6.

Spontaneous Neoplasms in Fishes. V. Acinar Adenocarcinoma of the Pancreas in a Hybrid Platyfish.

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(Plates I-VIII; Text-figure 1).

INTRODUCTION.

Pancreatic tumors in fishes have never been reported. Among thousands of viviparous killifishes bred and reared in the Genetics Laboratory of the New York Aquarium, and among thousands of others routinely autopsied, only a single adenocarcinoma of the pancreas was found. The histological details of this tumor are remarkably similar to those of comparable pancreatic neoplasms in man.

MATERIALS AND METHODS.

The tumor of the pancreas was found in a 16-months-old male hybrid platyfish, *Platypoecilus variatus* × *Platypoecilus xiphidium*. The genetic history of this fish is as follows. Its female parent was a Spike-tail Platyfish, *Platypoecilus xiphidium*, of a stock originally obtained from the Rio Purificacion, Tamaulipas, Mexico. Its male parent was a Variatus Platyfish, *Platypoecilus variatus*, of a stock originally obtained from a pool at El Nilo, probably associated with the Rio Tampaon, San Luis Potosi, Mexico. The female exhibited, at the anterior margin of the caudal fin, two heritable color patterns, called *crescent* and *cut-crescent* which may be referred to the dominant autosomal genes *C* and *Ct*, respectively (Text-fig. 1). With the recessive gene, here referred to by plus signs (+), these form a series of three multiple alleles. The male was heavily spotted with large melanophores, and this heritable trait may be referred to the independent, dominant, spotted gene *Sp*. As will be seen by the results obtained from the mating of these individuals, the male was apparently heterozygous for the spotted gene (*Sp*+):

P1

Platypoecilus xiphidium Female	*Platypoecilus variatus* Male
Crescent and Cut-Crescent	Black spotted
++ *CCt*	*Sp*+ ++

F1
(Pedigree h3)

	Daughters	Sons
Sp+ *C*+ (spotted, crescent)	5	3
Sp+ *Ct*+ (spotted, cut-crescent)	1	4
++ *C*+ (no spots, crescent)	8	6
++ *Ct*+ (no spots, cut-crescent)	4	1
Totals	18	14

The results indicate that the *C* and *Ct* genes are probably allelic, since they segregate approximately equally among the first generation hybrids (Plate I, Fig. 1). The spotted gene segregates independently and is found only in about half of the hybrids. The hybrid with the pancreatic tumor was one of the three males that were spotted and had a crescent pattern (Text-fig. 1).

The external manifestation of the tumor in this fish was a swollen belly, which had developed gradually. The behavior of the fish, however, did not indicate any functional disturbance up to and including the time at which it was sacrificed. Autopsy revealed a large tumorous growth between the folds of the intestine, the main mass being posterior and ventral in position. The intestine, liver and spleen were displaced and the gall bladder was greatly distended with a yellowish, watery bile. The kidneys were intact and typical in external appearance. Although the fish had a well developed gonopodium and was presumably a male, no testis or gonadal tissue were found. With the exception of the kidneys, all the viscera, including the tumorous growth, were removed and fixed in Bouin's solution, embedded in paraffin and sectioned at 6 microns. The mounted sections were stained with Delafield's haematoxylin-eosin, Heidenhain's iron-haematoxylin with and without eosin, and by the methods of

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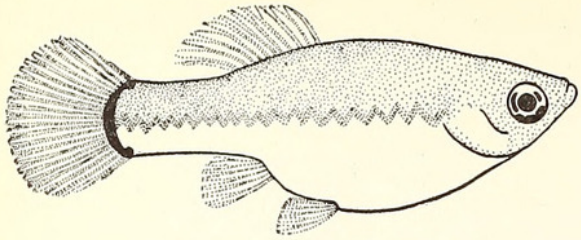
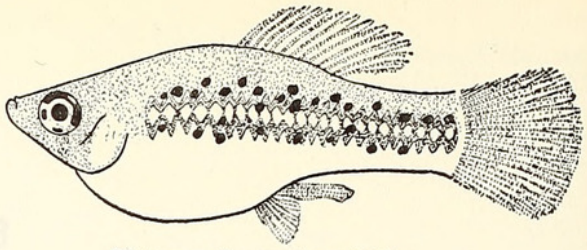
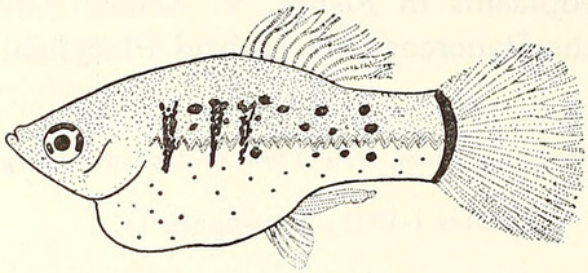


Platypoecilus xiphidium Female



Platypoecilus variatus Male



Platypoecilus xiphidium × *Platypoecilus variatus* Hybrid

TEXT-FIG. 1. The hybrid platyfish with an acinar adenocarcinoma of the pancreas was found among 32 fish from the mating of a female Spike-tail Platyfish, *Platypoecilus xiphidium*, and a male Variatus Platyfish, *Platypoecilus variatus*.

Giemsa, Mallory and Gomori. The pancreas and other organs of normal specimens of the closely related Common Platyfish, *Platypoecilus maculatus*, were similarly treated for purposes of comparison.

HISTOLOGY OF THE PANCREAS IN *Platypoecilus*.

The exocrine and endocrine parts of the pancreas in the normal platyfish are separate structures (Reiter & Nigrelli, 1949). The exocrine gland is a diffuse organ suspended in the mesentery between the folds of the intestine, extending from the stomach to the posterior end of the intestine. The main portion of the gland, however, is found in the duodenal region (Plate I, Fig. 2).

The lobules of the gland are held together by a loose connective tissue which also contains fat cells and blood vessels (Plate I, Fig. 2). Each acinus consists of a single row of epithelial cells, resting on a delicate reticular membrane and converging towards a central lumen (Plate I, Fig. 3; Plate II, Fig. 4). Centro-acinar cells are sometimes evident. In secreting groups, the inner zone of each cell is filled with acidophilic zymogen granules of varying size, while the outer basophilic portion contains the nucleus (Plate I, Fig. 3). Although the ducts were not traced completely, intercalary and secondary ducts are seen in certain regions (Plate II, Fig. 5). The main pancreatic duct, together with the bile duct, opens on a common papilla in the duodenum (Plate II, Fig. 6). Microscopically, some acinar tissue occurs in the folds of the liver, along the hepatic artery, portal vine, hepatic ducts and surrounding the endocrine lobe (Plate II, Figs. 5 and 6; Plate III, Fig. 7). In some regions, acinar tissue adheres to the serosa of the intestine.

The endocrine part of the pancreas is a single encapsulated organ situated in the region between the liver and the duodenum, at the level of the bile duct, Plate III, Fig. 7). The gland is highly vascular and the cells, when treated by Gomori's method, vary from a basophilic to an eosinophilic condition, the former reaction predominating (Plate III, Fig. 8).

STRUCTURE AND GROWTH OF THE ADENOCARCINOMA OF THE PANCREAS.

The neoplastic growth of the pancreas in the hybrid platyfish was identified as an adenocarcinoma of the acinar type. The major part of the growth formed a solid mass between the folds of the intestine (Plates IV and V); groups of acini were scattered in surrounding regions. The main mass and region of most proliferation was found towards the posterior end of the gut, ventral in position (Plate V, Figs. 13 and 14). Only a mild inflammatory reaction was present but necroses and haemorrhages were extensive in many areas (Plate V, Figs. 13 and 14); Plate VI, Fig. 16).

The tumor was massive and destructive to surrounding tissues (Plate V, Figs. 13 and 14) but no metastases were found. The primary and secondary ducts were obliterated by the neoplasm, but in regions where normal acini were present, intercalated elements were evident. The growth also penetrated the serosa and invaded the *muscularis* of the intestine in certain regions (Plate V, Fig. 12). Both the endocrine part of the pancreas and the testis were wanting. It is assumed that these structures were completely destroyed by the tumor, since the greatest proliferation of neoplastic cells was found in the regions where these organs are normally situated. The kidneys in the diseased

fish showed hydropic degeneration. This organ, the liver and the spleen, contained large amounts of haemosiderin.

Histologically, the growth consisted of acinar elements supported by a delicate vascular reticulum. An increase in connective tissue was found in certain areas (Plate VII, Fig. 18; Plate VIII, Fig. 20). In the anterior region of the body, at the level of the stomach, a few normal-appearing acini were present. At the level of the duodenum, the gland had a compact appearance (Plate IV, Fig. 9), which under high magnification revealed numerous degenerated acinar cells, closely packed but not arranged in acini (Plate VIII, Fig. 22). In more distal parts of the growth (Plate VI, Fig. 17), the cells showed evidence of considerable proliferative activity, with varying degrees of anaplasia. Some areas showed distinct lobule formation in which there seemed to be some attempt to form acini (Plate VII, Fig. 18). In most regions, however, the growth had a disorganized appearance with many isolated cells (Plate VII, Fig. 19). These were comparatively small, spherical or oval, but contained typical nuclei. Only a few mitotic figures were found. The distinct polarization of the cytoplasm, showing the basophilic basal zone and acidophilic inner zone, was lacking (Plate VII, Fig. 18; Plate VIII, Fig. 20); instead, the entire cell was filled with zymogen granules of various sizes, resulting in an overall acidophilic appearance.

The surrounding regions showed extensive areas filled with secretion-like coagulum (Plate V, Figs .13 and 14; Plate VI, Fig. 15), probably resulting from the liquefaction of the released zymogen granules (Plate VIII, Fig. 21) or from gelatinous changes of the tissues.

Discussion.

Schlumberger & Lucké (1948) pointed out that "Malignant tumors of gland-cell origin are the predominant cancers in man. By contrast, but few examples, 7 in all, have hitherto been reported in fishes." They noted, however, that "This fact does not permit us to conclude that this kind of cancer is uncommon in fishes; it may mean that an adequate search has not yet been made. This supposition is the more plausible because most adenocarcinomas originate in the viscera, and not on the body surface as do the epitheliomas." According to these authors, adenocarcinomas of the kidney, ovary, rectal gland, thyroid and the glandular elements of the skin, mouth and operculum have been described in fishes. Tumors of the pancreas have not been previously reported for either fishes or amphibians. Ratcliffe (1935, 1943) described several cases of a neoplastic-like disease of the pancreas in more than a dozen species of snakes, but he has recently (in Lucké & Schlumberger, 1949) questioned his previous interpretation.

The majority of pancreatic tumors of man (Ewing, 1940; Willis, 1948) and other mammals (Fox, 1923; Kresky & Barnett, 1939) usually arise from the epithelial elements of the pancreatic ducts or from the islands of Langerhans. Tumors in which acinar elements are involved are often difficult to recognize because of metaplastic changes. The cell morphology may be so completely altered that the presence of zymogen granules or enzymes is the only evidence on which to base the origin of the growth.

In the pancreatic tumor found in the hybrid platyfish, the growth occurred in a region which may correspond to the tail of the pancreas in mammals. The acinar elements are more or less typical but are irregularly arranged and the cells, often isolated, lack the characteristic polarization of the zymogen granules seen in normal pancreatic tissue.

No evidence was obtained which would indicate the cause of this pancreatic tumor. Although the genetic history of the fish involved is given above, hereditary factors as the cause of the tumor are not implied.

It has been suggested that inflammatory changes may often be the cause of human pancreatic carcinomas. Willis (1948), however, stated that "Inflammatory changes in the pancreas accompanying cancer are in most cases clearly secondary to duct obstruction caused by the growth." In this connection, the fat necrosis of the pancreas described by Plehn (1939) in certain Chinese carp kept in captivity is of interest. The gland in these fish was not only present as a typical structure, but in addition pancreatic elements were widely scattered in the mesentery as small aggregations and as isolated cells. Inflammatory reactions were accompanied by granulation tumors and infiltrative cancer-like growths. The secretion from the gland-like aggregations destroyed the fat tissue and blood vessels of the surrounding regions. Plehn concluded that conditions of captivity and over-feeding were probably responsible for this disease. It is probable, however, that the inflammatory changes were secondary to the cancer-like growths, as suggested by Willis for mammalian carcinomas.

The isolated aggregations of pancreatic cells seen by Plehn in diseased carp occur more or less frequently in normal fishes. Boldyreff (1935) and others have shown that the amount and distribution of normal acini in fishes vary considerably with the species and, from our own observations, even with individuals of the same species. In fishes the pancreas appears to be a highly plastic organ, and it is our belief that a more intensive study of the gland in these animals might reveal a higher incidence of hyperplasia and neoplasia. In addition, such a study would have phylogenetic importance since it would perhaps provide evidence as to the origin of the aberrant or heterotopic pancreatic tissue often encountered in humans which, according to Ewing (1940), probably gives rise to certain localized carcinomas of this gland.

SUMMARY.

An acinar type of adenocarcinoma of the pancreas in a 16-months-old, male hybrid platyfish, *Platypoecilus variatus* × *Platypoecilus xiphidium*, is described. No metastases were found, but there was evidence of invasion and destruction of the surrounding tissues. The comparative pathology of pancreatic tumors in vertebrates, including man, is discussed.

REFERENCES.

BOLDYREFF, EPHRAIM B.

1935. A Microscopic Study of the Pancreas in Fishes; Especially Those of the Orders Haplomi and Cyprinodontes. *Copeia*, **1935**: 23-34.

EWING, JAMES.

1940. Neoplastic Diseases. W. B. Saunders, Philadelphia. xii + 1160 pp.

FOX, HERBERT.

1923. Disease in Captive Wild Animals and Birds. J. B. Lippincott, Philadelphia. vii + 665 pp.

KRESKY, PHILIP J. & ROY N. BARNETT.

1939. Carcinoma of the Pancreas of Acinar Origin in a Bear. *Zoologica*, **24**: 285-288.

LUCKÉ, BALDUIN & H. G. SCHLUMBERGER.

1949. Neoplasia in Cold-blooded Vertebrates. *Physiol. Rev.*, **29**: 91-126.

PLEHN, MARIANNE.

1938. Pankreas-Fettnekrose bei karpfenartigen Fischen (Cypriniden). *Virchow's Arch. f. Path. Anat. u. Physiol.*, **302**: 9-38.

RATCLIFFE, HERBERT L.

1935. Carcinoma of the Pancreas in Say's Pine Snake, *Pituophis sayi*. *Amer. J. Cancer*, **24**: 78-79.

1943. Neoplastic Disease of the Pancreas of Snakes (Serpentes). *Amer. J. Path.*, **17**: 359-369.

REITER, GLADYS & ROSS F. NIGRELLI.

1949. The Morphology and Histology of the Pancreas of the Platyfish, Platypoecilus maculatus. *Anat. Rec.*, **105**: 130-131. (Abstract).

SCHLUMBERGER, H. G. & BALDUIN LUCKÉ.

1948. Tumors of Fishes, Amphibians and Reptiles. *Cancer Res.*, **8**: 657-753.

WILLIS, R. A.

1948. Pathology of Tumours. C. V. Mosby, St. Louis. xxiii + 992 pp.

EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. A normal brother and sister of the male *Platypoecilus xiphidium* × *Platypoecilus variatus* hybrid which developed a pancreatic tumor. The female, to the left, has the spotted pattern (*Sp* gene) and the cut-crescent pattern (*Ct* gene). The male, to the right, is unspotted (*sp* or + gene) and has the crescent pattern (*C* gene). Life size. Photographed by S. C. Dunton.

Figures 2-8 are photomicrographs of sections of normal *Platypoecilus maculatus*, showing the distribution and relations of the pancreas to surrounding organs.

Fig. 2. Pancreas at the level of the spleen and duodenum. The light areas in the gland are fat cells and blood vessels. Delafield's haematoxylin-eosin. 50 ×.

Fig. 3. Groups of acini showing structure and arrangement typical of vertebrate pancreas. Note nucleus in darker basophilic zone and zymogen granules in lighter inner zone. Delafield's haematoxylin-eosin. 2500 ×.

PLATE II.

Fig. 4. Acinar groups stained with Gomori's chrome alum haematoxylin-phloxin. Note centro-acinar cell in upper group. 2500 ×.

Fig. 5. Section at the level of the gall bladder and duodenum, showing typical pancreatic tissue around blood vessel. Note secondary ducts in cross section. Masson's. 1500 ×.

Fig. 6. Primary duct, together with the bile duct opening on a papilla in the duodenum. The dark-staining areas are groups of acinar tissue surrounding blood vessels in the region of the liver. Giemsa's. 750 ×.

PLATE III.

Fig. 7. Endocrine gland or islet tissue of the pancreas. This encapsulated structure is found at the level of the gall bladder. Masson's. 750 ×.

Fig. 8. Details of the cellular elements of the islet tissue. The cells grade in staining reaction from a basophilic to an eosinophilic condition, with the former predominating. Gomori's chrome alum haematoxylin-phloxin. 2000 ×.

Figures 9-22 are photomicrographs of sections of pancreatic tumor in a male hybrid, *Platypoecilus variatus* × *Platypoecilus xiphidium*.

Plate IV.

Fig. 9. Section at the level of the posterior part of the liver, showing a massive, encapsulated-appearing tumor. For details see Plate VIII, Fig. 22. Delafield's haematoxylin-eosin. 25 ×.

Fig. 10. Pancreatic tumor at the level of the mid-intestinal region. Note associated effects (atrophy) on the intestine. Delafield's haematoxylin-eosin. 25 ×.

Fig. 11. Pancreatic tumor at the level of the large intestine. Delafield's haematoxylin-eosin. 25 ×.

Plate V.

Fig. 12. Details showing infiltration into the *muscularis* of the intestine. In normal fish pancreatic tissue may adhere to the serosa. Masson's. 2000 ×.

Fig. 13. The main mass and region of most proliferation was found in the region ventral to the large intestine. Note extensive areas with secretion-like coagulum. Delafield's haematoxylin-eosin. 25 ×.

Fig. 14. Another section similar to the one illustrated in Fig. 13, showing effects on surrounding connective tissue. Delafield's haematoxylin-eosin. 25 ×.

Plate VI.

Fig. 15. Higher magnification of region at the periphery of the coagulum-like secretion, showing active secreting acinar elements. Giemsa's. 300 ×.

Fig. 16. Area within the pancreatic tumor, showing an extensive haemorrhage. Delafield's haematoxylin-eosin. 75 ×.

Fig. 17. Low power magnification of a central region of the pancreatic tumor, showing a compact but irregular arrangement. Delafield's haematoxylin-eosin. 500 ×.

Plate VII.

Fig. 18. Higher magnification of an area shown in Fig. 17, showing the arrangement of the acinar elements. Delafield's haematoxylin-eosin. 2000 ×.

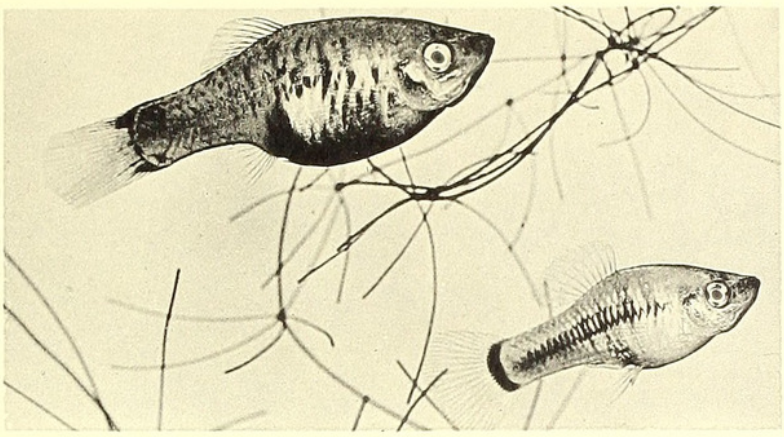
Fig. 19. Another area of the region shown in Fig. 17, showing isolated acinar cells with little or no zymogen granules. Delafield's haematoxylin-eosin. 2000 ×.

Plate VIII.

Fig. 20. Cytological details of cells of the pancreatic tumor. Note variability in size, loss of typical acinar arrangement, loss of polarization of cytoplasm. The nuclei are typical. Delafield's haematoxylin-eosin. 5000 ×.

Fig. 21. Area showing numerous zymogen granules released into surrounding region. Iron-haematoxylin. 1500 ×.

Fig. 22. Details, showing degenerate cells in the tumor mass shown in Fig. 9. Delafield's haematoxylin. 2500 ×.



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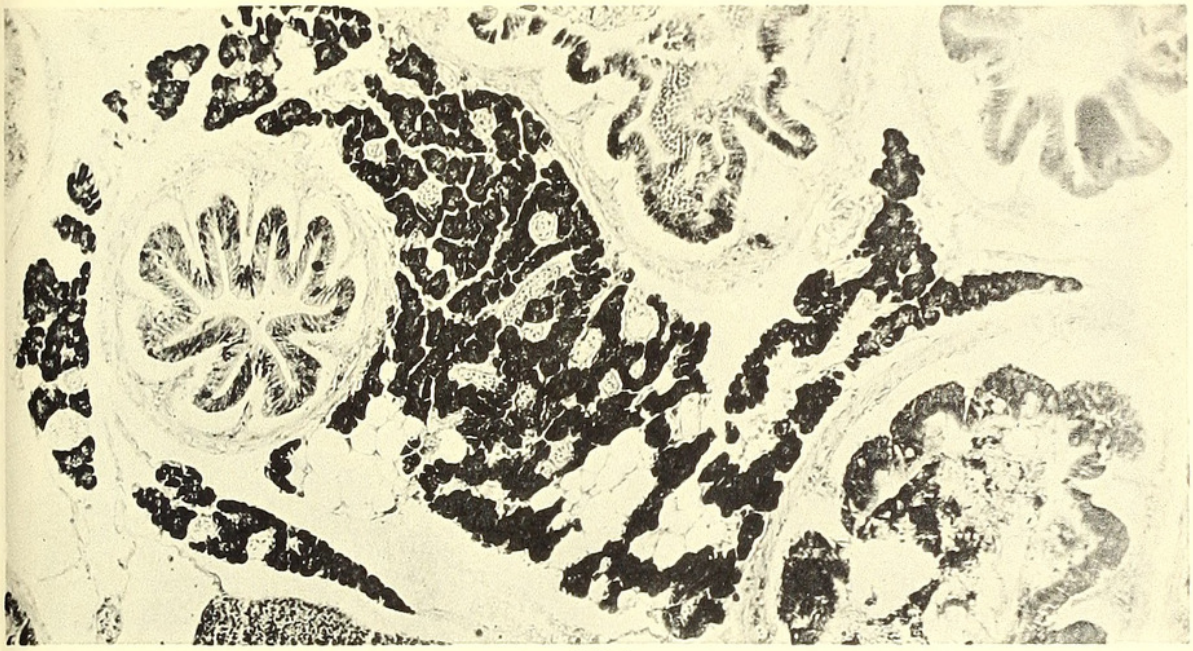

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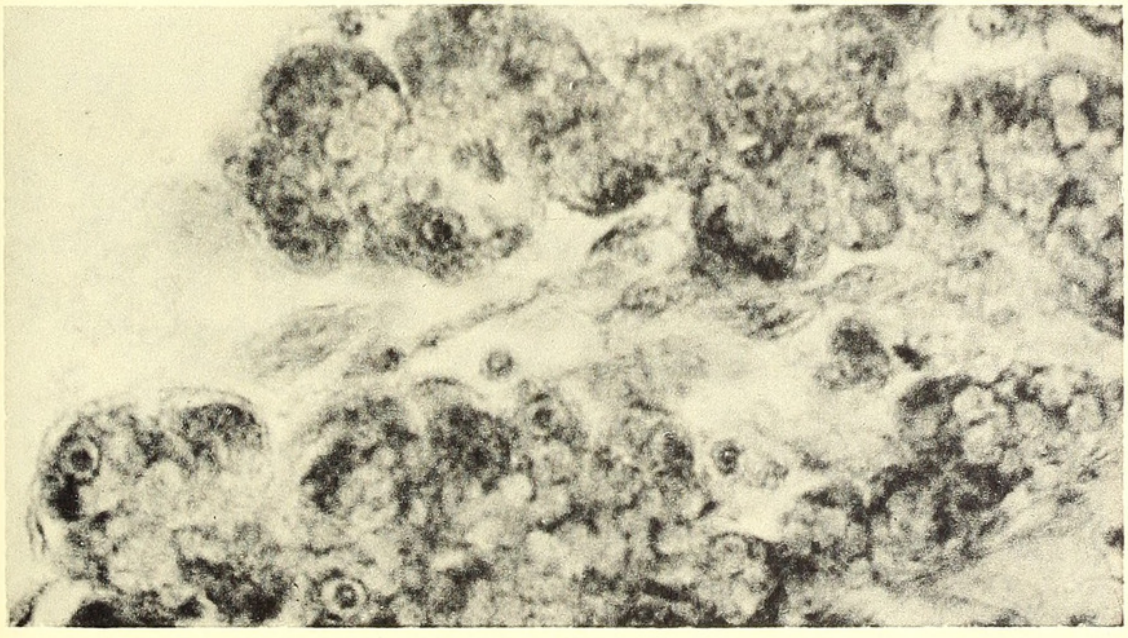
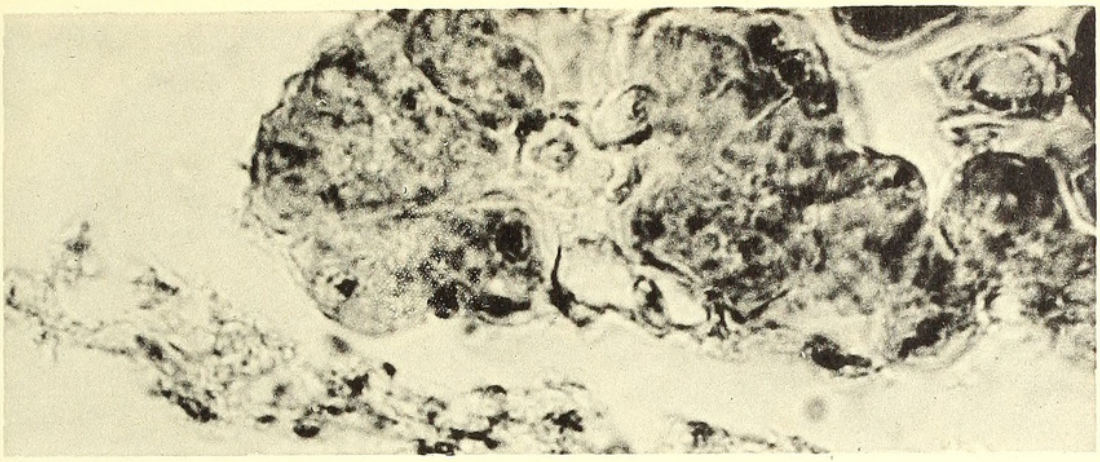


FIG. 3.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.



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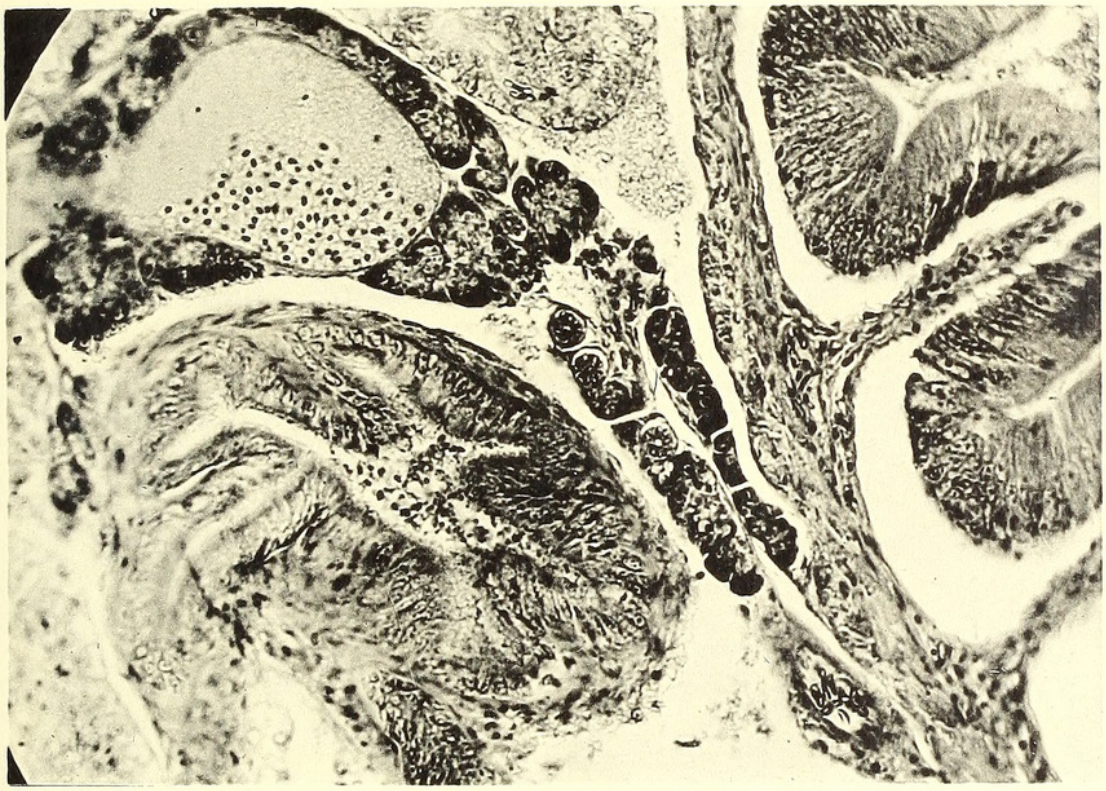

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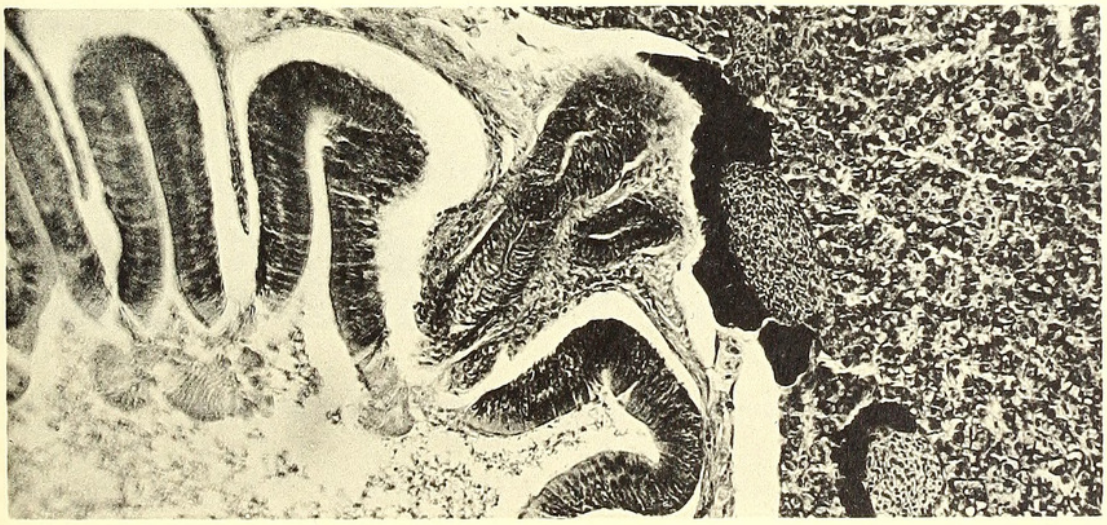
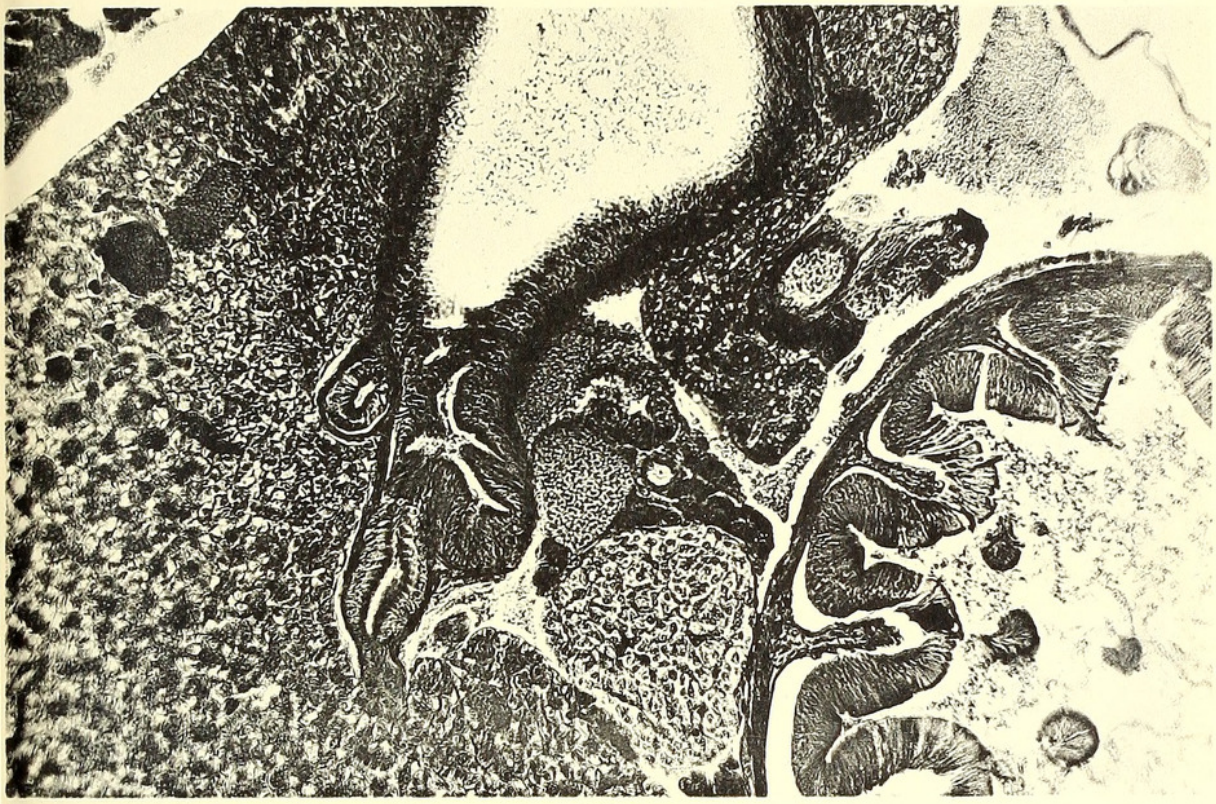


FIG. 6.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.



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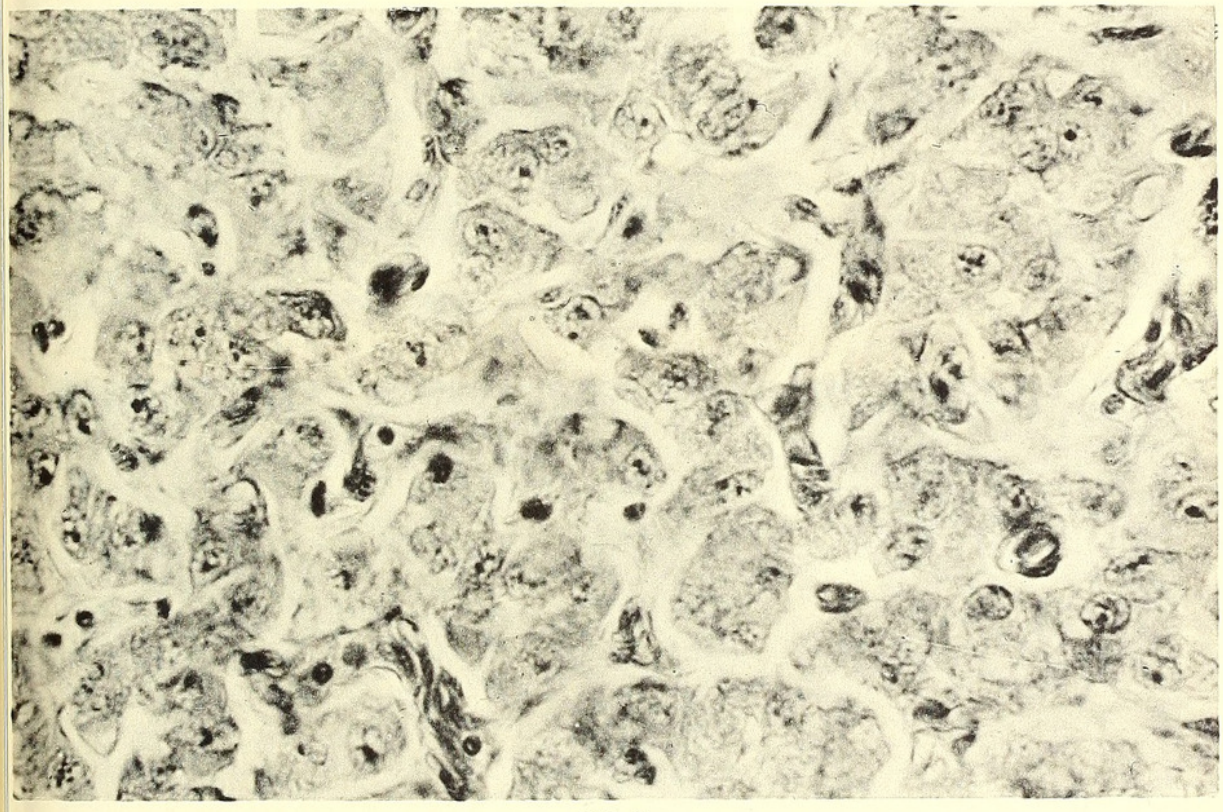
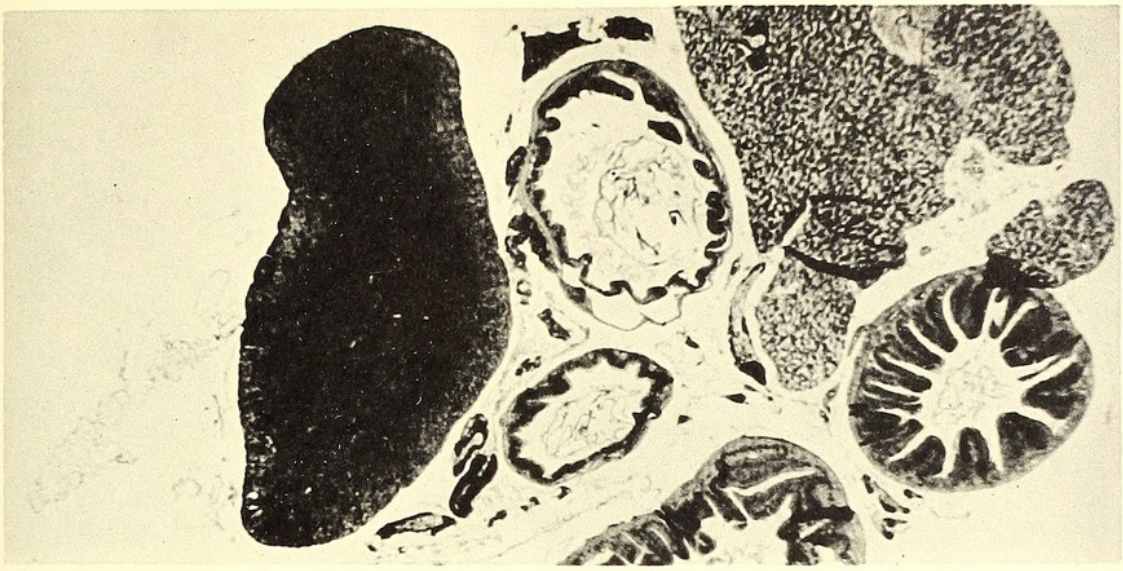


FIG. 8.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.



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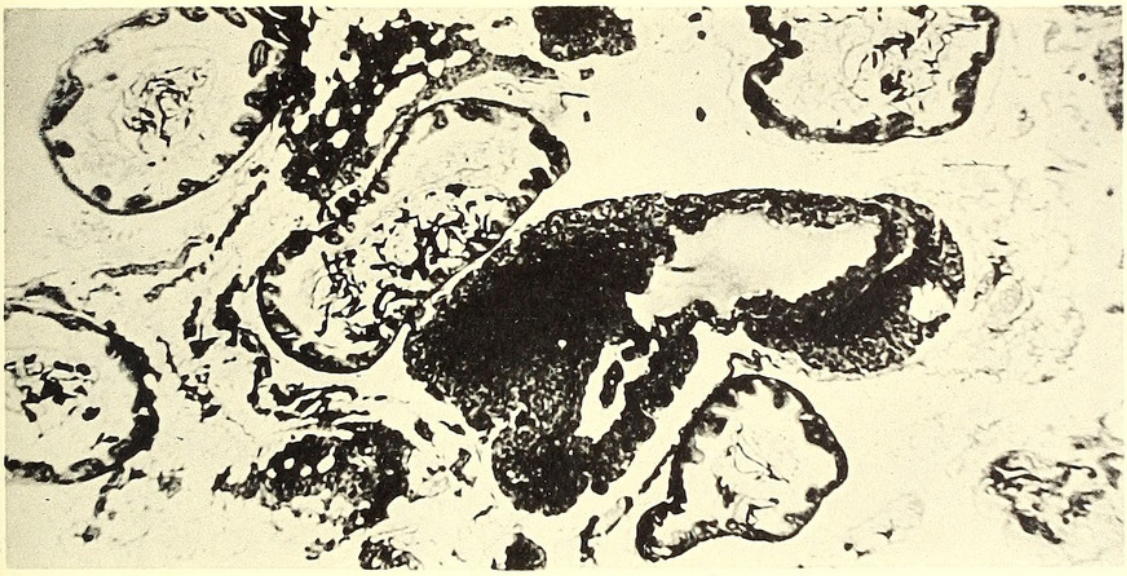

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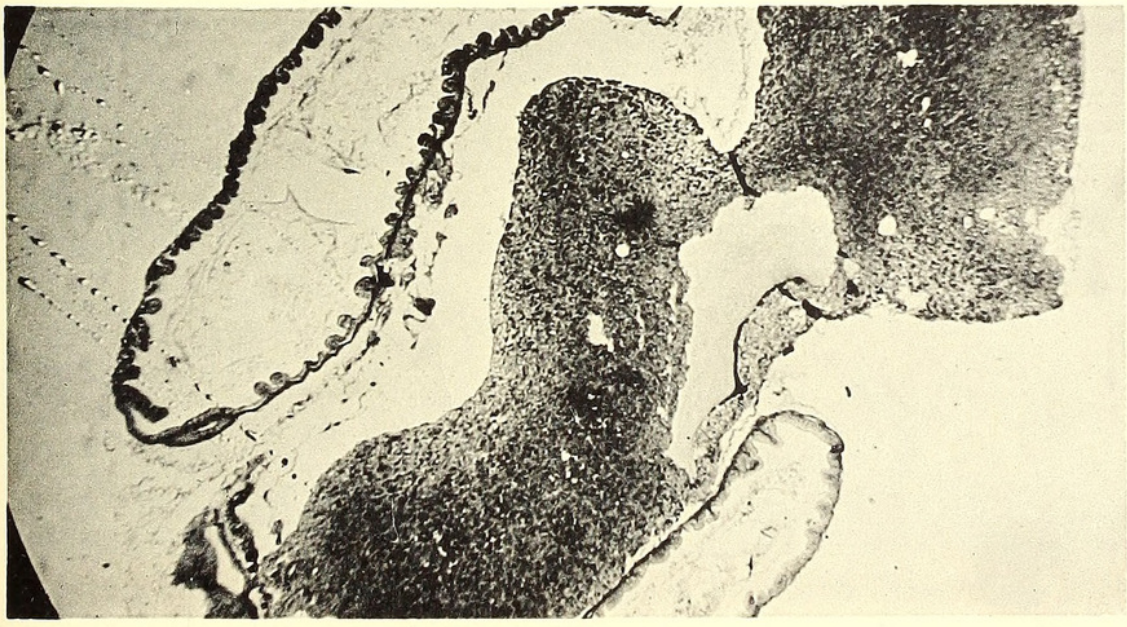
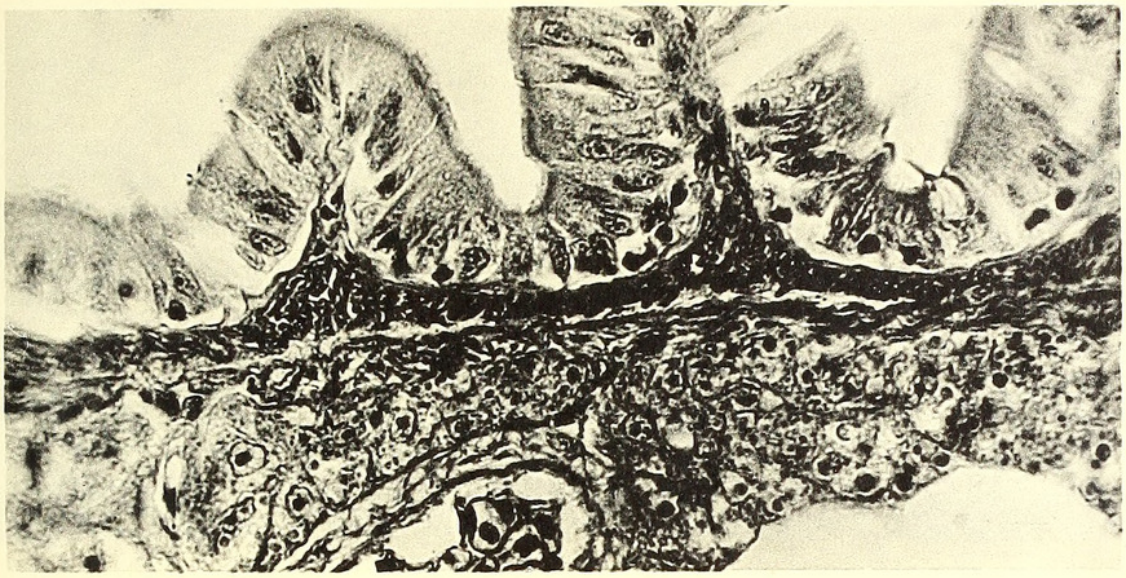


FIG. 11.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.



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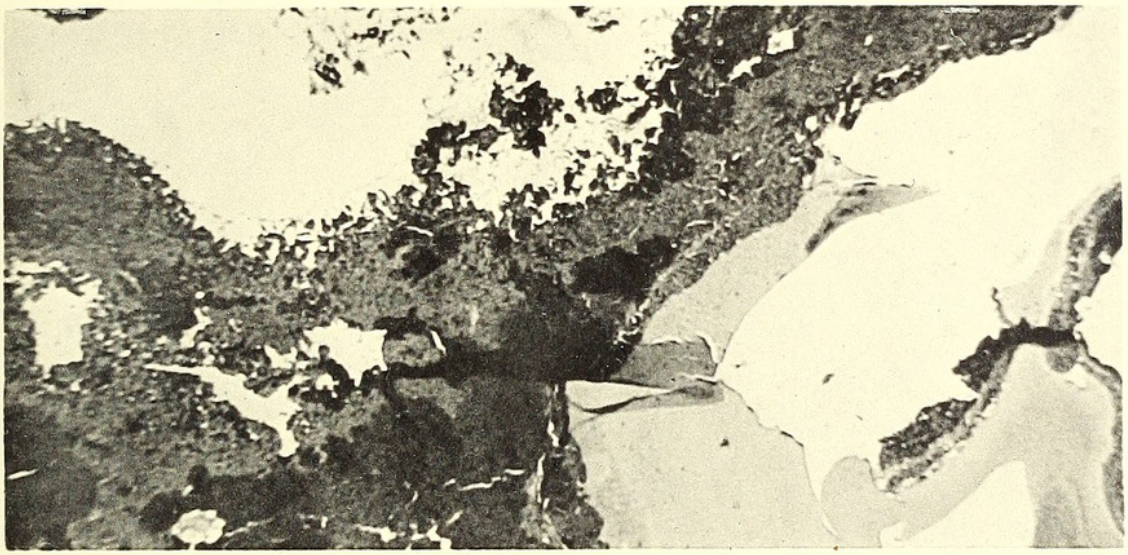

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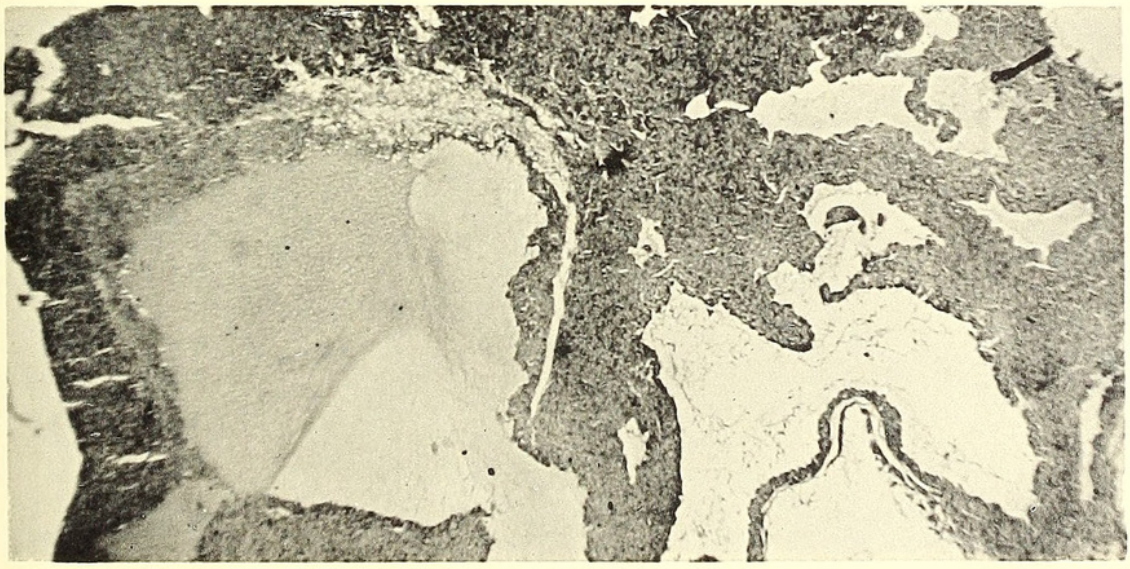
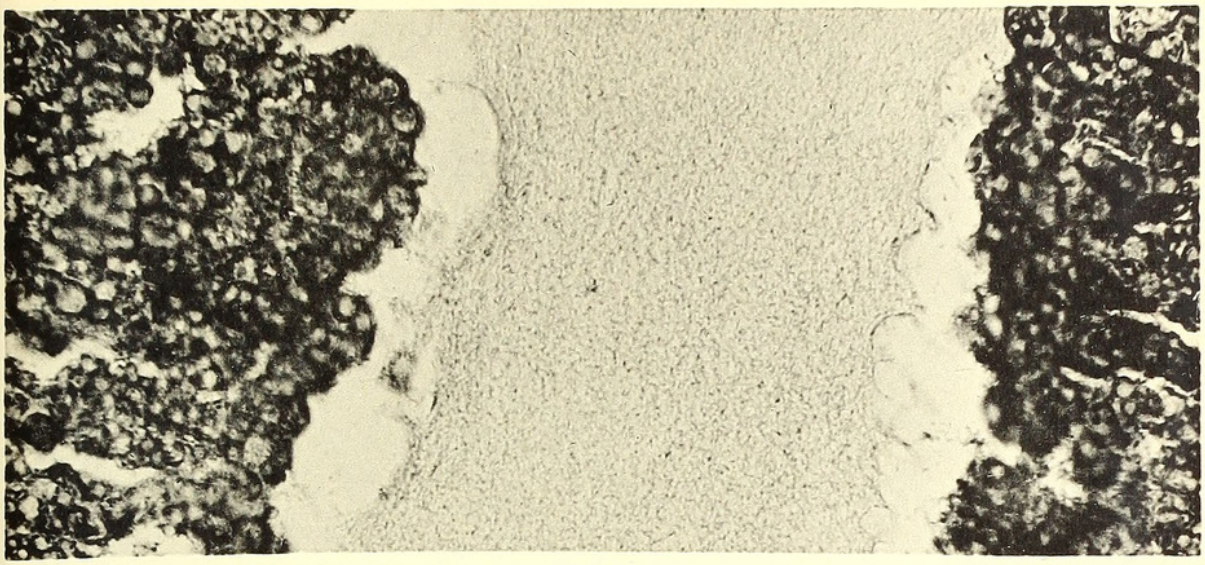


FIG. 14.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.



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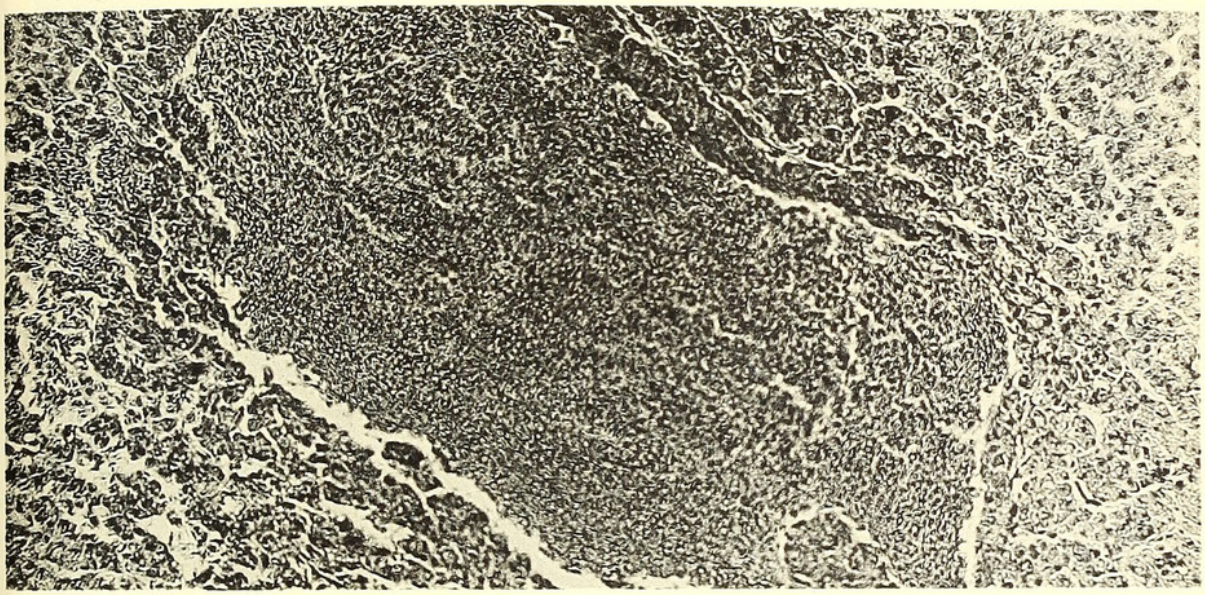

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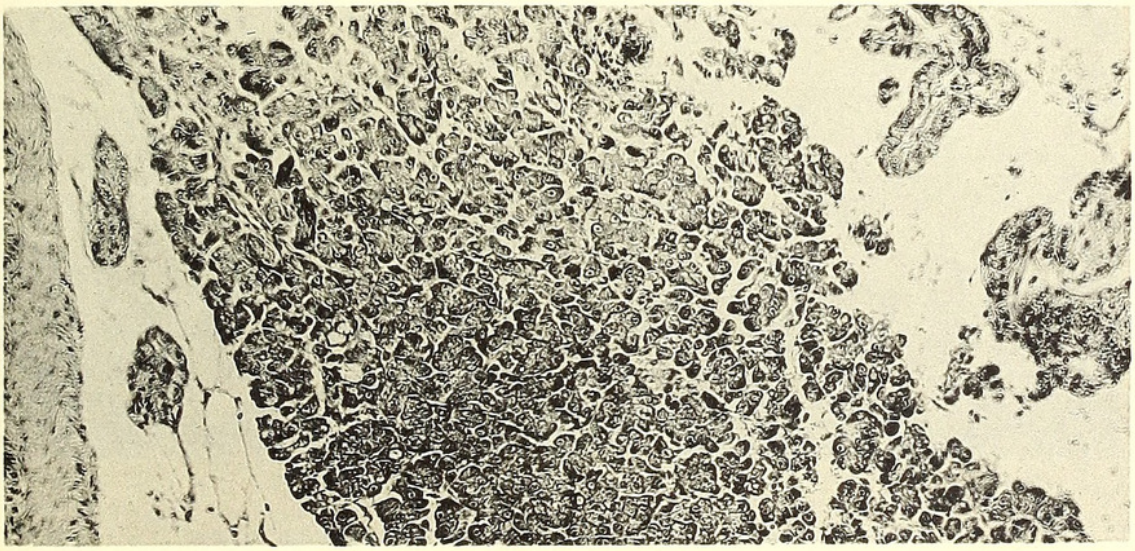
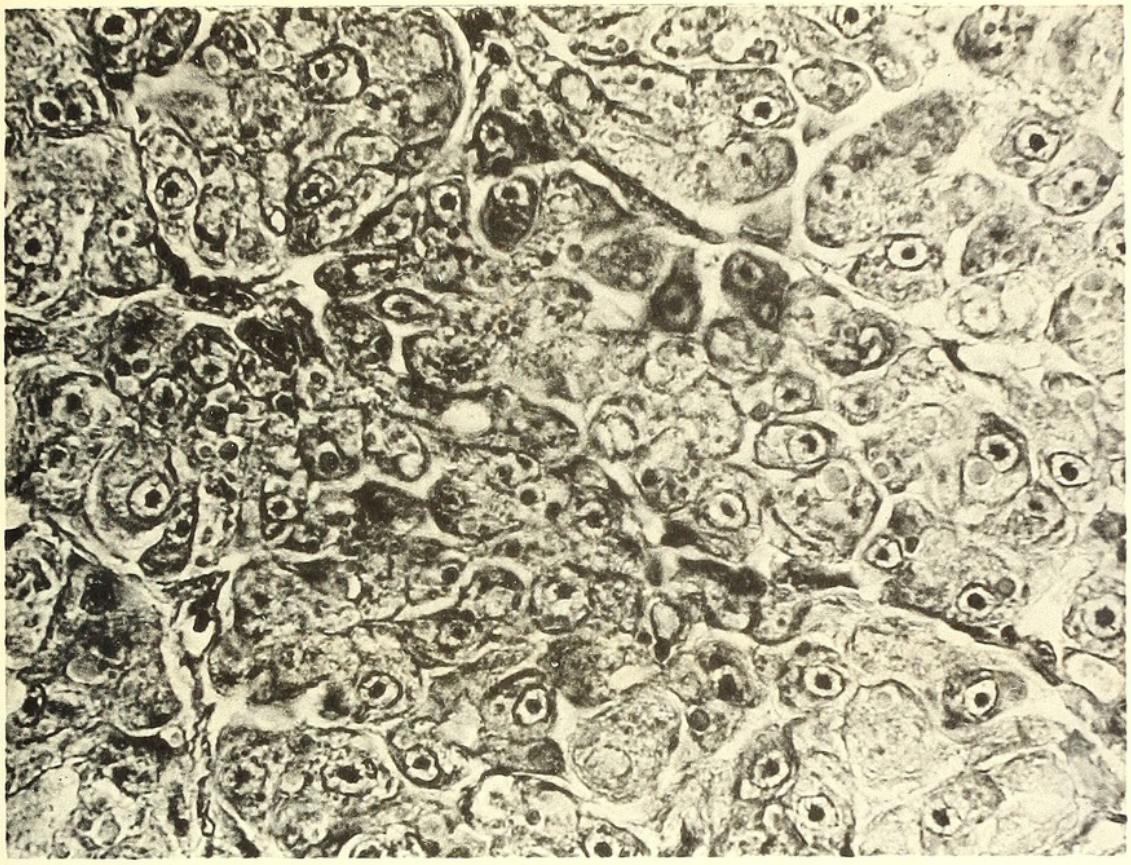


FIG. 17.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.



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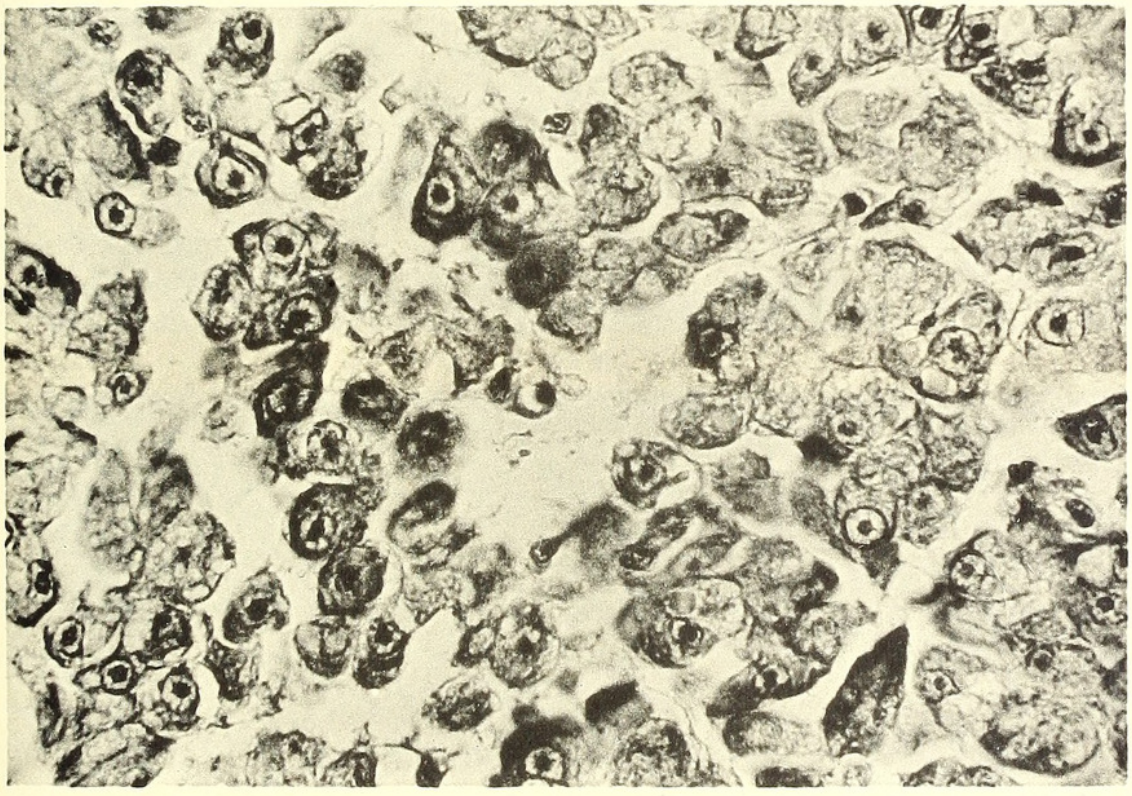

FIG. 19.

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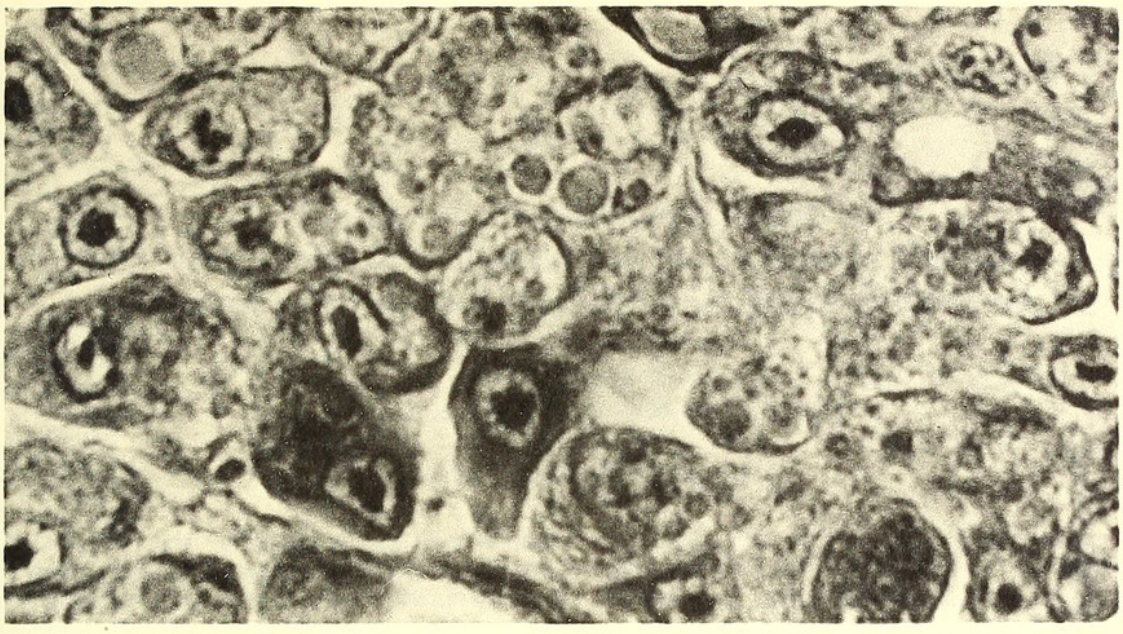

FIG. 20.

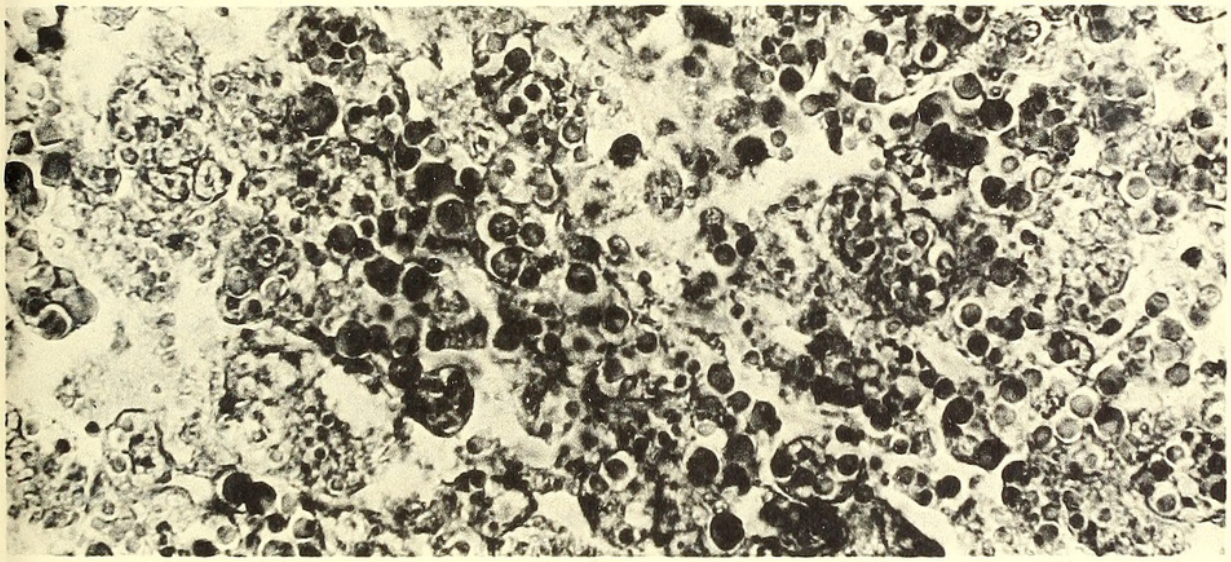

FIG. 21.

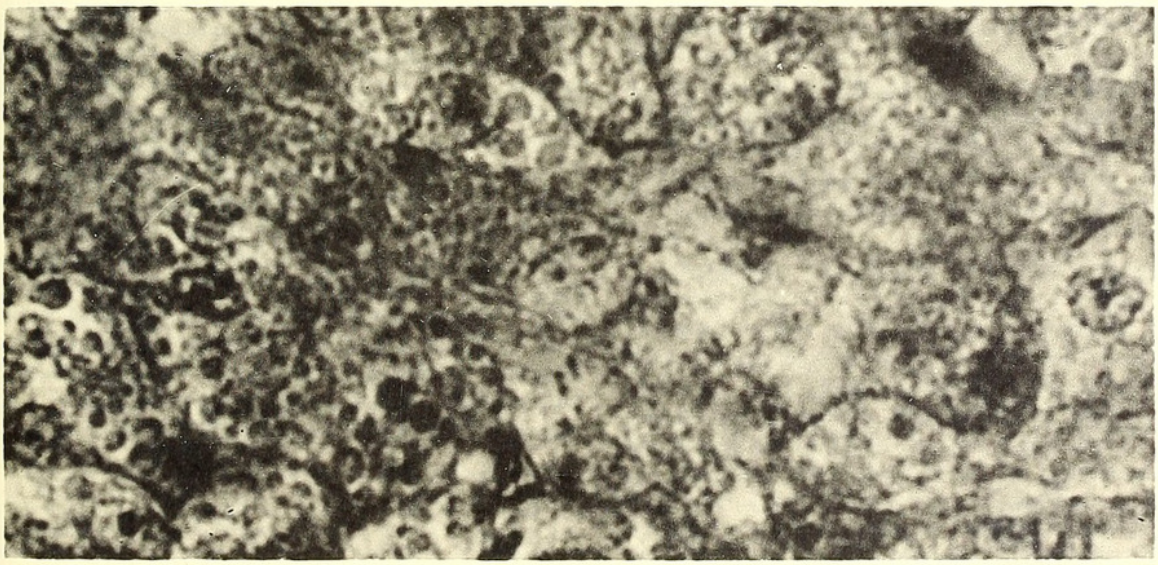

FIG. 22.

ACINAR ADENOCARCINOMA OF THE PANCREAS IN A HYBRID PLATYFISH.