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Response of a Spontaneous Fish Lymphosarcoma to Mammalian ACTH[1].

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(Plate I).

INTRODUCTION.

There are relatively few reports in the literature of diseases of lymphatic tissues in teleosts. The lines of experimental investigation which have been pursued in this field have not included any study of the relationship of the endocrine organs to lymphoid disorders.

Dougherty & White (1943, 1945, 1946) have demonstrated a close functional relationship between the pituitary and adrenal cortex and normal lymphoid tissue in mammals. Rasquin (1951) found a similar relationship in the teleost *Astyanax mexicanus*. Heilman & Kendall (1944), Pearson et al. (1949) and Sugiura et al. (1950) have shown that pituitary adrenotropic hormone and some of the adrenal cortical hormones have an inhibitory effect on lymphoid tumors in mammals.

In the light of the pituitary-adrenocortical-lymphoid tissue relationship, it was of interest to ascertain whether a spontaneous lymphosarcoma which appeared in a hybrid characin in this laboratory would undergo any regressive changes after stimulation of the adrenal cortex. A description of the metastasizing lymphosarcoma and its response to the administration of mammalian ACTH is given in this report.

MATERIALS AND METHODS.

Three fish were used for this investigation, all approximately six years old and all laboratory bred. The tumor-bearing fish was a male hybrid, a cross between *Astyanax mexicanus* (Filippi) and a blind cave derivative *Anoptichthys jordani* Hubbs & Innes. The second fish was a female of the same brood used as a normal untreated control. Both these fish showed the same pigmentation as the pure bred *Astyanax* although the eyes were somewhat smaller. As no more fish of this brood were available, the third fish used as an ACTH-injected control was a pure bred male *Astyanax* of the same age.

Three biopsies were made from the growth before injections were begun. The fish was anesthetized with 1% urethane and a piece was cut from the protruding tumor mass. The first biopsy was fixed in Bouin's fluid, sectioned at five microns and the sections were stained with Harris' hematoxylin and eosin, Masson's and Giemsa's stains. The second and third biopsies were taken two and one-half and four weeks later and were used for tissue culture, the results of which will be published subsequently.

Figure 1 is a photograph of the living fish showing the tumor protruding from the left branchial cavity. The photograph was taken 13 days after the third biopsy and prior to ACTH injection. From that day, 12 intraperitoneal injections of 0.1 mg. ACTH in 0.05 cc. of Holtfreter's solution were given, one per day, and one injection of 0.05 mg. in 0.03 cc. of solution was given, making a total of 1.25 mg. ACTH administered. The ACTH was a part of Lot 58-9(50) which had a biological potency of 120% of the Armour standard La-I-A and contained 0.03 units of oxytocin per milligram. The fish was sacrificed 24 hours after the last injection by direct fixation in Bouin's fluid. Immediately after death, the fish was rolled in blotting paper to remove excess moisture and the standard length and body weight were taken. The entire fish was decalcified, imbedded in paraffin and serial transverse sections were cut at five, seven and ten microns and then stained, as were the biopsy sections.

The ACTH used for the control injected fish was a part of Armour Lot 128-105R which had a potency 1.6 times the Armour La-I-A standard. To give a total concentration of ACTH biologically equivalent to the 1.25 mg. given to the tumorous fish, the control animal was given one daily intraperitoneal injection of 0.075 mg. ACTH in 0.05 cc. Holtfreter's solution for 12 successive days. The fish was sacrificed 24 hours after the last injection and the standard length and body weight were taken. This injected control and the normal untreated female control were fixed, decalcified, and imbedded as above. Sections were made of the pituitary region, the head-kidney with adrenal cortex, spleen, liver, pancreas and gonad.

Counts of mitotic figures per 10,000 lymphocytes were made on the injected tumorous

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fish from each of the three following regions: (1) the center of the tumor mass, (2) the periphery of the tumor, that is, the part which protruded from the branchial cavity and was equivalent to the tissue used for biopsy, and (3) the growing edge of the tumor directly invading the pharyngeal muscle. A Leitz ocular micrometer ruled in 100 equal squares was used for counting, and only sections cut at five microns and stained with hematoxylin and eosin were used for this technique. The results were compared with similarly-made counts on lymphoctyes in the untreated biopsy. Measurements of lymphocytes across the greatest diameter were made on 50 cells from each of the same regions in the treated fish and compared with measurements similarly made of lymphocytes from the untreated biopsy. Comparisons for statistical significance were made by using the following formula for the derivation of the value of $d/\sigma d$.

$$\sigma d = \sqrt{\sigma M_1^2 + \sigma M_2^2}$$

A Leitz ocular grid micrometer marked off in 100 equal squares was used for making the differential counts of the pituitary cells. Beginning with the first section that contained transitional lobe tissue, all the cells of the transitional lobe were counted in every fourth section through to the end of the transitional area. It was possible to count all the cells within the squared area, and by moving the counting area carefully, to count the remaining fields in the same section without duplicating any field already counted. Therefore no part of the transitional lobe was unrepresented in the final count. The total number of cells represents the number counted in each transitional lobe and is an index derived from counting every fourth section. It is not intended to signify the actual total number of cells in the transitional lobe.

Observations.

The growth when first observed was a gray-white, firm, resilient mass. It was well vascularized and of sufficient size to push the operculum almost at right angles to the body. Biopsy caused little bleeding. About three days after each biopsy the protruding mass had grown back to its original size, appearing first hyperemic, then grayish as melanophores appeared in the periphery. After the third biopsy, the tumor changed somewhat so that it projected posteriorly and ventrally rather than perpendicularly to the body. The fish ate very little, if at all, during the experimental period, but seemed otherwise undisturbed. The appetite of the ACTH-treated control was apparently unaffected by the experimental procedures.

Histologically, the tissue taken at biopsy was composed of small, closely packed lymphocytes supported by a fine reticular stroma. It corresponded to descriptions given for lymphosarcomas of other fishes as well as for lymphosarcomas of mammals described by Boyd (1939) and Ewing (1940).

Although lymph nodes are not found in fishes, regions of lymphoid concentration occur in the intestinal mucosa, spleen, thymus, kidney and head-kidney. Histological examination of the lymphosarcomatous fish showed that the growth originated in the thymus. Direct proliferation of the tumor extended anteriorly to about the level of the optic lobes, involving muscular, osseous, cartilaginous, nerve and epithelial tissues. Direct invasion of the brain or pituitary did not occur although these structures were surrounded by tumor cells in the intercranial spaces. The gills were not extensively involved. Direct proliferation posteriorly did not extend beyond the level of the esophagus and the head-kidney.

The growth was extended by metastases involving to some extent the kidney, corpuscles of Stannius, liver, pancreas, intestine, mesentery and peritoneum. The renal tubules were intact although pycnotic lymphocytes filled the spaces between them and in certain areas caused constriction of the tubules. However, the concentration of lymphocytes in the glomeruli rendered these structures for the most part invisible. The corpuscles of Stannius were heavily infiltrated in some regions. Metastasis to the liver was not extensive, occurring mostly in areas confined about the blood vessels and following the path of the interhepatic pancreas. In a few regions, the lymphocytes had broken up the perivascular arrangement of the pancreatic cells and replaced the glandular tissue. Diffuse portions of the pancreas embedded in the connective and fatty tissue of the mesentery were also surrounded by lymphocytes. The islet tissue was not involved. Although no metatases were found in the stomach, all the layers of the intestinal mucosa were involved to some extent. However, the lumen was not occluded. Metastatic growths were also seen in the mesentery and peritoneum; some regions were heavily infiltrated, while other areas contained only small patches of lymphocytes or none at all. The center of the tumor mass was poorly vascularized and was composed of densely packed lymphocytes lying in the meshes of a delicate reticulum. Some regions of the growth were necrotic and the cells were clumped together, leaving clear spaces around the aggregates.

The results of ACTH injection are divided in two categories: the effects on the tumor tissue, and the effects on normal tissues, both in the tumorous fish and in the ACTH-injected control.

After administration of ACTH, the protruding tumor mass changed in consistency from a firm, resilient mass to a softer, more flaccid one. Sections showed that this was probably a result of edema which appeared mainly in the periphery of the tumor. An

TABLE I.

Numbers of Mitotic Cells in the Lymphosarcoma Before and After ACTH Treatment.

Region	*% Mitotic figures per 10,000 lymphocytes*
Biopsy before ACTH treatment	3.38
Periphery of tumor after ACTH treatment	0.26
Center of tumor after ACTH treatment	0.48
Pharyngeal invasion after ACTH treatment	0.55

abrupt shift in staining reaction was exhibited by this part of the tumor. The center of the tumor mass was markedly basophilic when stained with hematoxylin and eosin, but where the tumor protruded from the operculum the lymphocytes took a strong eosin stain while the branchial epithelium which was stretched far out of normal position, stained normally. This protruding part of the tumor, which was equivalent to those portions cut for biopsy, differed histologically from both the untreated biopsy and the central part of the tumor. Lymphocytes were not densely packed. Some areas were very edematous and the reticular stroma was much more clearly seen. Figure 2 is a photograph of a section of the fish through the thoracic area, showing the extent of the tumor and the edematous portion at the periphery. Figure 3 is a photomicrograph under high power of the untreated biopsy which may be contrasted with Figure 4, a photomicrograph of the same peripheral area after ACTH administration. It seems improbable that this result was caused by lymphocytes falling out of the meshes of the reticulum at the time of biopsy, for the administration of ACTH was begun well after the cut edge had healed and the mass had regrown to its original size. Also the change in the consistency of the tumor on palpation occurred only after ACTH administration and not after biopsy.

Pycnotic lymphocytes were frequently seen as well as many others in various states of disintegration, shedding or extruding cytoplasm, or phagocytized by macrophages. These evidences of destruction were found in all parts of the tumor and in all the metastases with the exception of those to the liver and pancreas.

When comparisons are made between the tumor at biopsy and after ACTH-treatment, the lymphocytes in the latter case show a statistically significant decrease in size and numbers of mitotic divisions. Table I shows the decrease in percent of mitotic figures per 10,000 cells from 3.38% in the biopsy to as low as 0.26% in the tumor after ACTH administration. All phases of mitosis were observed in both tissues despite the decrease in numbers of dividing lymphocytes in the treated tumor. Table II shows a significant decrease in the size of the tumor lymphocytes after ACTH administration. The extreme diameters in the biopsy ranged from 3.0 to 5.0 microns as compared with 2.0 to 4.0 microns in the treated tumor.

The greatest effect of ACTH injection on normal tissues was noted in an enlargement of the anterior interrenal cells surrounding the cardinal veins. This tissue has been shown to be homologous with the mammalian adrenal cortex in this species (Rasquin, 1951). In both ACTH-treated fishes, individual cortical cells were greatly hypertrophied and cords of hyperplastic cells could

TABLE II.

Comparison of Lymphocyte Diameter for Statistical Significance Before and After ACTH Treatment.

Region	*Mean Lymphocyte Diameter in microns*	*Standard Deviation*	*Standard Error*	*Significance*
Biopsy before ACTH injection	4.04	.598	.08458	7.0
Center tumor mass after ACTH injection	3.29	.458	.06478	
Biopsy before ACTH injection	4.04	.598	.08458	4.5
Pharyngeal invasion after ACTH injection	3.46	.699	.09887	
Biopsy before ACTH injection	4.04	.598	.08458	6.5
Periphery of tumor after ACTH injection	3.34	.463	.06549	

be seen extending into the lumen of the cardinal vein as well as spreading into the parenchyma of the head-kidney. Granules which stained with methylene azure were seen within the cortical cells in Giemsa-stained sections. These basophilic granules were not specifically located in the cells either with respect to the nucleus or to the lumen of the blood vessel. They were not seen in the cortical cells of the normal untreated control.

Changes in the lymphoid tissues, whether normal or malignant, were undoubtedly a result of the stimulated cortical tissue. Most of the normal lymphocytes in the head-kidney showed greater destructive changes than did those tumor cells that had proliferated directly to this organ from the adjacent tumor mass. The parenchyma of the head-kidney of both ACTH-injected fishes appeared pitted with edematous areas and regions of greater or less lymphocyte concentration. Most of the lymphocytes had pycnotic nuclei and many had been phagocytized by macrophages. None of the cells of the hemopoietic series of either ACTH-injected fish showed any dividing forms. The head-kidney of the untreated control appeared normal; occasional mitoses were observed in the hemopoietic cells and there was less vascularization than in the injected fishes.

The spleens of both treated fishes were almost completely depleted of lymphoid elements. The vascular sinuses or pulp spaces were so engorged with mature red blood cells that superficially the organ appeared to be only an open sinus filled with blood. Close examination showed slender trabeculae from the capsule to be intact. Some small pycnotic lymphocytes were found concentrated in a ring around the periphery. The spleen of the untreated control fish showed the normal condition characterized by the presence of lymphoblasts and small lymphocytes, phagocytes and various types of granulocytes lying as irregular aggregates in the pulp, or arranged concentrically about the thick-walled ellipsoids, those arterioles common to the spleens of fishes.

The thymus in the tumor-bearing fish had been completely obliterated by the growth. The thymus of both the normal and ACTH-injected controls had undergone age involution and no apparent difference in degree of atrophy was discernible between them. The thymocytes, however, of the ACTH-injected control, were pycnotic and in some regions were clumped together in dense necrotic aggregates in contrast to the normal thymocytes of the untreated control.

No metastasis was observed in the testis of the lymphosarcomatous fish. In both ACTH-treated fishes, the sperm duct epithelium was greatly stimulated, giving the appearance of increased secretory activity. Spermatogenesis appeared normal in the tumorous fish, but was tremendously stimulated in the ACTH-injected control where the testicular lobules were completely filled with mature sperm.

The results of the differential analyses of the pituitary cells are given in Table III. According to work previously reported by Rasquin (1949, 1951) the proportions of cells shown by the two control fishes were normal for the sexually mature adult of this species. Histological study of the pituitary of the tumor-bearing fish indicates that the abnormal proportion of cells was a result of the loss of basophiles and not of an increase in acidophiles. In all three fishes the anterior lobes of the pituitaries were intact; the nuclei and cytoplasm were normal in form and granulation. The function of this part of the teleost pituitary is at present unknown. The transitional lobes, that are homologous to the mammalian anterior pituitary, showed excessive vacuolation of the basophiles. Granules differed in size and were clumped. Small sinuses or lacunae were present that appeared to have been formed by the union of adjacent vacuolations. In the tumor-bearing fish this process had advanced to such a state that the transitional lobe contained large numbers of lacunae and one had assumed such large proportions that it occupied approximately ⅛ to ¼ of the area of the sections. It was filled with an acellular, granular debris. Retrograde changes were also seen in the intermediate lobes of all three fishes.

Discussion.

Schlumberger & Lucké (1948), in a review of tumors of cold-blooded vertebrates, have described lymphosarcomas in 20 fishes and in a complementary paper the same authors (1949) reviewed the lines of experimental investigation which have been pursued. Five of these lymphosarcomas were non-metastatic. Two were reported by Johnstone, one arising in the eye of a female flounder, *Pleuronectes flesus* (1912), and the other in the body cavity of a herring of undetermined sex (1926). Another was described by Williams (1931), having its origin in the kidney of an adult female conger eel. Two more were reported by Haddow & Blake (1933), arising in the kidney of the salmon, *Salmo salar*, and at the base of the fins of a pike, *Esox lucius*. The work of Haddow & Blake was abstracted by Schlumberger & Lucké (1948).

The remaining descriptions of fish lymphosarcomas are all of metastatic tumors. Plehn (1924) described such a tumor originating in the kidney of a goldfish, which not only caused extensive damage to that organ but metastasized to the enlarged liver. Smith, Coates & Strong (1936) reported a lymphosarcoma from one *Rasbora lateristriata* which they believed had originated from lymphatic tissue near the peritoneum and which showed involvement of the roof of the oral cavity, the gills, the base of the brain and the left auditory sacculus. Nigrelli (1943,

TABLE III.

Standard Lengths and Weights of the Fishes and Statistical Analysis of the Cells of the Transitional Lobes of the Pituitaries.

	Lymphosarcomatous ACTH-injected fish	*ACTH-injected control fish*	*Normal untreated control fish*
Standard length in centimeters	6.7	7.1	—
Weight in grams	8.5	7.1	—
Total cells counted in pituitary	13,837	43,259	15,876
% pituitary basophiles	46.3	68.5	65.8
% pituitary acidophiles	52.1	29.9	32.6
% pituitary chromophobes	1.6	1.6	1.6

1947) described spontaneously occurring lymphosarcomas in 12 adult northern pike, *Esox lucius*. Both sexes were represented in this number. The tumor originated in the kidney where massive growths were characterized by lymphoblast-type cells supported by a reticular and fibrous stroma. Metastases occurred in the liver, spleen and retroperitoneal tissues.

Nigrelli (1947) also described an isolated case of lymphosarcoma in a four-year-old male *Astyanax mexicanus*. The fish appeared exophthalmic and the branchial region was enlarged. The swollen appearance was caused by a large mass of typical lymphoid cells which originated in the thymus-like lymphoid gland. There was considerable local proliferation as well as metastases to the skin, gills, submucosa of the intestine, liver, pancreas, kidney, spleen, testis and retroperitoneal tissues. This tumor occurred in one of the parental species of the fish with which the present report is concerned, and originated from the same organ. Slides kindly loaned to the authors by Dr. Nigrelli show the two tumors to be identical histologically. Four of these tumors have arisen spontaneously among approximately two thousand *Astyanax* bred in the laboratory. Three occurred in adult males, one in an adult female.

Various adrenocortical preparations as well as pituitary adrenocorticotropin have been used experimentally on mammalian lymphosarcomas. Compound E, used by Heilman & Kendall (1944), not only prevented development of the tumor in a 100% susceptible inbred strain of mice, but also, when administered in larger doses, caused marked regression of large tumors. The sex of the animals influenced the response of the tumor to Compound E. Female and young male mice showed quick and complete tumor absorption, whereas adult males responded only if estradiol propionate was administered simultaneously with Compound E, or if the mice were castrated. Sugiura et al. (1950) also found that Compound E markedly inhibited experimental mouse lymphosarcomas, but no sex difference in the response of the tumor was reported. Pearson et al. (1949) reported regression but not complete clinical remission with ACTH therapy in lymphoid tumors in man.

The structural similiarity between tumors in cold-blooded vertebrates and corresponding tumors of warm-blooded animals is well known. The inhibition of a fish lymphosarcoma by ACTH indicates a similiarity of physiological response between the fish and mammalian tumors. The dosages of ACTH used were necessarily arbitrary and it is conceivable that a more striking regression might have occurred had a greater amount of the hormone been employed.

The stimulation of the testis by ACTH administration is contrary to the results obtained by Baker et al. (1950). Such stimulation of the gonad would normally be associated with the administration of gonadotropic hormones. Since the ACTH supplied was contaminated only with a small fraction of posterior lobe hormone, the indication is either that the fishes did not react as specifically to the adrenocorticotropin as mammals do, or that the stimulated adrenal cortex elaborated androgenic substance.

The increased vacuolation in the transitional lobes of the pituitaries was probably associated with old age. The cytological changes were shown by all three fishes and old age was a common factor among them. The index of the total numbers of cells shown by the tumorous fish and the untreated control is common in this species, but the enormous number shown by the ACTH-injected control is unexplained since the gland showed no evidence of hyperplasia. It has been shown (Rasquin, 1951) that in normal fish, single injections of ACTH did not change the proportions of basophiles to acidophiles and chromophobes in the pituitary transitional lobes. This unaltered proportion also was noted here in the ACTH-treated control after repeated injections. It

therefore seems likely that the abnormal proportions of cells in the tumorous fish existed before ACTH treatment. Whether or not the lower per cent. of basophiles is associated with the etiology of the tumor is unknown.

Koneff (1944) found that continued administration of ACTH caused changes in the basophiles of the rat pituitary indicative of a depressed activity, while the acidophiles were unaltered. Also working with rats, Tuchmann-Duplessis (1950) found degranulation of both acidophiles and basophiles after adrenalectomy, and decrease in size and staining reaction of basophiles after ACTH administration. Hypertrophy and rich granulation of basophiles was noted after DCA administration. When assayed biologically, the hypophyses of animals rich in basophiles were reported to contain twice as much hormones as those of adrenalectomized animals. Rasquin (1951) has shown in younger *Astyanax* some indication of a decrease in total number of transitional lobe cells after a single injection of ACTH, suggestive of depressed activity. A study of the effects of adrenocortical stimulation on the pituitary of *Astyanax* is in progress.

Summary.

A six-year old male hybrid *Astyanax-Anoptichthys* bearing an extensive and metastatic lymphosarcoma was subjected to 12 daily injections of 0.1 mg. ACTH. Three biopsies were taken from the tumor before injection was begun. Two controls were used. One was a normal untreated female broodmate and the other was a six-year old male *Astyanax* subjected to the same injection routine.

Inhibition of the tumor after ACTH injections was shown grossly by a softening of the firm, resilient tumor mass. Histological examination showed edematous spaces and cellular debris in parts of the tumor. There was a marked decrease in the numbers of mitotic figures and a significant decrease in size of the lymphocytes of the tumor as compared with the untreated biopsy.

The adrenal cortex was hypertrophied in both ACTH-injected fishes. The lymphoid tissue of the head-kidney was in a state of disintegration and no mitotic figures were seen in the hemopoietic cells. The spleen was depleted of all lymphoid elements. The untreated control was normal in these respects.

All the fishes showed destructive changes in the hypophyses which were attributed to old age. Only the tumor-bearing fish showed an abnormal differential cell count in the pituitary, associated with a reduction in number of basophiles.

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References.

Baker, Burton L., Marjorie A. Schairer, Dwight J. Ingle, & Choh Hao Li

1950. The induction of involution in the male reproductive system by treatment with adrenocorticotropin. *Anat. Rec.*, 106: 345-359.

Boyd, William

1939. A Text-Book of Pathology. 3rd ed. Lea and Febiger, Philadelphia: 833-838.

Dougherty, Thomas F. & Abraham White

1943. Effect of pituitary adrenotropic hormone on lymphoid tissue. *Proc. Soc. Exper. Biol. & Med.*, 53: 132-133.

1945. Functional alterations in lymphoid tissue induced by adrenal cortical secretion. *Amer. Jour. Anat.*, 77: 81-116.

1946. Pituitary-adrenal cortical control of lymphocyte structure and function as revealed by experimental X-Radiation. *Endocrinology*, 29: 370-385.

Ewing, James

1940. Neoplastic Diseases. 4th ed. W. B. Saunders Co., Philadelphia and London: 421-433.

Haddow, A. & I. Blake

1933. Neoplasms in fish: A report of 6 cases with a summary of the literature. *Jour. Path. & Bact.*, 36: 41-47.

Heilman, F. R. & E. C. Kendall

1944. Influence of 11-dehydro-17-hydroxycorticosterone (Compound E) on growth of malignant tumor in the mouse. *Endocrinology*, 34: 416-420.

Johnstone, Jas.

1912. Internal parasites and diseased conditions of fishes. *Proc. & Trans. Liverpool Biol. Soc.*, 26: 103-144.

1926. Malignant and other tumors in marine fishes. *Ibid.*, 40: 75-98.

Koneff, A.

1944. Effect of adrenocorticotropic hormone on the anterior pituitary of the normal young male rat. *Endocrinology*, 34: 77-82.

Lucké, Balduin & H. G. Schlumberger

1949. Neoplasia in cold-blooded vertebrates. *Physiol. Rev.*, 29: 91-126.

Nigrelli, Ross F.

1943. Causes of diseases and death of fishes in captivity. *Zoologica*, 28: 203-216.

1947. Spontaneous neoplasms in fishes. III. Lymphosarcoma in *Astyanax* and *Esox*. *Ibid.*, 32: 101-108.

Pearson, O. H., L. P. Eliel, Rulon W. Rawson, Kondrad Dobriner, & C. P. Rhoads

1949. ACTH- and cortisone-induced regression of lymphoid tumors in man. A preliminary report. *Cancer*, 2: 943-945.

PLEHN, MARIANNE

1924. Praktikum der fischkrankheiten. In Handbuch der Binnenfischerei Mitteleuropas. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, Band I: 398-403.

RASQUIN, PRISCILLA

1949. The influence of light and darkness on thyroid and pituitary activity of the characin *Astyanax mexicanus* and its cave derivatives. *Bull. Amer. Mus. Nat. Hist.*, 93: 501-531.

1951. Effects of carp pituitary and mammalian ACTH on the endocrine systems of the teleost *Astyanax mexicanus*. *Jour. Exper. Zool.*, 117: (In Press).

SCHLUMBERGER, H. G. & BALDUIN LUCKÉ

1948. Tumors of fishes, amphibians and reptiles. *Cancer Research*, 8: 657-754.

SMITH, G. M., C. W. COATES, & L. C. STRONG

1936. Neoplastic diseases in small tropical fishes. *Zoologica*, 21: 219-224.

SUGIURA, KANEMATSU, C. CHESTER STOCK, & C. P. RHOADS

1950. The effect of cortisone and other steroids on experimental tumors. *Cancer Research*, 10: 244-245.

TUCHMANN-DUPLESSIS, H.

1950. L'action de la surrénale sur la structure et le fonctionnement de l'hypophyse du rat. *Bull. d'Hist. appl. et de Tech. Micros.*, 27: 89-99.

WILLIAMS, G.

1931. On various fish tumors. *Proc. & Trans. Liverpool Biol. Soc.*, 45: 98-109.

EXPLANATION OF THE PLATE.

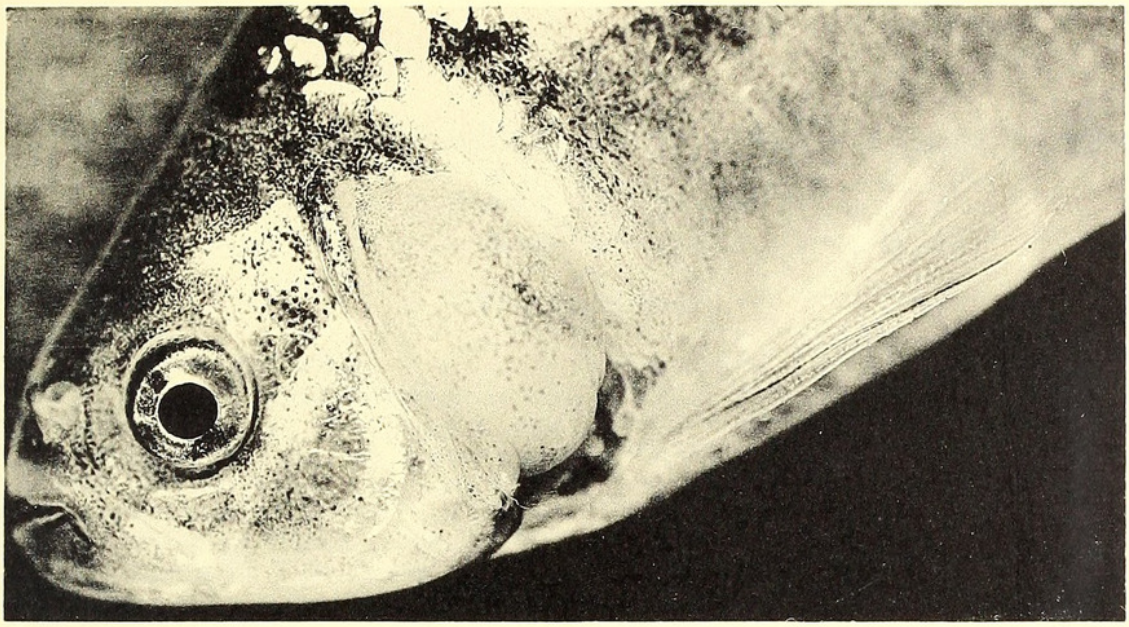
PLATE I.

Fig. 1. Photograph of the living fish prior to ACTH injections, showing the tumor protruding from the branchial cavity. The operculum has been deflected outward and forward by the tumor mass so that it is vertical to the plane of the photograph. Magnification 4×.

Fig. 2. Photomicrograph of a transverse section through the thoracic region of the fish, showing the extent of the tumor and the edematous periphery after ACTH administration. Magnification 83×.

Fig. 3. Photomicrograph of the untreated biopsy. Three mitotic figures are seen in the center of the field. Magnification 1000×.

Fig. 4. Photomicrograph of the peripheral region of the tumor after administration of ACTH. Magnification 1000×.



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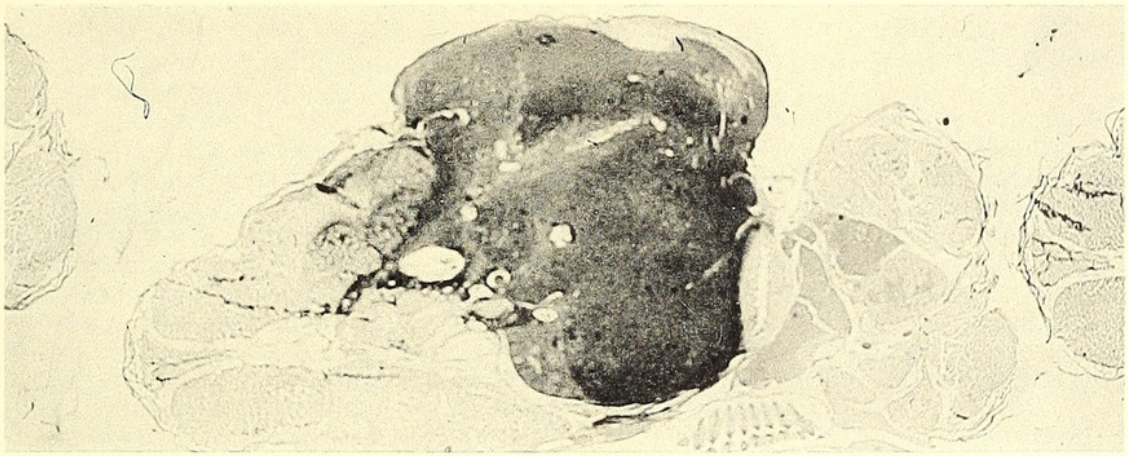

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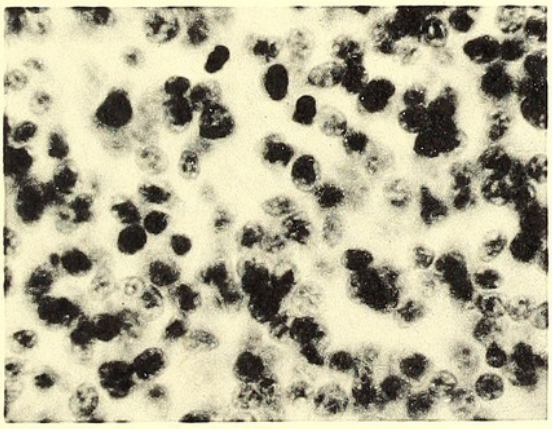

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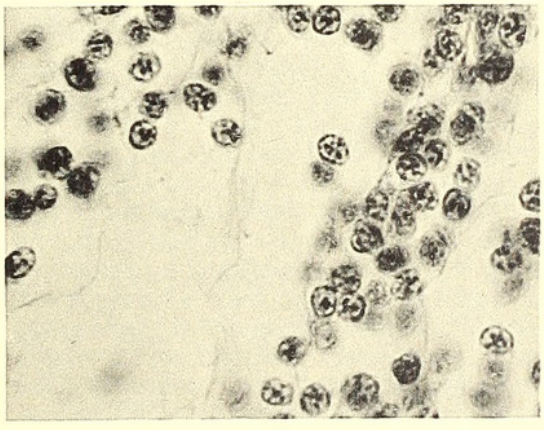

4

RESPONSE OF A SPONTANEOUS FISH LYMPHOSARCOMA TO MAMMALIAN ACTH.