave forms. Studies on the thyroid and pituitary glands of these fish sacrificed at monthly intervals reveal thyroid epithelial heights typical of eyed fish reared in the light and percentages of acidophiles, basophiles, and chromophobes in the pituitary also typical of eyed fish raised in the light. In other words the increase in activity caused by the blinding had no appreciable effect on the glands.

Etkin, Root, and Mofshin (1940), using oxygen consumption determinations, found that exposure to light seemed to raise the metabolism about 10 per cent in goldfish. Feeding thyroid to these fish had no effect on metabolism, and these authors are inclined to think that the thyroid of cold-blooded animals does not regulate the rate of metabolism. Matthews and Smith (1947) also found that injections of thiourea did not alter the oxygen consumption of *Fundulus*, nor was the oxygen consumption changed by the subsequent injection of thyroxin.

The blind fish from La Cueva Chica show a more usual reaction of physiological activity to light and darkness. The height of the thyroid epithelium is usually greater in light than in darkness, and the gland shows more evidence of secretory activity. In the dark, these fish store more fat than they do when kept in the light. They probably swim faster in the light than in the dark, for their oxygen consumption is increased in the light (Schlagel and Breder, 1947).

No determinations of oxygen consumption for the Sabinos form are yet available. It is probable that their metabolism is unchanged or not significantly changed by the transition from dark to light. No observable increase in guanin occurs; their activity, as judged subjectively by their swimming speed, appears to be the same under both conditions, and the activity of their thyroids is not increased.

The gonads of both blind varieties kept in the dark seem to be equal in size to those kept in the light, while the gonads of the river fish kept in the dark, although histologically normal, are much reduced in size. This is another indication that the cave varieties have adapted themselves endocrinologically to the dark environment in some manner not evident in the river fish. In the river fish the pituitary apparently has not kept up the same level of gonadotropic hormone in the dark that it is able to do in the light. The cave fishes, however, show no evidence of any lack of gonadotropic hormone, and are able to reproduce in the total darkness of their natural habitat.

The general adaptation syndrome of Selye (1946) was considered in reference to the effects of darkness on these river forms. The fish might be considered as being in the "stage of resistance," showing physiological reactions to prolonged stress imposed by continuous darkness. The pathological symptoms which Selye lists as characteristic of the adaptation syndrome were considered with the following results: No involution of the thymus-like tissue was observed. In fact one of the river fish which had been kept in the dark for three years has been described by Nigrelli (1947, fig. 39); this fish was discarded from the experiment because of a pathological condition characterized by such abnormal proliferation of lymphoid tissue that the thyroid area had been invaded and some of the thyroid follicles had been destroyed. No sign of gastrointestinal lesions were noted. No degranulation of acidophiles was noted in the transitional part of the pituitary; in fact these were the most prominent cells, both in number and in granulation, in the animals kept in the dark. The anterior lobe of the pituitary remained unchanged as far as could be determined. No involution of the pancreas was involved, nor was any atrophy of the thyroid exhibited. Adrenal cortical tissue has not yet been positively identified in these fish. The only symptoms that are identical with the adaptation syndrome in these fishes are the hyperplasia of the thyroid which sometimes follows on an initial involution, and the atrophy of the gonads of both sexes. In this case it may be that the pituitary is called upon to produce thyrotropic hormone (instead of the corticotropic hormone postulated by Selye) at the expense of the gonadotropic hormone, which would not be so necessary for the maintenance of physiological stability in the organism.

The hypothesis that the transitional lobe ("übergangsteil") of the teleost pituitary is homologous with the anterior lobe of other vertebrates has many supporters. Bock (1928) studied the seasonal variations of the hypophysis of *Gasterosteus aculeatus* Linnaeus throughout the year and found that there was no change in the anterior lobe but that a degranulation of acidophiles occurred in the transitional lobe during the spawning season. On a basis of cytology and anatomical relationships Bell (1938) concluded that the "übergangstiel" of the goldfish was really homologous with the anterior lobe of the higher vertebrates and that the so-called anterior lobe of the teleosts was homologous with the pars tuberalis of other forms. In the characin *Astyanax mexicanus* and its cave derivatives, with which this report is concerned, the changes that occurred in the hypophysis took place within this "übergangsteil," or transitional lobe, and the anterior portion remained unchanged.

There are many investigators who believe that the thyrotropic hormone is secreted by the basophile cells of the pituitary. This activity is attributed to the basophiles by Olivereau (1947) for the teleosts *Mullus barbatus* Linneaus and *Mullus surmuletus* Linnaeus. Woitkewitsch (1944a) unequivocally states that the function of the basophiles is to produce thyrotropic hormone. His work was done on amphibians. D'Angelo (1941) also attributes this function to the basophiles in frogs. For a review of the cellular changes in the hypophysis of mammals, see Severinghaus (1937). Griesbach and Purves (1945) added weight to this theory by correlating an increase in numbers and secretory activity of the basophiles with slight reductions in the thyroxine level in rats, which brought about a simultaneous increase in thyroid activity. The results of this report, however, indicate that river fish kept in total darkness, exhibiting stimulation of thyroid activity, show a reduction in number of basophiles and in the more advanced cases an almost complete degranulation of those remaining. Chromophobes are also significantly reduced in number, probably owing to the absence of light. When frogs are kept in darkness there is a reduction in the number and activity of basophiles, and after seven days no chromophobes can be found; when kept under constant light there is a reduction in number of acidophiles with a corresponding increase in chromophobes (Florentin and Stutinski, 1936; and Stutinski, 1936). Also using frogs (*Rana ridibunda*), Woitkewitsch (1944a) found that the number of basophiles increased under the influence of light and decreased appreciably in darkness. Working with reptiles (*Testudo horsfieldi*, 1944b) the same author attempted to correlate the activities of thyroid and basophile cells of the anterior lobe and believed that proper functioning of the thyroid depended upon adequate stimulation of the pituitary basophiles by light. Since exposure to constant light finally brought about exhaustion of the basophiles, he concluded that alternating light and dark, such as is produced by recurring day and night, was necessary to proper functioning of both glands. Again Woitkewitsch (1946) attempted to exhaust the anterior lobe of the pituitary in amphibians and reptiles by constant exposure to light for varying periods of time and kept numbers of the same species in darkness for a similar length of time. After an initial increase in the first days of exposure the number of the basophile cells remained unchanged throughout the experiment, but changes appeared in the structure of the cells, that is, they became smaller, were degranulated, and the thyrotropic hormone activity was apparently decreased more in those animals exposed to constant light than in those kept in darkness. Unfortunately this author publishes none of the techniques used nor any of the experimental data from which he draws his conclusions.

Pomerat (1942) subjected young female rats to continuous light and darkness over varying periods of time. He found the ovaries of the animals kept under both conditions to be reduced in size, the ones in the dark showing a greater reduction than those in the light, although this condition did not persist as the exposure time was lengthened. The pituitaries of the animals kept in the light showed no significant difference from those of the controls, but the pituitaries of the animals kept in the dark showed an increase in acidophiles, and the number of basophiles was doubled. The author relates this increased activity of the pituitary to the recovery of the size of the ovary in the animals kept for longer periods in darkness. No differences were noted in the thyroids of well-fed rats kept in light and darkness (Mayerson, 1935).

Of the fishes studied for this report only the river fish kept in darkness showed a reduction in the size of the gonads. This reduction in size persisted throughout the period of dark adaptation. Rats are nocturnal animals, and these river fish are not. It may be that nocturnal animals are more easily accommodated to constant darkness than diurnal ones. The thyroid of the dark-adapted catfish used in this report showed less increase in thyroid cell height than that of the diurnal *Astyanax*.

The pituitaries of the river fish maintained for a long period in darkness bear a rather startling resemblance to those of thyroidectomized rabbits (Bryant, 1930). In the latter, cells are palely stained, basophilia is considerably reduced, and cavities have appeared in the body of the gland which Bryant attributes to degeneration of the chromophobes. It seems possible that the pituitary of the river fish kept in the dark shows not genuine chromophobes, but rather degranulated chromophile cells—a gland either so overworked that storage of secretion cannot occur, or one functioning insufficiently through not receiving the stimulation of light. In those river fish that were raised in the dark from an early age, it is not a question of loss of basophilic cells but rather of an underdevelopment of that part of the gland that contains the basophilic cells.

The accumulation of fat, the reduced growth rate, and the underdeveloped gonads point to a condition of hypothyroidism in the river fish raised in the dark. The picture of great activity presented by the thyroids of these fish then may be indicative not of an overproduction of hormone, but of a gland unable to produce enough hormone for the physiological requirements of the animals. If the basophiles of the transitional part of the pituitary are responsible for the production of thyrotropic hormone, the reduced number of such cells found in the pituitaries of the dark-raised river fish could be responsible for the production of an insufficient supply of thyrotropic hormone. Thus the hypothyroid condition would result from insufficient stimulation of the pituitary by light. The association of reduced numbers of basophiles in the pituitary and reduced production of thyroxin by the thyroid, is, however, contrary to what other investigators have reported. Following thyroidectomy, Severinghaus, Smelser, and Clark (1934b) found a marked increase in basophiles and a decrease in acidophiles with degranulation of remaining acidophiles in rats. The same authors (1934a) reported increase in basophiles together with larger, more brilliantly staining acidophiles as a result of thyroid feeding and thyroxin injection. Griesbach and Purves (1945), again using rats, studied the effects of total and subtotal thyroidectomy and thiourea treatment on the cells of the pituitary. They found that even a slight drop in thyroxine level from the normal resulted in an increase in number and activity of basophiles, but only total thyroidectomy resulted in the degranulation of acidophiles. In rabbits, Marine, Rosen, and Spark (1935) demonstrated that thyroxine alone was able to prevent hypertrophy of the pituitary and degranulation of the acidophiles after thyroidectomy or occurrence of goiter. Severinghaus (1939) gives a review of the literature concerned with the cellular changes of the pituitary gland associated with thyroidectomy and hyperthyroidism. In both conditions basophiles increase in number and vacuolation, while acidophilia is decreased in thyroidectomy and increased in hyperthyroidism. It is extremely difficult to correlate the changes in this teleost pituitary with those of other vertebrates, for not only are the percentages of the cells different, but the cells themselves are unique. The normal pituitary of this species shows a greatly reduced number of chromophobes, compared with the glands of other forms, and a greatly increased number of basophiles. In the normal fish the basophiles are found in a vacuolated condition resembling the "castration" or "thyroidectomy cells" experimentally produced in some mammals. In the eyed river fish, no changes were noted in the percentage of basophiles and acidophiles in those animals subjected to treatment with thiourea or thyroid feeding, although the thyroids reacted in both cases. No hypertrophy of the pituitary was associated with the hyperplastic thyroids of the fish raised in the dark; in fact the pituitaries appeared underdeveloped. It should be noted that none of the literature quoted above concerns animals under the influence of darkness.

The effect of hypophysectomy on the thyroid is to reduce the height of the epithelial cells (Uotila, 1939; Adams, 1933; Zalesky, Wells, Overholser, and Gomez, 1941; Schaefer, 1933). The pituitaries of these dark-raised fish are therefore still producing thyrotropic hormone; otherwise the thyroid could not exhibit such obvious activation.

Innate modes of behavior evidently are responsible for the entrance into caves by the river fish. The facts that they are rheotropic and thermotropic and that they normally seek shelter in dark places would tend to make them enter the warmer water flowing from the cave into the river somewhere beneath the surface (Breder and Gresser, 1941b). The development of a disturbed endocrine system presented by the river fish kept in the dark would seem to indicate that the establishment of such a colony of fish in the caves is not so simple a procedure as might be thought. It is doubtful whether the river fish trapped in a dark cave can maintain the endocrine balance necessary to spawning for any considerable length of time. It is possible that only a few river fish out of a great many entering the cave are able to make the necessary adjustment to total darkness. These few adjusted individuals then would be the only fish able to carry out successful spawnings and might transmit to their offspring the ability to function at a normal physiological level in the cave environment. Fish reared from eggs in the dark might not suffer this kind of glandular difficulty which has been shown to obtain in fish transferred from the light. This possibility is, of course, subject to experimental verification which will be undertaken in the future. The eyed, pigmented fish taken from La Cueva Chica, which appear exactly similar to the eyed river form, must represent either some of the few adjusted individuals from the river or the progeny of these fish that had spawned in the cave. Although it is unknown just how the endocrine balance has been accomplished in the cave environment, populations of fish have been evolved in the various caves that are capable of carrying on all the life processes in perpetual darkness, and this capability has not been found in the ancestral river form.

SUMMARY

1. THE EYED, RIVER-DWELLING CHARACIN, *Astyanax mexicanus*, can live for apparently indefinite periods in total darkness, but under such conditions develops hyperplasia of the thyroid, reduction in the relative number of pituitary basophiles, reduction in the size of the gonads, and an alteration in body form caused by the accumulation of subdermal fat.

2. The blind, cave-dwelling derivatives of *Astyanax* thrive in either light or darkness, and under either condition show no reduction in size of the gonads nor any change in body form. The form from La Cueva Chica may exhibit a slightly increased thyroid activity in the light, and deposits additional quantities of subdermal guanin. The form from Cueva de los Sabinos shows neither of these reactions.

3. When either river or cave fish that have been raised in the light are placed in darkness, no reduction in guanin takes place.

4. For comparison, young *Ameiurus* were similarly treated and showed a slight rise in thyroid activity in darkness.

5. Blinded river fish kept in the light show no morphological changes like those exhibited by individuals kept in the dark. Therefore enforced activity caused by the impossibility of optical fixation in the river fish in the dark cannot be the cause of increased thyroid activity, since the blinded fish kept in the light are forced into the same activity without subsequent increase in the height of the thyroid epithelium.

6. Hyperplasia of the thyroid in the normal river fish in light may be produced by treatment with thiourea, and a decrease in thyroid activity in river fish kept in the dark may be produced by feeding desiccated mammalian thyroid. In the first case the condition approximates that found in the untreated fish living in the dark, and in the latter the decrease may exceed that of normal fish living in the light.

7. The development of aberrant features in river fish kept in the dark and the lack of these features in their cave-dwelling derivatives may indicate that the latter have developed some modification of the endocrine system that enables them to function normally in the total darkness of caves.

8. Because of these reactions of the river fish to a totally dark environment, the present extensive cave populations may have been established by a few fish from the river that were able to make the appropriate endocrine adjustment, or from the eggs of river fish spawned in the cave before the influence of darkness had disturbed the endocrine balance sufficiently to make reproduction impossible. None of the present experimental animals was able to make such an adjustment. The physiological behavior of fish produced from eggs hatched in darkness is yet to be determined.

BIBLIOGRAPHY

ADAMS, A. E.
1933. The effects of hypophysectomy and anterior lobe administration on the skin and thyroid of *Triton cristatus*. Jour. Exp. Biol., vol. 10, pp. 247–255.

ALBERT, A.
1945. The experimental production of exophthalmos in *Fundulus* by means of anterior pituitary extracts. Endocrinology, vol. 37, pp. 389–406.

BAST, T. H., J. S. SUPERNAW, R. LIEBERMAN, AND J. MUNRO
1928. Studies in exhaustion due to lack of sleep. V. Effect on the thyroid and adrenal glands with special reference to mitochondria. Amer. Jour. Physiol., vol. 85, pp. 135–140.

BELL, W. R.
1938. Morphology of the hypophysis of the common goldfish (*Carassius auratus* L.). Zoologica, New York, vol. 23, pp. 219–234.

BERGFELD, W.
1930. Der Einfluss des Tageslichtes auf die Rattenschilddruse mit Berucksichtigung des Grundumsatzes. Endokrinologie, vol. 6, pp. 269–298.

BOCK, F.
1928. Die Hypophyse des Stichlings (*Gasterosteus aculeatus* L.) unter besonderer Berucksichtigung der jahrescyklischen Veranderungen. Zeitschr. Wiss. Zool., vol. 131, pp. 645–710.

BREDER, C. M., JR.
1942. Descriptive ecology of La Cueva Chica with especial reference to the blind fish, *Anoptichthys*. Zoologica, New York, vol. 27, pp. 7–15.
1943. Problems in the behavior and evolution of a species of blind cave fish. Trans. New York Acad. Sci., ser. 2, vol. 5, pp. 168–176.

BREDER, C. M., JR., AND E. B. GRESSER
1941a. Correlations between structural eye defects and behavior in the Mexican blind characin. Zoologica, New York, vol. 26, pp. 123–131.
1941b. Further studies on the light sensitivity and behavior of the Mexican blind characin. *Ibid.*, vol. 26, pp. 289–296.

BREDER, C. M., JR., AND P. RASQUIN
1947a. Evidence for the lack of a growth principle in the optic cyst of Mexican cave fish. Zoologica, New York, vol. 32, pp. 29–33.
1947b. Comparative studies in the light sensitivity of blind characins from a series of Mexican caves. Bull. Amer. Mus. Nat. Hist., vol. 89, pp. 323–351.

BRETSCHNEIDER, L. H., AND J. J. DUVENE DE WIT
1941. Histo-physiologische Analyse der sexuell-endokrinen organisation des Bitterlingweibchens (*Rhodeus amarus*). Zeitschr. Zellforsch. Mikro. Anat., vol. 31, pp. 227–344.

BRYANT, A. R.
1930. The effect of total thyroidectomy on the structure of the pituitary gland in the rabbit. Anat. Rec., vol. 47, pp. 131–145.

D'ANGELO, S. A.
1941. An analysis of the morphology of the pituitary and thyroid glands in amphibian metamorphosis. Amer. Jour. Anat., vol. 69, pp. 407–437.

DAWSON, A. B., AND H. B. FRIEDGOOD
1938. Differentiation of two classes of acidophiles in the anterior pituitary of the female rabbit and cat. Stain Tech., vol. 13, pp. 17–21.

DEMPSEY, E. W., AND G. B. WISLOCKI
1945. Histochemical reactions associated with basophilia and acidophilia in the placenta and pituitary gland. Amer. Jour. Anat., vol. 76, pp. 277–301.

DESCLIN, L.
1940. Detection de substances pentosenucleiques dans les cellules du lobe anterieur de l'hypophyse du rat et du cobaye. Compt. Rendus Soc. Biol., Paris, vol. 133, pp. 457–459.

ETKIN, W., R. W. ROOT, AND B. P. MOFSHIN
1940. The effect of thyroid feeding on oxygen consumption of the goldfish. Physiol. Zool., vol. 13, pp. 415–429.

FLORENTIN, P., AND F. STUTINSKI
1936. Modifications cytologiques de la gland pituitaire des grenouilles maintenues a l'obscurité. Compt. Rendus Soc. Biol., Paris, vol. 122, pp. 674–676.

GAYLORD, H. R., AND M. C. MARSH
1912. Carcinoma of the thyroid in the salmonoid fishes Bull. U. S. Bur. Fish., vol. 32, pp. 313–524.

GOLDSMITH, E. D., R. F. NIGRELLI, A. S. GORDON, H. A. CHARIPPER, AND M. GORDON
1944. Effect of thiourea upon fish development. Endocrinology, vol. 35, pp. 132–134.

GORBMAN, A.
1940. Suitability of the common goldfish for

assay of thyrotropic hormone. Proc. Soc. Exp. Biol. Med., vol. 45, pp. 772–773.

GRIESBACH, W. E., AND H. D. PURVES
1945. The significance of the basophil changes in the pituitary accompanying various forms of thyroxine deficiency. Brit. Jour. Exp. Path., vol. 26, pp. 13–17.

GROBSTEIN, C., AND A. W. BELLAMY
1939. Some effects of feeding thyroid to immature fishes. (*Platypoecilus*.) Proc. Soc. Exp. Biol. Med., vol. 41, pp. 363–365.

GUDERNATSCH, J. F.
1911. The thyroid gland of the teleosts. Jour. Morph., vol. 21, pp. 709–782.

HUBBS, C.
1938. Fishes from the caves of Yucatan. Carnegie Inst. Washington Publ., no. 91, pp. 261–295.

KENYON, A. T.
1935. Thyroid hypertrophy in the rat with references to the effect of light. Proc. Soc. Exp. Biol. Med., vol. 32, pp. 697–700.

KERR, T.
1942. A comparative study of some teleost pituitaries. Proc. Zool. Soc. London, vol. 112A, pp. 37–56.

LANDGREBE, F. W.
1941. The role of the pituitary and the thyroid in the development of teleosts. Jour. Exp. Biol., vol. 18, pp. 162–169.

LEVENSTEIN, I.
1939. The cytology of the pituitary gland of two varieties of gold fish (*Carassius auratus*, L.) with some reference to variable factors in the gland which may possibly be related to the different morphological types. Zoologica, New York, vol. 24, pp. 47–59.

LILLIE, R. D.
1940. Further experiments with the Masson Trichrome modification of Mallory's connective tissue stain. Stain Tech., vol. 15, pp. 17–23.

LYNE, EVERETT
1945. Cell division in relation to excessive fatigue. Thesis, New York University, 168 pp. (University of Michigan microfilm no. 727.)

MACKAY, E. M., AND J. W. SHERRILL
1942. Influence of thyroidectomy on fat deposition in the rat. Endocrinology, vol. 28, p. 518.

MARINE, D., S. H. ROSEN, AND C. SPARK
1935. Effect of iodine and desiccated thyroid on anterior pituitary of goitrous and thyroidectomized rabbits. Proc. Soc. Exp. Biol. Med., vol. 32, pp. 803–810.

MATTHEWS, S. A.
1936. The pituitary gland of *Fundulus*. Anat. Rec., vol. 65, pp. 357–367.

MATTHEWS, S. A., AND D. C. SMITH
1947. The effect of thiourea on the oxygen consumption of *Fundulus*. Physiol. Zool., vol. 20, pp. 161–164.

MAYERSON, H. S.
1935. The effect of light and darkness on the thyroid gland of the rat. Amer. Jour. Physiol., vol. 113, pp. 659–661.

MAYERSON, H. S., AND C. H. H. BRANCH
1934. Effect of light and of darkness on thyroid. Proc. Soc. Exp. Biol. Med., vol. 31, pp. 650–652.

NIGRELLI, R. F.
1943. Causes of diseases and death of fishes in captivity. Zoologica, New York, vol. 28, pp. 203–216.
1947. Spontaneous neoplasms in fishes. III. Lymphosarcoma in *Asytanax* and *Esox*. *Ibid.*, vol. 32, pp. 101–108.

OLIVEREAU, M.
1947. Histologie comparée de l'hypophyse et de la thyroide des deux types de Rougets: *Mullus barbatus*, L. et *Mullus surmuletus*, L. Compt. Rendus Acad. Sci. Paris, vol. 224, pp. 596–598.

POMERAT, G. R.
1942. Cell changes in the pituitary and ovary of the white rat following exposure to constant light or darkness. Anat. Rec., vol. 82, pp. 531–542.

RASMUSSEN, A. T.
1929. The percentage of the different types of cells in the male adult human hypophysis. Amer. Jour. Pathol., vol. 5, pp. 263–274.

RASQUIN, P.
1947. Progressive pigmentary regression in fishes associated with cave environments. Zoologica, New York, vol. 32, pp. 35–42.

RAWSON, R. W., AND P. STARR
1938. Direct measurement of height of thyroid epithelium. Arch. Intern. Med., vol. 38, pp. 726–738.

SCHAEFER, W. H.
1933. Hypophysectomy and thyroidectomy of snakes. Proc. Soc. Exp. Biol. Med., vol. 30, pp. 1363–1365.

SCHLAGEL, S. R., AND C. M. BREDER, JR.
1947. A study of the oxygen consumption of blind and eyed cave characins in light and darkness. Zoologica, New York, vol. 32, pp. 17–27.

SCRUGGS, W. M.
1939. The epithelial components of the teleost pituitary gland as identified by a stand-

ardized method of selective staining. Jour. Morph., vol. 65, pp. 187–214.

SELYE, HANS
1946. The general adaptation syndrome and the diseases of adaptation. Jour. Clin. End., vol. 6, pp. 117–230.

SEVERINGHAUS, A. E.
1937. Cellular changes in the anterior hypophysis with special reference to its secretory activities. Physiol. Rev., vol. 17, pp. 556–588.
1939. Anterior hypophyseal cytology in relation to the reproductive hormones. *In* Allen, Edgar (ed.), Sex and internal secretions. Baltimore, Williams and Wilkins, chap. 19, pp. 1045–1087.

SEVERINGHAUS, A. E., G. K. SMELSER, AND H. M. CLARK
1934a. Anterior pituitary changes in adult male rats following thyroxin injections or thyroid feeding. Proc. Soc. Exp. Biol. Med., vol. 31, pp. 1125–1127.
1934b. Anterior pituitary changes in the adult male rat following thyroidectomy. *Ibid.*, vol. 31, pp. 1127–1129.

SIMPSON, G. G., AND A. ROE
1939. Quantitative zoology. New York, McGraw-Hill Book Company.

SMITH, D. C., AND G. M. EVERETT
1943. The effect of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish, *Lebistes reticulatus*. Jour. Exp. Zool., vol. 94, pp. 229–240.

STUTINSKI, F.
1936. Effets de l'éclairment continu sur la structure de la gland pituitaire de la grenouille. Compt. Rendus Soc. Biol., Paris, vol. 123, pp. 421–423.

TURNER, K. B., AND E. M. BENEDICT
1932. Thyroid hyperplasia produced in chickens by ultra-violet light deficiency. Jour. Clin. Invest., vol. 11, pp. 761–774.

UOTILA, U. U.
1939. The role of the cervical sympathetics in the regulation of thyroid and thyrotropic function. Endocrinology, vol. 25, pp. 63–70.

WOITKEWITSCH, A. A.
1944a. Influence of light upon thyrotropic activity of anterior lobe of pituitary body. Compt. Rendus Acad. Sci. U.R.S.S., vol. 45, pp. 357–360.
1944b. Function of thyroid as dependent on pituitary body activized by light. *Ibid.*, vol. 45, pp. 396–400.
1946. Phenomenon of functional exhaustion of certain endocrine glands (thyroid gland and anterior lobe of pituitary body). *Ibid.*, vol. 51, pp. 729–732.

ZALESKY, M. L., L. J. WELLS, M. D. OVERHOLSER, AND E. T. GOMEZ
1941. Effects of hypophysectomy and replacement therapy on the thyroid and adrenal glands of the male ground squirrel. Endocrinology, vol. 28, pp. 521–530.